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Editorial

Invitación a participar en la reunión de la Asociación para el Desarrollo de Cultivos Industriales (AAIC) en Termas de Chillán (Chile)

[Invitation to participate in the meeting of the Association for the Advancement of Industrial Crops (AAIC) in Termas de Chillán (Chile)]

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La Asociación para el Desarrollo de Cultivos Industriales (AAIC, por su siglas en ingles *Association for the Advancement of Industrial Crops*) es una organización internacional sin fines de lucro, fundada en Diciembre de 1988 con el objetivo de promover la investigación y desarrollo de los cultivos industriales. La AAIC está compuesta por miembros provenientes principalmente de universidades (40%), organismos gubernamentales (32%), sector privado (19%), entre otros. La asociación cuenta en la actualidad con cinco divisiones. (Fibras y Celulosa, Cultivos Generales y sus Productos, Gomas Naturales y sus Resinas, Oleaginosas, y Plantas Medicinales y Nutraceuticas), sirviendo en varios países (Argentina, Canadá, Chile, España, Estados Unidos y México).

Los objetivos de la AAIC son: 1) promover las actividades de producción, procesamiento, desarrollo y comercialización de los cultivos industriales; 2) educar e informar a sus miembros y aquellos interesados en los cultivos industriales a través de una *newsletter* cuatrimestral, reuniones internacionales, y su sitio de internet (www.aaic.org); 3) proveer al publico general, la industria, y el sector político con información experta sobre los aspectos científicos, ingenieriles, y de negocios de los productos industriales que derivan de la agricultura y la horticultura.

Desde sus inicios, la AAIC albergó a científicos latinoamericanos interesados en los cultivos industriales. Así, en 1995 la Asociación Latinoamericana de Jojoba y Nuevos Cultivos (ALAJO) se unió a la AAIC a través de la entonces "División de la Jojoba". Desde que se iniciaron las reuniones científicas en el 1989, tres reuniones han sido realizadas en países hispano parlantes, Argentina (1994), México (1997) y España (2005).

La división de Plantas Medicinales y Nutracéuticas (www.aaic.org/medicinal.htm), se inició a fines del 2005, para promover las actividades en los productos derivados de plantas medicinales, aromáticas y nutracéuticas. Con el objeto de incrementar los lazos con países latinoamericanos, esta división se ha asociado con el Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas (BLACPMA) para promover actividades de investigación y desarrollo en este grupo particular de plantas y sus productos.

Los días 14 al 19 de Noviembre del 2009, la AAIC tendrá su reunión anual en Termas de Chillán, Chillán, Chile. Nuestra división tendrá un día entero dedicado a las plantas medicinales y nutracéuticas. Hay varias razones para participar en esta interesante reunión.

En Latinoamérica, así como también a nivel mundial, hay un creciente interés en productos naturales derivados de plantas, lo que está generando un mercado multimillonario con impacto en el desarrollo de la región. Los países latinoamericanos tienen una rica tradición en el uso de plantas medicinales, tanto en medicina popular como en productos comerciales. La región cuenta con numerosas universidades e instituciones de investigación, que convierten a Latinoamérica en un líder en investigación y desarrollo en este campo. La región tiene un fuerte sector agrícola, y una gran diversidad de recursos naturales, que hacen de Latinoamérica un productor importante de productos que derivan de plantas medicinales, aromáticas, y nutraceuticas.

El orador principal en este día, será Mark Blumenthal, el fundador y director del *American Botanical Council (ABC)*. Blumenthal ha desempeñado un papel clave en la industria de

productos naturales en los Estados Unidos de América. ABC es una organización progresiva e independiente, cuyo objetivo es proveer a los consumidores, miembros del sector privado, científicos, educadores y medios de difusión con información confiable sobre los usos de plantas medicinales y aromáticas (www.herbalgram.org). ABC publica trimestralmente la revista Herbalgram, con información científica sobre estas plantas, una *newsletter* mensual (herbalClip) y un servicio bimensual de revisión de literatura con reciente información sobre los usos medicinales de estas plantas.

Por todas estas razones, estamos esperando una reunión exitosa en Termas de Chillan, con gran participación del sector público y privado. Por tal motivo, la AAIC y en particular la división de Plantas Medicinales y Aromáticas, invitamos a todos aquellas personas interesadas en el campo de las plantas medicinales a participar en esta interesante reunión en la pintoresca ciudad del sur de Chile. Para más información sobre la AAIC y la reunión en Termas de Chillán lo invitamos a visitar el sitio de internet de la asociación (www.aaic.org/2009_meeting.htm).

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Artículo Original | Original Article

Treatment of bronchial asthma with an aqueous extract of *Mangifera indica* L. (VIMANG®): two cases report

[Tratamiento del asma bronquial con un extracto acuoso de *Mangifera indica* L. (Vimang®): dos reportes de casos]

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Abstract

A case report was carried out in two asthmatic patients treated with a new health natural product named Vimang®, an aqueous extract of *Mangifera indica* L stem bark, which has been registered as antioxidant and anti-inflammatory. A 39 years-old female with persistent moderated asthma and a 43 years-old male with persistent severe asthma were treated orally with Vimang® (capsule 300 mg, one every 8 h) during three months. Respiratory functional tests and determination of total serum immunoglobulin E (IgE), eosinophil cationic protein (ECP) and metalloproteinase-9 (MMP-9) activity were done at times 0, 6 and 12 weeks, respectively. An improvement in the volume of force expiration (FEV1) was observed for both patients. The values of IgE, ECP and the enzymatic activity of MMP-9 decreased also for both patients. These results could open the way to further clinical researches about the use of Vimang® as an alternative for the treatment of bronchial asthma.

Keywords: Mangifera indica*; Vimang; Asthma; IgE; Eosinophil cationic protein; Force expiration volume.*

Resumen

Un reporte de caso fue realizado en dos pacientes asmáticos tratados con un nuevo producto natural de salud llamado Vimang®, un extracto acuoso de la corteza de *Mangifera indica* L. que ha sido registrado como antioxidante y antiinflamatorio. Una mujer de 39 años con asma moderada persistente y un hombre de 43 años con asma severa persistente fueron tratados por vía oral con Vimang® (cápsulas 300 mg, una cada ocho horas durante tres meses). En las semanas 0, 6 y 12 se realizaron pruebas funcionales respiratorias y determinaciones de inmunoglobulina E total en suero (IgE), proteína catiónica de eosinófilo (ECP) y la actividad de metaloproteinasa-9 (MMP-9). En ambos pacientes se observó una mejoría en el volumen de espiración forzada (FEV1). Los valores de IgE, ECP y la actividad de MMP-9 decrecieron en ambos pacientes. Estos resultados podrían sentar las bases para la realización de más investigaciones clínicas acerca del uso del Vimang® como una alternativa para el tratamiento del asma bronquial.

Palabras Clave: Mangifera indica; *Vimang; Asma; IgE; Proteína catiónica de eosinófilo; Volumen espiratorio forzado.*

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INTRODUCTION

Vimang® is the brand name of formulations from an aqueous extract of the stem bark of selected varieties of *Mangifera indica* L. (Anacardiaceae) traditionally used in Cuba for its anti-inflammatory, analgesic, antioxidant and immunomodulatory properties (Garrido et al., 2007). Chemical studies performed with this extract have enabled the isolation and identification of phenolic acids, phenolic esters, flavan-3-ols, mangiferin, which is the predominant component of this extract, and micronutrients as selenium (Nuñez-Sellés et al., 2002). Also, acute and chronic toxicology studied have demonstrated the safety of the extract (Gámez et al., 2002).

Recently, it was demonstrated Vimang® has antiallergic properties through the inhibition of IgE production, histamine release from rat mast cells and lymphocyte proliferative response (Rivera et al., 2006). Also, Vimang® reduces eosinophil migration into bronchoalveolar space and peritoneal cavity, and eosinophil generation in bone marrow and blood during an asthma murine model. This reduction was associated with inhibition of IL-5 production in serum and eotaxin in lung homogenates (Sa-Nunes, et al., 2006).

According to these experimental evidences, the aim of the present investigation was to describe the effects of Vimang® in the treatment of two patients with different asthma severity.

MATERIALS AND METHODS

Cases presentation

Two asthmatic patients were chosen. The patients were classified according to the Global Strategy for Asthma Management and Prevention (GINA, 2006). Their clinical conditions were as follow:

Persistent Moderated Asthmatic Patient: 39 years-old female. Standard treatment: salmeterol and fluticasone spray (Seretide® 25/50 µg) 3 puffs daily. Allergic potential: presence of animals, dust and perfumes. Symptoms included dry coughing and breath shortening.

Persistent Severe Asthmatic Patient: 43 years-old male. Standard treatment: salmeterol and fluticasone spray (Seretide® 25/50 µg) 3 puffs daily. Allergic potential: dust. Symptoms included repetitive breath shortening and pronounced dry coughing.

Capsules: Vimang® capsules were prepared with 300 mg of dry extract obtained from a standard *Mangifera indica* L. stem bark. This extract was prepared by decoction with water for 1 h and then it was concentrated by evaporation and spray-dried to obtain a fine homogeneous brown powder with a particle size of 30–60 mm. (Nuñez-Sellés et al 2002). The lot used in the study was 200402-E from Novatec Laboratory (La Habana, Cuba). The quality control analysis showed more than 50% of total polyphenols, according to the established specification.

Both patients received one capsule of Vimang®, 300 mg, every 8 h, during 3 months. Respiratory functional tests were done to both patients at times 0, 6 and 12 weeks of treatment as described. Blood samples were collected and the serum was obtained and stored at -20 ºC until the determination of IgE, ECP and MMP-9.

Experimental methods

Measurement of the Force Expiration Volume in one second (FEV1): Measurement of FEV1 was done using computerized spirometers (Modular Collins G.S., USA). The treatment was considered beneficial when FEV1 values pre- and post-aerosol improved equal or superior than 10% compared to 0, 6 and 12 weeks of treatment.

Determination of serum total IgE and ECP: ECP and IgE concentrations were measured by an immunofluorescence ELISA technique according with the manufacturer instructions (ImmunoCAP, Pharmacia-Upjohn, USA).

Determination of enzymatic activity of MMP-9: The enzymatic activity of MMP-9 in blood serum was determined by gelatin zymographs (Atkinson and Senior, 2003). Briefly, each serum sample was diluted (1:10) and subjected to electrophoresis on 10% polyacrylamide SDS gels containing 1 mg/ml of porcine skin gelatin (Sigma, USA). Gelatin digestion was identified as clear zones of lysis against a blue background. Molecular weights of gelatinolytic bands were estimated using SDS-polyacrylamide gel electrophoresis (PAGE) protein standards. Gelatinolytic activity was measured on zymography-digitalized images (Bioimaging System, Syngen, Canada).

Both patients were informed about the treatment and they gave written informed consent to be included in the study. The Ethics Committee of CIMEQ Hospital (La Habana, Cuba) approved the study, and it

was carried out in accordance with the current good clinical practice guidelines (GCP).

RESULTS

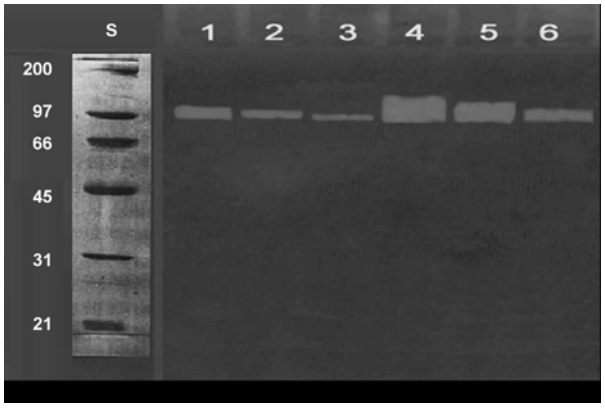
Serum concentrations of total IgE and ECP were reduced and FEV1 values were improved in both patients after Vimang® treatment (Table 1). MMP-9 activity in blood serum was also reduced (Fig. 1).

Table 1. Blood serum concentrations of IgE and ECP, MMP-9 activity and FEV1 values in asthmatic patients after Vimang® treatment.

Time (weeks)	IgE (kU/l)	ECP (ng/ml)	FEV1			
			Pre	Chg (%)	Post	Chg (%
A. Persistent moderated asthmatic patient						
0	1369	51.6	1.75	-	1.78	-
6	1174	40.4	2.23*	27	2.10	18
12	740	29.8	2.62*	50	2.23	25
B. Persistent severe asthmatic patient						
0	422	62.9	2.16	-	2.50	-
6	368	41.8	2.39 *	11	2.49	0
12	290	30.7	2.62*	21	2.74	10

* Represents the improvement of patients FEV1 with respect to the initial values. Chg- Change respect time 0. Pre and Post- values pre- and post-aerosol improved equal or superior than 10% compared to 0, 6 and 12 weeks of treatment.

Figure 1. MMP-9 activity (gelatinase) in blood serum of asthmatic patients after Vimang® treatment during three months, determined by Zymography using sera of both patients.



Lane S: protein standard, kD (inserted); Lane 1, 2, 3: persistent moderated asthmatic patient at time 0, 6 and 12 weeks, respectively; Lane 4, 5, 6: persistent severe asthmatic patient at time 0, 6 and 12 weeks, respectively.

The patient with persistent moderated asthma did not show any of the allergic symptoms as above described (to see Materials and Methods) during the 3 months of treatment with Vimang® capsules. Any other drug was administered during the study. On the other hand, the patient with persistent severe asthma didn't have acute symptoms during the treatment and he took only salbutamol at the second and fifth weeks, one time each. Significantly, the steroids never were consumed by neither the two patients during the three months of treatment. No adverse events were reported during the treatment.

DISCUSSION

In this study it was found that the treatment with Vimang® capsules (300 mg, orally, every 8 h during 3 months) of two patients with persistent moderated and persistent severe asthma improved the measured values of FEV1, IgE, ECP and MMP-9.

IgE, ECP and MMP-9 are important mediators involved in the physiopathology of bronchial asthma. IgE is the most important antibody implicated in this disease and some studies have shown the local production of IgE in allergic airway diseases (Smurthwaite and Durham, 2002). The activation of eosinophils gives rise to the extracellular release of a number of potent cytotoxic proteins such as ECP, which have been associated with the development of subacute and chronic symptoms of allergy (Venge, 2004). MMP-9 (gelatinase B, 92-kDa gelatinase) induces the migration of eosinophils, lymphocytes, neutrophils, dendritic cells and the deposition and degradation matrix (Atkinson and Senior 2003; Kelly and Jarjour, 2003)

Experimental studies in mice have demonstrated the capacity of Vimang® to reduce IgE and IL-5 production, and the maturation and migration of eosinophils (Rivera et al., 2006; Sa-Nunes et al., 2006), which support the described clinical results. Also, Vimang® has about 50% of polyphenols in its composition, and they are reported that possess *in vivo* anti-allergic activity (Cheong et al., 1998).

Recently, some evidences suggested that oxidative chemical species produced by the airway inflammatory cells plays an essential role in the pathogenesis of bronchial asthma. These results are in correspondence with other studies in which they have been utilized as alternative medicine products such as TJ-96 and Pycnogenol® (Nadeem et al., 2003). It has been also evidenced that the antioxidant endogenous capacity is decreased in asthmatic patients. Therefore, Vimang®, which has shown a high antioxidant capacity besides its anti-inflammatory effects (Martinez et al., 2000; Garrido et al., 2007), could be

an alternative or complementary antioxidant therapy for the treatment of asthmatic patients.

CONCLUSION

Both asthmatic patients included in this study reduced the total IgE and ECP concentrations, and MMP-9 activity in blood serum and improved the respiratory function (FEV1) after treatment with Vimang® capsules during 3 months avoiding the use of steroids. These evidences could open the way to further clinical trials about the use of Vimang® as an alternative or complementary therapy for the treatment of bronchial asthma.

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REFERENCES

Atkinson JJ, Senior RM. 2003. Matrix metalloproteinase-9 in lung remodeling. Am J Respir Cell Mol Biol 28:12-24

Cheong H, Ryu SY, Oak MH, Cheori SH, Yoo GS, Kim KM. 1998. Studies of structure activity relationship of flavonoids for the antiallergic actions. Arch Pharmacol Res 21:478-480

Gámez R, Más R, Noa M, Menéndez R, Arango E, González J, Mendoza N, García H, Rodríguez Y, Goicoechea E, Mendoza S, González R. 2002. Estudio de la Toxicidad Subcrónica Oral (90 días) del VIMANG en Ratas Sprague Dawley. Informe Técnico, Centro de Productos Naturales, 40 pp

Garrido-Garrido G, Martínez-Sánchez G, Pardo-Andreu G, García-Rivera D, Hernández-Casaña P, Rodeiro-Guerra I, Guevara-García M, Delgado-Hernández R and Núñez-Sellés AJ. 2007. Recent advances in the research & development of an aqueous stem bark extract obtained from *Mangifera indica* L. In Capasso A. (Ed) Recent Developments in Medicinal Plant Research 9, pp 169-192.

GINA-Global Strategy for Asthma Management and Prevention. 2006. National Institutes of Health. National Heart, Lung, and Blood Institute, USA.

Kelly EA, Jarjour NN. 2003. Role of matrix metalloproteinases in asthma. Curr Opin Pulm Med 9:28-33.

Martínez G, Delgado R, Pérez G, Garrido G, Núñez-Sellés AJ, León OS. 2000. Evaluation of the *in vitro* antioxidant activity of *Mangifera indica* L. extract (VIMANG). Phytother Res 14:424-427.

Nadeem A, Chhabra SK, Masood A, Raj HG. 2003. Increased oxidative stress and altered levels of antioxidants in asthma. J Allergy Clin Immunol 111:72-78.

Núñez-Sellés AJ, Vélez-Castro HT, Aguero-Aguero J, Gonzalez-Gonzalez J, Naddeo F, De Simone F, Rastrelli L. 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. J. Agric. Food Chem. 50:762–766

Rivera DG, Balmaseda IH, León AA, Hernández BC, Montiel LM, Garrido GG, Cuzzocrea S, Hernández RD. 2006. Anti-allergic properties of *Mangifera indica* L. extract (VIMANG) and contribution of its glucosylxanthone mangiferin. J Pharm Pharmacol 58:385-392.

Sa-Nunes A, Rogerio AP, Medeiros AI, Fabris VE, Andreu GP, Rivera DG, Delgado R, Faccioli LH. 2006. Modulation of eosinophil generation and migration by *Mangifera indica* L. extract (VIMANG). Int Immunopharmacol 6:1515-1523.

Smurthwaite L, Durham SR. 2002. Local IgE synthesis in allergic rhinitis and asthma. Curr Allergy Asthma Rep 2:231-239.

Venge P. 2004. Monitoring the allergic inflammation. Allergy 59:26-32.

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Artículo Original | Original Article

Acute and subchronic toxicological study of senna in rodents

[Toxicología aguda y subcrónica de una pasta de sen en roedores]

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Abstract

Senna crude herbal paste, CIRUELAX®, laxative used in America and Spain, was administered by oral gavage to mice and rats once daily in doses from 2 to 10 g/kg for 7 days. LD_{50} was rated as higher than 10 g/kg. To determine subchronic peroral toxicity (SPT), rats were treated with 1, 2.7 and 6 g/kg of CIRUELAX® for 90 days. Body weight (BW) data and weights of liver, spleen, kidney, heart, gonads and lung were determined each week until 90th day. No decrease in weight gain was observed; males BW increased about 10% after 60 days. Liver and gonad relative weight in males and females, respectively, showed a slight increase at lower doses. Organs were free from discernible lesions. Microscopical examination showed no morphological changes. Rat males treated with CIRUELAX® showed blood hypokalemia. Potassium decreased about 25% after 90 days. Low doses of CIRUELAX® decreased WBC count in 19.61%. Monocytes count increased about five times after 90 days with 2.7 g/kg of CIRUELAX®. Absence of hepatocellular injury was inferred by AST and ALT activities. Soluble alkaline phosphatase (SAP) activity was not modified. CIRUELAX® higher dose equivalent to 200 times the dose used to treat human constipation induced no acute or subchronic toxic effects in rodents.

Keywords: Cassia angustifolia, Senna alexandrina; *senna; laxative; anthranoids; Phytotherapy.*

Resumen

CIRUELAX®, es una pasta herbal cruda preparada a base de sen, *Senna alexandrina* Mill., ampliamente utilizada en América y España. Fue administrada oralmente a ratas y ratones una vez al día en dosis de 2 a 10 g/kg durante 7 días. La Dosis Letal 50, LD_{50}, fue estimada como superior a 10 g/kg. Para determinar la toxicidad subcrónica por vía oral, un grupo de ratas fueron tratadas con 1; 2,7 y 6 g/kg de CIRUELAX® durante 90 días. Se determinaron los datos de peso corporal, peso del hígado, bazo, riñón, corazón, gónadas y pulmones cada semana hasta el día 90. No se apreció disminución en peso corporal, los machos experimentaron 10% de aumento de peso tras 60 días. Los pesos relativos de hígado y gónadas en machos y hembras, respectivamente, mostraron un ligero aumento en dosis bajas de CIRUELAX®. Los órganos no presentaron lesiones visibles y el examen microscópico no mostró variación de las características morfológicas. Las ratas machos tratadas con CIRUELAX® mostraron hipokalemia sanguínea. El potasio disminuyó alrededor de 25% tras 90 días de tratamiento. Bajas dosis del laxante disminuyeron alrededor de 19 % el recuento de glóbulos blancos. Los monocitos aumentaron alrededor de 5 veces tras 90 días de tratamiento con 2,7 g/kg de CIRUELAX®. La ausencia de daño hepatocelular fue inferida de la determinación de actividades de enzimas hepáticas (AST y ALT). La actividad de fosfatasa alcalina soluble (SAP) no fue modificada. La dosis superior de CIRUELAX® equivalente a 200 veces la dosis que se emplea para tratar estreñimiento en humanos, no indujo efectos tóxicos en roedores.

Palabras Clave: Cassia angustifolia; Senna alexandrina; *sen; laxantes naturales; antranoides; Fitoterapia.*

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INTRODUCTION

Chronic constipation occurs in all age groups, but apparently increases with age. Modern style of living has increased the prevalence of chronic constipation due to bad alimentary habits, low-fiber diet, a higher degree of sedentarism, and medication. In Germany 1995, it was determined that 25% of women and 10% of men acknowledged to be suffering some kind of difficulty to defecate and constipation (Knopf et al., 1995).

Since its introduction by Arabians to occidental medicine in the 19th century, anthranoids laxatives have been widely used. Among the anthranoids containing plants (*Rhamnus frangula, Rhamnus purshiana Rheum palmatum*), Senna (*Senna alexandrina* ex *Cassia angustifolia*) is used worlwide to treat constipation and is the most studied anthranoid laxative (Leng-Peschlow, 1992).

Due to their natural origin, specificity, low oral toxicity, efficacy and because they do not require medical prescription, anthranoids are commonly used to treat constipation. Unfortunately, this popularity has led to abuse of natural anthranoids. In England, 1993, 2% of the population between 40 and 59 years and 20 to 30% of the people older than 60 years used laxants more than once a week (Cooke, 1981). In Germany, 80% of the people under chronic use of laxants preferred anthranoids (May, 1982). Although long-term daily use can produce severe diarrhoea that causes hyponatremia, hypokalemia and dehydration, use for two to three times per week has been considered safe and effective (Ralevic et al., 1990; Wald, 2000).

Numerous studies have characterized toxic status of anthranoids. Hietala et al. (1987) distinguished between pure sennosides and other fractions of senna, since it had been found that it was possible to differentiate between the laxative and toxic effects of various senna fractions; the typical mixed preparations contain impurities that are three- to five –folds more toxic than pure sennosides which are hardly toxic, but less laxative. Those studies however, considered only acute toxicity (24 h) and were performed using very high doses.

Sennosides are the main active metabolites of senna and exhibit a very low toxicity in rats (Hietala et al., 1987). Sennosides genotoxicity both in bacterial strains and mammal cells has been observed, to be very low (Sandnes et al., 1992; Mukhopadhyay et al., 1998; Heidemann et al., 1993; Mereto et al., 1996).

Toxicological and genotoxic status of crude extract of senna is considered to be different. While mean LD_{50} of sennosides at 98% purity is 4100 mg/kg, LD_{50} for senna extract standardized at 5.5% sennosides is 384 mg/kg (Hietala et al., 1987). Others anthranoids as chrysantine, hidroxyanthraquinones (emodin, aloe-emodin, rhein), which are present as minor components in senna, have a toxicologic and mutagenic status, different from that of the sennosides, and presently is a very controversial issue. Emodin and aloe-emodin resulted positive in genotoxic assays in *Salmonella typhimurium*, V79-HGPRT cells, rat hepatocytes and mice fibroblasts (Westendorf et al., 1990), while resulted negative in the study of Heidemann et al. (1993). Mori et al. (1990) observed formation of neoplasms in stomach, intestine and liver of rats before 480 days of dietary exposure to 1% hydroxianthraquinones. In addition, out from Siegers et al. (1993) clinical work, it has been speculated that chronic use of anthranoids laxative is a risk factor for colorectal cancer development. Others investigators disagreed with these observations (Sandnes et al., 1992; Heidemann et al., 1993; Mascolo et al., 1999; Mengs et al., 1999). The aim of this work was to determine acute and subchronic 13-weeks toxicity of CIRUELAX®, an herbal paste containing linseed, prune and senna leaves and pods, as laxatives compounds.

MATERIALS AND METHODS

Animal preparation

All animals were supplied by the animal house of the Universidad Católica of Chile, Santiago of Chile. All were individually identified by ear tatoo. Upon arrival, experimental and control animals were caged separately. A period of 7 days of acclimatization was allowed before experimentation. Animals received a high quality food made by Champion S.A., Santiago, Chile, with the following composition: Protein 20.5%; fiber 5.0% and humidity 4.0%. Water was given ad libitum. Environmental conditions were as followed: temperature 20-24 ºC, relative humidity 60% and light-dark cycle of twelve hours each. The cleansing procedures were daily and the bed (wood shavings) was changed daily too. All procedures were authorized by the ethical comitee of the Universidad Austral de Chile.

Substance preparation

CIRUELAX® herbal paste contained (per 100 g): Powdered senna, *Senna alexandrina* Mill. –Fabaceae, standardized to 0.12-0.22% of anthraquinones calculated as sennosides B, 6.6 g; fruit pulp 48.8 g; white sugar 18.0 g; honey 9.5 g; linseed (seeds of *Linum usitatissimum*), 2.5 g; caramel 1.9 g; citric acid 1.0 g; sodium benzoate 0.07 g; potassium sorbate 0.07 g; water q.s.f. 100 g.

Quantification of sennosides was performed by HPLC. Column LUNA 250 x 4.6 mm, temperature: 40 °C, flow rate 1.0 mL/min; pressure 160 bar. Mobile phase acetonitrile: buffer pH 2.77 TEA 15:85. Sennosides A and B were detected at 265 nm by UV detector. The respective peak areas were integrated by comparison with external standard calibration curves.

The sample of CIRUELAX® herbal paste was homogenized with destilled water in an ultraturrax and given by oral gavage using buco-oesophagial cannulae at different doses as shown below. Maximum dosage volumes were 1 mL in mice and 3 mL in rat.

Acute peroral toxicity in mice and rats

100 Rockefeller mice of both sexes; body weight 25-30 g and 100 Sprague-Dawley rats of both sexes body weights 150 ± 8 g were randomly assigned to different treatment groups. The animals were fasted for 24 h before the administration of a single dose of CIRUELAX®. Single increased doses were: 2, 4, 6, 8 and 10 g/kg.

Afterwards, the animals were observed daily for 7 days. Mortality LD_{50} was estimated by method of Miller and Tainter (1944), designed to assess the ED_{50} and its error by means of logaritmic-probit graph paper. This method allows estimating toxicity from all-nothing responses considering fixed doses given to a group, instead of individual doses. Clinical signs, time of onset, duration and reversibility of toxicity were recorded. Gross necropsies were performed in all animals after the observation period.

Subchronic peroral toxicity in rats

Four groups of 11-13 rats per dose and per sex, with an initial body weight of 140 ± 10 g, were randomly assigned to treatment and control groups. CIRUELAX® doses were 1.0 g/kg (Group 1), 2.7 g/kg (Group 2) and 6.0 g/kg (Group 3). Control group received distilled water. The duration of the study was 90 days. At the end of this period, animals previously anaesthetized with ether were sacrificed by decapitation and different organs dissected. Every 3-4 days the product was mixed in a know quantity of water and administered during the whole experiment. The doses were daily adjusted to body weight (weights of groups) and weekly by individual weight.

Body and organ weight

Body weight data was obtained at the beginning and every 7 days until the end of the 90 day period, by using an electronic scale (Soehnle).

At the end of the 90 days, liver, spleen, kidneys, heart, gonads, and lung obtained from control and treated groups were weighted, after removal of connective and fat tissue, in a Sartorius analytical Scale.

Hystopathological examination

Tissues from heart, lung, kidney, spleen, stomach, duodenum, colon, liver, ovary, uterus and testicles of control and CIRUELAX® treated groups were prepared for optical and electronic microscopical examination. To light microscopy, tissues were undergone to 10% formaldehyde and routine hematoxylin eosine stainining. To electron microscopy examination the tissues were fixed in 1% buffered osmium tetroxide and processed by standard procedures.

Haematological and blood chemistry tests

Sampling of blood from each animal was performed at the first, 7^{th}, 30^{th}, 60^{th} and 90^{th} day. The blood was drawn by retro-orbital puncture under light ether anesthesia. The following analyses were done: red blood cells (RBC), white blood cells (WBC), volume packed RBC, haemoglobin, differential count of lymphocytes, monocytes, neutrophil segmenters, neutrophil bands, eosinophils and basophils.

Blood samples were added (1:9) to a 1% ethylenediaminetetraacetic acid (EDTA) solution previous to the standard laboratory procedures.

Blood samples were kept during 2 h at 4ºC and then were centrifuged to obtain serum for analysis of the following parameters: urea-N, protein, alkaline phosphatase (SAP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, potassium, calcium.

Data analysis

All hematological and biochemical data were evaluated according to the following statistical

methods: basic statistics including mean (M), standard error of the mean (SEM), standard deviation (SD), number (N), minimum, maximum, range, variance, Skewness (G1), Kurtosis(G2), sum and coefficient of variation (C.V.), Bartlett test for homogeneity or variance, ANOVA ONE WAY and multiple Tukey's comparison test were used. After that a Kruskal-Wallis non-parametric test was utilized for all variables. Finally, an ANOVA TWO WAY (factors: treatment and sex) was used and, when significant a Scheffe's test was also used. Sequentially, a box–design graphic statistics was employed.

Same descriptive statistics as described above was used for the evaluation of body weight. An ANCOVA TWO WAY (covariance analysis) was utilized. All data were analyzed with the software SYSTAT 5.04 and the level of significance defined was $p< 0.05$.

RESULTS

Oral Acute Toxicity

The study for acute toxicity was performed with a total of 100 Sprague Dawley rats and 100 Rockefeller mice. Doses used were 2, 4, 6, 8 and 10 g/kg. Only at maximal doses animals showed a reduction in the stool consistence, i.e. this dose represents a laxative dose in rats. During the first week following a single administration of CIRUELAX® paste no animal died.

Subchronical Toxicity

Mean body weight of female rats remained without statistically significant changes with respect to control group throughout the study. After 60 days, mean body weight of males treated with 1.0 and 2.7 g/kg of CIRUELAX® increased about 10% compared to control group ($p< 0.05$), see Fig. 1. Two female and five male rats died during ether anaesthesia.

At the end of the treatment period, both liver and ovary relative weight of female rats exposed to 1 g/kg of CIRUELAX® increased 12 and 150% in comparison to control group, however, this effect was not observed with higher doses. In male rats treated with 1 and 2.7 g/kg, liver relative weight increased about 19 and 13% respectively, compared with control group (Table 1). This effect was not observed with 6 g/kg dosis of CIRUELAX®.

Transaminase enzymes level was determined as an indicator of hepatocellular injury. Aspartate aminotransferase (AST) activity of males exposed to CIRUELAX® was similar to control group (Fig. 2A). After 30 days, AST activity of treated female rats, with all doses, increased respect to control while at day 60th the AST activity increased only in rats treated with 1 and 2.7 g/kg. At day 90^{th}, no difference in AST activity between experimental and control group (Fig. 2B), was observed.

Alanine aminotransferase (ALT) activity increased about 9% in male rats treated with 1g/kg at day 30, with respect to control. No differences were observed either in ALT of males treated with higher doses or in ALT of females exposed at different doses (Fig. 3A and B).

Alkaline phosphatase (SAP) activity showed a great variation throughout the study, however no significant differences were found among experimental and control groups (Fig. 4).

Urea nitrogen and serum protein levels in both, males and females treated with CIRUELAX® remained unaltered with respect to control groups (data not shown).

Potassium levels decreased 31, 22 and 20% at day 90 in males but not in females treated with 1, 2.7 and 6 g/kg of CIRUELAX® (Fig. 5A and B). Sodium and calcium electrolytes in males and females were not statistically different from controls throughout the 90 days of treatment. In comparison to Control group, no changes were observed in red blood cells (RBC) count, packed volume of RBC (PVC), hemoglobin and leukocytes, in males treated with CIRUELAX® (data no shown). WBC count in females rats treated with 1 g/kg of CIRUELAX® decreased 19.6% at 90^{th} day. Also in females, monocytes count at day 90^{th} was 1.2% in the control group and 5.8% in the group treated with CIRUELAX® 2.7 g/kg (Figs. 6 and 7).

Examination of heart, lung, kidney, spleen, stomach, duodenum, colon, liver, ovary, uterus and testicles of control and treated groups showed them to be free from specific macroscopically discernible lesions under the described experimental conditions.

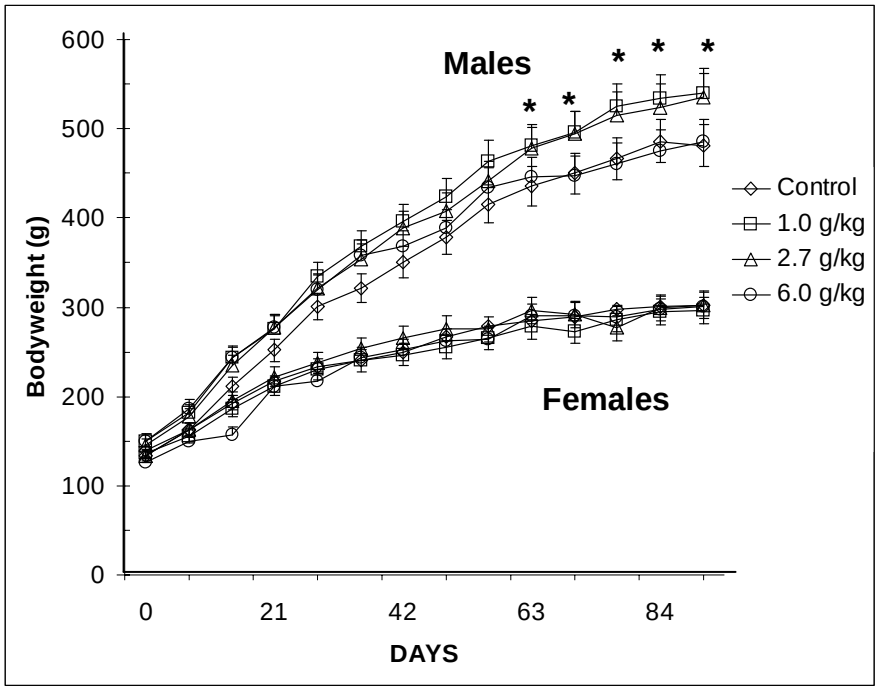
Optical and electronic microscopical examination of tissues from different organs, showed no microscopic morphological variations or differences among control and CIRUELAX® treated groups.

Table 1. Liver, kidney, heart and gonads relative weight, standardized to 100 g of body weight (somatic index) after 90 days of administration of CIRUELAX® paste.

		CIRUELAX® paste Doses (g/kg)		
	CONTROL	1	2.7	6
Males				
Liver	1.99± 0.037	2.37 ± 0.060 *	2.25 ± 0.058 *	2.08 ± 0.050
Kidney Right	0.30 ± 0.010	0.30 ± 0.001	0.29 ± 0.007	0.29 ± 0.008
Heart	0.29 ± 0.007	0.29 ± 0.008	0.28 ± 0.012	0.29 ± 0.008
Gonad Right	0.34 ± 0.011	0.32 ± 0.010	0.33 ± 0.008	0.35 ± 0.011
Gonad Left	0.34 ± 0.011	0.32 ± 0.008	0.32 ± 0.008	0.35 ± 0.016
Females				
Liver	2.84 ± 0.062	3.18 ± 0.090 *	3.06 ± 0.0120	2.82 ± 0.075
Kidney Right	0.33 ± 0.012	0.34 ± 0.009	0.33 ± 0.009	0.32 ± 0.010
Heart	0.33 ± 0.014	0.30 ± 0.015	0.33 ± 0.009	0.31 ± 0.013
Gonad Right	0.02 ± 0.002	0.05 ± 0.005 *	0.02 ± 0.001	0.02 ± 0.001
Gonad Left	0.02 ± 0.003	0.06 ± 0.009 *	0.02 ± 0.001	0.02 ± 0.002

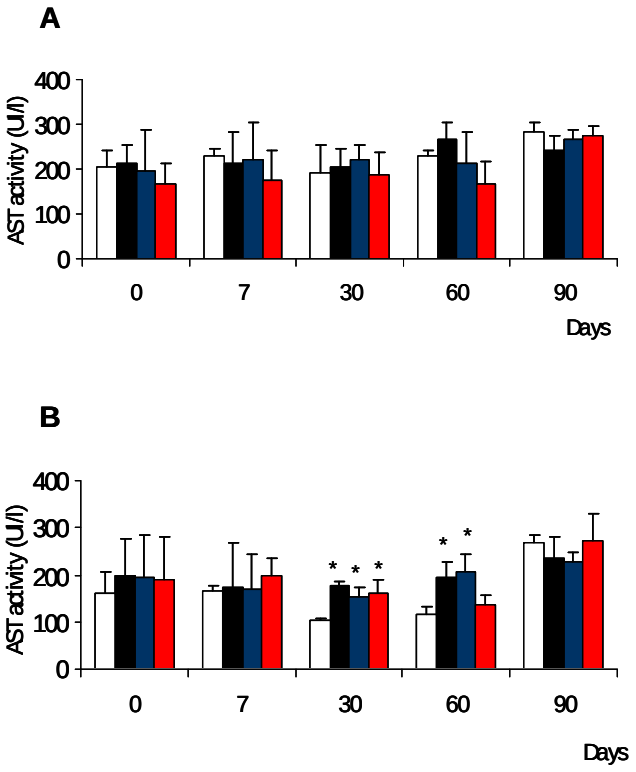
* p< 0.001, statistically significative with respect to control. Values are the mean ± standard deviation of 11-13 rats.

Figure 1. Effect of different doses of CIRUELAX® on male (A) and female (B) body weights throughout the study.



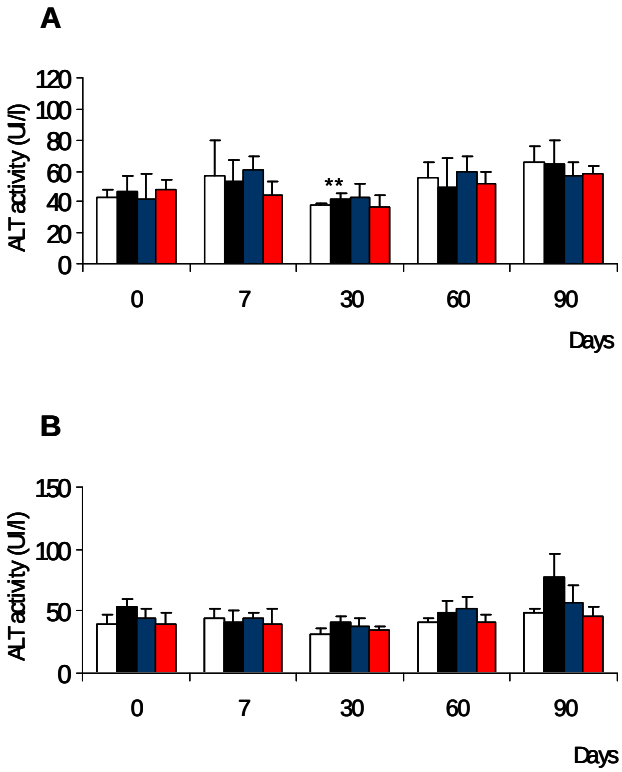
Each point represents the mean ± SEM of 11-13 rats. * p< 0.05 with respect to the control.

Figure 2. Effect of CIRUELAX® on male (A) and female (B) seric AST activity of rats at 0, 7, 30, 60 and 90 days of treatment.



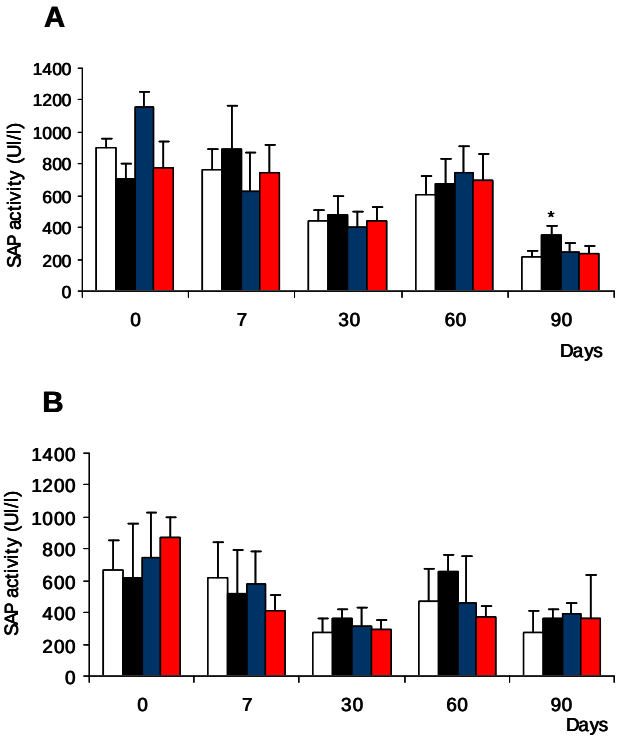
0 g/kg (□); 1 g/kg (■); 2.7 g/kg (■); 6.0 g/kg (■) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. * p< 0.05 with respect to the control.

Figure 3. Effect of CIRUELAX® on male (A) and female (B) seric ALT activity of rats at 0, 7, 30, 60 and 90 days of treatment.



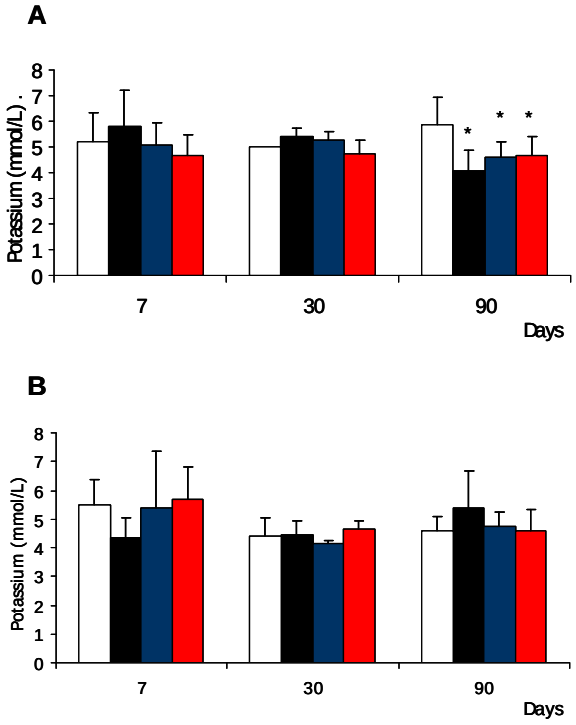
0 g/kg (□); 1 g/kg (■); 2.7 g/kg (■); 6.0 g/kg (■) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. ** p<0.01 with respect to the control.

Figure 4. Effect of CIRUELAX® on male (A) and female (B) seric SAP activity at 0.7, 30, 60 and 90 days of treatment.



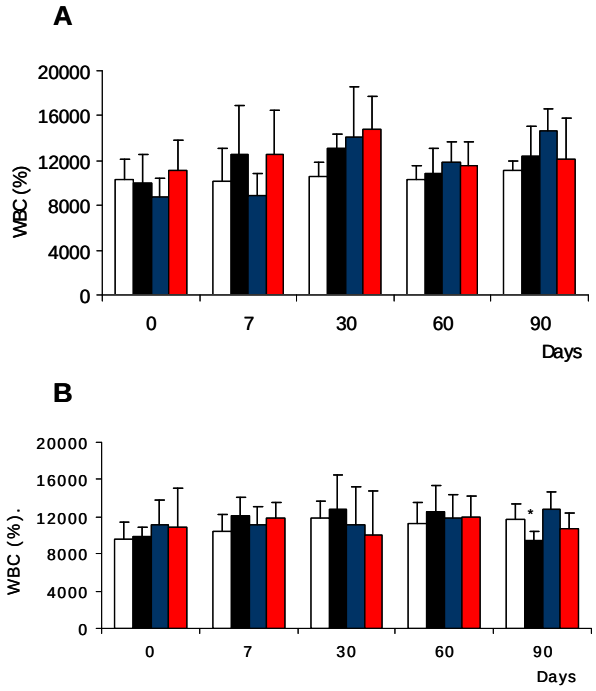
0 g/kg (□); 1 g/kg (▮); 2.7 g/kg (▮); 6.0 g/kg (▮) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats.* $p < 0.05$ with respect to the control.

Figure 5. Effect of CIRUELAX® on male (A) and female (B) plasmatic potassium levels at 7, 30 and 90 days of treatment.



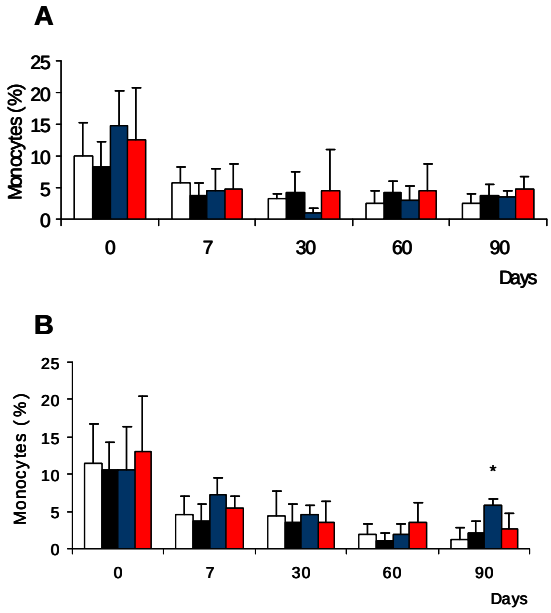
0 g/kg (□); 1 g/kg (▮); 2.7 g/kg (▮); 6.0 g/kg (▮) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. * $p < 0.05$ with respect to the control.

Figure 6. Effect of CIRUELAX® on male (A) and female (B) white blood cells (WBC) count at 0, 7, 30, 60 and 90 days of treatment.



0 g/kg (□); 1 g/kg (▮); 2.7 g/kg (▮); 6.0 g/kg (▮) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. * $p < 0.05$ with respect to the control.

Figure 7. Effect of CIRUELAX® on male (A) and female (B) monocytes count at 0, 7, 30, 60 and 90 days of treatment.



0 g/kg (□); 1 g/kg (▮); 2.7 g/kg (▮); 6.0 g/kg (▮) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. *$p < 0.05$ with respect to the control.

DISCUSSION

No deaths occurred in the rat oral acute toxicity study with CIRUELAX® doses 2, 4, 6, 8 and 10 g/kg, so that LD_{50} for both sexes has to be rated as higher than 10 g/kg. It is important to mention that at 10 g/kg, CIRUELAX® induced a definite reduction in the stool consistence only in some animals. It is known that acute toxicity of pure sennosides is very low: LD_{50} of sennosides 98% pure equals 4100 mg/kg in *i.v.* acute toxicity using mice (Hietala et al., 1987) while LD_{50} for a 93% sennosides extract as 5200 and 3530 mg/kg for males and females, respectively in oral toxicity study using Wistar rats (Mengs, 1988). LD_{50} for extracts of senna is different: LD_{50} of calcium sennosides 5.5% (senna extract) is 384 mg/kg in acute *i.v.* toxicity study in mice (Hietala et al., 1987). CIRUELAX® is a preparation that contains 6.6% of senna leaf, so that, maximal dosis of 10 g/kg of CIRUELAX® used in this work, could be equivalent to 660 mg/kg of senna powder, and to about 79 mg/kg of anthraquinones calculated as sennosides B. The low acute oral toxicity of CIRUELAX® observed in this study is consistent with observations from other studies (Mengs, 1988; Mengs et al., 2004). In spite of previously observations reported elsewhere (Mengs et al., 2004), in subchronic toxicity study, no decrease in weight gain was observed. In contrast, male body weight increased about 10% after 60 days of treatment. This body weight increase was not correlated with laxative dose. Liver relative weight increased lightly in groups treated with lower but no maximal doses of CIRUELAX®. In addition, gonads relative weight was increased in females at lower but no maximal doses of CIRUELAX®. Therefore, increase of liver and gonads relative weight were not dose correlated. No changes with respect to control group were observed in kidney

relative weight, contrary to previous reported results after treatment with pure sennosides and senna powder (Mengs, 1988; Mengs et al., 2004). This different result is probably due to lower senna doses used in the present work (maximal doses: 660 mg/kg) when compared to that used by Mengs et al. (2004) (maximal doses: 1500 mg/kg). Potassium level decreased in comparison to control group in males but not in females that received doses of 1, 2.7 and 6 mg/kg of CIRUELAX® at end of treatment. This result is consistent with hypokalemia due to abuse of stimulant laxatives (Knopf et al., 1995; Cummings, 1974). Sodium and chloride serum levels in treated animals were similar to control group. In other subchronic senna pod study (Mengs et al., 2004) it was described that sodium and chloride serum levels increased in rats treated with 750 and 1500 mg/kg of senna powder, while potassium levels exhibited no differences between treated and control groups. These discrepancies are probably due to lower doses of senna used in the protocol of subchronic toxicity in the present study (maximal dosis: 396 mg/kg). It has been described that chronic use or abuse of stimulant laxatives produces disturbance of electrolyte balance, especially potassium deficiency (Mengs et al., 2004; Mitchell, 2006).

Haematological evaluation resulted in a slight decrease of WBC count and an increase in monocytes count in rats treated with 1 g/kg and 2.7 g/kg with respect to control at day 90th of treatment. No other changes were observed. It has been described elsewhere that the invasion of colonic mucosa by monocytes is a result of stimulant laxatives use (Van Gorkom et al., 1998), but further studies are necessary to determine if the invasion of colonic mucosa by monocytes produces an increase of plasmatic monocytes count.

Many morphologic changes associated to anthranoids use have been reported in literature, although some of them are still controversial: pseudomelanosis coli, a reversible pigmentation of intestine considered harmless, apoptotic cells increment on colonic mucosa (Van Gorkom et al., 2001), higher proliferative activity in mucosa cells (Kleibeuker et al., 1995), swelling and basophilia of cortical tubular epithelial cells in kidney and epithelial hyperplasia in forestomach and large intestine (Mengs et al., 2004) and damage of the myenteric plexus (Smith., 1973; Defour and Gendre, 1984). Further hystopathologic research with chronic CIRUELAX® is necessary to determine probable effects of this laxative in long term abuse. A recently study of oral toxicity of senna in the rat reported no histo-pathological abnormalities after 13-week of treatment with 750 and/or 1500 mg/kg senna extract per day (Mengs et al., 2004).

CONCLUSION

From the results obtained in the present work and under the experimental procedures described above, no acute or subchronic toxic effects of CIRUELAX® have been observed. It is useful to emphasise that at present, there is no hard proof that short term or occasional use of this kind of laxative possesses a threat of toxic effects. If any, it could be attributed to chronic use of these substances.

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REFERENCES

Cooke WT. 1981. Laxative abuse. Acta Gastroenterol Belg 44:448-458.

Cummings JH.1974. Laxative abuse. Gut 15:758-766.

Defour P and Gendre P. 1984. Ultrastructure of mouse intestinal mucosa and changes observed after long term anthraquinone administration. Gut 25:1358-1363.

Heidemann A, Miltenburger HG, Mengs U. 1993. The genotoxicity status of senna. Pharmacology 47 (Suppl. 1):178-186.

Hietala P, Marvola M, Parviainen T, Lainonen H. 1987. Laxative potency and acute toxicity of some anthraquinone derivatives, senna extracts and fractions of senna extracts. Pharmacol Toxicol 61:153-156.

Kleiburker JH, Cats A, Zwart N, Mulder NH, Hardonk MJ, de Vries E. 1995. Excessively high cell proliferation in sigmoid colon after an oral purge with anthraquinone glycosides. J Natl Cancer Inst 87:452-455.

Knopf H, Braemer-Hauth M, Melchert HU, Thefeld W. 1995. Ergebnisse der nationalen Untersuchungs-Surveys zum Laxantiengebrauch. Bundesgesundhbl 38:459-467

Leng-Peschlow E. 1992.The importance of senna products Pharmacology 44(Suppl. 1):1-2

Mascolo N, Mereto E, Borrelli F, Orsi P, Sini D, Izzo A, Massa B, Boggio M, Capasso F. 1999. Does senna extract promote growth of aberrant crypt foci and malignant tumors in rat colon? Dig Dis Sci 44(11):2226-2230.

May H. 1982. Missbrauch von Abführmitteln, nachweisbare Schäden an Kolon, Anus und Stoffwechsel. Ärz Natur 7:365-371.

Mengs U. 1988. Toxic effects of sennosides in laboratory animals and *in vitro*. Pharmacology 36(Suppl. 1):180-187.

Mengs U, Grimminger W, Krumbiegel G, Schuler D, Silber W, Volkner W. 1999. No clastogenic activity of a senna extract in the mouse micronucleus assay. Mutat Res 444(2):421-426.

Mengs U, Mitchell J, McPherson S, Gregson R, and Tigner J. 2004. A 13-week oral toxicity study of senna in the rat with an 8-week recovery period. Arch Toxicol 78 (5):269-275.

Mereto E, Ghia M, Brambilla G. 1996. Evaluation of the potential carcinogenic activity of Senna and Cascara glycosides for the rat colon. Cancer Lett 101(1):79-83.

Miller LC, Tainter ML. 1944. Estimation of the ED50 and its error by means of logaritmic-probit graph paper. Proc Soc Exp Biol Med 57:261-264.

Mitchell JM, Mengs U, McPherson S, Zijlstra J, Dettmar P, Gregson R, Tigner JC. 2006. An oral carcinogenicity and toxicity study of senna (*Tinnevelly senna* fruits) in the rat. Arch Toxicol 80(1):34-44.

Mori H, Yoshimi N, Iwata H, Mori Y, Hara A, Tanaka T, Kawai K. 1990. Carcinogenicity of naturally occurring 1-hydroxyanthraquinone in rats: induction of large bowel, liver and stomach neoplasms. Carcinogenesis 11:799-802.

Mukhopadhyay MJ, Saha A, Dutta A, DE BY, Mukherjee A. 1998. Genotoxicity of sennosides on the bone marrow cells of mice. Food Chem Toxicol 36:937-940.

Ralevic V, Hoyle Ch, Burnstock G. 1990. Effects of long-term laxative treatment on rat mesenteric resistance vessel responses in vitro. Gastroenterology 89:1352-1357.

Sandnes D, Johansen T, Teien G, Ulsaker G. 1992. Mutagenicity of crude senna and senna glycosides in Salmonella typhimurium. Pharmacol Toxicol 71:165-172.

Siegers CP, Von Hertzberg-Lottin E, Otte M, Schneider B. 1993. Anthranoid laxative abuse – a risk for colorectal cancer? Gut 34:1099-1101.

Smith B. 1973. Pathologic changes in the colon produced by anthraquinone purgatives. Dis Colon Rectum 16:455-458.

Van Gorkom BAP, Karrenbeld A, Limburg AJ, Kleibeuker JH. 1998. The effect of sennosids on colonic mucosal histology and bowel preparation. Z Gastroenterol 36:13-18.

Van Gorkom BA, Karrenbeld A, van der Sluis T, Zwart N, de Vries EG, Kleibeuker JH. 2001. Apoptosis induction by sennoside laxatives in man; escape from a protective mechanism during chronic sennoside use? J Pathol 194(4):493-499.

Wald A. 2000. Constipation. Med Clin North Am 84:1231-1246.

Westendorf J, Marquardt H, Poginsky B, Dominiak M, Schmidt J, Marquardt H. 1990. Genotoxicity of naturally occurring hydroxyanthraquinones Mutation Res/Gen Toxicol 240(1):1-12.

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Uso de plantas medicinales cultivadas en una comunidad semi-rural de la estepa patagónica

[Use of medicinal plants cultivated in a semi-rural community of the Patagonian steppe]

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Abstract

In the present study, we evaluated the medicinal use of plants cultivated in home-gardens, gardens and green houses in a semi-rural population of the Patagonian steppe, Argentina. We analyzed richness of native and exotic species utilized, botanical families, life forms, plant part used and therapeutic effects. Ethnobotanical fieldwork was conducted by means of semi-structured interviews and visits to each cultivated area. We recorded a total of 30 medicinal cultivated species and we found a higher proportion of exotic herbaceous plants. The species most frequently cited were: oregano (*Origanum vulgare* L.), mint (*Mentha spicata* L.), onion (*Allium cepa* L.), and wormwood (*Artemisia absinthium* L.). The Pilcaniyeu inhabitants commonly utilize these plants for hepatic-intestinal and respiratory affections. They mainly use leaves, while flowers and branches are utilized in a lesser proportion.

Keywords: Ethnobotany; *medicinal plants*; *horticulture*; *Patagonia*; *Argentina.*

Resumen

En una población semi-rural de la estepa patagónica argentina, se estudió la utilización de plantas medicinales cultivadas en huertas, invernaderos y jardines. Se analizó la riqueza de especies, nativas y exóticas, usadas por los pobladores así como sus efectos terapéuticos más comunes. Asimismo, se estudiaron las especies medicinales más representativas, sus familias botánicas, sus formas de vida y partes utilizadas. El relevamiento etnobotánico se realizó por medio de entrevistas semi-estructuradas y visitas a cada espacio de cultivo. Se registró un total de 30 especies medicinales cultivadas, encontrándose una mayor proporción de plantas exóticas y herbáceas. Las plantas más citadas fueron: el orégano (*Origanum vulgare* L.), la menta negra (*Mentha spicata* L.), la cebolla (*Allium cepa* L.) y el ajenjo (*Artemisia absinthium* L.). Los habitantes de Pilcaniyeu utilizan estas plantas comúnmente para afecciones hepático-intestinales y respiratorias, haciendo uso principalmente de las hojas, y en una menor proporción flores y ramas.

Palabras clave: Etnobotánica; plantas medicinales; horticultura; Patagonia; Argentina.

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INTRODUCCIÓN

La horticultura es una de las innovaciones culturales más importantes de la humanidad. Pueblos que se desarrollaron como cazadores y recolectores por miles de años, cambiaron significativamente su forma de vida al adoptar a la horticultura como parte importante de su subsistencia (Casas et al., 1987). Uno de estos pueblos, fue el Mapuche, cuyos antiguos pobladores que habitaban el noroeste de la Patagonia poseían como base de sustentación tradicional tanto la producción hortícola, la caza como la recolección de especies silvestres (Pardo y Pizarro, 2005). En particular, su profundo conocimiento de la flora ha contribuido a la salud y el bienestar mediante el uso de recursos vegetales como medicinas, en un contexto cultural lleno de matices rituales, mágicos y religiosos (Citarella et al., 1995).

La conquista española quebró las modalidades de vida y de relaciones intergrupales entre estos pueblos, e impuso mecanismos y medidas de fuerte efecto demográfico (Citarella et al., 1995). Entre las numerosas transformaciones sufridas, se produjo la marginación o la desaparición de especies tradicionales cultivadas (ej. el maíz, la quinoa, la papa, la calabaza, etc.) y en general, un drástico cambio en el modelo de producción hortícola (Noggler, 1972; Parodi, 1999; Mösbach, 2000). De la misma manera, se fue abandonando la recolección de plantas silvestres con fines comestibles y medicinales, así como la transmisión de estas prácticas y costumbres a las generaciones más jóvenes (Ladio, 2001; Estomba et al., 2006; Lozada et al., 2006).

En la actualidad, comunidades rurales de la estepa patagónica argentina continúan con sus costumbres hortícolas en huertas y jardines, incorporando a su saber ancestral nuevas prácticas y patrones agrarios. Agentes externos de extensión como el Instituto Nacional de Tecnología Agropecuaria (INTA) y el Programa Social Agropecuario (PSA), ejercen una fuerte influencia en los pobladores, promoviendo nuevas tecnologías, como los invernaderos y el cultivo de plantas exóticas (obs. pers.).

Las plantas medicinales son de gran importancia aún en la atención primaria en comunidades rurales y semi-rurales alejadas de los centros urbanos (Estomba et al., 2006; Lozada et al., 2006; Ladio et al., 2007; Ladio y Lozada, 2008). Ellas son utilizadas en numerosas circunstancias, en las que la enfermedad es considerada como una pérdida de bienestar físico, emocional y espiritual (Ladio y Lozada, 2008). Se ha observado en numerosas investigaciones, que los ambientes próximos al hogar son espacios preferenciales para la recolección y el cultivo de especies medicinales (Etkin y Ross, 1982; Etkin, 1994; Ladio, 2001; Stepp y Moerman, 2001; Stepp, 2004; Ladio et al., 2007; Ladio y Lozada, 2008).

En el presente trabajo, se estudió en qué medida las huertas familiares constituyen aún reservorios de plantas medicinales. Se investigaron cuáles son las especies curativas que todavía se cultivan en huertas, jardines e invernaderos en una comunidad semi-rural de la estepa patagónica de la provincia de Río Negro, Argentina. Además, se analizaron los efectos terapéuticos más comunes, las familias botánicas, así como también las partes usadas y formas de vida más representativas de dicha herbolaria.

MATERIALES Y MÉTODOS

Sitio de estudio

El estudio se realizó en el paraje de Pilcaniyeu, ubicado a 75 km de San Carlos de Bariloche, en lo que se conoce como el Valle de Pilcaniyeu que corre en dirección Norte (Fig. 1), y cuenta aproximadamente con 1.445 habitantes. Posee una precipitación media anual de 125 mm y su temperatura media anual es de 7,3 ºC. Los vientos circulan predominantemente desde el oeste y su humedad relativa anual es del 66%.

Este paraje se caracteriza por un paisaje estepario de mesetas y valles, con afloramientos rocosos y humedales cordilleranos (llamados localmente “mallines”).

Desde el punto biogeográfico, esta región se encuentra dentro del Distrito Patagónico Occidental en la Provincia Patagónica. La vegetación está compuesta principalmente por arbustos y hierbas: el neneo (*Mulinum spinosum*), el charcao (*Senecio filaginoides*), el coirón amargo (*Stipa humilis*, *Stipa speciosa*) y *Poa huecu*, *Bromus macranthus*, *Poa ligularis*, *Festuca argentina* y otras hierbas (Cabrera, 1976). Gran parte de los pobladores se desempeñan como empleados públicos y otros en actividades agrícola-ganaderas.

Métodos etnográficos y análisis de datos

Se entrevistaron 20 mujeres y 10 hombres, entre 18 y 85 años, en el verano de los años 2006-2007. El relevamiento etnobotánico se realizó por medio de entrevistas semi-estructuradas. Se interrogó al miembro de la familia con mayor dedicación al cultivo de la tierra, sobre el uso de las especies cultivadas en huertas, invernaderos y jardines. En cuanto a las

plantas medicinales, se indagó acerca de las partes más usadas y sus efectos terapéuticos. Éstos últimos se agruparon en las siguientes categorías de acuerdo a Estomba et al. (2006): hepático-intestinal, antitusivo-respiratorio, analgésico-anti-inflamatorio, dermatológicos, circulatorios, sedante, febrífugo y ginecológico. Asimismo, se realizaron visitas a huertas, invernaderos y jardines donde se registraron, por sus nombres comunes, las especies medicinales presentes. Posteriormente, se determinaron las especies a partir del material herborizado y se cuantificaron las familias botánicas más representativas. Las plantas fueron clasificadas mediante la nomenclatura de Correa (1969; 1971; 1978; 1984; 1988; 1998; 1999), Marticorena y Quezada (1985); y Ezcurra y Brion (2005). El material herborizado fue depositado en el herbario del Centro Regional Universitario Bariloche, Universidad del Comahue, Argentina (BCRU)

Se comparó la similitud de las especies cultivadas en los diferentes espacios de cultivo (huerta, invernadero y jardín), mediante el índice de Jaccard (IJ) (Höft et al., 1999), utilizando la siguiente fórmula: **IJ: c/ (a + b+ c) x 100**, donde **c** es el número de las especies comunes, **a** es el número de especies únicas presentes en un área de cultivo dada y **b** es el número de especies únicas presentes en la otra área.

El consenso se estimó calculando el número de veces que una misma especie fue citada considerando la totalidad de personas entrevistadas.

RESULTADOS Y DISCUSIÓN

Riqueza y consenso de especies

Los pobladores de Pilcaniyeu cultivan en sus tres espacios de cultivo un total de 30 especies con fines medicinales, siendo Lamiaceae (30%) y Asteraceae (16,6%) las familias botánicas más representativas (Tabla 1, Fig. 2). Estas familias también fueron registradas como las más frecuentes a nivel global (Heinrich et al., 1998; Moerman et al., 1999; Leonti et al., 2003; Novais et al., 2004; Macía et al., 2005; De la Cruz et al., 2007) y en otras comunidades patagónicas de diferentes ambientes (Ladio et al., 2007; Ladio y Lozada, 2008).

De la totalidad de las plantas, el 80% (24 spp) es de origen exótico; mientras que el 20% restante (6 spp) es nativo (Tabla 1).

Las especies más frecuentemente citadas (Tabla 1), es decir con mayor consenso entre los informantes son: el orégano (*Origanum vulgare* L., 60%), la menta negra (*Mentha spicata* L. 56%), la cebolla (*Allium cepa* L., 46%) y el ajenjo (*Artemisia absinthium* L., 40%). Asimismo, las especies silvestres de origen exótico cultivadas son: el ajenjo (*Artemisia absinthium* L.), el llantén (*Plantago lanceolata* L.), la malva (*Malva sylvestris* L.), la malva rubia (*Marrubium vulgare* L.) y la palma (*Tanacetum vulgare* L.).

Sin embargo, ciertas especies nativas que naturalmente se encuentran en el ambiente de estepa, han sido introducidas en los espacios de cultivo, tal es el caso de: el pañil (*Buddleja araucana* Phil.), la paramela (*Adesmia boronioides* Hook.f.), la carqueja (*Baccharis sagittalis* (Less) DC) y el paico (*Chenopodium ambrosioides* L.). También se registraron otras especies nativas que crecen en ambientes más húmedos, como la zarzaparrilla (*Ribes magellanicum* Poir.) y el maitén (*Maytenus boaria* Molina).

Estos resultados corroboran la conspicua incorporación de especies medicinales exóticas a su conocimiento herbolario como se ha encontrado en otras investigaciones realizadas en la región patagónica argentino-chilena (Citarella, 1995; Estomba et al., 2006; Ladio et al., 2007). Esto puede deberse al carácter cosmopolita de la mayoría de las especies medicinales que son recolectadas, cultivadas o comercializadas en todo el mundo, como *Mentha* spp., *Plantago lanceolata* L. y *Artemisia absinthium* L. (comunic. pers.), las cuales han arribado a la zona hace unos 300 años con la conquista española (Ezcurra y Brion, 2005). Desde ese entonces, ha transcurrido tiempo suficiente para incorporarlas a la herbolaria tradicional, por lo que son cultivadas y consideradas como elementos curativos de gran importancia. Por otro lado, la fuerte influencia de agentes de promoción ha contribuido a la incorporación de numerosas especies exóticas en sus espacios de cultivo, incluyendo las medicinales (obs. pers.). Asimismo, en otras investigaciones se ha encontrado que el uso de las exóticas podría estar relacionado con la utilización directa de los ambientes antrópicos, como lo son las huertas, invernaderos y jardines, lo que refleja una continua interacción entre los seres humanos y los ambientes que ellos mismos generan (Bennett y Prance, 2000; Ladio y Lozada, 2000; 2001; Stepp, 2004; Albuquerque et al., 2005).

A pesar del mayor uso de especies exóticas, aún se conserva el uso medicinal de plantas nativas (e.g. Ladio, 2004; Estomba et al., 2006; Lozada et al., 2006; Ladio, 2007). Diversos estudios han demostrado que las especies medicinales nativas son recolectadas principalmente en ambientes no antrópicos y que los

pobladores locales recorren grandes distancias para acceder a estos valiosos recursos (Ladio, 2001, 2002, 2007; Estomba et al., 2006; Ladio et al., 2007).

En Pilcaniyeu, la incorporación de especies provenientes de otros ambientes más húmedos podría reflejar que este conocimiento tradicional sobre plantas medicinales tuvo su origen en antiguas poblaciones Mapuche asentadas en los bosques andino-patagónicos. Dicho conocimiento se ha mantenido durante generaciones mediante transmisión cultural, hecho que podría revelar la importancia de su historia y su inercia cultural (Ladio y Lozada, 2008). Sin embargo, este conocimiento local está decreciendo actualmente debido a marcados procesos de aculturación asociados a profundas transformaciones sociales y culturales por las que atraviesan estas comunidades (Ladio y Lozada, 2000; 2001; 2008; Ladio et al., 2007).

Efectos terapéuticos

El efecto terapéutico más citado es el hepático-intestinal (43%), seguido por el antitusivo-respiratorio (17%). Un 11,5% se refirió a efectos analgésico-anti-inflamatorio y dermatológicos, 8,5% a efectos circulatorios y en último lugar, febrífugo, ginecológico y sedante con un 2,85% (Tabla 1, Fig. 3). En otras investigaciones, el uso de plantas para dolencias gastrointestinales también fue el más frecuentemente citado (Schlage et al., 2000; El-Hilaly et al., 2003; Katewa et al., 2004; Novais et al., 2004; Estomba et al., 2006; Macía et al., 2005; Almeida et al., 2006; De la Cruz et al., 2007; Ladio y Lozada, 2008).

Especies como el orégano (*Origanum vulgare* L.), la menta (*Mentha spicata* L.) y el ajenjo (*Artemisia absinthium* L.) son usualmente empleadas para dolencias de tipo hepático-intestinal, en forma de infusiones. La menta y el ajenjo también fueron citadas para este tipo de afecciones en otro estudio en la región patagónica (comunic. pers.). En cuanto a las afecciones respiratorias, *Allium cepa* L. y *Artemisia absinthium* L. son las más utilizadas.

La alta frecuencia de plantas empleadas en afecciones hepático-intestinales, podría estar relacionado con el tipo de alimentación y hábitos de las comunidades de la región. Por un lado, en el último siglo, se ha observado un aumento en el consumo de harinas y azúcares refinados, carnes y grasas saturadas de origen animal, disminuyendo la ingesta de plantas silvestres y cultivadas; lo que ha conducido a una dieta no balanceada y esencialmente pobre en fibras y ácidos grasos esenciales (Ferrari et al., 2004). Adicionalmente, el agua potable es un recurso escaso en muchas comunidades. Los pozos se encuentran próximos a letrinas o son compartidos con los animales de crianza, lo cual aumenta el riesgo de incorporación de agentes patógenos.

Formas de vida

En su mayoría, las plantas cultivadas con fines medicinales son hierbas (67%), un 23 % son arbustos, 6,5 % árboles y 3,5% sub-arbustos (Tabla 1). Este hecho podría estar vinculado con la abundancia relativa de dichas formas de vida en la Patagonia (Ezcurra y Brion, 2005). Un mayor uso de hierbas también ha sido señalado en otros estudios, y estaría relacionado con la alta concentración de compuestos secundarios activos (Bennett y Prance, 2000; Stepp y Moerman, 2001; Stepp, 2004; Albuquerque et al., 2005).

Partes usadas

Las partes más empleadas con fines medicinales por los habitantes de Pilcaniyeu son las hojas (75%), como en el caso de: el orégano (*Origanum vulgare* L.), la menta (*Mentha* sp.), el ajenjo (*Artemisia absinthium* L.), el llantén (*Plantago lanceolada* L.), el paico (*Chenopodium ambrosioides* L.), entre otras. Generalmente, estas plantas son utilizadas en infusiones para uso interno o externo. También se utilizan flores (9,1%) destacándose el uso de la rosa (*Rosa* sp.) y el sauco (*Sambucus nigra* L.). Finalmente, las ramas son utilizadas en un 6,1% (Tabla 1), como por ejemplo en el caso de la paramela (*Adesmia boronioides* Hook.f.). El mayor uso de hojas como recurso medicinal pone en evidencia que las sustancias bio-activas se hallan principalmente en este órgano (Stepp, 2004; Voeks, 2004; Almeida et al., 2005). En otras comunidades patagónicas, así como de otras partes del mundo, los órganos aéreos, como hojas, corteza, ramas y flores, también son empleados con fines medicinales (Macía et al., 2005; Almeida et al., 2006; Ladio et al., 2007).

Similitud entre los espacios de cultivo según la riqueza de especies

Como se ha descrito previamente, los pobladores locales cultivan y utilizan recursos vinculados a su salud, que se encuentran próximos a sus hogares o en áreas peri-domésticas (Etkin y Ross, 1982; Etkin, 1994; Stepp y Moerman, 2001; Stepp, 2004; Ladio et al., 2007; Ladio y Lozada, 2008).

Tabla 1. Plantas medicinales cultivadas por los habitantes de Pilcaniyeu

Nombre científico	Nombre vulgar	Familia botánica	Origen	Lugar	Usos	E.T	Partes usadas	Formas de vida	Consenso
Origanum vulgare L. (E067)	Orégano	Lamiaceae	Exótico	h,i	c,m	H-I	hojas	hierba	0,60
Mentha spicata L. (E066)	Menta negra	Lamiaceae	Exótico	h,j,i	m	H-I	hojas	hierba	0,56
Allium cepa L. (E077)	Cebolla	Liliaceae	Exótico	h,i	c,m	A/R	bulbo	hierba	0,46
Artemisia absinthium L.(E012)	Ajenjo	Asteraceae	Exótico	h,j,i	m	H-I/R	hojas	hierba	0,40
Solanum tuberosum L.(E118)	Papa	Solanaceae	Exótico	h	c,m	R	tubérculo	hierba	0,30
Melisa officinalis L.(E073)	Toronjil	Lamiaceae	Exótico	h,j,i	m	C/H-I	hojas	hierba	0,30
Plantago lanceolata L.(E091)	Llantén	Plantaginaceae	Exótico	h,j,i	m	H-I	hojas	hierba	0,23
Rosa sp.(E106)	Rosa	Rosaceae	Exótico	h,j	m,o	A-A	flores	arbusto	0,23
Mentha rotundifolia (L.) Huds.(E065)	Menta blanca	Lamiaceae	Exótico	h,j	m	H-I	hojas	hierba	0,20
Salvia officinalis L.(E070)	Salvia	Lamiaceae	Exótico	h,j,i	c,m	H-I	hojas	hierba	0,16
Artemisia abrotanum L.(E017)	Éter	Asteraceae	Exótico	h,j	m	S	hojas	hierba	0,13
Chenopodium ambrosioides L.(E039)	Paico	Chenopodiaceae	Nativo	h,j	m	H-I	hojas	hierba	0,13
Sambucus nigra L.(E034)	Sauco	Caprifoliaceae	Exótico	h,j	c,m,o	A/R	flores	arbusto	0,13
Malva sylvestris L.(E083)	Malva	Malvaceae	Exótico	j	m	D/A-A	hojas	hierba	0,10
Rosmarinus officinalis L.(E069)	Romero	Lamiaceae	Exótico	j,i	c,m	H-I	hojas, flores	arbusto	0,10
Ruta graveolens L.(E108)	Ruda	Rutaceae	Exótico	j,i	m,r	H-I	hojas	subarbusto	0,10
Ribes magellanicum Poir.(E115)	Zarzaparrilla	Saxifragaceae	Nativo	h,j	c,m	H-I	hojas	arbusto	0,06
(?)(E041)	Rochela	Crassulaceae	Exótico	i	m	A-A/D	hojas	hierba	0,03
Baccharis sagittalis (Less.) DC(E014)	Carqueja	Asteraceae	Nativo	h	m	H-I	parte aérea	arbusto	0,03
Eucalyptus sp.(E085)	Eucaliptus	Myrtaceae	Exótico	j	m,s	R	hojas	árbol	0,03
Linum usitatissimum L.(E082)	Lino	Linaceae	Exótico	i	c,m	H-I	semilla	hierba	0,03
Maytenus boaria Molina (E036)	Maitén	Celastraceae	Nativo	h	m	F	hojas	árbol	0,03
Marrubium vulgare L.(E064)	Malva rubia	Lamiaceae	Exótico	h	m	A/R	hojas	hierba	0,03
Urtica dioica L.(E121)	Ortiga	Urticaceae	Exótico	h,j	m	C	hojas	hierba	0,03
Tanacetum vulgare L.(E024)	Palma	Asteraceae	Exótico	j	m,o	A-A	hojas	hierba	0,03
Buddleja araucana Phil.(E033)	Pañil	Buddlejaceae	Nativo	h,j	m	D	hojas	arbusto	0,03
Adesmia boronioides Hook.f.(E056)	Paramela	Fabaceae	Nativo	j	m	C/D	ramas, hojas	arbusto	0,03
Mentha pulegium L.(E068)	Poleo	Lamiaceae	Exótico	i	m	H-I	hojas	hierba	0,03
Nepeta mussini Henk.(E071)	Té del gato	Lamiaceae	Exótico	i	m	H-I	hojas	hierba	0,03
Tanacetum balsamita L.(E025)	Yerba de San Juan, menta turca	Asteraceae	Exótico	i	m	G	hojas	hierba	0,03

Abreviaturas: (**BCRU):** número de espécimen. **Lugar**: h – huerta, j – jardín, i- invernadero. **Usos:** c – comestible, m – medicinal, o – ornamental, r – fines rituales, s – sombra/cerca viva.
E.T: Efectos terapéuticos: H-I: Hepático-Intestinal, A: Antitusivo, R: Respiratorio, C: Circulatorio, A-A: Analgésico-anti-inflamatorio, D: Dermatológico, G: Ginecológico, F: febrífugo.

Figura 1. Ubicación de la comunidad de Pilcaniyeu

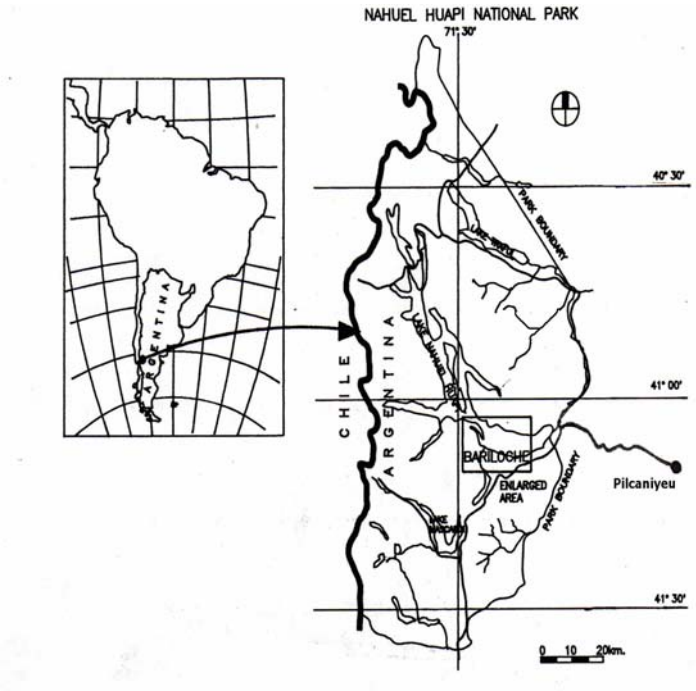


Figura 2. Familias botánicas a las que pertenecen las plantas medicinales cultivadas por los habitantes de Pilcaniyeu

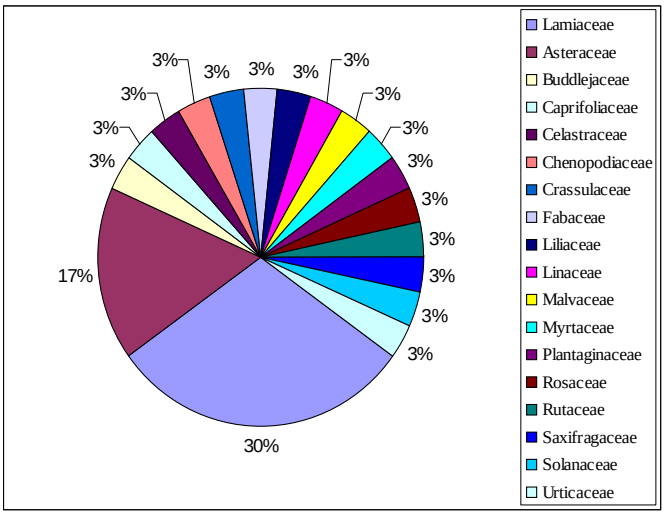
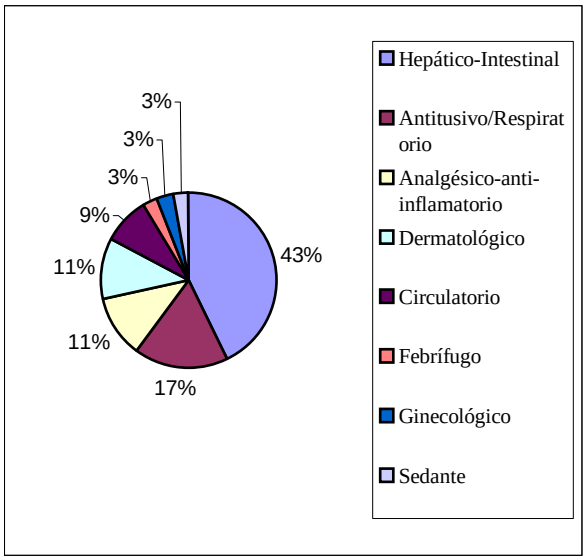


Figura 3. Efectos terapéuticos más representativos, citados por los habitantes de Pilcaniyeu



La huerta y el jardín se asemejan más entre sí de acuerdo con el número de especies medicinales cultivadas (52%), mientras que, en comparación, el invernadero diverge significativamente (27%). De la totalidad de las especies medicinales, 19 son cultivadas en la huerta, y el mismo número en el jardín; mientras que 14 fueron registradas en invernaderos (Tabla 1).

CONCLUSIONES

El uso de plantas medicinales cultivadas continúa siendo una costumbre importante en pobladores con ascendencia Mapuche, como fue encontrado en esta pequeña comunidad semi-rural del noroeste patagónico argentino. El notable poco uso de especies nativas sugiere que el conocimiento ancestral está sufriendo procesos de aculturación asociados a profundas transformaciones ocurridas en estas poblaciones (Ladio, 2002; Estomba et al., 2006; Ladio y Lozada, 2008). Estos procesos están relacionados, en parte, con una fuerte influencia occidental ejercida desde la medicina oficial, así como la intervención de agentes de extensión.

Este cuerpo de conocimientos en relación con el uso de plantas medicinales es dinámico, y refleja cómo los habitantes de Pilcaniyeu han respondido de manera flexible a cambios tanto ambientales como socio-culturales dando lugar a un tipo de conocimiento de carácter híbrido (Padoch et al., 1998; Sears et al., 2007). De esta manera, han incorporado a su herbolaria tradicional un elevado número de especies exóticas, en su mayoría pertenecientes a las familias Lamiaceae y Asteraceae; así como nuevas tecnologías y prácticas. El gran potencial curativo de las 30 plantas medicinales estudiadas, muy probablemente sea el resultado de complejos procesos de selección y de los efectos de la globalización.

Este estudio de caso pone en evidencia la relevancia de las huertas, los invernaderos y los jardines como espacios para el cuidado de la salud (Ladio y Lozada, 2008); no sólo a través del uso de las especies medicinales, sino probablemente también ligado al contacto con la naturaleza (Ulrich, 2002; Schultz et al., 2004).

BIBLIOGRAFÍA

Alburquerque UP, Cavalcanti Andrade L, Oliveira de Silva AC. 2005. Use of plant resources in seasonal dry forest (Northeastern Brazil). Acta Bot Bras 19(1):27-38.

Almeida CFCBR, Lima e Silva TC, de Amorin ELC, Maia MBS, Albuquerque UP. 2005. Life strategy and chemical compostion as predictors of the selection of medicinal plants from the Caatinga (Northeast Brazil). J Arid Environ 62:127–142.

Almeida CFCBR, de Amorim ELC, Albuquerque UP, Maia MBS. 2006. Medicinal plants popularly used in the Xingó region – a semi-arid location in Northeastern Brazil. J Ethnobiol Ethnomed 2:15.

Bennett BC, Prance GT. 2000. Introduced plants in the indigenous pharmacopeia of northern South America. Econ Bot 54(1):90-102.

Cabrera AL. 1976. Regiones fitogeográficas argentinas. In Enciclopedia Argentina de la Agricultura y Jardinería. Fascículo 1. Tomo II. Editorial Acme S.A.C.I. Buenos Aires, Argentina.

Casas A, Viveros J L, Katz E, Caballero J. 1987. Las plantas en la alimentación mixteca: una aproximación etnobotánica. Am Indíg 47(2):317-343.

Citarella L, Conejeros AM, Espinossa B, Jelves I, Oyarce AM, Vidal A. 1995. Medicinas y culturas en La Araucanía. Programa de Atención Primaria en Salud, Cooperación Italiana: 11-38.

Correa MN. 1969-1999. Flora Patagónica. Partes 1,2,3,4,5,6 y 7. Colección Científica, del Instituto Nacional de Tecnología Agropecuaria. Buenos Aires, Argentina.

De la Cruz H, Vilcapoma G, Zevallos PA. 2007. Ethnobotanical study of medicinal plants used by the Andean people of Canta, Lima, Peru. J Ethnopharmacol 111:284-294.

El-Hilaly J, Hmammouchib M, Lyoussi B. 2003. Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). J Ethnopharmacol 86:149-158.

Estomba D, Ladio AH, Lozada M. 2006. Medicinal wild plant knowledge and gathering patterns in a Mapuche community of North-western Patagonia. J Ethnopharmacol 103:109-119.

Etkin NL. 1994. Consuming a therapeutic landscape: a multicontextual framework for assessing the health significance of human-plant interactions. In: Flagler J, Poincelot RG (Eds.). People—Plant Relationships: Setting Research Priorities. Haworth Press, pp. 61–81.

Etkin NL, Ross P. 1982. Food as medicine and medicine as food. An adaptative framework for the interpretation of plant utilization among the Hausa of Northern Nigeria. Soc Sci Med 16:1559–1573.

Ezcurra C, Brion C. 2005. Plantas del Nahuel Huapi. Catálogo de la Flora Vascular del Parque Nacional Nahuel Huapi, Argentina. Universidad Nacional del Comahue. Red Latinoamericana de Botánica. Bariloche, Argentina.

Ferrari MA, Morazzani F, Pinotti LV. 2004. Patrón alimentario de una comunidad aborigen de la Patagonia Argentina. Rev Chil Nutr 30(2). Sitio Web: http://www.scielo.cl/scielo.php?pid=S0717-75182004000200005&script=sci_arttext (Consultado: Septiembre 20, 2008)

Heinrich M, Robles M, West JE, Ortiz de Montellano BR, Rodriguez E. 1998. Ethnopharmacology of Mexican Asteraceae (Compositae). Annu Rev Pharmacol Toxicol 38:539–565.

Höft M, Barik SK, Lykke AM. 1999. Quantitative Ethnobotany. Applications of multivariate and statistical analyses in ethnobotany. People and Plant Working Paper. Division of Ecological Sciences, UNESCO. Paris, France.

Katewa SS, Chaudhary BL, Jain A. 2004. Folk herbal medicines from tribal area of Rajasthan, India. J. Ethnopharmacol. 92:41-46.

Ladio AH. 2001. The maintenance of wild plants gathering in a Mapuche community of Patagonia. Econ Bot 55(3):243-254.

Ladio AH. 2002. Las plantas comestibles en el noroeste patagónico y su utilización por las poblaciones humanas: una aproximación cuantitativa. Tesis Doctoral, Universidad Nacional del Comahue. Bariloche, Argentina.

Ladio AH. 2004. El uso actual de plantas nativas silvestres comestibles en comunidades Mapuches del NO Patagonico. Bol Latinoam Caribe Plant Med Aromat 3(2):30-36.

Ladio AH. 2007. Plantas medicinales del noroestede la Patagonia: Aportes de la etnobotánica cuantitativa para la conservación biocultural. Bol Latinoam Caribe Plant Med Aromat 6(5):197-198.

Ladio AH, Lozada M. 2000. Edible wild plant use in a Mapuche community of northwestern Patagonia. Hum Ecol 28(1):53-71.

Ladio AH, Lozada M. 2001. Non-timber forest product use in two human populations from NW Patagonia: A quantitative approach. Hum Ecol 29(4):367-380.

Ladio AH, Lozada M, Weigandt M. 2007. Comparison of traditional wild plant knowledge between aboriginal communities inhabiting arid and forest environmentsin Patagonia, Argentina. J Arid Environ 69:695-715.

Ladio AH, Lozada M. 2008. Medicinal plant knowledge in rural communities of North-western Patagonia, Argentina. A resilient practice beyond acculturation. In Albuquerque Ulysses Paulino de and Alves Ramos Marcelo (Ed). Current Topics in Ethnobotany, pp 40-53.

Leonti M, Ramirez RF, Sticher O, Heinrich M. 2003. Medicinal flora of the Popoluca, México: A botanical systematical perspective. Econ Bot 57(2):218-230.

Lozada M, Ladio AH, Weigandt M. 2006. Cultural transmission of ethnobotanical knowledge in a rural community of northwestern Patagonia. Econ Bot 60(4):374-385.

Macía MJ, García E, Vidaurre PJ. 2005. An ethnobotanical survey of medicinal plants commercialized in the markets of La Paz and El Alto, Bolivia. J Ethnopharmacol 97:337-350.

Marticorena C, Quezada M. 1985. Flora vascular de Chile. Gayana Bot 42(1-2):1-157.

Moerman DE, Pemberton RW, Kiefer D, Berlin B. 1999. A comparative analysis of five medicinal floras. J Ethnobiol 19(1):49-67.

Mösbach EW de. 2000. Lonco Pascual Coña. Ñi tuculpazugun. Testimonio de un cacique mapuche. Texto dictado al padre Ernesto Wilhelm de Mösbach. Sexta Edición. Ed. Pehuen. Chile.

Noggler A. 1972. Cuatrocientos años de misión entre los Araucanos. Temuco: Imp. Wesaldi.

Novais MH, Santos I, Mendes S, Pinto-Gomes C. 2004. Studies on pharmaceutical ethnobotany in Arrabida Natural Park (Portugal). J Ethnopharmacol 93:183-195.

Padoch C, Harwell E, and Susanto A. 1998. Swidden, Sawah, and In-Between: Agricultural transformation in Borneo. Hum Ecol 26(1):3-19.

Pardo BO, Pizarro TJL. 2005. Especies botánicas consumidas por los chilenos prehispánicos. Colección Chile Precolombino. Primera edición. Ed. Mare Nostrum. Santiago, Chile.

Parodi LR. 1999. La agricultura aborigen argentina. 2da. Edición. Ed. Eudeba. Buenos Aires, Argentina

Schlage C, Mabula C, Mahunnah RLA, Heinrich M. 2000. Medicinal Plants of the Washambaa (Tanzania): Documentation and Ethnopharmacological Evaluation. Plant Biol 2:83-92.

Schultz PW, Shriver C, Tabanico JJ, Khazain AM. 2004. Implicit connections with nature. J Environ Psychol 24:31-42.

Sears RR, Padoch C, Pinedo-Vasquez M. 2007. Amazon Forestry Transformed: Integrating Knowledge for Smallholder Timber Management in Eastern Brazil. Hum Ecol 35(6):697-707.

Stepp JR. 2004. The role of weeds as sources of pharmaceuticals. J Ethnopharmacol 92:163-166.

Stepp JR, Moerman D. 2001. The importance of weeds in ethnopharmacology. J Ethnopharmacol 75:19-23.

Ulrich RS. 2002. Health benefits of gardens in hospitals. Paper for conference, Plants for People, International Exhibition Floriade. Center for Health Systems and Design. Colleges of Architecture and Medicine. Texas A & M University. College State, TX.

Voeks RA. 2004. Disturbance Pharmacopoeias: medicine and myth from the Humid Tropics. Ann Assoc Am Geog 94(4):868–888.

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Effects of *Nigella sativa* L. and *Cinnamomum zeylanicum* Blume oils on some physiological parameters in streptozotocin-induced diabetic rats

[Efectos de los aceites de *Nigella sativa* L. and *Cinnamomum zeylanicum* Blume sobre algunos parámetros fisiológicos en ratas diabéticas inducidas por estreptozotocina]

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Abstract

The effects of *Nigella sativa* L. and *Cinnamomum zeylanicum* Blume oils on some physiological parameters were investigated in streptozotocin (STZ)-induced diabetic male Wistar rats. STZ-induced diabetic rats showed significant increases in the levels of blood glucose, triglycerides, cholesterol, low density lipoprotein LDL-cholesterol, urea, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) while body weight, high density lipoprotein HDL-cholesterol, total protein and uric acid levels were significantly decreased compared to normal rats. Administration of the tested oils to diabetic rats resulted in a significant decrease in blood glucose, triglycerides, cholesterol, LDL–cholesterol and ALT while HDL–cholesterol level was markedly increased after three weeks compared to untreated diabetic rats. In addition, treating diabetic rats with *N. sativa* plus *C. zeylanicum* oils showed significant increases in the levels of total protein, uric acid and creatinine. In contrast, administration of *C. zeylanicum* oil to diabetic rats resulted in a significant decrease in ALT level compared to the untreated diabetic rats. The results of this study indicate that the tested oils possess hypoglycemic, hypolipidemic and antioxidant effects in STZ-induced diabetic rats.

Keywords: Nigella sativa; Cinnamomum zeylanicum*; Oil; Streptozotocin; Diabetes; Rats.*

Resumen

En este estudio fueron investigados los efectos de los aceites de *Nigella sativa* L. y *Cinnamomum zeylanicum* Blume sobre algunos parámetros fisiológicos en ratas Wistar machos diabéticos inducidas por estreptozotocina (STZ). Las ratas diabéticas inducidas por STZ mostraron incrementos significativos en las concentraciones de glucosa, triglicéridos, colesterol, lipoproteínas de baja densidad LDL-colesterol, urea, alaninoaminotransferasa (ALT) y aspartatoaminotransferasa (AST), mientras que fueron disminuidos significativamente el peso corporal, lipoproteína de alta densidad HDL-colesterol, proteína total y ácido úrico comparados con ratas normales. La administración de los aceites en estudio a las ratas diabéticas decreció significativamente la glucosa sanguínea, los triglicéridos, colesterol, LDL-colesterol y ALT mientras hubo un incremento de HDL-colesterol después de tres semanas comparado con las ratas diabéticas no tratadas. Además, las ratas diabéticas tratadas con los aceites de *N. sativa* más *C. zeylanicum* mostraron incrementos significativos en las concentraciones de proteínas totales, ácido úrico y creatinina. Por el contrario, la administración del aceite de *C. zeylanicum* a ratas diabéticas decreció significativamente las concentraciones de ALT comparado con las ratas diabéticas no tratadas. Los resultados de este estudio indican que los aceites probados poseen efectos hipoglicémicos, hipolipémicos y antioxidantes en ratas diabéticas inducidas por STZ.

Palabras Clave: Nigella sativa, Cinnamomum zeylanicum*, Aceite, Estreptozotocina, Diabetes, Ratas*

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INTRODUCTION

The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides (Larner, 1985). Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents have been successful in maintaining euglycaemia and controlling long-term microvascular and macrovascular complications (Larner, 1985; Stenman et al., 1990). Insulin therapy is used for management of diabetes mellitus but there are several drawbacks, which include insulin allergy, insulin antibodies, lipodystrophy, autoimmunity and other delayed complications like morphological changes in kidney and severe vascular complications (Defronzo et al., 1982; Jarvinen and Koivisto, 1984; 1986). The five types of oral anti-diabetic drugs, currently approved for the treatment of type 2 diabetes do not have a favorable effect on CV disease (Fisman et al., 2004), and some of these drugs are associated with serious adverse effects (El-Hilaly et al., 2006). Thus, new, relatively non-toxic, therapeutic agents are needed to treat hyperglycemia, which also would correct dyslipidemia to reduce the risk of CV complications of diabetes (El-Hilaly et al., 2006).

Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager. Many studies have confirmed the benefits of medicinal plants with hypoglycaemic effects in the management of diabetes mellitus. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities. Moreover, during the past few years some of the new bioactive drugs isolated from hypoglycaemic plants showed antidiabetic activity with more efficacy than oral hypoglycaemic agents used in clinical therapy (Bnouham et al., 2006). Presently, there is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (Holman and Turner, 1991; Kameswara et al., 1997; Kim et al., 2006). More than 400 plants with glucose lowering effect are known (Ernst, 1997). Also a number of plants have a hypolipidemic effect (Sharma et al., 2003). However, there is little information about plants with both hypoglycemic and hypolipidemic effects (Subash Babu et al., 2006).

N. sativa is a spice plant belonging to the family Ranunculaceae. It is cultivated in several countries in the Mediterranean region and Asia, known in vernacular as "sannouj, habbat el Baraka or habbah saouda" (Rchid et al., 2004). The seeds were used in the orient as condiments or flavourings and also in traditional medicine applications (Ballero and Fresu, 1993). It has been shown that *N. sativa* has bronchodilatory (El-Dakhakhny, 1965; Mahfouz and El-Dakhakhny, 1966), anti bacterial (Toppozada et al., 1965), hypotensive (Mahfouz et al., 1962), immuno-potentiating (El-Kadi and Kandil , 1987), antioxidant (Burits and Bucar, 2000), antitumoral (Worthen et al., 1998) and antidiabetic properties (Al-Awadi et al., 1985; Bamosa et al., 1997; Meral et al., 2001). The oil of *N. sativa* was potent analgesic and anti-inflammatory drug in rats (Houghton et al., 1995; Hajhashemi et al., 2004) and had *in vitro* and *in vivo* cytotoxic and immunosuppressive properties (Islam et al., 2004). The petroleum ether extract exerted lipid-lowering and insulin-sensitizing actions in rats (Mai Le et al., 2004).

N. sativa has been shown to contain >30% of a fixed oil and 0.4-0.45% w/w of a volatile oil (Hashim and El-Kicy, 1962; El-Alfy et al., 1975). The volatile oil has been shown to contain 18.4-24% thymoquinone, 5.8-11.6% carvacrol, 15.5-31.7% p-cymene, 9.3% α-pinene, 2-6.6% 4-terpineol, 1-8% longifolene and 0.25-2.3% t-anethole (Canonica et at., 1963; El- Dakhakhny, 1963; Aboutabl et al., 1986; El-Tahir et al., 1993; Burits and Bucar, 2000).

True cinnamon (*C. zeylanicum*) is among 300 species of *Cinnamomum* that belong to the Lauraceae family. The aromatic bark of the cinnamon tree is used worldwide for culinary purposes, but it is also used in Ayurvedic and traditional Chinese medicine for its hypoglycaemic, digestive, antispasmodic, and antiseptic properties (Battaglia 1995; Ody 1993). Animal studies have demonstrated that cinnamon, and its active constituent cinnamaldehyde, doses dependently improve glycaemic control and hyperlipidaemia in normal and Streptozotocin-induced diabetic rats (Kannappan et al., 2006; Kim et al., 2006; Subash Babu et al., 2006).

Major compounds present in cinnamon stem-bark oil and cinnamon root-bark oil are cinnamaldehyde (75%) and camphor (56%), respectively (Senanayake et al., 1978) identified 53 constituents along with the major component eugenol (81-84.5%) in cinnamon leaf oil. Thirty-four compounds have been previously identified in cinnamon fruit oil with *(E)*-cinnamyl acetate (42-54%) and caryophyllene (9-14%) as the major components (Jayaprakasha et al., 1997).

Twenty-six compounds constitutes 97% of the volatile oil from cinnamon flowers were characterized with *(E)*-cinnamyl acetate (42%), *trans*-α-bergamotene (8%) and caryophyllene oxide (7%) as the major compounds (Jayaprakasha et al., 2000).

Induction of diabetes in laboratory animals is a convenient and useful strategy in the understanding and treatment of the disease. An appropriate dose of streptozotocin was used to induce experimental diabetes. Streptozotocin selectively destroyed pancreatic β-cells, resulting in hypoinsulinemia (Szkudelski, 2001). Streptozocin-treated rats are often used as diabetic animals with insulin-deficiency resulting from damage of beta-cells caused by the drug. These rats are hyperglycemic and have reduced uptake of glucose in skeletal muscles (Wallberg-Harrison and Holoszy, 1985; Goodyear et al., 1988; Markun et al., 1999). It is generally considered that hyperglycemia is the major factor in the pathogenesis of diabetic complications (Odetti et al., 1996). In diabetes there is inability to store fat and protein along with breakdown of existing fat and protein stores. Streptozotocin induced diabetic rats showed significant increases in the levels of cholesterol, phospholipids, triglycerides, and free fatty acids (Ravi et al., 2005). These changes remain important in terms of explaining the accelerated atherosclerosis. In addition, there is a loss of body weight (Bolkent et al., 2005; Zari and Al-Attar, 2007). Impairment of kidney function is a prominent feature of diabetes. Elevated levels of urea and decreased concentrations of uric acid and creatinine were shown in diabetes (Gawronska-Szklarz et al., 2003; Yassin et al., 2004). Over time diabetic nephropathy will develop, characterized by proteinuria, a loss of renal function, and a rapid progression to end stage renal failure (Tesch and Nikolic-Paterson, 2006).

Little information exists concerning the effects of *N. sativa*, *C. zeylanicum* and *N. sativa* plus *C. zeylanicum* on physiological parameters in STZ-induced diabetic rats. Therefore, the aim of this study is to find if the administration of the oils of *N. sativa*, *C. zeylanicum* and *N. sativa* plus *C. zeylanicum* could have beneficial effects on some physiological parameters in STZ-induced diabetic rats.

MATERIALS AND METHODS

Materials

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from Al-Saggaf Est. (Jeddah, Saudi Arabia). The oils of *N. sativa* and *C. zeylanicum* were obtained from Dreams Essential Oils Est. (Jeddah, Saudi Arabia). *N. sativa* oil was extracted by steam distillation and *C. zeylanicum* oil was extracted by hydro-distillation. The major compounds of *N. sativa* oil were thymoquinone (29.7%), p-cymene (23%), carvacrol (11.5%), α-pinene (8.6%), 4-terpineol (3.7%), longifoline (2.8%), carvone (1.8%) and t-anethole (0.8%). The major compounds of *C. zeylanicum* oil were cinnamaldehyde (62.7%), β-caryophyllene (6.5%), eugenol (5.25%), α-terpineol (2.8%) and cinnamyl alcohol (0.18%).

Experimental animals

Male Wistar rats weighing (180–230 g) were obtained from The Animal Experimental Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia. The rats were housed in well-aerated individual cages in an animal room and maintained in a temperature-controlled room (24 ± 1 °C) with a 12 h light/12 h dark cycle, 55±10 % humidity. They were fed with normal commercial chow and water *ad libitum*. Throughout the experiments, animals were processed according to the suggested international ethical guidelines for the care of laboratory animals and all experimental procedures were approved by the Animal Care and Use Committee of King Abdul Aziz University.

Induction of diabetes

The experimental animals were fasted for 12 hours and then diabetes was induced by a single intraperitonial injection of streptozotocin (Sigma Chemical Co., St. Louis, MO, USA), dissolved in a freshly prepared physiological saline solution (0.9% NaCl) at a dose of 65 mg/kg body weight. While normal rats received only the saline solution (0.9% NaCl) in the same volume and through the same route. After injection, all animals were returned to their cages and given free access to food and water.

After 3 days, the fasting blood glucose levels were measured from tail blood samples by using an OneTouch Ultra® glucometer (Lifescan; Johnson & Johnson, Milpitas, CA, USA). Animals with blood glucose levels more than 277 mg/dl were considered diabetic and used for the experiment.

Experimental design

A total of 50 rats (40 surviving diabetic rats and 10 normal rats) were used in the experiment. The rats

were divided into 5 groups of 10 animals each as follows: Group 1: Normal control (Non-diabetic normal rats) received normal commercial chow and water *ad libitum*. Group 2: STZ-Control (diabetic control rats) received the same diet given in group 1. Group 3: STZ + *N. sativa* oil received diet containing 5% *N. sativa* oil. Group 4: STZ + *C. zeylanicum* oil received diet containing 5% *C. zeylanicum* oil. Group 5: STZ + *N. sativa* oil plus *C. zeylanicum* oil received diet containing 2.5% *N. sativa* oil plus 2.5% *C. zeylanicum* oil. All of the experimental groups received the treatments for a period of 3 weeks.

Body weight

Rats were weighed at the start of the experimental period and weekly for 3 weeks using a digital balance. These weights were determined at the same time during the morning.

Blood collection

After 3 weeks, the rats were fasted for 8 h before blood sampling, water was not restricted. Blood samples were collected from the orbital venous plexus of the rat under mild ether anaesthesia by heparinized capillary tube and into non-heparinized tubes, Plate et al. (1985) indicated that brief exposure and little amount of anesthetic used do not influence the activity of hepatic cytochrome P450 2E1 and P450 reductases in the rat. Clear serum samples were separated by centrifugation at 3000 rpm for 20 min and then collected and stored at -20 °C for different biochemical analyses, prior immediate determination of glucose, triglycerides, cholesterol, high density lipoprotein HDL-cholesterol (HDL-C), low density lipoprotein LDL-cholesterol (LDL-C), total protein, creatinine, urea, uric acid, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). All of these parameters were measured using an automatic analyzer (Architect c8000 Clinical Chemistry System, USA).

Statistical analysis

Statistical analyses were performed using SPSS package for Windows version 13.0. Data are expressed as mean±SE. One-way ANOVA and two-way ANOVA were used to analyze differences among groups. Post-hoc analyses of significance were made using least-significant difference (LSD) test. Differences between groups were considered statistically significant at $p<0.05$.

RESULTS

Body weight change

Table 1 shows mean body weight of both control and experimental groups after one week, two weeks and three weeks. There were significant effects for the treatment ($p<0.001$) and duration ($p<0.001$). The treatment x duration ($p<0.001$) interaction was also significant. It is obvious that control animals showed a progressive increase in body weight with the lapse of time. STZ-induced diabetic rats given the control diet had the lowest body weight change after three weeks ($p<0.001$). STZ-induced diabetic rats given diets containing *N. sativa* oil, *C. zeylanicum* oil or *N. sativa* plus *C. zeylanicum* oils had higher body weight changes than STZ-induced diabetic rats given the control diet after three weeks ($p<0.05$). STZ-induced diabetic rats given diets containing the oils of *N. sativa* or mixture of *N. sativa* and *C. zeylanicum* had higher body weight changes than STZ-induced diabetic rats given the diet containing *C. zeylanicum* oil after three weeks.

Blood glucose

The mean values of blood glucose of both control and experimental groups are presented in Table 2. STZ-induced diabetic rats showed a highly significant ($p<0.001$) increase in the levels of blood glucose, registering increases of 172.6% after three weeks compared to the controls. Administration of *N. sativa* oil, *C. zeylanicum* oil and *N. sativa* plus *C. zeylanicum* oils to diabetic rats resulted in a significant ($p<0.001$) decrease in blood glucose levels of 48.4%, 48.7% and 43.7% respectively, after three weeks, compared to untreated diabetic rats. On the other hand, STZ-induced diabetic rats given the diets containing the oils of *C. zeylanicum* and *N. sativa* had lower mean blood glucose than STZ-induced diabetic rats given the diet containing *N. sativa* plus *C. zeylanicum* oils.

Blood triglyceride, cholesterol, LDL-C and HDL-C

The changes in the levels of serum lipids in control and experimental groups are illustrated in Table 2. There was a significant ($p<0.001$) decrease in the level of HDL- cholesterol (22%) and significant ($p<0.001$) increases in the levels of cholesterol, LDL- cholesterol and triglycerides in STZ-induced diabetic rats, with percentages of 58.5%, 62.5% and 130.6% respectively, compared to the controls. However, treatment of STZ-induced diabetic rats with *N. sativa* oil, *C. zeylanicum* oil and *N. sativa* plus *C. zeylanicum* oils resulted in a

significant ($p<0.01$) decrease in the levels of triglycerides, cholesterol and LDL- cholesterol compared to untreated diabetic rats. While HDL-cholesterol level was significantly ($p<0.05$) increased. On the other hand, the treatment with *C. zeylanicum* oil and *N. sativa* plus *C. zeylanicum* oils noticeably returned the levels of cholesterol and LDL- cholesterol to near control levels.

Blood total protein, urea, uric acid and creatinine

The mean values of blood total protein, urea, uric acid and creatinine concentrations of both control and experimental groups are presented in Table 3. STZ-induced diabetic rats showed a significant ($p<0.05$) decrease in blood total protein and uric acid with percentage of 5.5% and 23.9% respectively, compared to control. In contrast, STZ-induced diabetic rats showed a significant ($p<0.001$) increase in blood urea by 135.7% compared to the control. There is no significant change in creatinine concentration in STZ-induced diabetic rats when compared with control. On the other hand, only the treatment with *N. sativa* plus *C. zeylanicum* oils lead to significant ($p<0.05$) increases in the levels of total protein, uric acid and creatinine in STZ-induced diabetic rats by 4.4%, 91.4% and 15.8% respectively, as compared with untreated STZ-induced diabetic rats.

Blood AST and ALT

Table 3 shows the mean values of AST and ALT activities of both control and experimental groups after 3 weeks. In STZ-induced diabetic rats the activities of blood AST and ALT were significantly ($p<0.001$) increased by 49.3% and 96.03%, respectively, compared to their normal levels. On the other hand, treatment of the STZ-induced diabetic rats with *N. sativa* oil, *C. zeylanicum* oil or *N. Sativa* plus *C. zeylanicum* oils caused reduction in the activity of these enzymes in blood by 27.2, 29.4 and 19.4%, respectively for AST and by 40.9, 34.2 and 37.4% respectively for ALT, compared to the mean values of untreated diabetic group. STZ-induced diabetic rats fed a diet supplemented with *C. zeylanicum* oil had the lowest AST. While STZ-induced diabetic rats fed a diet supplemented with *N. sativa* oil had the lowest ALT.

Table 1. Effects of *N. sativa*, *C. zeylanicum* and *N. sativa* plus *C. zeylanicum* oils supplementation on body weight in STZ-diabetic rats.

	Body weight (g)			
Treatment	week 0	week 1	week 2	week 3
Normal control	199.90 ± 3.7	242.90 ± 3.1 (21.64±1.0) ***	263.10 ± 3.8 (30.04±2.3) **	283.20 ± 4.7 (41.78±1.5) ***
STZ	190.20 ± 4.2	178.40 ± 6.7 (-6.05±2.93) ***	176.30 ± 6.8 (-7.16±3.5) ***	172.90 ± 8.1 (-9.00±4.1) ***
STZ + *N. sativa* oil	196.60 ± 4.8	183.50 ± 7.3 (-6.66±3.0) ***	172.40 ± 8.2 (-12.33±3.6) ***	204.80 ± 8.6 (4.17±3.6) *** ##
STZ + *C. zeylanicum* oil	198.10 ± 4.9	180.70 ± 8.8 (-9.13±3.0) ***	182.20 ± 8.7 (-8.30±3.2) ***	188.50 ± 6.3 (-4.37±3.9) *** ##
STZ + *N. sativa* plus *C. zeylanicum* oils	202.00 ± 5.3	188.30 ± 6.2 (-6.87±1.4) ***	180.30 ± 6.7 (-10.87±1.8) ***	209.50 ± 9.5 (3.39±2.7) *** ##

The number of animals was 10 for each treatment. Percent changes are included in parentheses.
All values are expressed as means ± SE.

(* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$) when compared to control values.

(## $p < 0.01$) when compared to STZ values.

Table 2. Effects of *N. sativa*, *C. zeylanicum* and *N. sativa* plus *C. zeylanicum* oils supplementation on blood glucose, triglyceride, cholesterol, LDL-C and HDL-C in STZ-diabetic rats.

	Treatment				
Parameters	Normal control	STZ	STZ + *N. sativa* oil	STZ + *C. zeylanicum* oil	STZ+*N. sativa* plus *C. zeylanicum* oils
Glucose (mg/dl)	130.14 ± 4.3	354.82 ± 8.4	183.08 ± 2.8***	182.00 ± 2.1***	199.68 ± 2.9***
Triglyceride(mg/dl)	65.92 ± 3.8	152.04 ± 2.7	95.63 ± 1.4***	128.16 ± 1.5*	131.73 ± 2.0**
Cholesterol (mg/dl)	64.59 ± 2.6	102.36 ± 5.9	84.53 ± 2.3**	76.34 ± 2.5***	69.63 ± 2.0***
HDL-C (mg/dl)	20.33 ± 0.8	15.86 ± 0.4	18.50 ± 0.2*	18.22 ± 0.2*	19.51 ± 0.3**
LDL-C (mg/dl)	32.45 ± 1.2	52.72 ± 1.0	45.30 ± 2.1**	32.95 ± 1.8***	26.27 ± 1.3***

The number of animals was 5 for each treatment except for the control and STZ, in which it was 10.
All values are expressed as means ± SE.
Significantly different from untreated STZ-induced diabetic rats (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

Table 3. Effects of *N. sativa*, *C. zeylanicum* and *N. sativa* plus *C. zeylanicum* oils supplementation on blood total protein, urea, uric acid. creatinine, AST and ALT in STZ-diabetic rats.

	Treatment				
Parameters	Normal control	STZ	STZ + *N. sativa* oil	STZ + *C. zeylanicum* oil	STZ+*N. sativa* plus *C. zeylanicum* oils
Total protein (g/L)	6.31 ± 0.1	5.96 ± 0.1	6.14 ± 0.1	6.00 ± 0.1	6.22 ± 0.1
Creatinine (mg/dl)	0.40 ± 0.0	0.38 ± 0.0	0.36 ± 0.0	0.40 ± 0.0	0.44 ± 0.0
Uric acid (mg/dl)	0.92 ± 0.1	0.70 ± 0.1	0.74 ± 0.1	0.51 ± 0.0***	1.34 ± 0.2***
Urea (mg/dl)	20.00 ± 0.6	47.14 ± 5.2	51.20 ± 5.8	41.74 ± 4.1	40.79 ± 3.6
AST (U/L)	79.90 ± 3.6	119.30 ± 16.3	86.80 ± 1.2*	84.20 ± 3.5**	96.20 ± 3.1
ALT (U/L)	45.30 ± 1.9	88.80 ± 9.3	52.40 ± 3.5***	58.40 ± 8.4***	55.60 ± 2.0***

The number of animals was 5 for each treatment except for the control and STZ, in which it was 10.
All values are expressed as means ± SE.
Significantly different from untreated STZ-induced diabetic rats (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

DISCUSSION

Results of the present study showed that diabetic rats exhibited a significant increase in blood glucose level. This result is in consistent with the finding of Augusti and Sheela (1996) and Campos et al. (2003) in rats, Kumar and Reddy (1999) in mice and Jain and Vyas (1975) in rabbits. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in STZ-induced diabetes mellitus rats (Ravi et al., 2004; Rajasekaran et al., 2005; Sekar et al., 2005). In the present study, the oils of *N. sativa*, *C. zeylanicum* or *N. sativa* plus *C. zeylanicum* significantly reduces blood glucose levels in STZ-induced diabetic rats after 3 weeks of treatment, which also demonstrates that there is significantly higher rate of glucose disposal. Similar observations were also obtained by Al-Awadi et al. (1985), who reported that plant mixture extract comprising of *N. sativa*, Myrrh, Gum Arabic, Gum Asafoetida and Aloe to have a blood glucose lowering effect. Also, the intraperitoneal administration of volatile oil of *N. sativa* to fasting normal and alloxan-

diabetic rabbits produced significant hypoglycemic effects (Al-Hader et al., 1993). Kim et al. (2006) observed that cinnamon extract significantly reduces blood glucose levels in db/db mice after 2 weeks of treatment. Similarly, Kham et al. (2003) had reported that blood glucose level decreased after administration of cinnamon extract in people with type II diabetes. However, the mechanism of these oils used has not been clearly defined. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due to the effects of the diabetogenic agent streptozotocin (Szkudelski, 2001). Previous studies demonstrated that the essential oils of cinnamon and black seed and their active constituents have proven free radical scavenging and antioxidant activities (Burits and Bucar, 2000; Tomaino et al., 2005; Jayaprakasha et al., 2007). Based on above mentioned reports, we suggest that the possible mechanism of action by the oils of *N. sativa*, *C. zeylanicum* or *N. sativa* plus *C. zeylanicum* could be related to antioxidants that aid to recover from impaired metabolism of glucose.

In the present study, STZ-induced diabetic rats given the control diet had the lowest body weight change after three weeks. Similarly, several studies showed that the diabetic rats had significantly lower weight gain than the controls (Ananthi et al., 2003; Howarth et al., 2004; AL-Rawi, 2007). In the study of Howarth et al. (2004), they found that body weight in diabetic rats declined from 271 g before the administration of STZ to 238 g at 30 days after STZ treatment, whereas the body weight of control rats increased significantly from 247 to 314 g. Moreover, AL-Rawi (2007) found at the end of the experiment that control rats gained about 38.91 % of the original body mass whereas the diabetic rats lost about 30.82%. A decrease in body weight of diabetic rats is possible due to catabolism of fats and protein, even though the food intake is more in diabetic rats than control. Due to insulin deficiency protein content is decreased in muscular tissue by proteolysis (Vats et al., 2004). The diabetes caused by streptozotocin administration increases fat mobilization in skeletal muscle (Stearns et al., 1979) inducing significant weight loss (Besse et al., 1993) as was observed in the present study. However, diabetic rats treated with *N. sativa* oil and *N. sativa* plus *C. zeylanicum* oils showed increase in body weight which may be explained by increased insulin secretion or increased food consumption (Fernstrom and Fernstrom, 1993; Farouque and Meredith, 2003).

Lipids play a vital role in the pathogenesis of diabetes mellitus. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Al-Shamaony et al., 1994). In our study, we have noticed significantly increased levels of serum total cholesterol, triglycerides and LDL- cholesterol but markedly decreased level of serum HDL- cholesterol in STZ- induced diabetic rats. These results are in agreement with those obtained by (Bolkent et al., 2004; Ravi et al., 2005; Singh et al., 2005; Rajasekaran et al., 2006). The abnormal high concentrations of serum lipids in diabetic animals are due mainly to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone-sensitive lipase (Pushparaj et al., 2000). Excess fatty acids in the serum of diabetic rats are converted into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins (Bopanna et al., 1997). The present study showed that *N. sativa*, *C. zeylanicum* or *N. sativa* plus *C. zeylanicum* oils had favourably modified serum lipid profile in rats with significant decreases in total cholesterol, LDL-cholesterol, triglycerides and increased HDL. On the other hand, the effect was more with *C. zeylanicum* oil. But the effect of the two oils in combination was more than that with either of the oils alone. Zaoui et al. (2002) reported that serum cholesterol, triglycerides and glucose levels were significantly decreased in *N. sativa* oil (1 ml/kg/day) for 12 weeks) treated rats. Subash Babu et al. (2006) found that Administration of cinnamaldehyde which are the main constituent of cinnamon lowers serum lipids, and also increases the serum HDL-cholesterol. The antilipidemic action of *N. sativa* oil, *C. zeylanicum* oil or *N. sativa* plus *C. zeylanicum* oils may reside in their ability to stimulate insulin secretion and action.

The data revealed significant elevations in blood urea in STZ-induced diabetic rats. A similar effect was recorded previously (Gawronska-Szklarz et al., 2003). The highly significant increase in serum urea concentrations of diabetic rats may be due to depletion of serum protein, increase in the rate of circulating amino acids and deamination takes place that consequently leads to the formation of large amount of ammonia which is eventually converted to urea. The

breakdown of amino acids during gluconeogenesis in the liver results in increased production of urea, fostering negative nitrogen balance (Ganong, 2003). In contrast, serum total protein, uric acid and creatinine were decreased in diabetic animals. The decrease in blood total protein observed in diabetic rats is coinciding with the findings of Peavy et al. (1985) and Wanke and Wong (1991). This decline may be due to the inhibited oxidative phosphorylation processes which lead to decrease of protein synthesis, increase in the catabolic processes and reduction of protein absorption (Tragl and Reaven, 1972; Jefferson et al., 1983). Possible defects in tubular reabsorption and probably increased excretion may explain such decreases in blood uric acid and creatinine. Creatinuria occurs in any condition associated with extensive muscle breakdown as in starvation and poorly controlled diabetes mellitus (Ganong, 2003). However, uric acid clearance has been associated with insulin resistance (Facchini et al., 1991). Previous changes in serum urea, uric acid and creatinine concentrations strongly suggested impairment of kidney function in diabetes. The treatment with *N. sativa* plus *C. zeylanicum* oils returned total protein level to near normal levels. While, the levels of uric acid and creatinine were higher than normal rats. The main effect of the *N. sativa* plus *C. zeylanicum* oils is presumably due to its ability to increase insulin secretion.

In STZ-induced diabetic rats the activities of blood AST and ALT were significantly increased compared to their normal levels. These results indicated that diabetes may be induced due to liver dysfunction. Ohaeri (2001) also found that liver was necrotized in STZ-induced diabetic rats. Therefore, increase in the activities of AST and ALT in blood may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993), which gives an indication on the hepatotoxic effect of STZ. On the other hand, treatment of the diabetic rats with *N. sativa* oil, *C. zeylanicum* oil or *N. Sativa* plus *C. zeylanicum* oils caused reduction in the activity of these enzymes in blood compared to the mean values of diabetic group and consequently may alleviate liver damage caused by STZ-induced diabetes. These results are in agreement with those obtained by Subash Babu et al. (2006) who reported that oral administration of cinnamaldehyde for 45 days significantly restores the enzyme levels to near normal in diabetic rats. A possible explanation for the differential effects of *N. sativa* oil, *C. zeylanicum* oil or *N. Sativa* plus *C. zeylanicum* oils on the activities of AST and ALT in blood is that these treatments may inhibit the liver damage induced by streptozotocin.

CONCLUSION

The results of this study indicate that the oils of *N. sativa*, *C. zeylanicum* or *N. sativa* plus *C. zeylanicum* possess hypoglycemic, hypolipidemic and antioxidant effects in STZ-induced diabetic rats and suggest that these oils may be a useful supplemental remedy in diabetes.

REFERENCES

Aboutabl B, El-Azzouny A, Hammerschtïiidt F. 1986. Aroma volatile of *Nigella sativa* seeds. Prog Essent Oil Res, Proc Int Symp Essent Oils 16th:49-55.

Al-Awadi F, Khattar M, Gumaa K. 1985. On the mechanism of the hypoglycemic effect of a plant extract. Diabetologia 28:432-434.

Al-Hader A, Aqel M, Hasan Z. 1993. Hypoglycaemic effects of the volatile oil of *Nigella sativa* seeds. Int J Pharmacognosy 31:96–100.

AL-Rawi M. 2007. Effect of *Trifolium* sp. flowers extracts on the status of liver histology of streptozotocin-induced diabetic rats. Saudi J Bio Sci 14:21-28.

Al-Shamaony L, Al-khazrajoi S, Twaij H. 1994. Hypoglycaemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. J Ethnopharmacol 43:167–171.

Ananthi J, Prakasam A, Pugalendi K. 2003. Antihyperglycemic activity of *Eclipta alba* leaf on alloxan-induced diabetic rats. Yale J Biol Med 76:97-102.

Augusti K, Sheela C. 1996. Antiperoxide effect of S-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. Experientia 52:115-120.

Ballero M, Fresu I. 1993. Le plante di uso officinale nella Barbagia di Scui (Sardegna Centrale). Fitoterapia 64:141-150.

Bamosa A, Ali B, Sowayan S. 1997. Effect of oral ingestion of *Nigella sativa* seeds on some blood parameters. Saudi Pharm J 5:126-129.

Battaglia S. 1995. The complete guide to aromatherapy. The Perfect Potion, Virginia, Queensland.

Besse S, Assayag P, Delcayre C, Carré F, Cheav SL, Lecarpentier Y, Swynghedauw B. 1993. Normal and hypertrophied senescent rat heart: mechanical and molecular characteristics. Am J Physiol 265:H183-H190.

Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. 2006. Medicinal plants with potential antidiabetic

activity - A review of ten years of herbal medicine research (1990-2000). Int J Diab Metab 14:1-25.

Bolkent S, Yanardag R, Karabulut-Bulen O, Ozsoy-Sacan O. 2004. The morphological and biochemical effects of glibornuride on rat liver in experimental diabetes. Hum Exp Toxicol 23:257-264.

Bolkent S, Yanardag R, Tunali S. 2005. Protective effect of vanadyl sulfate on the pancreas of streptozotocin-induced diabetic rats. Diabetes Res Clin Pract 70:103-109.

Bopanna K, Kannan J, Sushma G, Balaram R, Rathod S. 1997. Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. Indian J Pharmacol 29:162-167.

Burits M, Bucar F. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Res 14:323-328.

Campos K, Diniz Y, Cataneo A, Faine L, Alves J, Novelli E. 2003. Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. Int J Food Sci Nutr 54:241–246.

Canonica L, Jonuni G, Scolastico C, Boriati A. 1963. The pharmacologically active principle in *Nigella saliva*. Gazz Chim Ital 93:1404-1407.

Defronzo R, Hendeler R, Simonson D. 1982. Insulin resistance is a prominent feature of insulin dependent diabetes. Diabetes 31:795-801.

El-Alfy T, El-Fatatry H, Toama M. 1975. Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa L.* seeds. Pharmazie 30:109-111.

El-Dakhakhuy M. 1963. Studies on the chemical constitution of Egyptian *Nigtlla sativa* L. seeds. The essential oil. Planta Med 11:465-470.

El-Dakhakhny M. 1965. Egyptian *Nigella sativ*a. Arzneimittel-Forsch 15:1227-1229.

El-Hilaly J, Tahraoui A, Israili Z, Lyoussi B. 2006. Hypolipidemic effects of acute and subchronic administration of an aqueous extract of *Ajuga iva* L. whole plant in normal and diabetic rats. J Ethnopharmacol 105:441–448.

El-Kadi A, Kandil O. 1987. The black seed (*Nigella sativa*) and immunity: its effect on human T cell subset. Fed Proc 46:1222.

El-Tahir K, Ashour M, Al-Harbi M. 1993. The cardiovascular actions of the volatile otl of the black seed (*Nigella sativa*) in rats: Elucidation of the mechanism of action. Gen Pharm 24:1123-1131.

Ernst E. 1997. Plants with hypoglycemic activity in humans. Phytomedicine 4:73-78.

Facchini F, Chen Y, Hollenbeck C, Reaven G. 1991. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. JAMA 266:3008- 3011.

Farouque H, Meredith L. 2003. Effects of inhibition of ATP sensitive potassium channels on metabolic vasodilation in the human forearm. Clin Sci 104:39-46.

Fernstrom M, Fernstrom J. 1993. Large changes in serum free tryptophan levels do not alter tryptophan levels. Studies in streptozotocin, diabetic rats. Life Sci 52:907-916.

Fisman E, Tenenbaum A, Motro M, Adler Y. 2004. Oral antidiabetic therapy in patients with heart disease. A cardiologic standpoint. Herz 29:290–298.

Ganong W. 2003. Review of medical physiology, 21st Ed., Lange Medical Books, McGraw-Hill, New York.

Gawronska-Szklarz B, Musial D, Pawlik A, Paprota B. 2003. Effect of experimental diabetes on pharmacokinetic parameters of lidocaine and MEGX in rats. Pol J Pharmacol 55:619-624.

Goodyear L, Hirshman M, Knutson S, Horton E, Horton E. 1988. Effect of exercise training on glucose homeostasis in normal and insulin-deficient diabetic rats. J Appl Physiol 65:844–851.

Hashim F, El-Kicy M. 1962. *Nigella sativa* seeds of Egypt. Pharmac Sci. United Arab Rep 3:121-133.

Hajhashemi V, Ghannadi A, Jafarabadi H. 2004. Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. Phyto Res 18:195- 199.

Holman R, Turner R. 1991. Oral agents and insulin in the treatment of NIDDM. In Textbook of Diabetes. Blackwell. (Eds.), Pickup S, Williams G. Oxford, pp. 467-469.

Houghton P, Zarka R, Las Heras B, Hoult J 1995. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med 61:33-36.

Howarth F, Jacobson M, Naseer O, Adeghate E. 2004. Short-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Exp Physiol 90:237-245.

Islam S, Begun P, Ahsan T, Huque S, Ahsan M. 2004. Immunosuppressive and cytotocic properties of Nigella sativa. Phytother Res 8:395-398.

Jain R, Vyas C. 1975. Garlic in alloxan-induced diabetic rabbits. Am J Clin Nutr 28:684–685.

Jarvinen Y, Koivisto V. 1984. Insulin sensitivity in newly diagnosed type-I diabetes following ketoacidosis after a three month insulin therapy. J Clin Endocrinol Metab 59:371-378.

Jarvinen Y, Koivisto V. 1986. Natural course of insulin resistance in type-I diabetes. N Engl J Med 315:224-230.

Jayaprakasha G, Jaganmohan Rao L, Sakariah K. 1997. Chemical composition of the volatile oil from the fruits of *Cinnamomum zeylanicum* Blume. Flav Fragr J 12:331-333.

Jayaprakasha G, Jaganmohan Rao L, Sakariah K. 2000. Chemical composition of the flower oil of

Cinnamomum zeylanicum Blume. J Agric Food Chem 48:4294-4295.

Jayaprakasha G, Negi P, Jena B, Jagan Mohan Rao L. 2007. Antioxidant and antimutagenic activities of *Cinnamomum zeylanicum* fruit extracts. J Food Comp Anal 20:330–336.

Jefferson L, Liao W, Peavy D, Miller, Appel M, Taylor J. 1983. Diabetes-induced alterations in liver protein synthesis: Changes in the relative abundance of mRNA for albumin and other plasma proteins. J Biol Chem 258:1369-1375.

Kameswara Rao B, Giri R, Kesavalu M, Appa Rao Ch. 1997. Herbal medicine in the management of diabetes mellitus. Manphar Vaidhya Patrika 1:33-35.

Kannappan S, Jayaraman T, Rajasekar P, Ravichandran M, Anuradha C. 2006 Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed rat. Singapore Med J 47:858-63.

Kham A, Safdar M, Alikhan M, Khattak K, Anderson R. 2003. Cinnamon improves glucose and lipids of people with type 2 diabetes. Diab Care 26:3215–3218.

Kim S, Hyun S, Choung S. 2006. Anti- diabetic effect of cinnamon extract on blood glucose in db/db mice. J Ethnopharmacol 104:119–123.

Kumar G, Reddy K. 1999. Reduced nociceptive responses in mice with alloxan induced hyperglycemia after garlic (*Allium sativum*) treatment. Indian J Exp Biol 37:662–666.

Larner J. 1985. Insulin and oral hypoglycaemic drugs, glucagon. In The Pharmacological Basis of Therapeutics, (Eds.), Gilman A, Goodman L, Rall T, Murad F, Macmillan, New York, pp. 1490-1516.

Mahfouz M, El-Dakhakhny M. 1966. The isolation of a crystalline active principle from *Nigella sativa* seeds. J Pharmaceut Sci 1:9-19.

Mahfouz M, El-Dakhakhny M, Gemei A, Moussa H. 1962. Choleretic action of *Nigella sativa* seed oil. Egyp Pharmacol Bull 44:225-229.

Mai Le P, Benhaddou-Andaloussi A, Elimadi A, Settaf A, Cherrah Y, Haddad P. 2004. The petroleum ether extract of *Nigella sativa* exerts lipid-lowering and insulin-sensitizing actions in the rat. J Ethnopharmacol 94:251-259.

Markun J, Napoli R, Hirshman M, Davalli A, Cheatham B, Goodyear L. 1999. Effect of streptozocin-induced diabetes and ilst cell transplantation on insulin signaling in rat skeletal muscle. Endocrinol 140:106–111.

Meral I, Yener Z, Kahraman T, Mert N. 2001. Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damaga in experimentally-induced diabetic rabbits. J Vet Med 48:593-599.

Navarro C, Montilla P, Martin A, Jimenez J, Utrilla P. 1993. Free radicals scavenger and antihepatotoxic activity of Rosmarinus. Planta Med 59:312–314.

Odetti P, Travers O, Cosso L, Noberasco G, Pronzato M, Marinariu M. 1996. Good glycemic control reduces oxidation and glycation end- products in collagen of diabetic rats. Diabetologia 39:1440-1447.

Ody P. 1993. The complete medicinal herbal. Viking Books. Ringwood.

Ohaeri O. 2001. Effect of garlic oil on the levels of various enzyme in the serum and tissue of streptozotocin diabetic rats. Bio Sci Rep 21:19–24.

Peavy D, Taylor J, Jefferson L. 1985. Time course of changes in albumin synthesis and mRNA in diabetic and insulin-treated diabetic rats. Am J Physiol 248:656-663.

Plate A, Crankshaw D, Gallaher D. 2005. The effect of anesthesia by diethyl ether or isoflurane on activity of cytochrome P450 2E1 and P450 reductases in rat liver. Anesth Analg 101:1063-1064.

Pushparaj P, Tan C, Tan B. 2000. Effects of *Averrhoe bilimli* leaf extract on blood glucose and lipids in streptozotocin diabetic rats. J Ethnopharmacol 72:69-76.

Rajasekaran S, Ravi K, Savgananam K, Subramanian S. 2006. Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clin Exp Pharmacol Physiol 33:232-237.

Rajasekaran S, Sivagnanam K, Subramanian S. 2005. Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats. Pharmacol reports 57:90–96.

Ravi K, Ramachandra B, Subramanian S. 2004. Protective effect of *Eugenia jambolana* seed kernel on tissue antioxidants in streptozotocin induced diabetic rats. Biol Pharm Bull 27:1212-1217.

Ravi K, Rajasekaran S, Subramanian S. 2005. Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats. Food Chem Toxicol 43:1433-1439.

Rchid H, Chevassus H, Nmila R, Guiral C, Petit P, Chokaïri M, Sauvaire Y. 2004. *Nigella sativa* extracts enhance glucose-induced insulin release from rat-isolated Langerhans islets. Fund Clin Pharmacol 18:525-529.

Sekar D, Sivagnanam K, Subramanian S. 2005. Antidiabetic activity of Momordica charantia seeds on streptozotocin induced diabetic rats. Pharmazie 60:383-7.

Senanayake U, Lee T, Wills R. 1978. Volatile constituents of cinnamon (*Cinnamomum zey- lanicum*) oils. J Agric Food Chem 26:822-824.

Sharma S, Nasir A, Prabhu K, Murthy P, Dev G. 2003. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits. J Ethnopharmacol 85:201–206.

Singh N, Kamath V, Rajini P. 2005. Protective effect of potato peel powder in ameliorating oxidative stress in streptozotocin diabetic rats. Plant Foods Hum Nutr 60:49-54.

Stearns S, Tepperman H, Tepperman J. 1979. Studies on the utilization and mobilization of lipid in skeletal muscles from streptozotocin-diabetic and control rats. J Lipid Res 20:654–662.

Stenman S, Groop P, Laakkonen K, Wahlin-Boll E, Melander,A. 1990. Relationship between sulfonylurea dose and metabolic effect. Diabetes 39:108A.

Subash Babu P, Prabuseenivasan S, Ignacimuthu S. 2006. Cinnamaldehyde-A potential antidiabetic agent. Phytomedicine 14:15-22.

Szkudelski T. 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50:537-546.

Tesch G, Nikolic-Paterson D. 2006. Recent insights into experimental mouse models of diabetic nephropathy. Nephron Exp Nephrol 104:57-62.

Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A, Saija A. 2005. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem 89:549–554.

Toppozada H, Mazloum H, El-Dakhakhny M. 1965. The antibacterial properties of *Nigella sativa* seeds:active principle with some clinical application. J Egypt Med Assoc 48:187-202.

Tragl K, Reaven G. 1972. Effect of insulin deficiency on hepatic ribosomal aggregation. Diabetes 21:84-88.

Vats V, Yadav S, Grover J. 2004. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. J Ethnopharmacol 90:155–160.

Wallberg-Harrison H, Holoszy J. 1985. Activation of glucose transport in diabetic muscle:response to contraction and insulin. Am J Physiol 249:233–237.

Wanke L, Wong N. 1991. Diabetes mellitus decreases the activity of the albumin promotor *in vitro*. J Biol Chem 266:6068-6072.

Worthen D, Ghosheh O, Crooks P. 1998. The in vitro anti-tumor activity of some crude and purified components of Black seed *Nigella sativa* L. Anticancer Res 18:1527-1532.

Yassin M, Ashour A, Elyazji N. 2004. Alterations in body weight, protein profile, non-protein nitrogen constituents and kidney structure in diabetic rats under glibenclamide treatment. J Islam Univ Gaza 12:65-82.

Zaoui A, Cherrah Y, Alaoui K, Mahassine N, Amarouch H, Hassar M. 2002. Effects of *Nigella sativa* fixed oil on blood homeostasis in rat. J Ethnopharmacol 79:23-26.

Zari T, Al-Attar A. 2007. Effects of ginger and clove oils on some physiological parameters in streptozotocin-diabetic and non-diabetic rats. J Med Sci 7:267-275.

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Genotoxicity assessment of water extracts of *Ocimum gratissimum, Morinda lucida* and *Citrus medica* using the *Allium cepa assay*

[Evaluación de la genotoxicidad de extractos acuosos de *Ocimum gratissimum, Morinda lucida* y *Citrus medica* mediante el ensayo de A*llium cepa*]

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Abstract

Herbal preparations may be with a single or combination of herbs. With continuous and uncontrolled use of medicinal plants, there may be sufficient exposure to permit the expression of genotoxicity. In this study, the genotoxicity of water extract of *Ocimum gratissimum* (Linn.) alone, and in combination with *Morinda lucida* (Benth.); and combination of *Morinda lucida* extract and *Citrus medica* (Linn.) juice were investigated using the *Allium cepa* test. Leaves of *O. gratissimum* and *M. lucida* and juice of *C. medica* were used to prepare the extracts as practiced locally. Roots of *Allium cepa* were exposed to 1, 2.5, 5, 10 and 25 % concentrations (v/v) of each of the extracts for 24 and 48 h, respectively for cytological analysis. Tap water was utilized as the control. All the extracts caused a statistically significant ($p < 0.05$) concentration dependent suppression of mitotic activity in root tip cells of *Allium cepa.* The extract of *O. gratissimum* induced binucleated cells at different concentrations. The mixture of the extracts of *O. gratissimum* and *M. lucida* totally inhibited cell division at the highest tested concentration indicating blockage of the cell cycle at the interphase stage. Data on the combination of extracts indicate interaction of the herb constituents. These observations suggest cytotoxic and mitodepressive effects of the extracts on *A. cepa* cells.

Keywords: *Allium*, *Ocimum, Morinda, Citrus,* genotoxicity, herbs, recipes, mitodepressive.

Resumen

Las preparaciones a base de hierbas pueden ser simples o con hierbas en combinación. Con el uso continuo e incontrolado de estas preparaciones, puede haber suficiente exposición para permitir la expresión de genotoxicidad. En este estudio, la genotoxicidad del extracto acuoso de *Ocimum gratissimum* (Linn.) solo y en combinación con *Morinda lucida* (Benth.), y la combinación de los extractos de *Morinda lucida* y el jugo de *Citrus medica* fueron investigados mediante la prueba de *Allium cepa*. Las hojas de *O. gratissimum* y *M. lucida* y jugo de *C. medica* se utilizaron para preparar los extractos como se utilizan a nivel local. Las raíces de *Allium cepa* se expusieron, para el análisis citológico, a concentraciones de 1; 2,5; 5; 10 y 25% (v/v) de cada uno de los extractos 24 y 48 h, respectivamente. El agua del grifo se utilizó como control. Todos los extractos causaron diferencias estadísticamente significativas ($p < 0,05$) dependientes de la concentración, causando supresión de la actividad mitótica en las células radicular de *Allium cepa.* El extracto de *O. gratissimum* indujo células binucleadas a diferentes concentraciones. La mezcla de extractos de la *O. gratissimum* y *M. lucida* inhibieron la división celular en las concentraciones más altas indicando el bloqueo del ciclo celular en la interfase. Los datos de las combinaciones indican las interacciones de los constituyentes herbarios. Estas observaciones sugieren efectos citotóxicos y mitodepresivos sobre las células de *A. cepa.*

Palabras Clave: *Allium*, *Ocimum, Morinda, Citrus,* genotoxicidad, hierbas, mezclas, inhibidores de la mitogénesis.

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INTRODUCTION

The use of medicinal plants by the general population is an old and still widespread practice. The world Health Organization (W.H.O.) estimates that 80% of the world population uses herbal medicines for some aspects of primary health care (Farnsworth et al., 1985). In African ethnomedicine, it is well known that traditional healers make use of varieties of herbs in the treatment of diseases and a wide proportion of these herbal remedies are widely believed to be effective (Kirby, 1997; Warrell, 1997). Brinkmann and Brinkmann (1991) stated that only 8.25% of people with malaria visit health services, indicating that the rest uses alternative medicine which involves the use of medicinal herbs with antimalarial properties.

Medicinal plants and indeed plants in general, synthesize toxic substances, which in nature act as a defense against infections, insects and herbivores, but which often affect the organisms that feed on them (Teixeira et al., 2003). Reports of unwanted side effects and potential toxicity of phytomedicines have been on the increase in recent years (Effraim et al., 2001; Ruffa et al., 2002; Amida et al., 2007; Konan et al., 2007; Sowemimo et al., 2007; Nwafor et al., 2007). Moody et al. (1999) stated that since some of the herbal medicines are needed for the management of chronic diseases, long-term use might evoke mutagenicity and carcinogenicity. This is of importance in Nigeria where a large percentage of the populace cannot afford to pay for orthodox medical service (Fasola and Egunyomi, 2005; Obi et al., 2006) and thus resort to the use of herb extracts most of the time. Traditional practices in the country also encourage the use of herbal medicine, the preparation of which may be with a single herb or recipe of herbs. With the use of these herbs, there may be sufficient exposure to permit the expression of genotoxicity. Evidences on potential genotoxic and mutagenic effects of some herb extracts have been documented (Patterson et al., 1987; Magda, 2001; Gadano et al., 2000; 2002; 2006; Paes-Leme et al., 2005; Barcelos et al., 2007; Konan et al., 2007; Sowemimo et al., 2007).

In view of these, evaluating the potential cytotoxic and genotoxic effects of herb extracts in use in Nigeria is essential for standardization, safety purposes and to understand the mechanism and broad range of action of these herbs. *Ocimum gratissimum* (Linn.), *Morinda lucida* (Benth.) and *Citrus medica* (Linn.) are medicinal plants that are widely used in unison or in combination in the treatment of febrile illnesses in southwestern Nigeria (Ajaiyeoba et al., 2003). The present study sought to evaluate the potential effect of extracts of *O. gratissimum* alone; recipes containing *M. lucida* and *O. gratissimum* and recipes containing *M. lucida* and *C. medica* on the chromosome and cell division of *Allium cepa*. This is with the objective of investigating possible interactive effects of these herbs on the genetic material of *A. cepa*. The *Allium* root system which has been in use since 1938 (Levan, 1938) for investigating environmental pollution factors, toxicity of chemical compounds, and evaluating potential anticancer properties (Podbielkowska et al., 1981; 1995; Keightley et al., 1996; Bakare et al., 2000; Majewska et al., 2003; Babatunde and Bakare, 2006; Kura's et al., 2007) is very comfortable as it is easy to make preparations of onion roots. The test is a fast and inexpensive method, allowing the investigation of universal mechanisms for meristematic plant cells and extrapolation on animal cells.

MATERIALS AND METHODS

Collection of medicinal plants

The medicinal plants used for this study were selected based on their ethnobotanical uses and availability. *O. gratissimum* (Labiatae/Lamiaceae), commonly called basil or scent leaves and *M. lucida* (Rubiaceae) also called brimestone were collected within the premises of the University of Ibadan, Nigeria, while *C. medica* (Rutaceae) was obtained commercially at the Bodija market, Ibadan, Nigeria. These were taken to the University of Ibadan Herbarium (UIH located in department of Botany) for identification.

Preparation of extracts

Squeezed extracts of leaves of *O. gratissimum*, combination of *M. lucida* and *C. medica* and combination of *M. lucida* and *O. gratissimum* were prepared with water separately as practiced locally. Briefly, 150g of the leaves of *O. gratissimum* were washed with tap water in order to remove dirt, and then air-dried. These were then squeezed in 1 L of tap water. For the recipe containing *M. lucida* and *O. gratissimum*, 270 g of each were washed with tap water and air-dried. The leaves of the two herbs were then crushed in a mortar with pestle. The crushed leaves were then squeezed in 1 L of tap water. For the recipe containing *M. lucida* and *C. medica*, 270 g of *M. lucida* leaves were given the same treatment as for

the previous recipe. 400 ml of the extract was then mixed with 400 ml of *C. medica* fruit juice. All the extracts were filtered with a 2.5µm filter (Whatman® no. 42) to remove the suspended particles and kept at 4◦C until use. Each of these preparations was considered as the stock solution (100%). Serial dilutions were used in the experiment as the stock did not permit onion root growth.

Experimental design

Experiments were planned as per standard protocol for *Allium* test (Fiskesjo, 1985; Grant, 1982; 1994; Mosuro and Bakare, 1998). Onion bulbs (*Allium cepa*, 2n=16, Family *Amaryllidaceae*) were obtained commercially from Ibadan, Nigeria. They were sun dried for two weeks so as to reduce the moisture content and to facilitate root growth. For each herb/recipe extract, five concentrations (v/v) viz: 1, 2.5, 5, 10 and 25 % were considered. Tap water was used as the negative control. Two exposure periods of 24 h (Group I) and 48 h (Group II) were utilized in this study. At each concentration for each group and the control, 5 onion bulbs were grown in tap water in 100 ml beakers (27±1 ^{o}C) for 2 days in the dark. After 48 h of root sprouting, the bulbs were transferred into the respective concentration of the extracts and allowed to grow for time intervals of 24 h (Group I) and 48 h (Group II) respectively. Samples from the experimental and the control groups were changed every 24 h. At the end of the treatment period for each group, root tips were cut and fixed in ethanol: glacial acetic acid (3:1 v/v). Thereafter, these were hydrolyzed in 1N HCL at 60 °C for 5 min after which they were washed in distilled water. Two root tips were then squashed on a microscope slide and stained with acetocarmine for 10 min. Excess stain was removed and cover slips were carefully placed on the smear. The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant (1982). Six slides were prepared for each concentration and the control out of which four (at 1000 cells / slide) were scored for induction of aberrant cells at x1000 magnification. The mitotic index was calculated as the number of dividing cells per total cells scored at each concentration. The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored for each concentration of the extract (Bakare et al., 2000). The mitotic inhibition was obtained as follows:

Mitotic inhibition = (mitotic index in control – mitotic index in treated group) ×100 / (mitotic index in control)

Statistics

The SAS statistical package was used for this analysis. All data were subjected to Chi-square analysis to test the difference in percentage inhibition of cell division and aberration between the control and the individual dosage group of each extract. Level of significance was set at $p<0.05$.

RESULTS

At tested concentrations of aqueous extract of *O. gratissimum* at the two time intervals of 24 and 48 h, there was a concentration dependent reduction in the mitotic index of root tip cells of *A. cepa* when compared with the control (Table 1). Binucleate cell formation was observed at the tested concentrations (except at the 1% concentration at the two exposure periods and 25% concentration at the 48 h exposure period) of this extract within the two exposure periods (Table 1, Fig. 1). The highest frequency of binucleated cells at 24 h exposure period (r = 0.80) was observed at the 10% concentration, while the lowest was observed at the 2.5% concentration. At the 48 h exposure period (r = -0.08) the highest frequencies of binucleated cells were recorded at the 5% concentrations respectively.

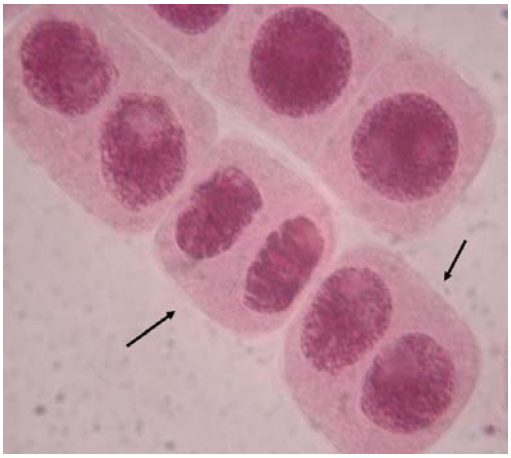
No aberrant cells were evident after 24 and 48 h exposure of the onion roots to extracts of the recipes. The number of dividing cells at the tested concentrations however, decreased compared to the control values at the two exposure periods respectively. The lowest and highest mitotic index of 5.0% and 10.35% resulting to 67.8% and 33.2% mitotic inhibitions were observed at the 25% and 1% concentrations of the *O. gratissimum* and *M. lucida* combinations after 24 h exposure. While the lowest mitotic index of 2% and highest mitotic inhibition of 80.95% were observed at the 10% concentration of the extract after 48 h exposure (Table 1). There were no dividing cells after 48 h at the 25% concentration of the extract, all of the cells being at the interphase stage of the cell cycle. For the combination of *M. lucida* and *C. medica* with a 24 h exposure period, the lowest and highest MI were 7.6% and 10.5% at the 25% and 1% concentrations (51% and 32.3% mitotic inhibition, respectively).

Table 1. Cytological effects of water extracts of *O. gratissimum*, recipes of *M. lucida* and *O. gratissimum*, and *M. lucida* and *C. medica* on cells of *A. cepa* after 24 and 48 hours exposure, respectively.

Test extracts	Exposure period	24 hr						48 hr					
	Conc. (%)[+]	0	1	2.5	5	10	25	0	1	2.5	5	10	25
Ocimum gratissimum	No of dividing cells	620	465	450	346	325	215	422	335	290	185	220	115
	Mitotic index	15.5	11.63	11.25	8.65	8.13	5.4	10.5	8.38	7.3	4.6	5.5	2.9
	Mitotic inhibition (%)	0	24.96	27.4	44.2*	47.5*	65.2*	0	20.2	30.5*	56.2*	47.6*	72.4*
	Frequency of aberrant cells (%)	0	0	0.42*	0.28*	1.16*	0.64*	0	0	0.86*	0.60*	0.36*	0
Morinda lucida and *Ocimum. gratissimum*	No of dividing cells	620	414	239	220	338	200	422	247	165	200	77	0
	Mitotic index	15.5	10.35	6	5.5	8.5	5	10.5	6.2	4.1	5	2	0
	Mitotic inhibition (%)	0	33.2*	61.3*	64.5*	45.2*	67.8*	0	40.95*	61.0*	52.4*	80.95*	100*
Morinda lucida and *Citrus medica*	No of dividing cells	620	420	417	409	397	304	422	385	362	303	289	142
	Mitotic index	15.5	10.5	10.4	10.2	9.9	7.6	10.5	9.6	9.05	7.6	7.2	3.55
	Mitotic inhibition (%)	0	32.3*	32.9*	34.2*	36.1*	51*	0	8.6	13.81*	27.62*	31.4*	66.19*

* Significant difference at P< 0.05 [+] 4000 cells/concentration of each extract

Figure 1. Binucleate cells induced in root tip cells of *Allium cepa* by water extracts of *Ocimum gratissimum*



After the 48 h exposure period, the MI values were 3.55% at the 25% concentration and 9.6% at the 1% concentration with 66.19% and 8.6% mitotic inhibitions respectively. The responses at the two exposure periods were concentration dependent and statistically significant for both recipes ($p<0.05$) (Table 1).

DISCUSSION

In this study, microscopic observation revealed normal chromosomal phases of mitotic division in onion root tip cells indicating that the extracts did not induce adverse chromosomal effect. Overall, the results showed that at the 2 exposure periods, the water extracts caused a concentration dependent reduction in the mitotic index compared with the control. There were no dividing cells at the highest concentration (%) of water extracts of the recipes of *M. lucida* and *O. gratissimum.* The mitotic index (MI) which is used as an indicator of adequate cell proliferation biomarkers measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be interpreted as cellular death. The mito-depressive effect suggests that the extracts had some effects on cell division. *Citrus medica*, *O. gratissimum* and *M. lucida* have been reported to contain alkaloids, tannins, flavonoids, anthraquinones and saponoside (Adegoke et al., 1968; Burkill, 1997) and these might be responsible for the inhibitory effects observed here.

When tested alone, the water extract of *M. lucida* inhibited root growth and the mitotic index, and caused disturbance of the mitotic spindle in *Allium cepa* (Akinboro and Bakare, 2007). In this study, combination of *M. lucida* with *O. gratissimum* induced a similar effect which was more pronounced at 48 h exposure period where there was no dividing cell at the 25% concentration, an observation that was recorded at the 20% concentration of *M. lucida* (alone) on *A. cepa* (Akinboro and Bakare, 2007). This total inhibition of cell division indicated blockage of the cell cycle at the interphase stage and might be attributed to the activity of *M. lucida* as treatment of *A. cepa* with *O. gratissimum* alone did not produce similar results. The combination of *M. lucida* with *C. medica* did not induce total arrest of cell division but only a reduction of MI dose-dependently. This may mean that *C. medica* probably has constituents that diluted the activities of the components of *M. lucida*. Many other investigations showed that the reduction in cell division activity could be due to change in the duration of the mitotic cycle. Van't Hoff (1968) suggested that the inhibition of mitotic activity by chemical compounds is due to an increase in the G2 period. This view was supported by the results of Bruneri (1971) who obtained a complete arrest of mitotic cycle at the G2. Other authors (Webster and Davidson, 1969; Macleod, 1969) attributed the inhibition of mitosis to an increase in the duration of the S-phase.

Tannins are good enzyme inhibitor and act as antidotes for certain types of alkaloids (Martins et al., 1995). Alkaloids like vincristine and vinblastine have been reported to be mutagenic (Ene-Obomg and Osuala, 1990). The effects of alkaloids in the extract of *O. gratissimum* might have been neutralized by other components such as tannins in the extracts of *M. lucida* with which it was combined; hence there was no observable chromosomal defect in *A. cepa* resulting from the combination. The toxic effect of the extracts on cell division of *A. cepa* was also time-dependent. The effect observed at 24 h was lower than those at 48 h of exposure. This might be due to accumulation of inhibitory substances present in the extracts over time.

The only cellular aberration observed in the cells of *A. cepa* treated with an aqueous extract of *O. gratissimum* was the presence of binucleated cells. This usually arises as a consequence of the inhibition of cell plate formation (Grant, 1978), which might be due to the suppression of phragmoplast formation in the early telophase (Soliman, 2001) by the alkaloids in the extract. Induction of binucleate cells consequently leads to the formation of multinucleate cells in succeeding generations. The reduction in the number of dividing cell at tested concentrations suggests that the tested extracts have a mitodepressive effect on the cell division of *A. cepa*. Mitodepressive effects of some plant extracts resulting from their interaction with DNA nucleotides thus inhibiting DNA synthesis (DNA/nucleoprotein equilibrium) and subsequent mitotic inhibition have been reported (Mercykutly and Stephen, 1980; Schulze and Kirscher, 1996; Soliman 2001). They may not even allow the initiation of their biosynthesis and such an action, occurring in the interphase nucleus, apart from influencing the ultimate structure of the chromosome during cell division, could also cause reduction of the number of other stages.

CONCLUSION

The results obtained in this study suggest that the tested extracts possess inhibitory and mitodepressive effects on the cell division of *Allium cepa*. The inhibitory and mitodepressive activities of these extracts may probably be part of the mechanism and mode of actions utilized in the treatment of febrile illness. Evidence in support of this has been documented on some plant extracts with anticancer therapy (Sheng et al., 2000; Kura's et al., 2007). There is a need for a closer look at the genotoxicological effects of the tested extracts in animal test systems for human welfare.

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REFERENCES

Adegoke EA, Akinsanya A, Naqai HZ. 1968. Study of Nigerian medicinal plants I. A preliminary survey of plants alkaloids. J West Afr Sci Assoc 13:13-33.

Ajaiyeoba EO, Oladepo O, Fawole OI, Bolaji OM, Akinboye DO, Ogundahunsi OAT, Falade CO, Gbotosho GO, Itiole OA, Happi TC, Ebong OO, Ononwu IM, Osowole OS, Odutola OO, Ashid JS, Odunola AM. 2003. Cultural categorization of febrile illnesses in correlation with herbal remedies used for treatment in southwest Nigeria. J Ethnopharmacol 85:179-185.

Akinboro A, Bakare AA. 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* (Linn.). J Ethnopharmacol 112:470–475.

Amida MB, Yemitan OK, Adeyemi OO. 2007. Toxicological assessment of the aqueous root extract of *Sanseviera liberica* Gerome and Labroy (Agavaceae). J Ethnopharmacol 113 (1):171-175.

Babatunde BB, Bakare AA. 2006. Genotoxicity screening of wastewaters from Agbara industrial estate, Nigeria evaluated with the *Allium* test. Poll Res. 25(2):227–234.

Bakare AA, Mosuro AA, Osibanjo O. 2000. Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa* (L). J Environ Biol 21(3):263–271.

Barcelos GRM, Shimabukuro F, Mori MP, Maciel MAM, C´olus IMS. 2007. Evaluation of mutagenicity and antimutagenicity of cashew stem bark methanolic extract *in vitro*. J Ethnopharmacol 114(2):268-273.

Brinkmann U, Brinkmann A. 1991. Malaria and health in Africa, the present situation and epidemiological trends. Trop Med Parasitol 42:204-213.

Bruneri. 1971. Synthesis of DNA and mitosis in relation to cell differentiation in the roots of *Vicia faba* and *Lactuva sativa*. Cytologia 36:229-247.

Burkill HM. 1997. The useful plants of west Tropical Africa, families J-L 2nd edition. Vol. 3, Pp.25-27.

Effraim KD, Jacks TW, Sodipo OA. 2001. Histopathological studies on the toxicity of *Ocimum gratissimum* leaves extract on some organs of rabbit. Afr J Biomed Res 6:21-25.

Ene-Obong EE, Osuala CL. 1990. The mutagenic potentials of water extract of *Borreria filiformis* (Hiern) Hutch and Dalz and *Vinca rosea* Linn. Nig J Bot 3:35-40.

Farnsworth NR, Akerele OO, Bingel AS, Soejarta DD, Eno Z. 1985. Medicinal plants in Therapy. Bull World Health Organ 63(6):965-981.

Fasola TR, Egunyomi A. 2005. Nigerian usage of bark in phytomedicine. Ethnobot Res Applicat 3:73–77.

Fiskesjo G. 1985. The *Allium* test as a standard in environmental monitoring. Hereditas 102:99-112.

Gadano AB, Gurni AA, Lopez Nigro M, Gralli P, Van Baren A, Ferraro G, Carballo MA. 2000. Cytogenetic effects of aqueous extracts of paico (*Chenopodium multifidum*) a medicinal plant. Pharm Biol 38:7–12.

Gadano AB, Gurni AA, Lopez P, Ferraro G, Carballo MA. 2002. *In vitro* genotoxic evaluation of the medicinal plant *Chenopodium ambrosioides* L. J Ethnopharmacol 81:11–16.

Gadano AB, Gurni AA, Carballo MA. 2006. Argentine folk medicine: genotoxic effects of Chenopodiaceae family. J Ethnopharmacol 103:246–251.

Grant WF. 1978. Chromosome aberrations in plants as a monitoring system. Environ. Health Perspect 27:37-43.

Grant WF. 1982. Chromosome aberrations assay in *Allium*. A report of the U.S. Environmental protection Agency. Gene-Tox programme. Mut Res 99:273-291.

Grant WF. 1994. The present status of higher plant bioassay for the detection of environmental mutagens. *Mut. Res.* 310: 175-185.

Keightley AM, Dobrzynska M, Podbielkowska M, Renke K, Zobel AM. 1996. Coumarin and its 4- and 7-substituted derivatives as retardants of mitoses in *Allium* root promeristem. Int J Pharmacol 34:105-113.

Kirby GC. 1997. Plants as sources of antimalarial components. Trop Doct 27(Suppl. 1):7-11.

Konan NA, Bacchi EM, Lincopan N, Varela SD, Varanda EA. 2007. Acute, subacute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (*Anacardium occidentale* L.). J Ethnopharmacol 110:30-38.

Kura´s M, Augustynowicz J, ´ Sliwi´nska E, Pilarski R, Ilasz R, Tykarska T, Zobel A, Gulewicz K. 2007. Changes in chromosome structure, mitotic activity and nuclear DNA content from cells of *Allium* test induced

by bark water extract of *uncaria tomentosa* (willd.) DC. J Ethnopharmacol 107(2):211-221.

Levan. A. 1938. The effect of colchicine on root mitoses in *Allium*. Hereditas 24:471-486.

Macleod RD. 1969. Some effects of 2, 4, 5, trichlorophenoxy acetic acid on the mitotic cycle of lateral root apical meristems of *V. faba*. Chromosoma 27:227-237.

Magda IS. 2001. Genotoxicity testing of Neem plant (*Azadirachta indica* A. juss) using the *Allium cepa* chromosome aberration assay. J Biol Sci 1(11):1021-1027.

Majewska A, Wolska E, Śliwińska E, Furmanowa M, Urbańska N, Pietrosiuk A, Zobel A, Kuraś M. 2003. Antimitotic effect, G2/M accumulation, chromosomal and ultrastructure changes in meristematic cells of *Allium cepa* L. root tips treated with the extract from *Rhadiola rosea* roots. Caryologia 56:337–351.

Martins ER, Castro DM, Castelani DC, Dias JE. 1995. Plantas medicinals imprensa universitaria, Vigosa, MG, Basil. P. 20.

Mercykutly VC, Stephen J. 1980. Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test. *Cytologia* 45: 769–777.

Moody JO, Ajaiyeoba EA, Adeboye JO, Ogundipo OO. 1999. Standardization and utilization of herbal medicines, *Proceedings of 1st International workshops on herbal medicinal products*, Ibadan. Pp. 6-8.

Mosuro AA, Bakare AA. 1998. Cytogenetic effects of lambdacyalothrin and paraquat on *Allium cepa.* J Sci Eng Tech 5(1):1055–1063.

Nwafor PA, Egwu G, Jahn GA, Deis RP. 2007. Effects of methanolic extract of *Asparagus pubescens* root on sexual behavior and pituitary hormone secretion on Wistar rats during pregnancy and lactation. J Ethnopharmacol 113(3):492-497.

Obi RK, Iroagba II, Ojiako OA. 2006. Virucidal potential of some edible Nigerian vegetables. Afr J Biotechnol 5:1785–1788.

Paes-Leme AA, Motta ES, De Mattos JCP, Dantas FJS, Bezerra RJAC, Caldeira-de-Araujo A. 2005. Assessment of *Aloe vera* (L) genotoxic potential on *Escherichia coli* and plasmid DNA. J Ethnopharmacol 102:197–201.

Patterson RM, Keith LA, Stewart J. 1987. Increase in Chromosomal abnormalities in Chinese hamster ovary cells treated with butylated hydroxytoluene *in vitro*. Toxicol In Vitro 1:55–57.

Podbielkowska M, Waleza M, Zobel AM. 1981. Influence of methotrexate on respiration and ultrastructure of meristematic cells from *Allium cepa* roots. Acta Soc Bot Pol 50:563-566.

Podbielkowska M, Piwocka M, Waszkowska E, Waleza M, Zobel AM. 1995. Effect of coumarin and its derivatives on mitosis and ultrastructure of meristematic cells. Int J Pharmacol 33:7-15.

Ruffa MJ, Ferraro G, Wagner ML, Calcagno ML, Campos RH, Cavallaro L. 2002. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J Ethnopharmacol 79:335–339.

Schulze E, Kirscher S. 1996. Microtubule dynamics in interphase. Cell J Cell Biol 2:1020–1021.

Sheng Y, Bryngelsson C, Pero RW. 2000. Enhanced DNA repair, immune function and reduced toxicity of C-MED.-100, a novel aqueous extract from *Uncaria tomentosa*. J Ethnopharmacol 69:115-126.

Soliman MI. 2001. Genotoxicity testing of neem plant (*Azadirachta indica* A. Juss) using the *Allium cepa* chromosomeaberration assay. Online J Biol Sci 1(11):1021-1027.

Sowemimo AA, Fakoya FA, Awopetu I, Omobuwajo OR, Adesanya SA. 2007. Toxicity and mutagenic activity of some selected Nigerian plants. J Ethnopharmacol 113(3):427-432.

Teixeira RD, Camparoto ML, Mantovani MS, Vicentini VEP. 2003. Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millifolium* L., in *in vitro* and *in vivo* assays. Genet Mol Biol 26(4):551-555.

Van't Hoff J. 1968. The action of IAA and kinetin on the mitotic cycle of proliferative and stationary phase exised root meristems. Exp H Cell Res 51:167-176.

Warrell DA. 1997. Herbal remedies for malaria. Trop Doct 27(Suppl. 1):5–6.

Webster PL, Davidson D. 1969. Changes in the duration of the mitotic cycle induced by colchicine and indol-3yl acetic acid in *V. faba* roots. J Exp Bot 20:671-685.

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Comunicación | Communication

Control biológico de *Colletotrichum fusarioides* O´Gara, patógeno de semillas de albahaca (*Ocimum basilicum* L.) con *Trichoderma* Rifai

[Biological control of *Colletotrichum fusarioides* O´Gara, pathogen of basil seeds (*Ocimum basilicum* L.) whit *Trichoderma* Rifai]

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Abstract

The effectiveness of *Trichoderma* isolate for the biological control of *Colletotrichum fusarioides* was studied.Trials were performed in the greenhouse by sowing pre-treated basil seeds with a bioproduct (liquid and semi liquid) of *Trichoderma* + *C. fusarioides.* The antagonistic effect was evaluated through: number of germinated seeds and healthy plants. Statistically significant differences ($p<0.05$) were observed in relation to: increase of germination rate (69% liquid multiplication), seeds treated with *Trichoderma* + *C. fusarioides* and a higher number of healthy plants (84% liquid, 26% semi liquid) obtained from seeds inoculated with the antagonist + pathogen compared to those only inoculated with *C. fusarioides.*

Keywords: *Antagonism; seed-borne pathogens; antagonists´multiplication; treatment of seeds; physiological quality.*

Resumen

Se estudio la efectividad de un aislamiento de *Trichoderma* para el control biológico de *Colletotrichum fusarioides.* En ensayos en invernáculo se sembraron en macetas semillas de albahaca previamente tratadas con el bioproducto (líquido y semilíquido) de *Trichoderma* + *C. fusarioides*. El antagonismo se evaluó a través de: número de semillas germinadas y plantas sanas. Se observaron diferencias significativas ($p<0,05$) en: aumento de germinación (69% multiplicación líquida) semillas tratadas con *Trichoderma* + *C. fusarioides* y mayor número de plantas sanas (84% líquida, 26% semilíquida), procedentes de semillas inoculadas con antagonista + patógeno en comparación con aquellas inoculadas con *C. fusarioides* en forma individual.

Palabras Clave: *Antagonismo; patógeno de semillas; multiplicación de antagonistas; tratamiento de semillas; calidad fisiológica.*

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INTRODUCCIÓN

La albahaca (*Ocimum basilicum* L.) es un planta anual perteneciente a la familia *Lamiaceae*, nativa de India y naturalizada en África y las Islas del Pacífico (Hooker, 1885), que se consume fresca y como un ingrediente importante demandado por la industria alimenticia.

El cultivo de esta hierba originalmente tenía importancia económica en países europeos y asiáticos (Garibaldi et al., 1997). Sin embargo, el creciente interés por la "dieta mediterránea" incrementó el consumo y la demanda de albahaca en numerosos países, que incluye Argentina.

Uno de los factores que limitan la producción de albahaca es la acción de enfermedades de etiología fungosa, entre las cuales se destaca la causada por *Colletotrichum fusarioides* O´Gara (Garibaldi et al., 1995; Bailey, et al., 1998) cuyo inóculo se encuentra presente en numerosas oportunidades en las semillas de esta especie. Este patógeno deteriora no solo el estado sanitario y comercial de la semilla si no también su comportamiento fisiológico posterior, reduciendo el número de plantas, y puede también afectar otros órganos de la planta. Cuando infecta hojas y tallos produce lesiones necróticas delimitadas, comúnmente llamadas "antracnosis", o, dependiendo de factores ambientales y de las condiciones de nutrición del hospedador, lesiones necróticas extensas denominadas tizones o "die back" (Agrios, 2005). Además, puede permanecer en semillas, tallos, hojas y flores como patógeno quiescente (Neergaard, 1977). La utilización de fungicidas para manejar el problema enfrenta crecientes restricciones impuestas por las autoridades sanitarias y el mercado internacional, que exige la ausencia de contaminación de origen químico y microbiológico (Carrillo, 2003; Mandeel, 2005).

En estudios preliminares sobre la micoflora asociada a semillas de albahaca *C. fusarioides* fue la especie patógena aislada con mayor frecuencia. Esta situación y la escasa investigación sobre patógenos de semilla de especies aromáticas en Argentina, a diferencia de lo registrado en otros países (Gamliel and Yarden, 1998; Deepak et al., 2008) guiaron el presente trabajo. El mismo fue realizado con el objetivo de obtener información preliminar sobre las posibilidades de aplicación de control biológico, como una estrategia alternativa de manejo del patógeno. El antagonista seleccionado con esta finalidad fue una cepa nativa de *Trichoderma sp.* caracterizada por la rápida producción de biomasa en distintos sustratos (Sandoval et al., 2001). *Trichoderma* es un hongo utilizado para el control biológico de numerosos patógenos (Tronsmo, 1986; Harman, 2000; Monte, 2001; Stefanova et al., 2004; Sandoval et al., 2006) que incluyen a *Colletotrichum* en semillas de distintos cultivos (Taylor et al., 1991, Shovan et al., 2008). Sin embargo, *Trichoderma* aún no ha sido evaluado como agente de control biológico de patógenos de semillas de albahaca.

MATERIALES Y MÉTODOS

Patógeno

La cepa *C. fusarioides* para el estudio proviene de semillas de albahaca altamente contaminadas y almacenada en el cepario de la Cátedra de Fitopatología. FCA-UNLZ/2003. Para la obtención del inóculo para las pruebas de invernáculo se procedió a cultivar el hongo en tubos en un medio de cultivo de agar papa glucosado (APG) al 2% p/v y en caldo de papa glucosado (CPG) al 2% p/v.

Antagonista

Se utilizó una cepa de *Trichoderma* aislada de la rizósfera de plantas sanas de albahaca (N° cepario 331/2000), mantenida por sucesivos repiques en APG. Para las pruebas en invernáculo el hongo fue multiplicado durante 17 días a 26 ºC y 16 horas de fotoperíodo, en frascos de vidrio de 500 mL, en dos medios de cultivo (semilíquido y líquido) pH 6,2. El medio semilíquido consistió en una mezcla de 200 g de granos enteros de maíz (*Zea mays* L.) y 1,5 g de extracto de levadura comercial, suspendida en una solución de 3 mL de jarabe de maíz y 1000 mL de agua destilada. Como medio líquido se empleó una mezcla de 250 mL de jarabe de maíz y 75 g de levadura de cerveza suspendida en 2000 mL de agua destilada. Previa a la inoculación con 5 mL de un cultivo de *Trichoderma*, con una concentración de 2 x 10^8 conidios, micelio, clamidosporas/mL (determinada con hematocímetro), los medios fueron esterilizados en autoclave 1 hora a 100 °C, durante tres días consecutivos. Durante la incubación los frascos inoculados fueron colocados en la bandeja de un agitador mecánico (80 r.p.m.). El medio líquido recibió agitación interna adicional a través del caudal de aire del aereador Aquafish.

Pruebas en invernáculo

Las semillas de albahaca fueron sembradas en macetas de 5 L, conteniendo una mezcla de arena y

tierra (procedente de un suelo molisol) esterilizada en autoclave. Las macetas se dispusieron al azar en las mesadas del invernáculo del Instituto Fitotécnico de Santa Catalina. Con 16 horas de fotoperíodo y temperaturas entre 24 y 27 ºC. Previo a la siembra, las semillas fueron desinfectadas con hipoclorito de sodio al 2% p/v (durante 3 minutos) y enjuagadas tres veces con agua destilada estéril. Los tratamientos efectuados, luego de la desinfección, consistieron en: i) Inoculación con un cultivo líquido (CPG al 2%) del patógeno (concentración $2x10^8$ conidios/mL, determinada mediante hematocímetro); ii) Inoculación, por separado, con los cultivos semilíquido y líquido del antagonista (concentración $2x10^8$ conidios, micelio, clamidosporas/mL); iii) Inoculación con la mezcla(1/1) del patógeno y del antagonista multiplicado en medio semilíquido; iv) Inoculación con la mezcla (1/1) del patógeno y del antagonista multiplicado en medio líquido; y v) Testigo sin inocular. El tiempo de contacto entre las semillas y los cultivos fue de 4 h. Para eliminar el exceso de humedad de estas semillas, las mismas se colocaron entre papeles de filtro estériles durante toda la noche. El ensayo se realizó con tres repeticiones. Se sembraron 50 semillas por repetición. Las variables medidas fueron: número de semillas germinadas, estado definido por la aparición del primer par de hojas cotiledonales y de plantas sanas (a los 10 y 21 días, respectivamente) luego de la siembra. La determinación del número de planas sanas fue realizada a través de la inspección visual de cada planta por separado. Esta inspección permitió realizar una clasificación preliminar entre plantas: asintomáticas y con sintomatología de antracnosis y/o tizón de infección (presuntiva) por *C. fusarioides*. A continuación, las muestras procedentes de cada ejemplar sintomático fueron sometidas a pruebas de rutina en fitopatología: desinfección, siembra, incubación e identificación (Agrios, 2005) para confirmar o descartar la presencia del patógeno. Con los datos obtenidos se procedió a calcular el porcentaje absoluto de germinación y el porcentaje relativo de plantas sanas. Para lo cual se utilizaron las siguientes fórmulas: Porcentaje de germinación = número de semillas germinadas / número total de semillas sembradas x 100. Porcentaje de plantas sanas= número de plantas sanas / número de plantas desarrolladas a partir del total de semillas germinadas (tratadas con el antagonista) x 100. Los datos fueron transformados mediante la expresión $\sqrt{x+0,5}$ (Sokal and Rolfh, 2002) y, luego sometidos al análisis de la varianza y a la prueba de comparación de medias de Tukey, con un nivel de significación del 5% utilizando el programa Statistix.

RESULTADOS Y DISCUSIÓN

Los valores de semillas germinadas, a los 10 días, estuvieron comprendidos en un rango de 90-82% (semillas inoculadas con *Trichoderma* multiplicación líquida y semilíquida, respectivamente) a 18% (semillas inoculadas con *C. fusarioides* solo). El análisis de los resultados obtenidos en esta prueba mostró diferencias estadísticamente significativas para los valores promedio de germinación (Tablas 1 y 3) entre las semillas inoculadas con el patógeno en comparación con el resto de los tratamientos. Se observó un aumento en la germinación de semillas inoculadas con la multiplicación líquida del antagonista + patógeno (69%) en comparación con las inoculadas sólo con *C. fusarioides* (18%) (Tabla 1). Con la multiplicación semilíquida el incremento en la germinación fue de solo 10% (Tabla 3), porcentaje inferior al registrado en la germinación del testigo (semillas sin inocular).

Al aplicar el bioproducto, cultivo líquido y semilíquido de *Trichoderma*, en semillas antes de la germinación aumentó la efectividad promoviendo la germinación de semillas tratadas (con el antagonista en forma individual), en comparación con la efectividad alcanzada como antagonista del patógeno (inoculación de antagonista + patógeno). La detección del nivel de eficiencia de la promoción de germinación y crecimiento de la cepa ensayada no era un objetivo en los ensayos realizados. Sin embargo, el comportamiento registrado resulta auspicioso y coincide con numerosas investigaciones realizadas en distintos cultivos (Taylor et al., 1991; Harman, 2000). En ellas se señala la conveniencia de recurrir a aislamientos nativos del antagonista (Tronsmo, 1986) como el utilizado en el presente trabajo.

En cuanto a las diferencias registradas en el nivel de efectividad del bioproducto como antagonista de *C. fusarioides* según método de multiplicación, los mejores resultados se registraron con *Trichoderma* obtenido en sustrato líquido. Esto se debería a que este sustrato favoreció la adherencia y la infectividad de los propágulos del antagonista en las semillas inoculadas en forma conjunta con el patógeno (Sandoval et al., 1995)

El rango del número de plantas sanas a los 21 días fue de 99–95% (plantas desarrolladas a partir de semillas inoculadas con sólo la multiplicación

semilíquida y líquida de *Trichoderma*, respectivamente) a 4% (semillas inoculadas con solo *C. fusarioides*) (Tablas 2 y 4). El análisis de los resultados alcanzados mostró diferencias estadísticamente significativas para los valores promedio del número de plantas sanas entre las semillas inoculadas con *C. fusarioides* en comparación con el testigo sin tratar, las semillas inoculadas con *Trichoderma* (multiplicación, liquida y semilíquida) y las tratadas en forma conjunta con *C. fusarioides* y la multiplicación líquida y semilíquida del antagonista. El incremento observado en el número de plantas sanas, desarrolladas a partir de las semillas inoculadas con *C. fusarioides* + *Trichoderma*, alcanzó valores de 84 y 26% (multiplicación líquida y semilíquida del antagonista, respectivamente) en comparación con el número de plantas desarrolladas a partir de las semillas inoculadas con el patógeno en forma individual (Tabla 2 y 4). Para la comparación entre *C. fusarioides* + multiplicación líquida y semíliquida de *Trichoderma* y, el testigo (plantas desarrolladas de semillas sin inocular) se observó aumento (56%) de plantas sanas para la multiplicación líquida y, disminución (5%) para la multiplicación semilíquida.

La mayor protección conferida por *Trichoderma* obtenido por multiplicación en medio líquido durante el desarrollo ulterior de las plantas, indicaría que la misma incidió también en la efectividad del antagonista como inhibidor de infecciones quiescentes del patógeno (Bailey et al., 1998), tipo de efectividad que fue escasa a nula en *Trichoderma* obtenido por multiplicación en medio sólido.

Por otro lado, los mejores resultados alcanzados con la multiplicación de *Trichoderma* en medio líquido están relacionados con el procedimiento de aireación adicional utilizado durante su preparación. Producto de la aireación lograda con el dispositivo mecánico (Aquafish) hubo una agitación más vigorosa del sustrato, aspecto recomendado en los distintos métodos de preparación de bioproductos a partir del hongo *Trichoderma* (Miura et al., 2003).

Tabla 1. Prueba de germinación de semillas de albahaca tratadas con la multiplicación líquida de *Trichoderma sp.*

Tratamiento	Semillas germinadas[1] Media ± DS[2]	Semillas germinadas Media: datos originales	Porcentaje de germinación[3]
Trichoderma	6,76 ± 0,08 [a]	45,33	90
Trichoderma + *C. fusarioides*	6,64 ± 0,17 [a]	43,66	87
Testigo	4,09 ± 0,14 [ab]	16,33	32
C. fusarioides	3,05 ± 0,41 [c]	9,00	18
	C.V.[4]: 9,39		

[1] Datos transformados según la expresión √x+0,5. [2] Medias seguidas de letras iguales, en la columna, no difieren significativamente (p=0,05). [3] Calculado según datos originales. [4] Coeficiente de variación.

Tabla 2. Promedio del número de plantas sanas (a los 21 días) de albahaca desarrolladas a partir de semillas tratadas con la multiplicación líquida de *Trichoderma sp.*

Tratamiento	Plantas sanas [1] Media ± DS[2]	Plantas sanas Media: datos originales	Porcentaje de plantas sanas[3]
Trichoderma	6,61 ± 0,17 [a]	43,33	95
Trichoderma + *C. fusarioides*	6,38 ± 0,1 [a]	40,33	88
Testigo	3,87 ± 0,42 [ab]	14,66	32
C. fusarioides	1,42 ± 0,83 [c]	2,00	4
	C.V.[4]: 15,27		

[1] Datos transformados según la expresión √x+0,5. [2] Letras iguales en la columna no difieren entre sí (p<0,05). [3] Calculado según datos originales. [4] Coeficiente de variación.

Tabla 3. Prueba de germinación de semillas de albahaca tratadas con la multiplicación semilíquida de *Trichoderma sp.*

Tratamiento	Semillas germinadas [1] Media ± DS[2]	Semillas germinadas Media: datos originales	Porcentaje de germinación[3]
Trichoderma	6,43 ± 0,07 [a]	41,00	82
Trichoderma + C. fusarioides	3,79 ± 0,26 [ab]	14,00	28
Testigo	4,07 ± 0,14 [ab]	16,33	32
C. fusarioides	3,05 ± 0,26 [c]	9,00	18
	C.V.[4]: 11,75		

[1] Datos transformados según la expresión √x+0,5. [2] Letras iguales en la columna no difieren entre sí (p<0,05). [3] Calculado según datos originales. [4] Coeficiente de variación.

Tabla 4. Promedio del número de plantas sanas (a los 21 días) de albahaca desarrolladas a partir de semillas tratadas con la multiplicación semilíquida de *Trichoderma sp.*

Tratamiento	Plantas sanas [1] Media ± DS[2]	Plantas sanas Media: datos originales	Porcentaje de plantas sanas[3]
Trichoderma	6,41 ± 0,83 [a]	40,66	99
Trichoderma + C. fusarioides	3,61 ± 0,41 [ab]	12,66	30
Testigo	3,87 ± 0,42 [ab]	14,66	35
C. fusarioides	1,42 ± 0,83 [c]	2,00	4
	C.V.[4]: 18,78		

[1] Datos transformados según la expresión √x+0,5. [2] Letras iguales en la columna no difieren entre sí (p<0,05). [3] Calculado según datos originales. [4] Coeficiente de variación.

CONCLUSION

La cepa nativa de *Trichoderma sp.* ensayada posee efecto antagónico sobre el hongo *C. fusarioides* patógeno de semillas de albahaca. La multiplicación líquida de esta cepa promueve la germinación y el crecimiento de las plantas desarrolladas a partir de semillas de albahaca tratadas con *Trichoderma + C. fusarioides*. Este mismo efecto es causado por el tratamiento de las semillas con la multiplicación líquida y semilíquida del antagonista en forma individual. Estos resultados indican que la utilización de *Trichoderma* es una estrategia promisoria para el control de *C. fusarioides* patógeno de albahaca y que su aplicación incide de manera positiva en la calidad fisiológica y sanitaria de las semillas.

REFERENCIAS

Agrios GN. 2005. Plant Pathology. Elsevier Academic Press, San Diego, USA, pp. 487-493

Bailey JA, O´Connell RJ, Pring RJ, Nash C. 1998. Infection Strategies of *Colletotrichum* species. In: Bailey JA. and Jeger MJ (eds.) *Colletotrichum*: biology, pathology and control. Oxford University Press, Oxford, UK. pp. 88-108.

Carrillo L. 2003. Los hongos de los alimentos y forrajes. Pub. Universidad Nacional de Salta, Argentina, pp. 81-86

Deepak P, Saran L, Lal G. 2008. Control of wilt and blight diseases of cumin throught antagonistic fungi under in vitro and field conditions. Not Bot Hort Agrobot Cluj 36(2):91-96

Gamliel A, Yarden O. 1998. Diversification of diseases affecting herb crops in Israel acompannies the increase in herb crop production. Phytoparasitica 26(1):1-6

Garibaldi A, Minuto G, Aloi C, Gullino ML. 1995. *Colletotrichum gloesporioides* Penz. nuovo parassita del basilico. Inf Fitopatol 45(2):34-35.

Garibaldi A, Gullino ML, Minuto G. 1997. Diseases of basil and their management. Plant Dis. 81(2):124-132

Harman GE. 2000. Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis 84:377-393

Hooker JD. 1885. Flora of British India. Vol 4. Reeve and Co., London. pp 14.

Mandeel QA. 2005. Fungal contamination of some imported spices. Mycopathol 159:291-298

Miura S, Armura T, Hoshino M, Kojima M, Dwiartil L, Okabe M. 2003. Optimization and scale-up of l-acid-lactil fermentation by mutant strains *Rhizopus sp.* MK-96-96-1196 in airflit reactors. J Biotechnol 9(1):65-69

Monte E. 2001. Understanding *Trichoderma:* between biotechnology and microbial ecology. Int Microbiol 4:1-4

Neergaard P. 1977. Seed Pathology. Vol II. MacMillan Press, London. pp 1191

Sandoval I, López MO, García D, Mendoza I. 1995. *Trichoderma harzianum* (Cepa A-34): un biopreparado de amplio espectro para micopatologías del tomate y del pimiento. Boletín Técnico CID-INISAV 3. 36 pp.

Sandoval MC, Fálico L, Atlas E, Noelting MC. 2001. Velocidad de formación de biomasa de *Trichoderma harzianum* en distintos substratos. Bol Soc Arg Bot 36:124-125

Sandoval MC, Fálico LM, Noelting MC, Corcuera VR, Cid P, Raggio G. 2006. Estrategia de control biológico de *Alternaria alternata* Keisller patógeno de *Coriandrum sativum* con *Trichoderma harzianum* Rifai. Rev Prot Veg 21(1):31-36

Shovan LR, Bhuiyan MKA, Begum JA, Pervez Z. 2008. *In vitro* control of *Colletotrichum dematium* causing anthracnose of soybean by fungicide, plant extracts and *Trichoderma harzianum.*Int. J Sustain Crop Prod 3(3):10-17.

Sokal RR, Rohlf, FJ. 2002. Supuestos teóricos del análisis de la varianza. p. 210. En: Sokal RR, Rohlf FJ. Introducción a la bioestadística. Reverte, Barcelona.

Stefanova M, Sandoval I, Martínez ML, Heredia I, Ariosa MD, Areválo R. 2004. Control de hongos fitopatógenos del suelo en semilleros de tabaco con *Trichoderma harzianum.* Fitosanidad 8(2):35-38.

Taylor AG, Min TG, Harman GE, Jin X. 1991. Liquid coating formulation for the applications of biological seed treatments of *Trichoderma harzianum.* Biol Control 1:16-22

Tronsmo A. 1986. Use of *Trichoderma spp.*in biological control of necrotrophic pathogens. pp. 348-362. In: Fokkema NJ, Van den Heuvel J (eds). Microbiology of the phyllosphere. Cambridge University Press, Cambridge.

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Artículo Original | Original Article

Evaluation of the antioxidant properties and effects on the biotransformation of commercial herbal preparations using rat liver endoplasmic reticulum

[Evaluación de la actividad antioxidante y los efectos sobre biotransformación de productos herbales comerciales en retículo endoplásmico hepático de rata.]

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Abstract

The antioxidant herbal ingredients are xenobiotics for animals, many of them must be biotransformed to be eliminated, especially lipophilic compounds. Biotransformation of such xenobiotics- occurs mainly in the hepatic endoplasmic reticulum, a process that may generate ROS and trigger oxidative stress. Therefore, we used rat liver microsomes to test the antioxidant capacity and the effects on biotransformation enzymes of five commercial herbal preparations containing *Silybum marianum, Tilia cordata, Crataegus oxyacantha, Avena sativa, Melissa officinalis, Valeriana officinalis; Passiflora incarnata, Foeniculum vulgare, Cassia senna, Peumus boldus* and *Opuntia ficus-indica*, as ingredients -alone or in combination-. With the exception of the aqueous preparation of *Opuntia ficus-indica*, all the hydro-alcoholic commercial preparations inhibited the oxidation of microsomal lipids and thiols and prevented the oxidative alterations of several microsomal biotransformation enzymes activities, all phenomena induced by Fe^{3+}/ascorbate. They also inhibited the UDP-glucuronyltransferase (UDPGT), GSH-transferase (GST), and N-demethylating, and Monooxygenase cytochrome P450 activities to a lower extent in the absence of oxidative stress. This last phenomenon might be due to the presence of lipophilic substrates in the herbal preparations. Thus, microsomes could be a good biological system to evaluate both the antioxidant properties and the possible interactions of herbal preparations with the enzymes involved in xenobiotics biotransformation.

Keywords: Microsomes; Polyphenols; Oxidative stress; Biotransformation-enzymes; Herbal-antioxidants; Herbal-ingredient biotransformation.

Resumen

Dependiendo de su lipofilicidad, los principios herbales antioxidantes que son xenobióticos para los organismos animales, deben biotransformarse para ser eliminados. Este proceso ocurre principalmente en el retículo endoplásmico hepático y puede generar ROS induciendo, paradójicamente, estrés oxidativo. En este trabajo hemos utilizado microsomas hepáticos de rata para evaluar la capacidad antioxidante y los efectos sobre la biotransformación de xenobióticos de varios extractos comerciales conteniendo *Silybum marianum, Tilia cordata, Crataegus oxyacantha, Avena sativa, Melissa officinalis, Valeriana officinalis; Passiflora incarnata, Foeniculum vulgare, Cassia senna, Peumus boldus and Opuntia ficus-indica, solos o en combination-*. Excepto el preparado acuoso de *Opuntia ficus-indica*, el resto de extractos comerciales –mayoritariamente hidro-alcohólicos- inhibieron la oxidación de los lípidos y los tioles microsómicos, las modificaciones oxidativas de varias actividades de enzimas microsómicas biotransformantes de xenobióticos, todos estos fenómenos inducidos experimentalmente por Fe^{3+}/ascorbato. En ausencia de estrés oxidativo estas preparaciones también inhibieron las actividades de UDP-glucuroniltransferasa (UDPGT) GSH-transferasa (GST), N-demetilasa y Monooxigenasa citocromo P450, aunque este efecto fue menos marcado que el antioxidante. Este último fenómeno puede deberse a la presencia de sustratos lipofílicos en estas preparaciones herbales. Así, los microsomas hepáticos podrían ser un buen sistema biológico para evaluar simultáneamente las propiedades antioxidantes y las interacciones que sobre la biotransformación de xenobióticos podrían ejercer las preparaciones herbales.

Palabras Clave: Microsomas; polifenoles; estrés oxidativo; enzimas de biotransformación; antioxidantes herbales; biotransformación de principios herbales.

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INTRODUCTION

Evidence exists for the antioxidant role of herbal products in the prevention of pathologies such as cardiovascular, neurodegenerative diseases and cancer. All these pathologies are especially associated to oxidative stress, a phenomenon triggered when generation of reactive oxygen species (ROS) is excessive and/or its removal deficient (Lee et al., 2003; Leone et al., 2003). In this condition, modifications of biomolecules occur, changing its biological functions, leading to cell death.

Several enzymes which participate in redox processes and are located in different subcellular organelles catalyze the generation of ROS (xantine-oxidase, monoamine oxidase, cytochrome P450 oxidative system) (Drögue 2002). In addition, transition metals such as iron and copper, in its redox-active forms can generate oxygen free radical through Haber-Weiss and/or Fenton reactions (Halliwell and Gutteridge, 1999). All organisms have developed different non-enzymatic and enzymatic antioxidant mechanisms to control ROS actions, accounting for the cellular antioxidant capacity; i.e. GSH and vitamin E are direct scavengers of oxygen free radicals and other ROS and, superoxide-dismutase, catalase and GSH-peroxidase catalyze the reduction of ROS (Drögue, 2002).

Phenolic compounds are the main herbal derivates involved in the antioxidant properties of herbal food and medicinal plants. The expression "phenolic compounds" involves a considerable range of substances whose structures vary from simple molecules (phenolic acids) to highly polymerized compounds (condensed tannins) (Urquiaga and Leighton, 2000). Numerous studies exist about the properties of natural polyphenols antioxidants, which are exercised through several mechanisms; i.e. they scavenge free oxygen radicals, induce superoxide-dismutase, catalase and GSH-peroxidase, and they are metal chelating agents (Halliwell and Gutteridge, 1999; Du et al., 2007). Moreover, *in vitro*, flavonoids inhibit xanthine-oxidase, cyclooxygenase and mitochondrial NADH-oxidase, enzymes that participate in the production of $O_2^{\cdot-}$. Besides, flavonoids induce the GSH-transferase (GST) that catalyzes the conjugation of lipophilic-highly electrophilic compounds with GSH. Although polyphenols act by different antioxidant mechanisms, they are not necessarily complementary, exerting synergistic antioxidant effects (Middleton et al., 2000).

Furthermore, data exist about different effects of herbal products on xenobiotics biotransformation enzymes. Thus, flavones induce epoxyhydrolase, GST, UDPGT and cytochrome P450-dependent mono-oxygenase system. Moreover, flavonoids induce the specific cytochrome P450 monooxygenase iso-enzymes; these enzymes bind the substrate in the first catalytic step of the cytochrome P450 system, which catalyses the biotransformation of lipophilic xeno-biotics (Healy et al., 2002; Siess et al., 1992).

Herbal preparations differ in their biological antioxidant capacity. These differences are due to several factors such as species variety, light, and the extent of ripeness, processing and storage of vegetal drug as well as of the extraction process used. Moreover, the herbal compounds biotransformation depends on their physicochemical properties, especially of their lipophilicity. Thus, in this work, we tested several blind natural commercial preparations with respect to their biological antioxidant properties and their capacity to inhibit UDPGT, GST and cytochrome P450 system catalytic activities, all related to xenobiotics biotransformation pathways. For these purposes, we used rat liver microsomes as biological system and Fe^{3+}/ascorbate as generator of oxygen free radicals. Our results show that a tight relation between the lipophilicity of herbal compounds and its antioxidant properties on microsomal components (lipids and proteins) and the effects on bio-transformation pathways exist. Moreover, polyphenol concentrations of hydro-alcoholic herbal preparations but not the aqueous preparation correlated in an exponential manner with their capacity to inhibit the lipid peroxidation and oxygen consumption. Therefore, microsomes could be a good biological system to evaluate the antioxidant capacity and the possible interactions on the xenobiotics bio-transformation of therapeutic and nutritional herbal products.

MATERIALS AND METHODS

Chemicals

5,5-dithiobis(2-nitrobenzoic acid) (DTNB), GSH, *p*-Nitrophenol (PNP), UDP-glucuronic acid (ammonium salt) (UDPGA), aminopyrine, NADP, glucose-6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase, Tris-HCl, bovine albumin Fraction IV were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloracetic acid (TCA), thiobarbituric acid (TBA), sodium ascorbate, $FeCl_3$, $MgCl_2$, Folin-

Ciocalteu's reagent were purchased from Merck Co. Chile. 1-chloro-2,4-dinitrobenzene was purchased from ACROS Organics (New Jersey, NJ, USA). Other chemicals were analytical grade. Hydro-alcoholic preparations I-02, I-08, R-01, R-03 and aqueous preparation S-01 were the commercial herbal preparations tested (Table 1), which were donated by Laboratorios Ximena Polanco, Santiago, Chile.

Table 1. Commercial herbal preparations tested in this study.

Blind name*	Commercial name	Herbal Ingredients
I - 02	Cardus Marianus T.M.®	*Silybum marianum* (L.)
I - 08	Melinerv®	*Tilia cordata* Mill.; *Crataegus oxyacantha* (L.); *Avena sativa* (L.); *Melissa officinalis* (L.).
R - 01	Passikit®	*Crataegus oxyacantha* (L.); *Melissa officinalis* (L.); *Valeriana officinalis(* L).; *Passiflora incarnata* (L.)
R - 03	Trique compuesto®	*Foeniculum vulgare* M.; *Cassia senna* (L.); *Peumus boldus* Mol..
S - 01	Naturpep-C®	*Opuntia ficus-indica* (L.).

*The term "blind name" was assigned by the manufacturer to the commercial preparations. The commercial names and vegetal ingredients were provided at the end of this study.

Animals

We used adult male Sprague-Dawley rats (200-250 g) maintained with free access to pellet food, controlled temperature (22 ^{o}C) and photoperiod (lights on from 07:00 to 19:00 h). All procedures were developed according to protocols approved by the Institutional Ethical Committee of the Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Liver microsomal fraction

To obtain microsomal fraction we used the method described in Letelier et al. (2005). Animals were fasted for 15 h with water *ad libitum* and sacrificed by decapitation. Livers were perfused in situ with 4 volumes of 25 mL 0.9% w/v NaCl, excised, and placed on ice. All homogenization and fractionation procedures were performed at 4 ºC and all centrifugations were performed using either a Suprafuge 22 Heraeus centrifuge or an XL-90 Beckmann ultracentrifuge. Liver tissue (9–11 g wet weight), devoid of connective and vascular tissue, was homogenized with five volumes of 0.154 M KCl, with eight strokes in a Dounce Wheaton B homogenizer. Homogenates were centrifuged at 9,000*g* for 15 min; sediments were discarded and supernatants were centrifuged at 105,000*g* for 60 min. Sediments (microsomes, enriched in endoplasmic reticulum) were stored at -80ºC until use. Microsomal protein was determined according to Lowry et al. (1951).

Polyphenols Determination

The principal antioxidant property of polyphenols is based in their capacity to scavenge free radicals, phenomenon which will be evaluated in this work. Thus, the total polyphenols concentration of several commercial herbal preparations was determined by the method described in Letelier et al. (2008). In a final volume of 5 mL, herbal extract 50 µL, Folin Ciocalteau reagent 250 µL, 20% w/v sodium carbonate 750 µL and distilled water 3,950 µL. Blanks contained all the reagents with the exception of herbal extract. Then, reaction mixtures were incubated for 2 h under darkness. At the end of this period, the absorbance of the samples was determined at 760 nm in a UV3 Unicam UV–VIS spectrophotometer, using their respective blanks as reference. Catechin, a polyphenol compound was used as reference standard. Results were expressed as nmol of catechin/µL of herbal preparation.

Oxidative stress conditions

The oxidative stress conditions used to evaluate the antioxidant properties of the commercial preparations assayed were 600 µM $FeCl_3$, 1 mM sodium ascorbate and 1 mg of microsomal protein. Iron and copper generate oxygen free radicals through Haber-Weiss and/or Fenton reactions (Halliwell and Gutteridge, 1999).

Microsomal lipid peroxidation assay

The extent of lipid peroxidation following Fe^{3+}/ascorbate microsomal pre-treatment was estimated determining the thiobarbituric acid reactive species (TBARS), according to Letelier et al. (2005). Mixtures (1 mL final volume) contained 1 mg/mL microsomal protein, 600 µM $FeCl_3$, 1 mM sodium ascorbate, 4 mM $MgCl_2$, in 50 mM phosphate buffer, pH 7.4. Blanks contained all the reagents but microsomal protein. Blanks and samples were incubated for 10 min at 37 ^{o}C with constant agitation.

Afterwards, 250 µL of 0.24 M TCA (4 °C) were added and all mixtures were centrifuged at 10,000*g* during 10 min and 4 °C using a Suprafuge 22 Heraeus. Then, mixtures of 500 µL of the supernatants and 500 µL of 35 mM TBA were incubated at 50 °C for 1 h. At the end of this period, the absorbance at 532 nm of samples was measured in a UV3 Unicam UV–vis spectrophotometer, using their respective blanks as reference. To evaluate the antioxidant effects of herbal preparations, microsomes (1 mg of microsomal protein/mL) were incubated 5 min with herbal compounds and then 10 min with Fe^{3+}/ascorbate before determining microsomal lipid peroxidation. Results are expressed in nmols of TBARS conjugated/min/mg of microsomal protein using the extinction coefficient 156 mM/cm of malon-dialdehyde.

Oxygen consumption

The extent of oxygen consumption generated by 50 µM $CuSO_4$ and 1 mM sodium ascorbate was continuously polarographically determined during 10 min with a Clark electrode Nº5331 (Yellows Springs instrument) in a Gilson 5/6 oxygraph. I-02, I-08, R-01, R-03 concentrations used were 1/5 of microsomal lipid peroxidation values. Because the IC_{50} lipid peroxidation of S-01 was not reached, a range between 1 and 20 µL/mL were assayed to determine oxygen consumption IC_{50} value (9 µL/mL). The herbal preparations effects were determined calculating the decrease of Cu^{2+}/ascorbate oxygen consumption slope after adding the herbal preparations.

Microsomal thiol content

Thiol groups were titrated with DTNB as described by Letelier et al. (2005). Microsomes (1 mg/mL total protein) were incubated with 600 µM $FeCl_3$, 1 mM ascorbate, in 50 mM phosphate buffer, pH 7.4. Blanks contained all the reagents but microsomal protein. Blanks and samples were incubated for 30 min at 37◦C with constant agitation. Afterwards, microsomal thiol content was titrated with DTNB. I-02, I-08, R-01, R-03 concentrations used were 1/5 of oxygen consumption IC_{50} values. To evaluate the antioxidant effects of herbal preparations, microsomes (1 mg of microsomal protein/mL) were incubated 5 min with herbal compounds and then 10 min with Fe^{3+}/ascorbate before determining microsomal thiol content. Thiol concentration was estimated by the equimolar apparition of 5-thio-2-nitrobenzoic acid (ε_{410} = 13,600 M/cm).

***p*-Nitrophenol conjugation with UDPGA**

This reaction catalyzed by UDP-glucuronyl-transferase (UDPGT) was measured according to Letelier et al. (2005). I-02, I-08, R-01, R-03 concentrations used were 1/5 of oxygen consumption IC_{50} values. Activity was assayed determining the remaining *p*-nitrophenol after 15 min incubation at the following conditions: 0.5 mM *p*-nitrophenol; 2 mM UDPGA, 100 mM Tris HCl, pH 8.5, 4 mM $MgCl_2$ and 2 mg/ml microsomal protein. Control samples were performed in absence of UDPGA. Reactions were stopped adding trichloroacetic acid (5% final concentration); samples were then centrifuged at 10,000*g* for 10 min. in a Suprafuge 22 Heraeus centrifuge and NaOH was added to the mixture in order to achieve a 0.5 M final concentration. Remaining *p*-nitrophenol was determined at 410 nm using control samples of known *p*-nitrophenol initial concentration as standards. Reaction rates were determined at conditions where product formation were linearly-dependent to time and protein concentration. To evaluate the antioxidant effects of herbal preparations, microsomes (1 mg of microsomal protein/mL) were incubated 5 min with herbal compounds and then 10 min with Fe^{3+}/ascorbate before determining UDPGT activity.

1-Chloro-2,4-dinitrobenzene conjugation with GSH

GSH-transferase (GST) activity to conjugate 1-chloro-2,4-dinitrobenzene was assayed according to Letelier et al., 2006. The reaction mixture contained 0.1 mg/mL microsomal protein, 1mM 1-chloro-2,4-dinitrobenzene, and 4 mM GSH in 100 mM phosphate buffer, pH 6.5. To evaluate the antioxidant effects of herbal preparations, microsomes (1 mg of microsomal protein/mL) were incubated 5 min with herbal compounds and then 10 min with Fe^{3+}/ascorbate before determining GST activity. Conjugated product apparition was recorded continuously for 3 min at 25 °C, at 340 nm (ε_{340} = 9.6×10^{-3} M/cm) in a UV3 Unicam UV–vis spectrophotometer. I-02, I-08, R-01, R-03 concentrations used were 1/5 of oxygen consumption IC_{50} values.

Aminopyrine *N*-demethylation

The cytochrome P450 system ***N***-demethylating activity was determined according to Letelier et al. (1985), measuring the absorbance of the formaldehyde formed in this enzymatic reaction. The reaction mixture contained 1mg of microsomal protein; 5 mM aminopyrine; 35 mM TRIS pH 8.0; 3.5 mM $MgCl_2$;

0.1 M G-6-P; 10 mM NADP; 5 Units of G-6-P dehydrogenase. Blank contained all reagents except G-6-P dehydrogenase. All mixtures were incubated for 15 min at 37 ºC. Reactions were stopped adding trichloroacetic acid (5% final concentration); samples were then centrifuged at 10,000*g* for 10 min. in a Suprafuge 22 Heraeus centrifuge. To develop the colorimetric reaction, 1 mL of supernatant, 0.5 mL of a mixture formed by 0.1 mL of 2,4-pentanodione and 25 mL of 4 M ammonium acetate were incubated for 2 h under darkness. At the end of this period, the absorbance of the samples was determined at 400 nm in a UV3 Unicam UV–VIS spectro-photometer, using their respective blanks as reference. I-02, I-08, R-01, R-03 concentrations used were 1/5 of oxygen consumption IC_{50} values.

Monooxygenase cytochrome P450 spectrum

Monooxygenase cytochrome P450 spectrum was determined according to Omura and Sato (1964). This method uses the ability of the carbon monoxide to coordinate with the monooxygenase; the conjugate formed has a peak of maximum absorbance at 450 nm and its ε is 91 mM/cm. The reaction mixture (blank and sample) contained 1 mg/mL microsomal protein, 5 mM sodium dithionite and 50 mM phosphate buffer, pH 7.4. The spectrum was developed after adding carbon monoxide to the sample. I-02, I-08, R-01, R-03 concentrations used were 1/5 of oxygen consumption IC_{50} values. Interaction of the commercial herbal preparations with Cyt-P450 oxidase was estimated by the decrease in the control absorption at 450 nm of Cyt-P450 monooxygenase measured in their absence.

Statistical analysis

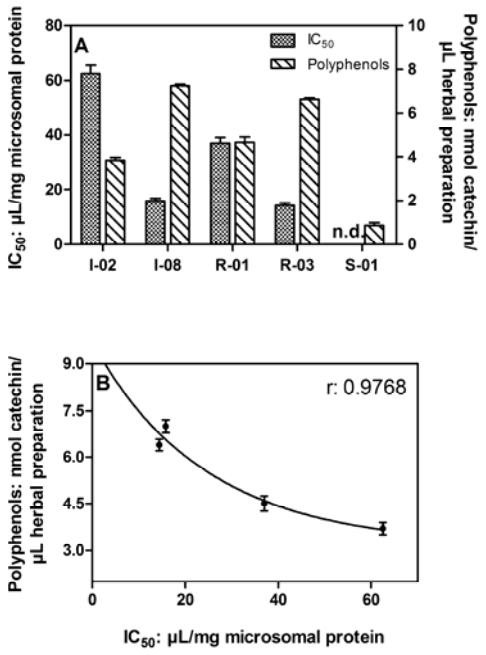
Data presented correspond to the arithmetical mean of at least four independent experiments ± SEM values. GraphPad Prism 5.0 software was used to develop statistical significance (ANOVA) and regression analyses. Differences were considered significant when $p<0.05$.

RESULTS

Antioxidant effects of herbal preparations on microsomal lipid peroxidation and the correlation with its polyphenol concentration

I-02, I-08, R-01 and R-03, hydro-alcoholic preparations, inhibited to different extent the microsomal lipid peroxidation, phenomenon induced by Fe^{3+}/ascorbate. Thus, R-03 and I-08 containing the higher polyphenol concentration had the lowest lipid peroxidation IC_{50} values, 14.4 and 15.8 µL/mg of microsomal protein, respectively. In contrast, R-01 and I-02, which contained the lowest polyphenol concentration, had the highest IC_{50} values, 37.0 and 62.5 µL/mg of microsomal protein, respectively. The polyphenol concentration of aqueous S-01 preparation was very low (0.84 nmol of catechin/µL of herbal preparation). Although quantities higher than 200 µL of this preparation were tested, its IC_{50} value was not obtained (Fig. 1A). Moreover, polyphenol content and lipid peroxidation IC_{50} values of the hydro-alcoholic preparations showed an exponential decay correlation, $r = 0.9768$ (Fig. 1B).

Figure 1. Antioxidant Effects of herbal preparations on microsomal lipid peroxidation and the correlation with its polyphenol concentration.



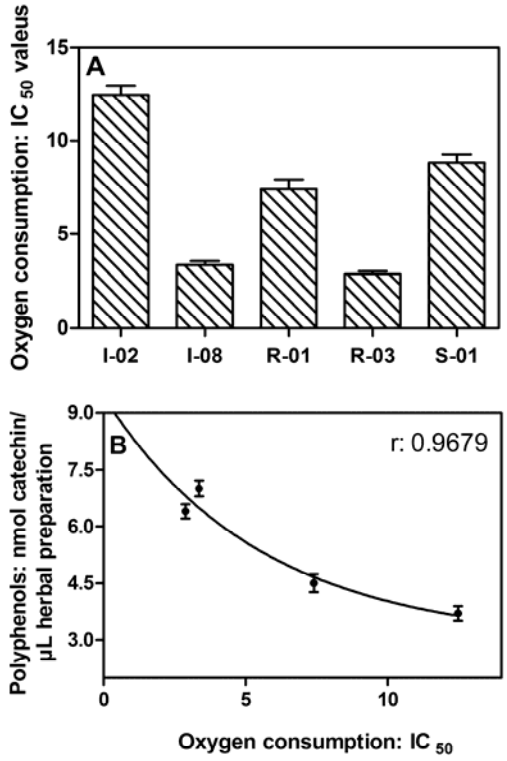
A: Microsomal lipid peroxidation and polyphenol concentrations were determined according to Methods. **B:** Correlation analysis between the polyphenol content and lipid peroxidation IC_{50} values of the hydro-alcoholic preparations. n.d.: IC_{50} value no determined. Each value represents the means of at least four independent experiments ± S.D.

Oxygen consumption developed by Cu^{2+}/ascorbate and effects of herbal preparations

In these assays, Cu^{2+}/ascorbate was used because the slope of oxygen consumption developed by this oxygen free radicals generator system was much greater than that of Fe^{3+}/ascorbate. The hydro-alcoholic preparations caused a great effect on oxygen

consumption; although 1/5 of lipid peroxidation IC_{50} values had to be used to compare their inhibitory effect, all these preparations inhibited 50% of the slope of oxygen consumption. Furthermore, the aqueous preparation S-01 showed an IC_{50} value equivalent to 9 µL (Fig. 2A). Moreover, only polyphenol concentrations of the hydro-alcoholic preparations correlated to oxygen consumption IC_{50} values as an exponential decay manner, r = 0.9679 (Fig. 2B).

Figure 2. Oxygen consumption generated by Cu^{2+}/ascorbate and the effects of herbal preparations.

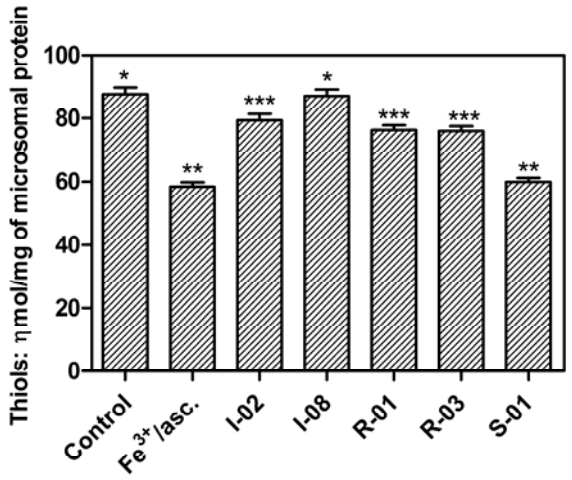


A: Herbal preparations effects on the reduction of the slope of Cu^{2+}/ascorbate oxygen consumption were determined according to Methods. **B:** Correlation analysis between polyphenol content of the hydro-alcoholic preparations and oxygen consumption reduction values. Each value represents the means of at least four independent experiments ± S.D.

Antioxidant effects of herbal preparations on microsomal thiol content

Fe^{3+}/ascorbate decreased microsomal thiol content 33% and the hydro-alcoholic herbal preparations (quantities equivalent to oxygen consumption IC_{50} values) prevented Fe^{3+}/ascorbate-derived microsomal thiol oxidation to different extents. Thus, I-08 totally prevented this phenomenon, I-02, R-01 and R-03 decreased it in the same extent, 11.8% ($p>0.05$) and S-01 had no effect (Fig. 3).

Figure 3. Fe^{3+}/ascorbate-derived oxidative effect on microsomal thiol content and the herbal preparations antioxidant capacity.



Control: microsomes incubated without Fe^{3+}/ascorbate. **Fe^{3+}/Asc.:** microsomes preincubated for 10 min with Fe^{3+}/ascorbate according to Methods. **I-02, I-08, R-01, R-03, S-01:** microsomes preincubated for 5 min with respective herbal preparations and then 10 min with Fe^{3+}/ascorbate before determining microsomal thiol content. Each value represents the means of at least four independent experiments ± S.D. *****: Values not statistically different ($p>0.05$).

Herbal preparations effects on xenobiotics biotransformation activities. UDPGT activity

The hydro-alcoholic preparations (quantities equivalent to oxygen consumption IC_{50} values) inhibited significantly and to different extents the *p*-nitrophenol conjugation with UDPGA, reaction catalyzed by UDPGT; but the aqueous preparation S-01 poorly inhibited this enzymatic activity (12.8%) (Table 2). Fe^{3+}/ascorbate increased the UDPGT activity ~3 fold and all the hydro-alcoholic herbal preparations prevented this phenomenon. In order to estimate the herbal preparations antioxidant effects on Fe^{3+}/ascorbate-derived UDPGT oxidative activation, the ratios between the UDPGT activity measured in the presence of herbal preparation and Fe^{3+}/ascorbate (V_2) and in the presence of herbal preparation alone (V_1), were calculated. All ratios were ~2.00, value significantly lower than that of control ratio 2.91 ($p<0.05$), calculated between UDPGT activity measured in the presence of Fe^{3+}/ascorbate (V_2) and in the absence of this oxidative system (V_1). The exception was the aqueous preparation S-01, whose

ratio was 2.89, value not significantly different to the control ratio (p>0.05).

Table 2. Effect of herbal extracts on UDPGT activity in the absence and presence of Fe^{+3}/ascorbate.

	UDPGT activity (nmol of conjugate/min/mg of microsomal protein)		
Conditions	**V_1**	**V_2**	**Activation**
Control	0.94 ± 0.08	2.74 ± 0.13	2.91*
I-08	0.55 ± 0.04	0.94 ± 0.05	1.71
R-01	0.51 ± 0.03	0.96 ± 0.05	1.88
I-02	0.76 ± 0.04	1.45 ± 0.09	1.91
R-03	0.56 ± 0.03	1.13 ± 0.07	2.02
S-01	0.82 ± 0.06	2.37 ± 0.11	2.89*

Control: UDPGT activity measured in the absence of herbal preparations. **V_1**: microsomes preincubated for 5 min with herbal preparations before determining UDPGT activity according to Methods**.** **V_2**: microsomes preincubated for 5 min with herbal preparation and then 10 min with Fe^{+3}/ascorbate before determining UDPGT activity. **Activation:** corresponds to ratios V_2/V_1. Each value represents the means of at least four independent experiments ± S.D. *: Values not statistically different (p>0.05).

GSH-transferase activity

The hydro-alcoholic preparations (quantities equivalent to oxygen consumption IC_{50} values) inhibited to different extent the conjugation of 1-chloro-2,4-dinitrobenzene with GSH, reaction catalyzed by microsomal GST, but aqueous preparation S-01 had no effect (Table 3). Fe^{3+}/ascorbate inhibited this enzymatic activity 59.0%. The pre-treatment of microsomes with the hydro-alcoholic preparations and then with Fe^{3+}/ascorbate, significantly increased the GST inhibition in a similar percentage, ~31% (Table 3). However, if the GST activity, measured in the presence of each herbal preparation alone, is considered as the GST activity 100%, the respective inhibition percentages were similar (~39%) and significantly lower than that observed by Fe^{3+}/ascorbate alone, 59% (p<0.05). However, S-01 aqueous preparation did not modify the Fe^{3+}/ascorbate-derived GST inhibition (Table 3).

Table 3. Effect of herbal extracts on GSH-transferase activity in the absence and presence of Fe+3/ascorbate.

	GSH-transferase Activity (GST) (nmol of conjugate/min/mg of microsomal protein)		
Conditions	**V_1**	**V_2**	**Inhibition (%)**
Control	132.3 ± 1.7*	54.2 ± 1.05	59.0*
R-03	87.4 ± 0.84	56.2 ± 1.48	35.7
I-02	75.4 ± 1.20	46.5 ± 1.21	38.3
I-08	89.9 ± 0.83	53.1 ± 1.31	40.9
R-01	63.5 ± 1.56	36.5 ± 1.52	42.5
S-01	124.3 ± 5.6*	50.0 ± 1.50	59.8*

Control: GST activity measured in the absence of herbal preparations. **V_1**: microsomes preincubated for 5 min with herbal preparations before determining GSHT activity according to Methods**.** **V_2**: microsomes preincubated for 5 min with herbal preparation and then 10 min with Fe^{+3}/ascorbate before determining GST activity. **Inhibition (%):** percentages calculated considering as 100% the GST activity to V_1 values. Each value represents the means of at least four independent experiments ± S.D. *: Values not statistically different (p>0.05).

Cytochrome P450 system

The hydro-alcoholic preparations (quantities equivalent to oxygen consumption IC_{50} values) also inhibited this enzymatic reaction, but S-01 had not effect. I-02 showed the highest inhibition (73.5%) and I-08, the lowest (11.5%). Fe^{3+}/ascorbate inhibited the aminopyrine *N*-demethylating activity of cytochrome P450 system 52.2% and the pre-treatment of microsomes with the hydro-alcoholic preparations and then with Fe^{3+}/ascorbate, significantly increased this inhibition (Table 4). However, if the cytochrome P450 activity, measured in the presence of each herbal preparations alone, is considered as the aminopyrine *N*-demethylating activity 100%, the inhibition percentages were significantly lower than that obtained by Fe^{3+}/ascorbate alone, 52% (p<0.05). Moreover, I-02 prevented fully this oxidative phenomenon, I-08, R-01 and R-03 reduced it to ~20% and aqueous preparation S-01 had not effect (Table 4).

Table 4. Effect of herbal extracts on the cytochrome P450 activity in the absence and presence of Fe^{+3}/ascorbate.

	Cytochrome P450 activity (nmol of HCHO/min/mg of microsomal protein)		
Conditions	**V_1**	**V_2**	**Inhibition (%)**
Control	1.13 ± 0.05	0.54 ± 0.03	52.2*
I-02	0.30 ± 0.02**	0.28 ± 0.01**	0
I-08	1.00 ± 0.05	0.80 ± 0.04	20.0
R-01	0.69 ± 0.04	0.54 ± 0.03	21.7
R-03	0.92 ± 0.03	0.71 ± 0.02	22.8
S-01	1.17 ± 0.06	0.55 ± 0.04	52.9*

Control: Aminopyrine *N*-demethylating activity measured in the absence of herbal preparations. **V_1**: microsomes preincubated for 5 min with herbal preparations before determining cytochrome P450 system activity according to Methods**. V_2**: microsomes preincubated for 5 min with herbal preparation and then 10 min with Fe^{+3}/ascorbate before determining cytochrome P450 system activity. **Inhibition (%):** percentages calculated considering as 100% the cytochrome 450 activities V_1 values. Each value represents the means of at least four independent experiments ± S.D. *: Values not statistically different ($p>0.05$).

Effect of herbal preparations on the absorbance spectrum of cytochrome P450 monooxygenase

Cytochrome P450 system catalyzes the biotransformation of lipophilic xenobiotics; the cytochrome P450 monooxygenase in its Fe^{3+}-hemin form binds the substrates in the first step of the catalytical cycle of this system, changing its absorbance spectrum. To evaluate the lipophilicity of herbal compounds present in the commercial preparations and its interaction with this enzyme, its maximum absorbance to 450 nm was determined in two conditions. Thus, microsomes were treated with sodium dithionite (reducing agent for Fe^{2+}-hemin form) after adding herbal preparations (Condition A) and before adding it (Condition B), prior to determining the monooxygenase absorbance to 450 nm according to Methods. The differences between the decreasing percentages obtained in condition A and B allowed estimating the binding to monooxygenase of lipophilic herbal compounds present in the hydro-alcoholic preparations. In both conditions the maximum absorbance of this enzyme decreased, but in condition A this decrease was significantly higher ($p<0.05$); the only exception was observed with the aqueous preparation S-01 whose monooxygenase absorbance values measured in both condition were not significantly different, $p>0.05$ (Table 5). In the condition A, I-02, I-08, R-01, R-03 and S-01 decreased the monooxygenase absorbance 26.2, 25.6, 21.7, 18.4 and 7.7%, respectively; but in the condition B the percentages were 10.4%, 12.8%, 15.0%, 10.0% and 7.1%, respectively. The difference values for I-02, I-03, R-03 and R-01 were 15.8, 12.8, 8.4 and 6.7, respectively (Table 5).

DISCUSSION

Food and medicinal herbal compounds possessing relevant antioxidant properties are xenobiotics for the human being. The hepatic endoplasmic reticulum is the main cellular organelle responsible of the xenobiotics biotransformation and one of the sites in which reactive oxygen species (ROS) are generated. Thus, in this cellular organelle the biotransformation of herbal compounds and the expression of their antioxidant properties may occur. As expected, the commercial herbal preparations assayed inhibited significantly the microsomal lipid peroxidation, phenomenon induced by Fe^{3+}/ascorbate as showed by their IC_{50} values. The only exception was the aqueous herbal preparation S-01; its IC_{50} value could not be obtained although up to 200 µL were assayed (Fig. 1A). Why S-01 microsomal anti-lipoperoxidative effect (IC_{50} value) could not be determined? This may be due to its very low polyphenol concentration (0.84 nmol of catechin/µL of preparation) and the presence of polar antioxidant compounds in this aqueous herbal preparation. Interestingly, polyphenol concentrations and IC_{50} values of hydro-alcoholic preparations correlated as an exponential decay manner (Fig. 1B); the presence of lipophilic compounds in these preparations and the ability of polyphenols to scavenge oxygen free radicals, one of the mechanisms through which they express its antioxidant properties could explain these antioxidant effects.

In addition, all herbal preparations including the aqueous preparation S-01 decreased the oxygen consumption caused by Cu^{2+}/ascorbate (Fig. 2). Interestingly, the effect of hydro-alcoholic preparations was higher than that obtained in the lipid peroxidation assays; only 1/5 IC_{50} value obtained from lipid peroxidation assays were needed to decrease 50% of the oxygen consumption induced by Cu^{2+}/ascorbate. This oxygen consumption depends on free copper ions concentration (Letelier et al., 2008). Polyphenols are chelating agents and hydro-alcoholic preparations showed a high concentration of these compounds.

Table 5. Effects of herbal extracts on the cytochrome P450 monooxygenase spectrum.

Blind name	Condition A		Condition B		Difference A-B
	nmol of monooxygenase/mg of microsomal protein	Decreasing %	nmol of monooxygenase/mg of microsomal protein	Decreasing %	
I-02	0.486 ± 0.023	26.2	0.590 ± 0.029	10.4	15.8
I-08	0.490 ± 0.026	25.6	0.575 ± 0.026	12.8	12.8
R-03	0.538 ± 0.029	18.4	0.593 ± 0.032	10.0	8.4
R-01	0.516 ± 0.024	21.7	0.560 ± 0.029	15.0	6.7
S-01	0.608 ± 0.052*	7.7	0.612 ± 0.034*	7.1	0.6
Control: 0.659 ± 0.032 nmol of monooxygenase/mg of microsomal protein					

Control: nmol of cytochrome P450 monooygenase measured in the absence of herbal preparations. **Condition A**: microsomes preincubated for 5 min with herbal preparations before adding sodium dithionite. **Condition B**: microsomes plus sodium dithionite and then incubated for 5 min with the herbal preparations before measuring the mono-oygenase according to Methods. **Difference A-B**: difference between the decreasing percentages calculated in the conditions A and B. Each value represents the means of at least four independent experiments ± S.D. *: Values not statistically different ($p>0.05$).

Moreover, oxygen consumption IC_{50} values of hydro-alcoholic preparations correlated in an exponential decay manner to polyphenol concentrations indicating that the metal chelating ability of these compounds could be involved in this phenomenon. Likewise, S-01 is a preparation enriched in mucilages (information given by the industry), which are also chelating agents (Wang et al., 2007). Therefore, mucilages chelating ability and in a minor grade, the S-01 polyphenol concentration could explain the decreasing of oxygen consumption caused by this aqueous herbal preparation (Fig. 2).

In contrast to the antioxidant effects on lipid peroxidation and oxygen consumption, the prevention of microsomal thiol oxidation did not correlate to the polyphenol content of herbal preparations. Thus, I-08 completely prevented the microsomal thiol oxidation induced by Fe^{3+}/ascorbate, I-02, R-01 and R-03 partially reduced it to the same extent and S-01 had no effect (Fig. 3). The cysteinyl residues of globular proteins are not equally reactive and the accessibility of herbal ingredients to proteins inserted in the microsomal membrane depends on its lipophilicity. Therefore, both factors could explain the differences in the antioxidant effects on microsomal thiol groups Fe^{3+}/asorbate-derived oxidation.

In addition, the hydro-alcoholic preparations alone inhibited all the enzymatic activities assayed, probably because substrates of these enzymes are present in these preparations (Tables 2, 3 and 4). UDPGT metabolizes alcoholic and phenolic compounds and all herbal preparations inhibited the catalytic activity of this enzyme measured through *p*-nitrophenol conjugation with UDPGA. However, polyphenol concentration of herbal preparations did not correlated to the extent of UDPGT inhibition. In the same way, the hydro-alcoholic preparations inhibited to different extent the GST and cytochrome P450 system activities, but aqueous preparation S-01 had no effect. GST and cytochrome P450 system metabolize lipophilic/electrophilic and lipophilic compounds, respectively, which could explain the absence of inhibitory effect of the aqueous preparation S-01.

In addition, hydro-alcoholic preparations prevented the Fe^{3+}/asorbate-derived oxidative damage on UDPGT, GST and cytochrome P450 system activities, but aqueous preparation S-01 had no effect. UDPGT, GST and cytochrome P450 monooxygenase are thiol proteins and the ROS-derived thiol group's oxidation alters its catalytic activities (Letelier et al*.,* 2005; Alterman et al., 1981; Letelier et al., 2006). Therefore, the antioxidant effects of hydro-alcoholic preparations on Fe^{3+}/ascorbate-derived microsomal thiol oxidation could explain the antioxidant effects observed on UDPGT, GST and cytochrome P450 system activities (Tables 2, 3, and 4). Interestingly, polyphenol concentration of hydro-alcoholic preparation did not correlate to the prevention of Fe^{3+}/ascorbate-derived oxidative damage on the catalytic activities of these biotransformation enzymes. Polyphenol concentrations of hydro-alcoholic preparations neither correlated to its microsomal thiol antioxidant effects (Fig. 3),

confirming that the antioxidant effects on the catalytic activities of these enzymes could relate to the protection of its thiol groups. Besides, the hydro-alcoholic preparation I-02 exerted the highest inhibition of cytochrome P450 system activity, the highest reduction of the monooxygenase absorbance and, totally prevented the Fe^{3+}/ascorbate-derived oxidative damage on the catalytic activity of this system. Instead, the aqueous herbal preparation S-01 had no effect on cytochrome P450 system and GST activities. Besides, it did not alter significantly the absorbance of the monooxygenase and did not prevent the Fe^{3+}/ascorbate-derived oxidative damage on cytochrome P450 system catalytic activity (Table 5). Thus, the lipophilicity of herbal ingredients present in hydro-alcoholic preparations could be another factor involved in the prevention of Fe^{3+}/ascorbate-derived oxidative damage on microsomal enzymes.

Another point to discuss is the biological advantages of total herbal extracts. Very little quantities of different active principles are present in these preparations, which could provoke multiple and synergistic antioxidant effects (Siess et al., 1992). This is evidenced by the ability of hydro-alcoholic preparation to prevent the Fe^{3+}/ascorbate-derived oxidative damage on microsomal membrane components (lipid, thiol groups and xenobiotics bio-transformation enzymes) and the inhibition of oxygen consumption induced by Cu^{2+}/ascorbate. Moreover, all hydro-alcoholic preparations inhibited the microsomal GST activity, which could indicate the presence of lipophilic/electrophilic substrates, physicochemical characteristics of highly reactive metabolites, which can induce oxidative stress or bind covalently to biomolecules. However, all of them showed a high antioxidant activity, so a balance that favours the antioxidant compounds on electrophilic compounds seems to exist in these hydro-alcoholic preparations. We cannot discard other mechanisms that could be involved in the GST activity inhibition; i.e. the binding of some components of herbal extracts to thiol group of microsomal GST, avoiding the formation of its catalytic active dimer may also occur (Aniya et al., 1993)

In general, these results show that in order to evaluate the herbal preparations antioxidant properties, the extraction method used in the manufacture of herbal preparations should be considered. It is necessary to note that ROS are the main biological oxidant species; synthetic nitrogen radicals, different to nitric oxide however, are commonly used as oxidant agents to evaluate antioxidant activity: important physicochemical differences between ROS and those synthetic radicals exist (Letelier et al., 2008). Furthermore, when food and medicinal plants extracts are going to be included in the processed foods or in the formulation of phytomedications, assays that investigate the protection of biomolecules against oxidative damage should be useful. Finally, rat liver microsomes are a good biological target to evaluate not only the biological antioxidant properties of herbal preparations, but also their pharmacokinetic inter-actions with other xenobiotics such as allopathic drugs.

CONCLUSIONS

Biotransformation is one of the main metabolic routes through which herbal active products may generate adverse effects, especially when herbal ingredients are metabolized through cytochrome P450 system. This system catalyzes mainly the bio-transformation of lipophilic compounds, so the extraction method used is very important in the herbal preparations conducing to phytomedications and/or food additives. We hereby put together a battery of tests -all based on the use of liver microsomes- to determine how some commercial herbal preparation can exert antioxidant activities -which are likely part of the mechanisms of their therapeutic activities- without compromising too much the oxidative metabolism of xenobiotics.

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REFERENCES

Alterman MA, Marekushev SA, Levchenko LA, Raevskii AV, Devichenskii VM. 1981. Cytochrome P450 distribution in endoplasmic reticulum membranes of rat liver. Biokhimiia 46(2):222-229.

Aniya Y, Shimaji M, Naito A. 1993. Increase in liver microsomal glutathione S-transferase activity by phenobarbital treatment of rats. Possible involvement of oxidative activation via cytochrome P450. Biochem Pharmacol 46, 1741-1747.

Dröge W. 2002. Free radicals in the physiological control of cell function. Physiol Rev 82(1):47-95.

Du Y, Guo G, Lou H. 2007. Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. J Agric Food Chem 55(5):1695-1701.

Halliwell B. and Gutteridge JM. 1999. Free radicals in Biology and Medicine (3rd edn). Claredon Press, Oxford.

Healy FG, Krasnoff SB, Wach, M, Gibson DM, Loria R. 2002. Involvement of a cytochrome P450 monooxygenase in Thaxtomin A. biosynthesis by *Streptomyces acidiscabies*. J Bacteriol 184(7):2019-2029.

Lee H, Yang L, Wang Ch, Hu S, Chang H, Lee Y. 2003. Differential effects of natural polyphenols on neuronal survival in primary cultured central neurons against glutamate- and glucose deprivation-induced neuronal death. Brain Res 986:103-113.

Leone M, Zhai D, Sareth S, Kitada S, Reed JC, Pellecchia M 2003. Cancer prevention by Tea polyphenols is linked to their direct inhibition of antiapoptotic Bcl-2-family proteins. Cancer Res 63:8118-8121.

Letelier ME, Del Villar, E., Sánchez E. 1985. Drug tolerance and detoxicating enzymes in *Octodon degus* and Wistar rats. A comparative study. Comp Biochem Physiol 80(1):195-198.

Letelier M.E, Martínez M, Gonzalez-Lira V, Faúndez M, Aracena-Parks P. 2006. Inhibition of cytosolic Glutathione *s*-transferase activity from rat liver by copper. Chem Biol Interact 164:39-48.

Letelier ME, Molina-Berríos A, Cortés-Troncoso J, Jara-Sandoval J, Holst M, Palma K, Montoya M, Miranda D, González-Lira V. 2008. DPPH and oxygen free radicals as pro-oxidant of biomolecules. Toxicol in Vitro 22:279-286.

Letelier ME, Pimentel A, Pino P, Lepe AM, Faúndez M, Aracena P, Speisky H. 2005. Microsomal UDP-Glucuronyltransferase in rat liver: Oxidative activation. Basic Clin Pharmacol Toxicol 96:480-486.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-273.

Middleton E, Kandaswami C, Theoharides T. 2000. The effects of plants flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673-751.

Omura T, Sato R. 1964. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. J Biol Chem 239(7):2370-2378.

Siess MH, LeBon AM, Suschetet M. 1992. Dietary modification of drug-metabolizing enzyme activities: Dose-response effect of flavonoids. J Toxicol Environ Health 35:141-152.

Urquiaga I, Leighton F. 2000. Plant polyphenol antioxidants and oxidative stress. Biol Res 33(2):55-64.

Wang TS, Lii CK, Chang JY, Kuo CI, Chen HP. 2007. Anti-Fenton reaction activity of three taxa of water yam (*Dioscorea alata* L.). Int J Food Sci Tech 42(9):1107-1113.

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Hepatoprotective activity of *Piper longum* traditional milk extract on carbon tetrachloride induced liver toxicity in Wistar rats

[Actividad hepatoprotectora del extracto tradicional lácteo de *Piper longum* en ratas Wistar]

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Abstract

Piper longum Linn. (Piperaceae) (fruits and roots powder) is given with boiled milk in the Indian traditional system of medicine for the treatment of liver ailments and jaundice. However, the biochemical basis and mechanism of hepatoprotective action of *Piper longum* milk extract is not scientifically studied. Thus, the present study was designed to investigate the hepatoprotective activity of *Piper longum* milk extract. Carbon tetrachloride (CCl_4) was used as a hepatotoxin at a dose of 0.5 ml/kg p.o. with olive oil (1:1) thrice a week for 21 days to produce the chronic reversible type of liver necrosis. Following treatment with *Piper longum* milk extract (200 mg/day p.o. for 21 days), a significant hepatoprotective effect was observed in CCl_4 induced hepatic damage as evident from decreased level of serum enzymes, total bilirubin and direct bilirubin. The hepatoprotective effect of *Piper longum* is comparable to the standard drug silymarin (25 mg/kg/day p.o. for 21 days).

Keywords: *Antioxidant enzymes; carbon tetrachloride; hepatoprotective activity;* Piper longum.

Resumen

En el sistema de medicina tradicional india, *Piper longum* Linn. (Piperaceae) (frutos y polvo de la raíz) es dado con leche hervida para el tratamiento de ictericia y dolencias del hígado. Sin embargo, las bases bioquímicas y el mecanismo de acción hepatoprotectora del extracto lácteo de *Piper longum* no se ha estudiado científicamente. El presente estudio fue designado para investigar la actividad hepatoprotectora del extracto lácteo de *Piper longum*. El tetracloruro de carcono (CCl_4) se usó como hepatoxina a una dosis de 0.5 ml/kg p.o. en aceite de oliva (1:1) tres veces a la semana por 21 días para producir la necrosis hepática crónica reversible. El tratamiento con el extracto lácteo de *Piper longum* (200 mg/día p.o. por 21 días) tuvo un efecto hepatoprotector significativo evidenciado por el decrecimiento en suero de enzimas, bilirrubina directa y bilirrubina total. El efecto hepatoprotector de *Piper longum* es comparable al fármaco de referencia silimarina (25 mg/kg/día p.o. por 21 días).

Palabras Clave: Cassia angustifolia; Senna alexandrina; *sen; laxantes naturales; antranoides; Fitoterapia.*

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INTRODUCTION

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years. Because of its unique metabolic functions and relationship to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics, oxidative stress, ethanol and toxic chemicals (antibiotics, chemotherapeutics, aflatoxins, carbon tetrachloride, chlorinated hydrocarbons, etc.). There are numerous plants and polyherbal formulations claimed to have hepato-protective activity. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. (Subramonium and Pushpangadan, 1999) *Piper longum* Linn. (Piperaceae), is a well known traditional medicine, promotes physical and mental health and also improves defense mechanisms of body. Nearly two-thirds of all traditional ayurvedic formulas contain a special blend of ingredients, which includes *Long pepper (Piper longum)* for this purpose. (Kirtikar and Basu, 1980). It is advised in Ayurveda that the fruits of this plant should be extracted as a milk decoction. The milk extract is reported to be nearly 27 times more active than the aqueous extract (Shankar et al., 2007, Sudha et al., 2004). Ethanolic extract of the fruits of *Long pepper* (*Piper longum*) was used for its hepatoprotective activity (Jalalpure et al., 2003). Also the main active constituent of *Piper longum*, Piperine is reported to have hepatoprotective property (Koul and Kapil, 1993, Khare, 2004). However, no systematic attempts have been made to establish the scientific basis of these beneficial effects of *Piper longum* milk extract. Hence, the aim of the present study was to investigate the hepatoprotective activity of *Piper longum* milk extract on carbon tetrachloride induced liver toxicity in rats.

MATERIALS AND METHODS

Animals

Healthy untreated wistar rats of either sex weighing 200-300 g were selected. The animals were housed 4 per cage, under standard environmental conditions like controlled light and dark cycle every 12 hours, temperature 22 ± 2 ˚C, 60-70% relative humidity. They were fed with standard rodent diet and water ad libitum. The animals were acclimatized for 5 days before starting experiment. All the experiments were conducted in accordance with the CPCSEA guidelines (Rule 5 (a) of the "Breeding of and Experiments on animals, Rules 1998", Government of India).

Plant material

Authenticated crude dried powder of *Piper longum linn.* was collected from Ms. Lallu Vrajlal Gandhi Ayurvedic store, Ahmedabad. The drug was authenticated by Prof. Vimalkumar, Head of Pharmacognosy Division. Voucher Specimen (PL-02/05-06) has been retained in the Institute of Pharmacy, Nirma University, India. It was mainly the dried powder of fruits and roots part of the herb. The powder was stored in polyethylene bags at room temperature until needed.

Extraction of plant material

Powdered drug was mixed with milk which was diluted with equal volume of water and then boiled to reduce the volume to half of the original. *Piper longum* (200 mg/kg p.o. for 21 days) was administered as milk extract along with the undissolved particles present in the vehicle. The extract was prepared fresh everyday for the entire treatment period of 21 days.

Experimental procedure

In the preliminary toxicity study, none of the animals showed any signs of toxicity up to 2 g/kg, p.o. dose and hence $1/10^{th}$ of the maximum dose administered (i.e. 200 mg/kg, p.o.) was selected for the present study.

The rats were divided into five groups of 7 animals each. Group I served as control 1 group and received only boiled milk (0.25 ml/kg/day, p.o. for 21 days; milk was diluted with equal volume of water and then boiled to reduce the volume to half of the original). Group II rats served as control 2 group and only olive oil (Vehicle for CCL_4) was given (0.05 ml/kg/day, p.o.) for 21 days. Group III rats received CCL_4 (0.05 ml/kg p.o. thrice a week) in olive oil (5:5) for 21 days. Group IV animals received CCl_4 (as in Group III) and standard reference drug silymarin (25 mg/kg/day, p.o.) for 21 days. Group V animals received CCl_4 (as in Group III) and P*iper longum* milk extract (200 mg/kg/day, p.o.) for 21 days.

After the completion of treatment period, blood samples were collected from the retro-orbital plexus under light ether anesthesia in anticoagulant free vials. 15 min later, samples were centrifuged at 3500-4000 rpm for 20 minutes to separate serum which was used for various liver function tests. All the animals were sacrificed under light ether anesthesia and livers were

quickly excised, rinsed in cold saline, blotted, weighed and immediately stored at -2 to -8 ˚C and used for various biochemical estimations (like Serum SGOT, SGPT, ALP, total and direct bilirubin levels, antioxidant parameters, etc.). A portion of liver was stored separately in formalin buffer after washing with ice-cold saline for histopathology studies.

Statistical analysis

Results are presented as mean ± standard error of mean. Statistical difference between the means of various groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A value of $P<0.05$ was used as the criterion of significance. The calculations were made with computer-assisted analysis using the SPSS software.

RESULTS

Administration of CCL_4 produced significant hepatotoxicity in experimental animals, as is evident by an elevation of the serum marker enzymes namely SGOT, SGPT and ALP in CCL_4 treated rats. Treatment of the animals with either boiled milk or olive oil, alone, did not influence either of the serum marker enzymes and hence acted as respective control groups (refer Figs. 1-3).

Effect on serum Enzymes

Effect on serum glutamate pyruvate transaminase (SGPT) levels

As shown in Fig. 1A, CCl_4 administered rats (0.5 ml/kg p.o. thrice a week for 21 days) showed significant increase in SGPT levels (159.30 U/L ± 6.86) when compared to both - control 1 (71.73 U/L ± 4.69) and control 2 animals (69.67 U/L ± 4.25), respectively. Administration of *Piper longum* milk extract (200 mg/kg/day p.o. for 21 days) significantly decreased SGPT levels (78.86 U/L ± 2.43), which were comparable with the standard drug silymarin (25 mg/kg/day p.o. for 21 days) (70.19 U/L ± 2.09).

Effect on serum glutamate oxaloacetate transaminase (SGOT) levels

As shown in Fig. 1B, CCl_4 administered rats (0.5 ml/kg p.o. thrice a week for 21 days) showed significant increase in SGOT levels (104.09 U/L ± 10.42) when compared to both, control 1 (52.68 U/L ± 3.21) and control 2 animals (50.58 U/L ± 1.72), respectively. Administration of *Piper longum* milk extract (200 mg/kg/day p.o. for 21 days) significantly decrease SGOT levels (67.89 U/L ± 3.69), which were comparable to silymarin (25 mg/kg/day p.o. for 21 days) (61.29 U/L ± 6.89).

Effect on serum alkaline phosphatase (ALP) levels

Fig. 1C shows that CCl_4 administered rats (0.5 ml/kg p.o. thrice a week for 21 days), exhibited significant increase in alkaline phosphatase levels (393.30 U/L ± 3.08) when compared to control 1 (163.0 U/L ± 4.32) and control 2 rats (157.25 U/L ± 2.11), respectively. Administration of *Piper longum* milk extract (200 mg/kg/day p.o. for 21 days) significantly decreased alkaline phosphatase levels (192.77 U/L ± 1.36) which were comparable with silymarin (25 mg/kg/day p.o. for 21 days) (170.58 U/L ± 1.16).

Effect on serum total bilirubin and direct bilirubin levels

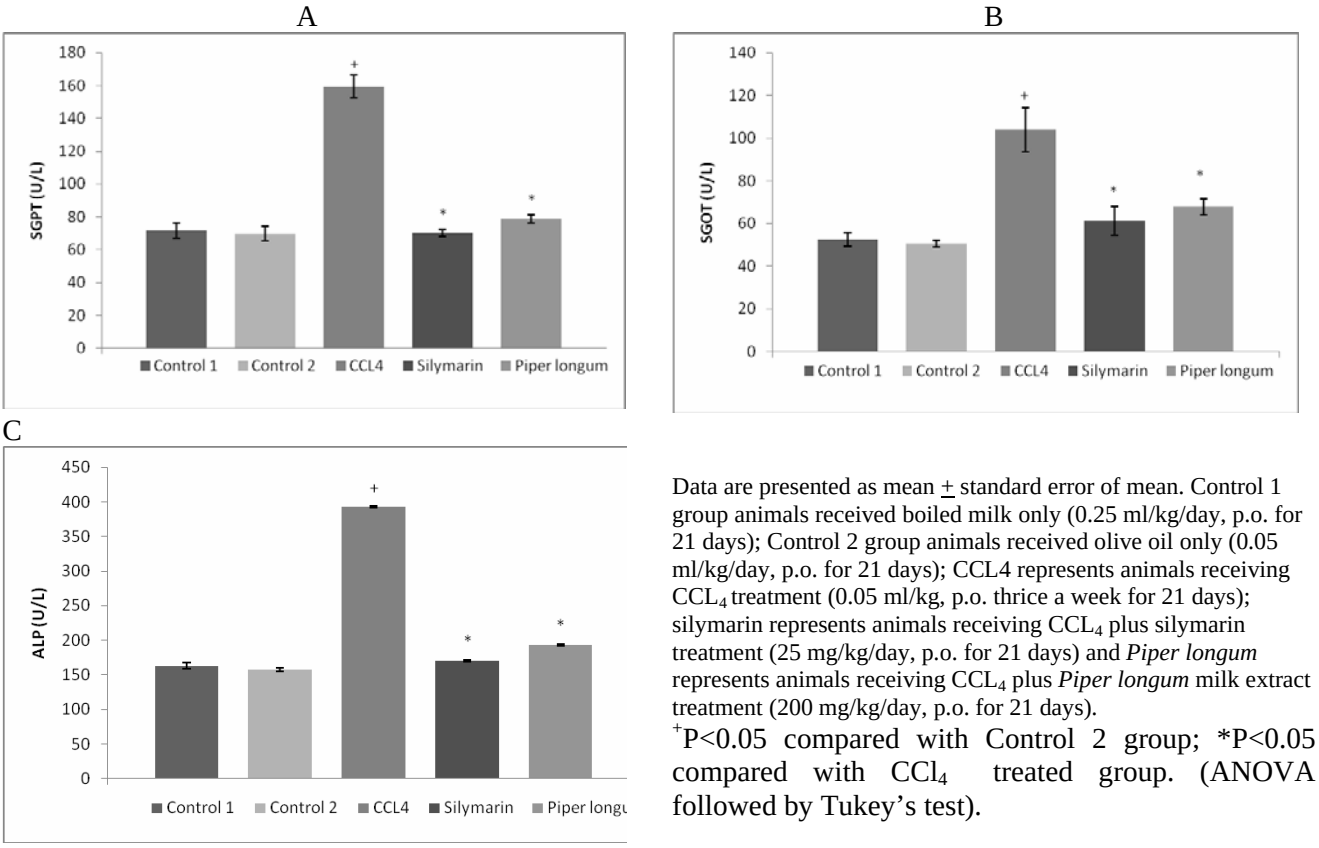
Effect on serum total bilirubin levels

Fig. 2A exhibits that CCl_4 administered rats (0.5 ml/kg p.o. thrice a week for 21 days) significantly elevated serum total bilirubin levels (3.27 mg/dl ± 0.15) when compared to control 1 rats (0.61 mg/dl ± 0.02) and control 2 rats (0.47 mg/dl ± 0.02) respectively. *Piper longum* milk extract (200 mg/kg/day p.o. for 21 days) significantly reduced total bilirubin levels (0.93 mg/dl ± 0.02) as compared to CCl_4 treated animals. Silymarin (25 mg/kg/day p.o. for 21 days) also significantly reduced total bilirubin levels (0.65 mg/dl ± 0.02).

Effect on serum direct bilirubin levels

In Fig. 2B can be observed that CCl_4 administered rats (0.5 ml/kg p.o. thrice a week for 21 days) significantly elevated serum direct bilirubin levels (0.854 mg/dl ± 0.02) as compared to control 1 group (0.41 mg/dl ± 0.01) and control 2 rats (0.32 mg/dl ± 0.02), respectively. *Piper longum* milk extract (200 mg/kg/day p.o. for 21 days) significantly reduced direct bilirubin levels (0.60 mg/dl ± 0.01) as compared to CCl_4 treated group. Silymarin (25 mg/kg/day p.o. for 21 days) also significantly reduced direct bilirubin levels (0.50 mg/dl ± 0.02).

Figure1. Effects of *Piper longum* milk extract on serum SGPT levels (A), serum SGOT levels (B); and serum alkaline phosphatase levels (C).



Data are presented as mean ± standard error of mean. Control 1 group animals received boiled milk only (0.25 ml/kg/day, p.o. for 21 days); Control 2 group animals received olive oil only (0.05 ml/kg/day, p.o. for 21 days); CCL4 represents animals receiving CCL_4 treatment (0.05 ml/kg, p.o. thrice a week for 21 days); silymarin represents animals receiving CCL_4 plus silymarin treatment (25 mg/kg/day, p.o. for 21 days) and *Piper longum* represents animals receiving CCL_4 plus *Piper longum* milk extract treatment (200 mg/kg/day, p.o. for 21 days).
$^{+}P<0.05$ compared with Control 2 group; $^{*}P<0.05$ compared with CCl_4 treated group. (ANOVA followed by Tukey's test).

Effect on antioxidant enzyme activity in liver

Effect on malondialdehyde contents in liver

Fig. 3A shows taht CCl_4 significantly increased malondialdehyde (MDA) levels in liver (42.47 µM/mg of protein ± 0.57) compared to control 1 group (14.78 M/mg of protein ± 0.51) and control 2 group (13.60 µM/mg of protein ± 0.2), respectively. *Piper longum* milk extract significantly reduced the levels of MDA (22.47 µM/mg of protein ± 0.98). Silymarin also reduced MDA levels (16.76 µM/mg of protein ± 0.46).

Effect on superoxide dismustase levels in liver

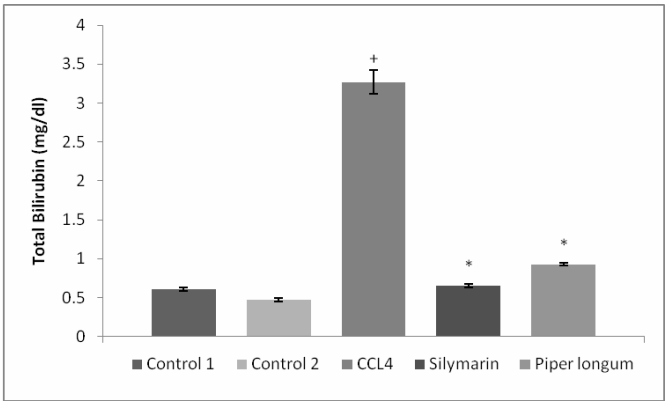
In Fig. 3C is observed that CCl_4 significantly reduced superoxide dismustase (SOD) levels (0.08 Units/mg of protein ± 0.02) in liver compared to control 1 group (0.24 units/mg of protein ± 0.01) and control 2 group (0.25 Units/mg of protein ± 0.01) respectively. *Piper longum* milk extract increased SOD levels (0.16 Units/mg of protein ± 0.01) as compared to CCl_4 treated group. Silymarin also increased SOD levels significantly (0.20 Units/mg of protein ± 0.01).

Effect on catalase levels in liver

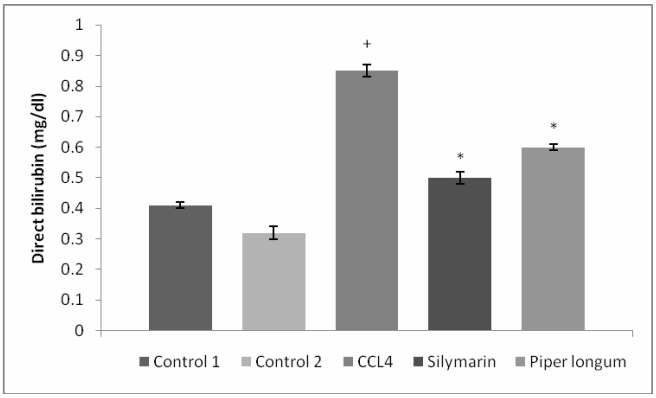
According with Fig. 3C, CCl_4 also reduced catalase levels (2.31 Units/min/mg of protein ± 0.11) as compared to control 1 group (7.88 Units/min/mg of protein ± 0.36) and control 2 group (8.55 Units/min/mg of protein ± 0.15) respectively. *Piper longum* milk extract and silymarin increased catalase levels significantly (3.86 Units/min/mg of protein ± 0.47) and (4.54 Units/min/mg of protein ± 0.20), respectively.

Figure 2. Effects of *Piper longum* milk extract on serum total bilirubin levels (A) and serum direct bilirubin levels (B).

A



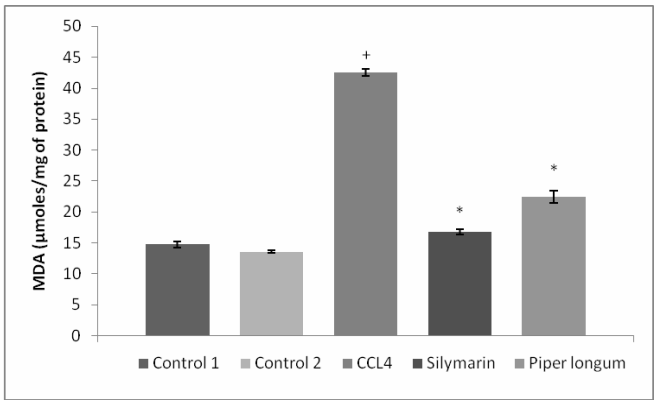
B



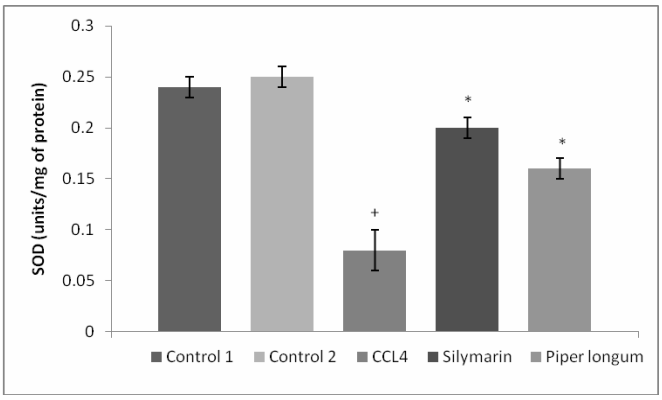
Data are presented as mean ± standard error of mean. Control 1 group animals received boiled milk only (0.25 ml/kg/day, p.o. for 21 days); Control 2 group animals received olive oil only (0.05 ml/kg/day, p.o. for 21 days); CCL4 represents animals receiving CCL_4 treatment (0.05 ml/kg, p.o. thrice a week for 21 days); silymarin represents animals receiving CCL_4 plus silymarin treatment (25 mg/kg/day, p.o. for 21 days) and *Piper longum* represents animals receiving CCL_4 plus *Piper longum* milk extract treatment (200 mg/kg/day, p.o. for 21 days).
$^+$ P<0.05 compared with Control 2 group; *P<0.05 compared with CCl_4 treated group (ANOVA followed by Tukey's test).

Figure 3. Effects of *Piper longum* milk extract on malondialdehyde contents in liver (A), superoxide dismustase levels in liver (B), and catalase levels in liver (C)

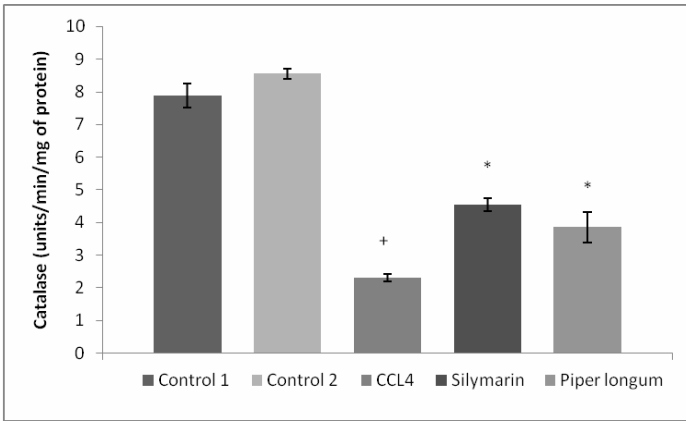
A



B



C



Data are presented as mean ± standard error of mean. Control 1 group animals received boiled milk only (0.25 ml/kg/day, p.o. for 21 days); Control 2 group animals received olive oil only (0.05 ml/kg/day, p.o. for 21 days); CCL4 represents animals receiving CCL_4 treatment (0.05 ml/kg, p.o. thrice a week for 21days); silymarin represents animals receiving CCL_4 plus silymarin treatment (25 mg/kg/day, p.o. for 21 days) and *Piper longum* represents animals receiving CCL_4 plus *Piper longum* milk extract treatment (200 mg/kg/day, p.o. for 21 days).
$^+$ P<0.05 compared with Control 2 group; *P<0.05 compared with CCl_4 treated group. (ANOVA followed by Tukey's test).

Figure 4. Microarchitecture of liver sections of control 1 rats (only boiled milk treated; 0.25 ml/kg/day, p.o. for 21 days). It shows central vein and normal parenchymal cells. Photographs to 10X.

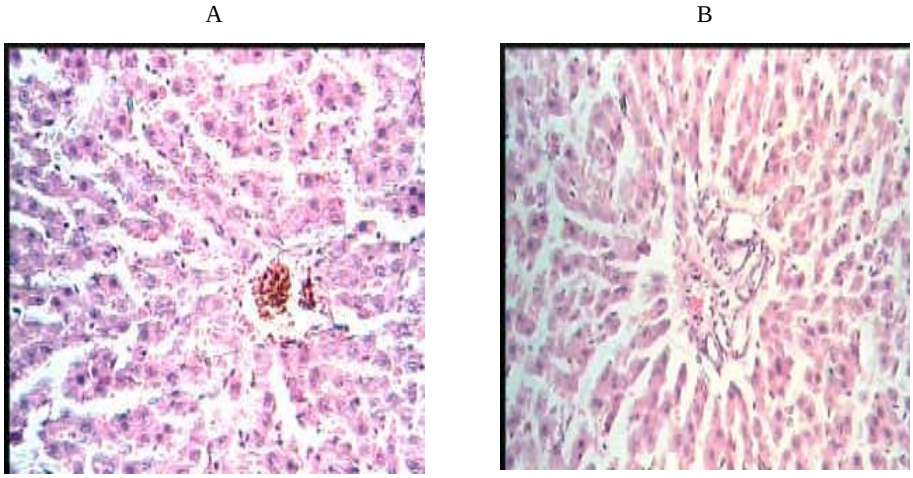


Figure 5. Microarchitecture of liver sections of control 2 (only olive oil treated; 0.05 ml/kg/day, p.o. for 21 days) rats. It shows central vein and normal parenchymal cells. Photographs to 40X (A) and 10X (B)

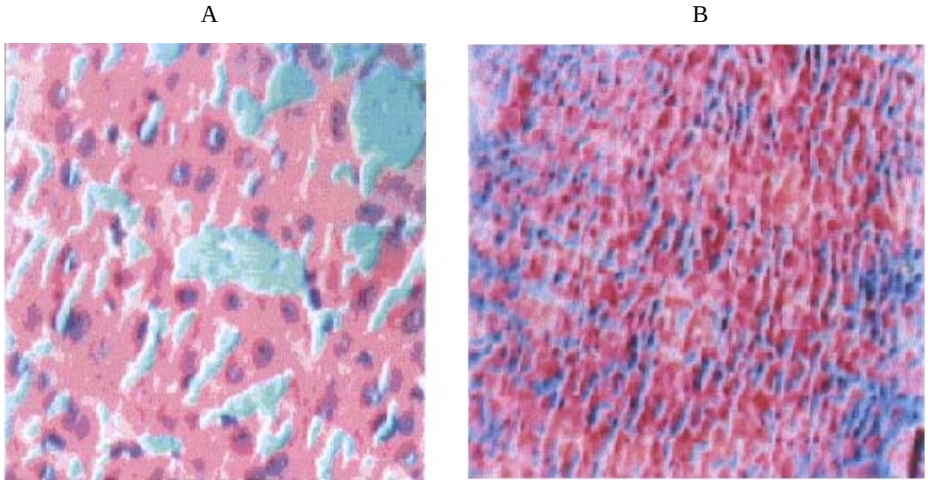


Figure 6. Microarchitecture of liver sections of CCl4 treated (0.05 ml/kg, p.o. thrice a week for 21 days) rats. It shows development of large septa of connective tissue flowing together and penetrating into parenchyma, resulting into extensive necrosis. Photographs to 10X.

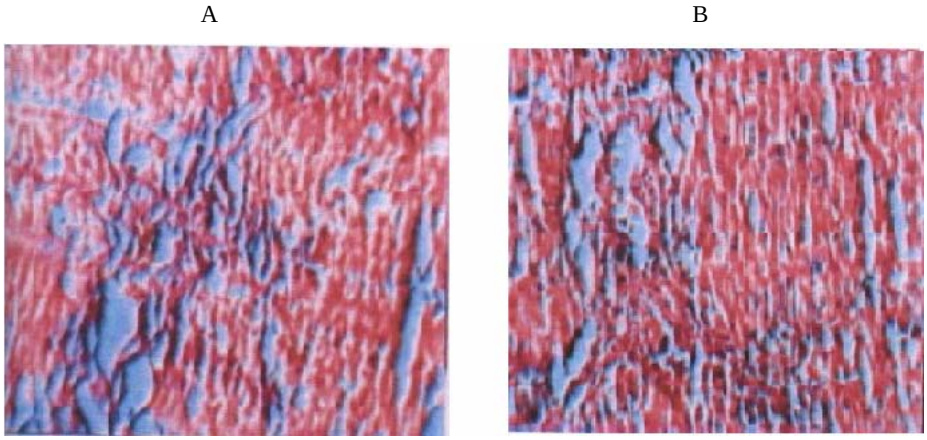


Figure 7. Microarchitecture of liver sections of silymarin treated (25 mg/kg/day, p.o. for 21 days) rats. It shows more number of regenerating liver cells around the necrotic area. Photographs to 40X.

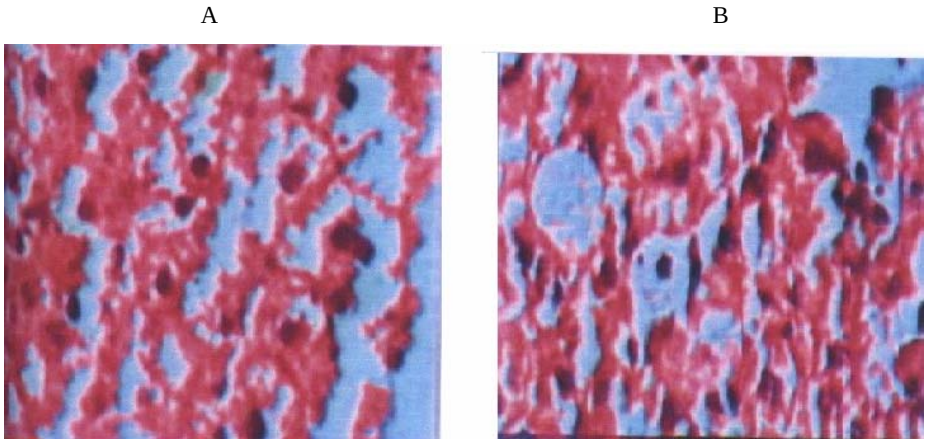
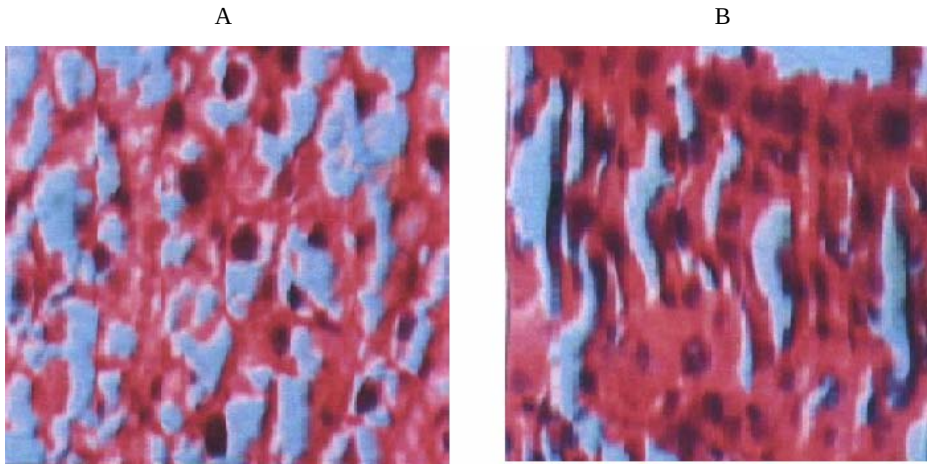


Figure 8. Microarchitecture of liver sections of *Piper longum* treated (200 mg/kg/day, p.o. for 21 days) rats. It shows regenerating liver cells around the necrotic area. Photographs to 40X.



Histopathological studies in liver

Histopathological findings indicated that administration of *piper longum* milk extract offered protection to the hepatocytes from damage induced by CCL_4, with mild fatty changes in the hepatic parenchymal cells, which corroborated the changes observed in the hepatic enzymes. It also showed regenerating liver cells around the necrotic area (Figs. 4-8).

DISCUSSION

Oral administration of CCl_4 is one of the easiest, fastest and reliable techniques to develop liver toxicity and can be used to screen hepatoprotective agents. CCl_4 gets accumulated in the hepatocytes, where it is activated by oxidases that are involved in the chemolytic breakage of C-Cl bond. This activation occurs in the hepatic endoplasmic reticulum via an enzyme system of electron transport from reduced nicotinamide adenine dinucleotide phosphate to oxygen. CCl_4 is activated by cytochrome CYP2E1, CYP2B1, or CYP2B2 and possibly CYP3A to form highly reactive and toxic metabolite trichloromethyl radical. The free radicals locally cause auto-oxidation of the polyenic fatty acids present within the membrane phospholipids. So, organic peroxides are formed after reacting with oxygen (lipid peroxidation). This reaction is autocatalytic in nature so that new radicals are formed from the peroxide radicals themselves. Thus, rapid breakdown of the structure and function of the endoplasmic reticulum is due to the decomposition of lipids. Within less than 30 minutes, there is decline in hepatic protein synthesis and within 2 h, there is swelling of smooth endoplasmic reticulum and dissociation of ribosomes from the rough

endoplasmic reticulum. (Farber et al., 1971). Lipid export from the hepatocytes is reduced owing to their inability to synthesize apoprotein to complex with triglycerides and thereby facilitate lipoprotein secretion. This results in a fatty liver due to CCl_4 poisoning. Mitochondrial injury then occurs and this is followed by progressive swelling of the cells due to increased permeability of the plasma membrane. Plasma membrane damage is thought to be caused by relatively stable fatty aldehydes, which are produced by lipid peroxidation in the smooth endoplasmic reticulum but are able to act at distant sites. This is followed by massive influx of calcium and cell death (Robbins, 2003). The lipid peroxide causes breakdown of the biomembranes at cellular and subcellular levels. As a consequence, the microsomal enzyme activities are found to be decreased and water soluble enzymes leak into the plasma from liver. SGPT is thought to be one of the indices of the degree of cell membrane damage while SGOT is an indicator for mitochondrial damage since mitochondria contains 80% of the enzyme (Dabba and Abdel-Rahman 1998). The increased activity of the liver marker enzymes such as SGPT, SGOT and ALP, as shown in Fig. 1 in the serum of CCl_4 induced rats indicate damage to hepatic cells. Both *Piper longum* and silymarin treated rats possess significantly lower SGPT, SGOT and ALP levels as shown in Fig. 1 as compared to CCl_4 treated animals. So we can speculate that the protective effect is on both mitochondria and hepatocytes. The normalized levels of the enzymes SGPT, SGOT and ALP after the treatment with *Piper longum* in CCl_4 intoxicated rats demonstrated its hepatoprotective action.

Increase in serum bilirubin levels may be found in hepatocellular damage, hemolytic jaundice or hepatitis. CCl_4 injury causes significant degeneration of hepatocytes and blockade of the bile ducts which results into significant increase in the serum total bilirubin and direct bilirubin levels (Saraswat et al., 1993). An increase in the levels of serum bilirubin reflected the depth of jaundice caused by CCl_4 intoxication as shown in Fig. 2. Pretreatment with *Piper longum* milk extract normalized the elevated total bilirubin and direct bilirubin levels.

The cellular infiltration of activated neutrophilic leukocytes amplifies inflammatory response and cellular injury or depth due to release of superoxide anions and other toxic mediators (Comportti, 1985). The antioxidant enzymes SOD, catalase and peroxidases constitute a mutually supportive team of defense against reactive oxygen species (Bandhopadhay et al., 1999). A decrease in activity of SOD in the liver of CCl_4 treated rats in our study might be due to the increased lipid peroxidation or inactivation of the enzyme by cross linking with malondialdehyde (Tabatabaie and Floyd, 1994). This may cause an increased accumulation of superoxide radicals, which could further stimulate lipid per-oxidation. In our study, elevation in the levels of end products of lipid peroxidation in CCl_4 treated animals was observed. The increase in MDA levels, decrease in catalase levels and superoxide dismustase levels in liver as seen in Fig. 3, following CCl_4 treatment indicates the damage and failure of antioxidant defense mechanism. Treatment with *Piper longum* and silymarin significantly prevented these changes.

Hepatotoxins develop hypoxic conditions which can damage the perivenular zone of the hepatic acinus. The highest expression of cytochrome CYP2E1 in the perivenular region produces oxy-radicals that contribute to the injury. Moreover, hepatocytes in the perivenular area contain less antioxidant factors and enzymes (Kera et al., 1987). Thus, while the lipid peroxidation mediated by oxy-radicals is likely to be the highest in the perivenular area, the detoxifying capacity of the hepatocytes here is reduced, therefore, the production may exceed the detoxification of the perivenular area (Rajesh and Latha 2004). Histopathological studies, as can be seen in Figs. 4 and 5 showed that control 1 and 2 animal groups had normal central vein and liver parenchymal cells. As shown in Fig. 6, the CCl_4 intoxicated animals show extensive necrosis, inflammation and infiltration by lymphocytes. In the *Piper longum* treated group (Fig. 8) the areas of regeneration are seen around the necrotic focus. There is more amount of regeneration with mild inflammation and some lymphocytic infiltration in the necrotic area. Standard drug silymarin treated rats showed more number of regenerating liver cells around the necrotic area as shown in Fig. 7. Thus, the histopathological studies substantiated the hepatoprotective effects observed in the biochemical studies and also pinpointed that the beneficial effects are comparable to that of the standard drug silymarin.

CONCLUSION

The results from the present study demonstrated a significant hepatoprotective and antioxidant activity of *Piper longum* milk extract. Our findings support the reported therapeutic use of this herb in tribal medicine

for liver ailments and jaundice. Further pharmacological investigations are underway to identify the specific constituents of the plant extract responsible for these activities.

REFERENCES

Bandhopadhay U, Das D, Banerjee KR. 1999. Reactive oxygen species: Oxidative damage and pathogenesis. Curr Sci 77:658-665.

Comportti M. 1985. Biology of diseases: Lipid peroxidation and cellular damage in toxic liver injury. Lab Invest 53:599-602.

Dabba MH, Abdel-Rahman M.S. 1998. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol Lett 95:23-29.

Farber E, Liang H, Schinozuka H. 1971. Dissociation of effects on protein synthesis and ribosomes from membrane changes induced by carbon tetrachloride. Am J Pathol 64:601-606.

Jalalpure SS, Patil MB, Prakash NS, Hemalata K. 2003. Hepatoprotective activity of the fruits of *Piper longum* Linn. Ind J Pharm Sci 65(4):363-366.

Kera Y, Sippel HW, Penttila KE, Lindros KO. 1987. Acinar distribution of glutathione dependent detoxifying enzymes. Low glutathione peroxidase activity in perivenous hepatocytes. Biochem Pharmacol 36:2003-2006.

Khare CP. 2004. Encyclopedia of Indian Medicinal Plants In: C.P. Khare editors. Piper. Rational Western Therapy, Ayurvedic and other Traditional Usage, Botany. 2nd ed. Springer. pp: 367-369.

Kirtikar KM and Basu BD. 1980. Indian Medicinal Plants. Vol-3 In: Colonel LR, I.C.S., editors. N.O. Piperaceae. International Book Distributors.2nd ed. New Delhi: M/S periodical experts. pp: 267-269.

Koul IB, Kapil A. 1993. Evaluation of the hepatoprotective potential of Piperine, an active principle of black and Long peppers. Planta Med 59(5):413-417.

Rajesh MG, Latha MS. 2004. Protective activity of *Glycyrrhiza glabra* Linn. on carbon tetrachloride induced peroxidative damage. Ind J Pharmacol 36:284-287.

Robbins. 2003. Pathological Basis of Disease. In: Cotran, Kumar, Collins, editors. Cellular pathology I: Cell injury and Cell death. 6th ed. W.B.Saunders Company pp: 1-30.

Saraswat B, Visen PK, Patnaik GK, Dhawan BN. 1993. Anticholestic effect of picroliv, active hepatoprotective principle of *Picrorhiza kurroa*, against carbon tetrachloride induced cholestasis. Ind J Expt Biol 31:316-371.

Shankar D, Unnikrishnan PM, Venkatasubramanian P. 2007. Need to develop inter-cultural standards for quality, safety and efficacy of traditional Indian Systems of medicine. Curr Sci 92:1499-1505.

Subramonium A, Pushpangadan P. 1999. Development of phytomedicines for liver diseases. Ind J Pharmacol 31:166-175

Sudha PVB, Geeta UG, Venkatasubramanian P. 2004. Bioactivity of traditional preparation of *Piper longum L.* (Piperaceae). J Trop Med Plants 5:179-182.

Tabatabaie T. Floyd Ra.1994. Susceptibility of glutathione peroxidase and glutathione reductase to oxidative damage and the protective effect of spin trapping agents. Arch Biochem Biophys 314:112-119.

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Benzylcarbamothioethionate from root bark of *Moringa oleifera* Lam. and its toxicological evaluation

[Bencilcarbamotioetionato de la raíz de *Moringa oleifera* Lam. y su evaluación toxicológica]

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Abstract

A new compound, benzylcarbamothioethionate (**1**), was isolated from the chloroform soluble fraction of the ethanolic extract of the root bark of *Moringa oleifera* Lam. Its structure was established on the basis of 1D and 2D-NMR and mass spectroscopy. The acute toxicity studies of chloroform extract and compound **1** were performed on Long Evan's rats using four groups (two controls and two experimental). The hematological parameters such as the total RBC (red blood cell), total WBC (white blood cell), differential count of WBC, platelet count, hemoglobin and ESR (erythrocytes sedimentation rate) remained unchanged in both experimental and control groups. In case of biochemical study, SGPT (serum glutamate pyruvate transaminase), SGOT (serum glutamateoxaloacetate transaminase), SALP (serum alkaline phosphatase), bilirubin, creatinine and blood urea of experimental groups were also similar to that of control groups. Histopathology of liver, kidney, heart and lung did not reveal acute toxicity. So this study revealed that both the chloroform soluble fraction and compound **1** of *Moringa oleifera* Lam. had no toxic effects in our experimental models.

Keywords: Moringa oleifera; *Moringaceae; Benzylcarbamothioethionate;Toxicity.*

Resumen

Un nuevo compuesto, bencilcarbamotioetionato (**1**), fue aislado de la fracción soluble clorofórmica del extracto etanólico de la corteza de la raíz de *Moringa oleifera* Lam. La estructura fue establecida sobre las bases de 1D y 2D-RMN y espectroscopia de masa. Los estudios de toxicidad aguda del extracto clorofórmico y el compuesto 1 fueron realizados en cuatro grupos de ratas Long Evans (dos controles y dos experimentales). Los parámetros hematológicos tales como TBC total (eritrocitos), WBC total (leucocitos), conteo diferencial de WBC, conteo de plaquetas, hemoglobina y eritrosedimentación permanecieron inalterables tanto en todos los grupos. En los estudios bioquímicos, SGPT (glutamato piruvato transaminasa sérica), SGOT (glutamato oxaloacetato transaminasa sérica), SALP (fosfatasa alcalina sérica), bilirrubina, creatinina y urea sanguínea de los grupos experimentales también fueron similares a los controles. La histopatología del hígado, riñón, corazón y pulmón no reveló toxicidad aguda. Este estudio reveló que tanto la fracción clorofórmica soluble como el compuesto **1** de *Moringa oleifera* Lam. no tuvieron efectos tóxicos.

Palabras Clave: Moringa oleifera; *Moringaceae; Bencilcarbamotioetionato;Toxicidad.*

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INTRODUCTION

Moringa oleifera Lam. (Fam. Moringaceae) (Local name- Shajna) is a monotypic species that is widely distributed in the Indian subcontinent and cultivated throughout the tropical belt (Sastri, 1962). Different parts of this plant are used in the indigenous systems of medicine for the treatment of a variety of human ailments and are also eaten as vegetables (Kirtikar and Basu, 1984). Traditionally, the plant is used as stimulant, carminative, stomatic, diuretic and is effective in articular pain, tetanus and paralysis (Yusuf et al., 1994). The gum of the tree is also used in dental carries while the seeds are used in venereal affections (Yusuf et al., 1994). Previous phytochemical studies on different parts (root, root bark, leaves and stem) of *M. oleifera* resulted in the isolation of alkaloids such as moringine and moringinine (Chopra, 1958), carbamates and thiocarmates derivatives (Faizi et al., 1995; Faizi et al., 1997; Murakami et al., 1998; Francis et al., 2004), flavonoids (Stavros and John, 2002; Bennett et al., 2003), steroids (Saluja et al., 1978; Giovanna, 2002), fatty acids and amino acids (Ramiah and Nair, 1977; Farooq and Bhanger, 2003; Abdulkarim et al., 2005), vitamins (Ching and Mohamed, 2001; Rangaswamy et al., 2005) and pterygospermin (Kurup and Rao, 1954). As far as the pharmacological activities are concerned, *M. oleifera* has been reported for hypotensive property (Ahmed et al., 1986; Faizi et al., 1998), antimicrobial activity (Caceres et al., 1991), anti-fertility activity (Prakash, 1988; Shukla et al., 1988), anticancer and antitumor activity (Guevara et al., 1999), the spasmolytic and anti-inflammatory and diuretic properties (Ezeamuzie et al., 1996; Caceres et al., 1992), antioxidant activity (Siddhuraju et al., 2003), CNS depressant nature on mice (Gupta et al., 1999), inhibition of systemic and local anaphylaxis (Mahajan and Mehta, 2007) and reduced locomotor activity on rats (Kausik et al., 2003). As a part of our research focused bioactive compounds from indigenous medicinal plants, we here report the isolation and identification of a new compound (**1**) from the chloroform soluble fraction of an ethanolic extract of *Moringa oleifera* as well as toxicological evaluation of the chloroform extract and compound (**1**).

MATERIALS AND METHODS

General experimental procedures

The ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz respectively with TMS as internal standard using a 500 MHz Bruker Drx NMR instrument. The IR spectra were recorded with a Perkin-Elmer Lambda spectrophotometer and the Mass spectra were recorded with LC-MS (APCI) in positive mode. Melting points were determined using a Digital Melting point Apparatus (model IA 8103, Electrothermal Engineering LTD, Southend-on-Sea, Essex, UK) and are uncorrected. All solvents used in this study were of analytical grade and purchased from the BDH and Merck.

Plant material

Fresh root bark of *Moringa oleifera* Lam. was collected from the adjoining areas of Rajshahi University Campus, Bangladesh in March, 2003 and identified by Professor A.T.M. Nadiruzzaman, Department of Botany, University of Rajshahi where a voucher specimen (FN/03/001) of this collection has been maintained.

Extraction and isolation of compound (1)

The powdered root bark (1.5 kg) of *Moringa oleifera* Lam. was macerated at room temperature with ethanol (5 L) for a week and then filtered and concentrated by using a rotary evaporator at 40 °C and under reduced pressure. The ethanol extract (50.0 g) was fractionated by solvent-solvent partitioning with petroleum ether (40-60 °C), then chloroform yielding petroleum ether fraction (5.8 g) and chloroform soluble fraction (10.6 g), respectively. The chloroform soluble fractions (5.0 g) were subjected to column chromatography over silica gel (Merck) eluting with n-hexane and ethyl acetate of increasing polarity which yielded a total of 47 fractions. Among these, fractions 26-30 eluted with n-hexane-ethyl acetate (50:50) were combined based on TLC analysis and further subjected to column chromatography eluting with n-hexane and chloroform of increasing polarity. From the second column chromatography, fractions 7-11 eluted with n-hexane-chloroform (75:25) were combined together based on TLC analysis. Concentration of the fractions followed by re-crystallization from petroleum ether (40-60 ^{o}C) afforded compound 1 (31.4 mg) as needles. Structure of compound 1 was confirmed by analysis of its IR, ^{1}H-NMR ^{13}C-NMR and Mass

(APCI) spectral data at the Centre for Phytochemistry, Southern Cross University, Australia.

Compound 1: Needles; mp. 58-60 ^{o}C; IR (KBr): 3294, 2872~3032, 1639,1525, 1217, 1195 cm^{-1}; ^{1}H-NMR(500 MHz, $CDCl_3$): δ 7.29 (1H, m, H-2), 7.35, (1H, m, H-3), 7.29, (1H, m, H-4), 7.35, (1H, m, H-5), 7.29 (1H, m, H-6), 4.48 (2H, d, *J*=4.9 Hz, H-7), 2.96 (2H, q, *J*=7.4 Hz, H-9), 1.32 (3H, t, *J*=7.4Hz, H-10), 5.63 (1H, br s, NH); ^{13}C-NMR (125 MHz, $CDCl_3$): 138.0 (C-1), 127.9 (C-2), 129.0 (C-3), 127.8 (C-4), 129.0 (C-5), 127.9 (C-6), 45.5 (C-7), 167.6 (C-8), 24.6 (C-9), 15.9 (C-10); LCMS (APCI) $[M+H]^+$ *m/z* 196 and fragment ions at *m/z* 166,132 and 105.

Animals

Long Evan's rats (103.2 - 104.2 g) were collected from the Animal Resources Branch of the International Center for Diarrhoeal Research, Dhaka, Bangladesh. The experiment was performed at the Department of Pathology, Rajshahi Medical College. The rats were kept in numbered iron cages for two weeks before treatment. They were fed a balanced diet (Hawk et al., 1954) and water, under standard conditions of a 12 h dark-light cycle, 60 ± 10% humidity and a temperature of 21.5 ± 1.0 °C. These protocols were approved by the Institutional Animal Care and Use Committee of UNICAMP, which follows the recommendations of the Canadian Council on Animal Care (Olfert et al., 1993)

Grouping and administration

The rats were divided into four groups (two controls and two experimental), four animals per group.

Control group: The rats were kept undisturbed and allowed free access to food and water.

Vehicle group: 0.2 mL vehicle (5.6 mL distilled water plus 5 drops of Tween-20) was administrated to rats intra-peritoneally once a day for 7 consecutive days.

Experimental group 1: 581.0 mg chloroform soluble fractions dissolved in distilled water (5.6 mL) with the help of Tween-20 in such a way that 0.2 mL contained 200 mg/kg body weight/day of chloroform fractions. This was administrated to rats intra-peritoneally once a day for 7 consecutive days.

Experimental group 2: compound 1 (14.175 mg) dissolved in distilled water (5.6 mL) with the help of Tween-20 so that 0.2 mL contained 5 mg/kg body weight/day of compound 1. This was administrated to rats intra-peritoneally once a day for 7 consecutive days.

Experimental procedure

A measured amount of fresh food was supplied daily at 10.00 A.M. The general well-being and behavior of the animals were observed daily throughout the study. For the hematological study, blood was collected from the tail vein of all the groups before and after the experimental period to estimate the total and differential blood count, platelet count, percent haemoglobin and ESR by standard procedure (Schalm et al., 1975). For the biochemical study, blood was collected from each rat sacrificed at day 7 from the jugular veins of each of the animals. Serum glutamic-oxaloacetic trans-aminase, serum glutamate pyruvate transaminase, serum alkaline phosphatase, urea, bilirubin and creatinine were determined using standard procedures and reagents supplied by Boehringer Mannheim GmbH Diagnostica. Histopathological studies of the liver, kidney, heart and lung were performed using haematoxylin, eosin stain and D. P. X mounting fluid (Gaur, 1962). The samples were observed under a microscope at the Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh.

Statistical analysis

Results are presented as the mean ± S.D. Student's *t*-test was used for comparison between the experimental and control groups (both normal diet and vehicle). $P < 0.05$ was considered to be statistically significant.

RESULTS

Identification of compound 1

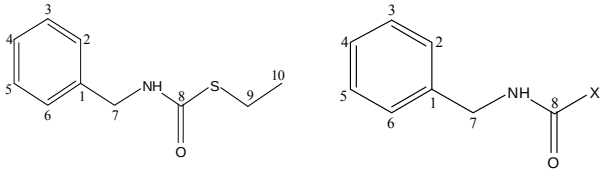
Compound **1** was isolated from the chloroform soluble fraction of ethanolic extract of *Moringa oleifera* Lam. as needles, mp. 58-60 ^{o}C. The LCMS (APCI) showed a $[M+H]^+$ peak at *m/z* 196 corresponding to the molecular formula $C_{10}H_{13}NOS$. Two fragment ions at *m/z* 166 and 133 were observed due to the elimination of C_2H_5 and C_2H_5-SH groups, respectively, from the molecule. The latter fragmentation pattern suggested the presence of a –S-C_2H_5 group, rather than -O-C_2H_5 group in compound **1**.

The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$, Table 1) exhibited aromatic protons as multiplate at δ 7.29 (3H) and 7.35 (2H) supporting the presence of a

mono-substituted benzene ring. It also showed signals at δ 5.63 (1H, br s), 4.48 (2H, *d*, *J*=4.9 Hz), 2.96 (2H, q, *J*=7.4 Hz) and 1.32 (3H, t, *J*=7.4 Hz). The *J* modulated ^{13}C-NMR spectrum (125 MHz, $CDCl_3$, Table 1) revealed the presence of carbonyl (δ_C 167.6), a quaternary (δ_C 138.0), one methyl (δ_C 15.9), two methylene (δ_C 45.5 and 24.6) and the remaining signals (δ_C 127.8, 127.9 and 129.0) for aromatic methine carbons.

In the ^{1}H-^{1}H COSY 45^o experiment, methyl protons (δ_H 1.32) coupled to methylene (δ_H 2.96), while the proton at δ_H 5.63 (NH) showed correlation to other methylene (δ_H 4.48). The latter methylene protons showed ^2J correlation to a quaternary carbon at δ_C 138.0 (C-1) and ^3J correlation to carbonyl at δ_C 167.6 (C-8) and methine carbons at δ_C 127.9 (C-2, 6; δ_H 7.29 from HSQC) in the HMBC experiment (Table 1). The aromatic methine protons at δ_H 7.35 (H-3, 5) showed ^3J correlation to the quaternary carbon at δ_C 138.0 (C-1). The above data suggested the partial structure of compound **1** as **1a** (Fig. 1).

Figure 1. Estructure of isolated compounds.



1 **1a**

The methylene protons at δ_H 2.96 (δ_C 24.6 from HSQC) showed connectivity to methyl carbon (δ_C 15.9) by ^2J and carbonyl (δ_C 167.6) by ^3J. The carbon chemical shift of carbonyl at δ_C 167.6 and existence of thioethyl group (as revealed by mass fraction pattern) suggested that, "X" must be SC_2H_5. Thus compound **1** was identified as benzylcarbamothioethionate, a new compound.

Gross general observation

The controls (both normal diet and vehicle), chloroform soluble fraction and compound **1** treated rat showed no signs of tremor, convulsion and reflex abnormalities. No muscular numbness of the hind and fore legs, salivation or diarrhoea was observed.

Body weight

Table 2 shows the average body weights of all the rats before and after the treatment and the data were compared. No significant changes in body weights of all the rats were observed**.** Percentage changes in body weights were due to normal body growth for the 7 days.

Hematological profiles

Table 3 reveals the hematological profiles such as total RBC, total WBC, differential count of WBC, platelet, hemoglobin and ESR observed at 1st day and 7th day of treatment. In controls (both normal diet and vehicle) and experimental (chloroform soluble fraction and compound 1 treated rat) groups, almost all hematological parameters were slightly increased after 7 days and no abnormality was observed in both controls and experimental rats.

Biochemical parameters of blood

Table 4 shows the biochemical parameters such as SGPT, SGOT, SALP, bilirubin, creatinine and blood urea of controls (both normal diet and vehicle) and experimental rats (both chloroform soluble fraction and compound **1**). Almost all the parameters of chloroform soluble fraction and compound **1** treated rat were changed slightly in comparison to control rats (both normal diet and vehicle), but the changes were statistically insignificant.

Histopathological observations

Histopathological examinations of liver, heart, lung and kidney of the controls (both normal diet and vehicle) and experimental (both chloroform soluble fraction and compound **1**) rats were observed after the experimental period. No detectable changes in the histopathology of these organs were examined under microscope.

Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz) and HMBC data of **1** in $CDCl_3$

Position	^{1}H	^{13}C	HMBC	
			^{2}J	^{3}J
1	-	138.0	-	-
2	7.29, m	127.9	C-3,5	C-7
3	7.35, m	129.0	C-2,6	C-1
4	7.29, m	127.8	C-3,5	C-7
5	7.35, m	129.0	C-2,6	C-1
6	7.29, m	127.9	C-3,5	C-7
7	4.48, d, *J*= 4.9 Hz	45.5	C-1	C-2,6, C-8
8	-	167.6	-	-
9	2.96, q, *J*= 7.4 Hz	24.6	C-10	C-8
10	1.32, t, *J*= 7.4 Hz	15.9	C-9	-
NH	5.63, br s	-	-	-

Table 2. Change of body weights of controls (both normal diet and vehicle) and experimental rats (chloroform soluble fraction and compound **1**).

Group of rats	Treatment	Body weight (g) before experiment	Body weight (g) after experiment	Change (%)
Controls	Normal diet	103.25 ± 0.50	109.75 ± 1.26	6.3
	Vehicle (0.2 mL)	104.25 ± 0.96	111.50 ± 2.38	6.9
Experimental	Chloroform fraction (200 mg/kg/day)	103.75 ± 0.96	112.00 ± 1.41	7.9
	Compound 1 (5 mg/kg/day)	104.00 ± 0.50	110.75 ± 1.30	6.4

The treatments were applied by i.p. pathway. Values are mean mean ± S.D. n=4.

Table 3. Hematological parameters of control (both normal diet and vehicle) and experimental rat (chloroform soluble fraction and compound **1**).

Parameters	Control (normal diet)		Control (vehicle)		Experimental 1 (Chloroform soluble fraction)		Experimental 2 (compound 1)	
	1st day	7th day	1st day	7th day	1st day	7th day	1st day	7th day
RBC Cells/mL $\times 10^6$	4.95±1.52	5.00±0.08	4.80±0.18	5.05±0.06	4.85±0.13	5.10±0.16	4.75±0.06	5.25±0.06*
WBC Cells/mL $\times 10^6$	6.60±0.18	7.02±0.05	6.77±0.12	7.15±0.12	6.87±0.09	7.12±0.09	6.50±0.08	7.05±0.09
Neutrophil Cells/mL $\times 10^6$	54.00±1.83	54.75±2.06	54.50±1.73	56.75±1.50	54.00±1.83	54.75±2.06	54.50±1.73	56.75±1.50
Lymphocyte Cells/mL $\times 10^6$	31.50±1.29	31.75±1.26	32.25±1.26	32.75±0.96	31.50±1.29	31.75±1.26	32.25±1.26	32.75±0.96
Monocyte Cells/mL $\times 10^6$	4.00±0.82	4.75±0.96	3.75±0.50	4.00±0.82	4.00±0.82	4.75±0.96	3.75±0.50	4.00±0.82
Eosinophil Cells/mL $\times 10^6$	1.50±0.54	1.75±0.50	1.75±0.50	1.75±0.50	1.50±0.54	1.75±0.50	1.75±0.50	1.75±0.50
Platelet Cells/mL $\times 10^6$	302.50±1.2	305.00±1.0	308.5±9.9	310.00± 9.1	298.20±2.3	305.00±5.7	305.0±5.7	310.0±9.1
Hemoglobin (%)	12.75±0.50	13.25±0.50	13.00±0.00	13.50±0.58	13.00±0.82	13.75±0.96	12.75±0.50	13.00±0.00
ESR (mm/1st hour)	13.75±1.26	14.25±1.26	14.00±0.82	14.75±0.50	14.00±0.82	14.50±1.29	14.00±0.82	14.25±1.26

*P<0.05, experimental (Compound **1**) vs control (normal diet). Values are mean ± S.D.

Table 4. Biochemical parameters of control (both normal diet and vehicle) and experimental rat (chloroform soluble fraction and compound **1**).

Parameters	Control (normal diet)	Control (vehicle)	Experimental 1 (chloroform fraction)	Experimental 2 (compound 1)
SGPT (IU/L)	12.25 ± 0.50	12.50 ± 0.58	12.75 ± 0.50	12.50 ± 0.50
SGOT (IU/L)	14.00 ± 0.82	14.25 ± 0.50	14.75 ± 0.00	13.75 ± 0.50
SALP (IU/L)	10.00 ± 0.00	10.25 ± 0.50	10.00 ± 0.00	10.50 ± 0.58
Bilirubin (mmol/dL)	0.37 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.37 ± 0.01
Creatinine (mg/dL)	0.59 ± 0.01	0.59 ± 0.05	0.61 ± 0.03	0.60 ± 0.07
Blood urea (mg/dL)	17.75± 0.96	17.50± 2.08	17.75± 1.50	17.50± 1.29

Values are mean ± S.D.

DISCUSSION

The use of traditional medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Interest in medicinal plants as a reemerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being and the bio-prospecting of new plant-derived drugs (Lucy and Edgar, 1999).

In this study a new compound, benzylcarbamothioethionate (**1**) was isolated from the chloroform soluble fraction of ethanolic extract of the powdered root bark of *Moringa oleifera* Lam. The structure of this compound was established on the basis of IR, ^{1}H and ^{13}C NMR and mass spectroscopy. Compound **1** and chloroform soluble fraction were then used for acute toxicity test on rats. From the biochemical, hematological and histopathological examinations, it was confirmed that chloroform soluble fractions (200 mg/kg body weight/day) and compound **1** (5 mg/kg body weight/day) had no toxic effects on cellular structure, i.e., they do not cause degeneration of the cells of these organs. It was previously reported that the methanolic extract root of *M. oleifera* had low toxicity on rats at low to moderate dose, but high dose affects liver and kidney functions and hematological parameters (Mazumder et al., 1999).

CONCLUSION

The aim of this study was to determine the acute toxicity and did not attempt to identify the specific mechanisms involved. This study revealed that both the chloroform soluble fraction and compound **1** of *Moringa oleifera* Lam. had no toxic effects in our experimental models. However, further toxicological studies of compound **1** (subacute and chronic toxicity) are needed in order to establish its safety. Therefore, these findings provide a support to the safe use of *Moringa oleifera* Lam. in indigenous system of medicine.

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REFERENCES

Abdulkarim SM, Long K, Lai OM, Muhammad SKS, Ghazali HM. 2005. Some physico-chemical properties

of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods. Food Chem 93:253-263.

Ahmed M, Rashid MA, Choudhury SAR, Choudhury AKA. 1986. Hypotensive effect of methanol extract of *Moringa oleifera* in rats. Bangladesh J Physiol Pharmacol 4:27-32.

Bennett RN, Mellon FA, Nikolaus F, John PH, Susan DM, Lionel P, Paul KA. 2003. Profiling glucosinolates and phenolics in vegetative and productive tissues of the multipurpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala* L. J Agric Food Chem 51:3546-3553.

Caceres A, Alvarez AV, Freire V, Giron LM, Pacheco JG. 1991. Pharmacological properties of *Moringa oleifera*: Preliminary screening for antimicrobial activity. Economic Bot 45:522-526.

Cáceres A, Saravia A, Rizzo S, Zabala L, Leon ED, Nave F. 1992. Pharmacological Properties of *Moringa oleifera*: Screening for antipasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol 36:233-237.

Ching LS, Mohamed S. 2001. Alpha-tocopherol content in 62 edible tropical plants. J Agric Food Chem 49:3101-3105.

Chopra RN. 1958. Indigenous drugs of India. U.N. Dhar and sons (pvt.) Ltd., Calcutta, p 364.

Ezeamuzie IC, Ambakederemo AW, Shode FO, Ekwebelem SC. 1996. Antiinflammatory effects of *Moringa oleifera* root extract. Int J Pharmacog 34:207-212.

Faizi S, Siddiqui BS, Saleem R, Aftab K, Shaheen F, Gilani AH. 1998. Hypotensive constituents from the pods of *Moringa oleifera*. Planta Med 64:225-228.

Faizi S, Siddiqui BS, Saleem R, Noor F, Husnain S. 1997. Isolation and structure elucidation of a novel glycoside niazidin from the pods of *Moringa oleifera*. J Nat Prod 60:1317-1321.

Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AUH. 1995. Fully acetylated carbamate and hypotensive thiocarbamate glycosides from *Moringa oleifera*. Phytochemistry 38:957-963.

Farooq A, Bhanger MI. 2003. Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. J Agric Food Chem 51:6558-6563.

Francis JA, Bolleddula J, Olson LK, Muraleedharan NG. 2004. Insulin secretagogues from *Moringa oleifera* with cyclooxygenase enzyme and lipid peroxidation inhibitory activities. Helv Chim Acta 87:317-326.

Gaur E. 1962. Staining, Animal Tissues, Leonard Hill (Books) Ltd. London, p 631.

Giovanna V, Kiprono CP, Ndalut PK. 2002. ^{13}C NMR characterization of triacylglycerols of *Moringa oleifera* seed oil: An "oleic-vaccenic acid" oil. J Agric Food Chem 50:970-975.

Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, Maoka T, Kozuka M, Ito Y, Tokuda H, Nishino H. 1999. An antitumor promoter from *Moringa oleifera* Lam. Mutat Res 440:181-188.

Gupta M, Mazumder UK, Chakrabarti S. 1999. CNS activities of methanolic extract of *Moringa oleifera* root in mice. Fitoterapia 70:244-250.

Hawk PB, Oser L, Summerson WH. 1954. Practical Physiological Chemistry,13th edn., McGraw Hill Book Company, USA. pp 136-139.

Kausik R, Rimi H, Debjani G. 2003. Central inhibitory effect of *Moringa oleifera* root extract: possible role of neurotransmitters. Indian J Exp Biol 41:1279-1284.

Kirtikar KR, Basu K. 1984. Indian Medicinal Plants. Lalit Mohan Basu MB. Allahabad, India, vol. 1, pp 677-680.

Kurup PA, Rao KL. 1954. An antibiotic substance, pterygospermin from the root of *Moringa oleifera*. Indian J Med Res 42:85-88.

Lucy H, Edgar JD. 1999. Medicinal plants: a re-emerging health aid. Electronic J Biotechnol 2:1-15.

Mahajan SG, Mehta AA. 2007. Inhibitory action of ethanolic extract of seeds of *Moringa oleifera* on systemic and local anaphylaxis. J Immunotoxicol 4:287-294.

Mazumder UK, Gupta M, Chakrabarti S, Pal D. 1999. Evaluation of hematological and hepatorenal functions of methanolic extract of *Moringa olefera* Lam. root treated mice. Indian J Exp Biol 37:612-614.

Murakami A, Kitazono Y, Jiwajinda S. 1998. Niaziminin, a thiocarbamate from the leaves of *Moringa oleifera,* holds a strict structural requirement for inhibition of tumor-promotor induced Epstein-Barr virus activation. Planta Med 64:319-323.

Olfert ED, Cross BM, McWillian AA. 1993. Guide to the Care and Use of Experimental Animals. Canadian Council on Animal Care, Ottawa, Ontario.

Prakash AR. 1988. Ovarian response to aqueous extract of *Moringa oleifera* during early pregnancy in rats. Fitoterapia 59:89-96.

Ramiah N, Nair GN. 1977. Amino acid and sugars in the flowers and fruits of *Moringa oleifera* Lam. J Instn Chem 49:163.

Rangaswamy L, Marisiddaiah R, Parthasarathy KT, Vallikannan B. 2005. Determination of major carotenoids in a few Indian leafy vegetables by high-performance liquid chromatography. J Agric Food Chem 53:2838-2842.

Saluja MP, Kapil RS, Popli SP. 1978. Studies on Medicinal Plants. Part vi. Chemical constituents of *Moringa oleifera* Lam. (hybrid variety) and isolation of 4-hydroxymellein. Indian J Chem 16:1044.

Santos AFS, Argolo ACC, Coelho LCBB, Paiva PMG. 2005. Detection of water soluble lectin and antioxidant component from *Moringa oleifera* seeds. Water Res 39: 975-980.

Sastri BN. 1962. The wealth of India. Council of Scientific and Industrial Research, New Delhi. p 425.

Schalm OW, Jain NC, Carrol GH. 1975. Veterinary Haematology 3rd (ed), Wadsworth Publication Co. Inc., Belmont, California.

Shukla S, Mathur R, Prakash AO. 1988. Antifertility profile of the aqueous extract of *Moringa oleifera* roots. J Ethnopharmacol 22:51- 62.

Siddhuraju P, Becker K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem 51:2144-2155.

Stavros L, John T. 2002. Extraction and identification of natural antioxidant from the seeds of the *Moringa oleifera* tree variety of Malawi. J Am Oil Chem Soc 79:677-683.

UNESCO. 1996. Culture and Health, Orientation Texts-World Decade for Cultural Development 1988-1997. Documents CLT/DEC. PRO-1996, Paris, France, p 129.

Yusuf M, Chowdhury JU, Wahab MA, Begum J. 1994. Medicinal plants of Bangladesh, BCSIR Laboratories, Chittagong, Bangladesh, p 171.

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Actividad de *Ugni molinae* Turcz. frente a microorganismos de importancia clínica

[Activity of *Ugni molinae* Turcz. against microorganisms with clinical importance]

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Abstract

Ugni molinae Turcz. (*Myrtaceae)*, "Murtilla", is a Chilean plant whose medicinal use has indigenous origin and is based on the astringent properties of leaves that are administered in the form of infusion. Previous phytochemical studies of a leaf infusion demonstrated the presence of phenolic compounds and saponins with recognized antimicrobial activity. The aim of this study was to determine the antimicrobial activity of *U. molinae* extracts against microorganisms with clinical importance such as *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Candida albicans.* The extracts of *U. molinae* showed a significant antimicrobial activity against the studied microorganisms. The antimicrobial activity of the leaves of this species is a contribution in the search for new antimicrobial drugs and for ethnomedicine and may constitute a source of future studies to validate the knowledge and practices in ethnomedicine.

Keywords: Ugni molinae*; Myrtaceae; Antimicrobial activity.*

Resumen

Ugni molinae Turcz. (*Myrtaceae)*, "Murtilla", es una planta chilena cuyo empleo medicinal es de origen indígena y está basado en las propiedades astringentes de las hojas que se administran en forma de infusión. Estudios fitoquímicos previos en la infusión de las hojas, permitieron conocer la presencia de sustancias de tipo fenólico y saponínico que son compuestos con reconocida actividad antimicrobiana. El objetivo de este trabajo fue determinar la actividad antimicrobiana en extractos de *U. molinae* frente a microorganismos de importancia clínica tales como *Pseudomonas aeruginosa, Enterobacter aerogenes, Staphylococcus aureus y Candida albicans.* Los extractos de *U. molinae* mostraron actividad antimicrobiana significativa frente a los microorganismos estudiados. La actividad antimicrobiana de las hojas de esta especie es una contribución en la búsqueda de nuevas drogas antimicrobianas y para la etnomedicina, pudiendo constituir en el futuro una fuente de estudios para la validación de los conocimientos y de los usos etnomédicos.

Palabras Clave: Ugni molinae*; Myrtaceae; Actividad antimicrobiana.*

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INTRODUCCIÓN

Ugni molinae Turcz. es un arbusto chileno perteneciente a la familia *Myrtaceae* llamado vulgarmente "Murtilla", "Murta" o "Uñi" (Hoffmann, 1991). El empleo medicinal de la planta es de origen indígena y está basado en las propiedades astringentes de las hojas. Mapuches, Puelches y Pehuenches utilizan infusiones de las hojas en el tratamiento de diarreas y disenterías (Hoffmann, 1991). Estudios fitoquímicos previos en la infusión de las hojas, permitieron conocer la presencia de sustancias de tipo fenólico (ácidos fenólicos, flavonoides y taninos hidrolizables), y heterósidos saponínicos que son compuestos con reconocida actividad antimicrobiana (Avello, 2005*;* Suwalsky et al., 2006, 2007).

Bajo la observación de campo, *Ugni molinae* es una especie que no constituye un blanco habitual de patógenos, pudiendo relacionarse este hecho con mecanismos de defensa altamente efectivos que incluyen metabolitos secundarios como compuestos fenólicos y heterósidos saponínicos, que no son parte de las cadenas defensivas de la especie, pero que le confieren protección innata (Gudesblat, 2007). Las propiedades antimicrobianas e insecticidas de los vegetales son relevantes en la defensa del vegetal mismo y en la proyección de un uso aplicable frente a patógenos en diversas áreas. Por ejemplo, en la búsqueda de nuevas fármacos antimicrobianos, en la conservación de diversos productos y como aleloquímicos. De acuerdo con los resultados químicos previos de las hojas de *Ugni molinae*, esta especie podría tener una aplicación innovadora en estas áreas. Por lo tanto, el objetivo del siguiente trabajo fue determinar la actividad antimicrobiana de extractos de *Ugni molinae* frente a diferentes especies microbianas de importancia clínica, como *Staphylococcus aureus, Enterobacter aerogenes, Pseudomonas aeruginosa y Candida albicans.* Estos microorganismos fueron seleccionados para el estudio debido a la resistencia antibiótica que han desarrollado a través del tiempo y a las infecciones intrahospitalarias que causan, en particular *Pseudomonas aeruginosa y Candida albicans,* en pacientes inmunodeprimidos.

MATERIALES Y MÉTODOS

Material vegetal y obtención de extractos

Se elaboraron extractos de hojas de *Ugni molinae* colectadas en la caleta Maule, Coronel, en marzo de 2008. La especie se identificó en el Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas de la Universidad de Concepción, (CONC 146511). Las hojas se deshidrataron a la sombra (temperatura ambiente), y se redujeron hasta un tamaño de partícula de 0,5 cm.

Se procesaron 50 g del material seco y molido en aparato Soxhlet para la obtención de los extractos. Se usaron los siguientes disolventes: agua 100% (H_2O), mezclas metanol-agua (40-60%) ($MeOH/H_2O$), y metanol 100% (MeOH), con muestras vegetales diferentes en cada extracción. Cada extracción se llevó a cabo hasta agotar el material vegetal, lo cual se determinó mediante la medición del contenido de sólidos en el extracto (H_2O 18,6%, $MeOH/H_2O$ 42,1%, MeOH 45,5%).

Cada extracto se concentró en rotavapor y se llevó a sequedad en liofilizador. Para cada extracto se determinó el rendimiento total en base seca. Se almacenaron en lugar seco, a temperatura ambiente y protegidos de la luz hasta el momento de su utilización.

Determinación de fenoles totales

Cada extracto se estandarizó por su contenido fenólico en Equivalentes de Ácido Gálico (EAG), medido y estandarizado por el método de Folin-Ciocalteu (Velioglu et al., 1998). El protocolo fue el siguiente, a 0,5 mL de muestra se le adicionó 25 mL de agua destilada, 2,5 mL de Reactivo Folin-Ciocalteu (Merck) y 10 mL de Carbonato de Sodio 20%, en el mismo orden. Luego se enrasó a 50 mL con agua destilada. Se agitó para homogenizar y se dejó reposar por 30 minutos. Paralelamente, se preparó un blanco analítico con agua destilada. La absorbancia de la muestra fue medida a una longitud de onda de 765 nm en espectrofotómetro. La determinación de fenoles totales se realizó por triplicado.

Determinación cuantitativa de saponinas

Cada extracto fue disuelto en 2 mL de agua, se le adicionó 1 mL de fenol (5% v/v) y 5 mL de H_2SO_4 (c). La determinación cuantitativa se efectuó después de 20 min en frío a 490 nm, por triplicado. Como estándar se usó saponina pura blanca, triterpénica (Merck) (Hideaki et al., 1978).

Actividad antimicrobiana

La actividad antimicrobiana se determinó por el método de difusión en agar tripticasa para bacterias y agar Sabouraud para hongos, utilizando *Staphylococcus aureus* (ATTC 6538P), *Pseudomonas*

aeruginosa, *Enterobacter aerogenes y Candida albicans* (aisladas desde productos patológicos que se mantienen en el cepario del Departamento de Microbiología, Facultad de Ciencias Biológicas, Universidad de Concepción), correspondientes a una concentración de 10^6 ufc/mL. En orificios de 7 mm de diámetro se depositaron 100 µL de los extractos a ensayar (4 mg/mL) en suero fisiológico. Las placas se incubaron a 37 °C durante 24 h y se realizaron lecturas del diámetro del halo de inhibición del crecimiento microbiano.

El parámetro de selección fue un diámetro del halo de inhibición superior a 15 mm frente a los microorganismos. Se utilizaron como controles amoxicilina (25 µg/mL, Lab. Chile) y caspofungina (5 µg/mL, Lab. Neo-Sensitabs TM).

RESULTADOS Y DISCUSIÓN

Los extractos se estandarizaron en Equivalentes de Ácido Gálico (EAG). Para los extractos metanólico y metanol/H_2O, el contenido fue de 0,036 (M EAG) y para el extracto acuoso fue de 0,032 (M EAG).

La cuantificación de saponinas se basó en la ecuación: $y = 0{,}0214\ x + 0{,}3485$, $r^2 = 0{,}999$; obtenida de la curva de calibración realizada con el estándar (saponina pura blanca, triterpénica) y el valor R cuadrado del gráfico. Se expresó en gramos de saponinas/100 g de fracción seca y fue en promedio 8,86 ± 0,02 mg de saponinas/g de planta seca.

Los extractos de *Ugni molinae* mostraron ser activos frente a los microorganismos estudiados, según se puede apreciar en la Tabla 1.

En la búsqueda de nuevas drogas los productos naturales se perfilan como un recurso para la experimentación como alternativas eficientes ante microorganismos de importancia clínica que hayan desarrollado resistencia. De acuerdo los resultados obtenidos, los extractos de *Ugni molinae* presentan actividad antimicrobiana frente a especies microbianas de importancia clínica, como *Pseudomona aeruginosa*, que se caracteriza por presentar multiresistencia frente a los antimicrobianos de uso corriente y sobre *Candida albicans*, agente infeccioso en pacientes inmunodeprimidos. Este marco referencial constituye una importante fuente de investigación para futuras aplicaciones de los extractos de esta especie.

Tabla 1. Actividad antimicrobiana, determinada por el diámetro del halo de inhibición (mm) por exposición durante 24 h a extractos de *Ugni molinae*, frente a *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Staphylococcus aureus* y *Candida albicans*.

	Diámetro de halo de hnhibición (mm ± SD)			
Extracto (4 mg/mL)	*Pseudomonas aeruginosa*	*Enterobacter aerogenes*	*Staphylococcus aureus*	*Candida albicans*
MeOH	25,0 ± 0,1	25,0 ± 0,1	26,0 ± 0,1	25,0 ± 0,1
MeOH/H_2O	25,0 ± 0,1	21,0 ± 0,1	21,0 ± 0,1	20,0 ± 0,2
H_2O	23,0 ± 0,1	20,0 ± 0,1	25,0 ± 0,1	25,0 ± 0,2
Amoxicilina	S/A	S/A	30,0 ± 0,1	-
Caspofungina	-	-	-	12,0 ± 0,1

Las concentraciones utilizadas para los controles fueron: Amoxicilina 25 µg/mL y Caspofunfina 5µg/mL. n =3.

CONCLUSION

Los extractos de *Ugni molinae* ejercen actividad antimicrobiana frente a microorganismos de importancia clínica: *Pseudomonas aeruginosa, Enterobacter aerogenes, Staphylococcus aureus y Candida albicans.* Estos resultados proponen a la especie *U. molinae* como recurso natural para la experimentación en busca de alternativas eficientes ante microorganismos de importancia clínica.

La capacidad antimicrobiana de las hojas de esta especie es una contribución importante a la etnomedicina, sobre todo por el concepto de la población chilena acerca de los grandes beneficios terapéuticos de sus frutos, pudiendo constituir en el futuro una fuente de estudios y trabajo importante, y la validación de los conocimientos y medicinas de nuestras etnias puesto que este estudio nace de la observación del uso de las hojas de "Murtilla" por parte de las comunidades pehuenches, puelches y mapuches.

REFERENCIAS

Avello M, Pastene E. 2005. Actividad antioxidante de infusos de *Ugni molinae* Turcz. (Murtilla). Bol Latinoam Caribe Plant Med Aromat 4(2):33–39.

Gudesblat G. 2007. Señalización en la interacción entre bacterias fitopatógenas y su hospedador. Rev Quím Viva 6(2):70-81.

Hideaki O, Shigeo K, Shoji S. 1978. Separation and determination of saponins of Bupleuri Radix by Droplet Counter Current Chromatography CDCC. Planta Med 33:152-59.

Hoffmann JA. 1991. Flora Silvestre de Chile Zona Araucana. Ed. II Editorial Claudio Gay, Santiago, Chile, pp. 160.

Suwalsky M, Orellana P, Avello M, Villena F, Sotomayor C. 2006. Human erythrocytes are affected *in vitro* by extracts of *Ugni molinae* leaves. Food Chem Toxicol 44:1393–1398.

Suwalsky M, Orellana P, Avello M, Villena F. 2007. Protective effect of *Ugni molinae* Turcz against oxidative damage of human erythrocytes. Food Chem Toxicol 45:130–135.

Velioglu Y, Mazza G, Gao L, Oomah B. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agr Food Chem 46:4113-4117.

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Validación antibiótica de plantas medicinales del noroeste de Colombia contra *Staphylococcus aureus*[*]

[Antibiotic validation of medicinal plants of the Northwest of Colombia against *Staphylococcus aureus*]

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Abstract

Finding new antibacterial medicaments for treating infectious illnesses is a worldwide concern. Upon *Staphylococcus aureus* and other bacteria's proved resistance to some antibiotics, total extracts from 27 plants used against infections are evaluated *in vitro* by using the dilution in agar technique in order to validate the behaviour of different doses under *Staphylococcus aureus* presence, streptomycin sulphate 10 µg/mL positive control, Mueller Hinton agar negative control, 24 h measures. Tests for each extract are taken in three treatments by triplicate each. Results show 52% (14) of the *S. aureus* total inhibition samples.

Keywords: *Medicinal plants; Antibacterial activity; Ethanolic extracts; Colombian pacific.*

Resumen

A escala mundial hay interés en la búsqueda de nuevos medicamentos antibacterianos para tratamientos de enfermedades infecciosas. Ante la comprobada resistencia de *Staphylococcus aureus* y otras bacterias a varios antibióticos se evalúan *in vitro* los extractos totales de 27 plantas usadas contra infecciones, mediante la técnica de dilución en agar, para convalidar la actividad de diferentes dosis frente a *Staphylococcus aureus*, control positivo sulfato de estreptomicina 10 µg/mL, control negativo agar Mueller Hinton, lecturas a 24 horas. Las pruebas se realizan en tres tratamientos para cada extracto probado por triplicado cada uno. Los resultados muestran 52% (14) de las muestras con inhibición total frente *a S. aureus*.

Palabras Clave: *Plantas medicinales; Actividad antibacteriana; Extractos etanólicos; Pacífico colombiano.*

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INTRODUCCIÓN

En la esquina nor-occidental de Sur América se encuentra el departamento del Chocó o parte norte del pacífico colombiano, lugar en el que se combina la extraordinaria belleza de su biodiversidad reconocida por varios autores (Forero & Gentry, 1989; Rangel y Lowy 1993, Rangel y Rivera, 2004) y las diversas culturas de las etnias negras, indígenas y mestizas que lo conforman, aunque su abundante flora silvestre es usada frecuentemente en la medicina tradicional, no se ha convalidado la información ya que ha sido poco estudiada química y biológicamente. Dado que la atención primaria de salud, en gran parte de estas comunidades depende del uso de plantas medicinales, lo anterior además de ser preocupante, es una limitante para un real manejo de la diversidad biológica regional como reserva mundial, por ello, con este trabajo se busca corroborar la actividad antibiótica frente a *S. aureus* y otras bacterias. *S. aureus* es una bacteria que posee muestras evolutivas de resistencia a antibióticos. Se espera con estos resultados determinar cuales plantas poseen real actividad frente a esta bacteria y generar mejores alternativas de uso en la región, debido aprovechamiento y tendencia a probable explotación racional, sustentable y sostenible, previos estudios específicos que garanticen calidad, eficacia y seguridad.

MATERIALES Y MÉTODOS

Área de estudio

El material vegetal fue recolectado en horas de la mañana, en diferentes localidades del departamento del Chocó, el cual está localizado en el eje de las calmas ecuatoriales, caracterizado por baja presión atmosférica, alta nubosidad y precipitación; lo anterior es la esquina nor-occidental de América del Sur, entre los 4° 10' y los 8° 10' de latitud Norte, y entre los 76° y 78° de longitud Oeste, única región con costas sobre dos océanos, Atlántico (por el Mar Caribe) al Norte y el Pacífico al Occidente. Sus variables climáticas con índices pluviométricos que alcanzan valores mayores de 11.000 mm anuales, temperatura promedio de 27ºC y humedad relativa de 88% (IGAG 2006).

Recolección y procesamiento del material vegetal

En la metodología de trabajo, se tuvo en cuenta la selección de material vegetal para muestras de herbario, a las cuales se les realizó proceso de secado, montaje, identificación y radicación en el herbario en el herbario CHOCÓ de la UTCH con duplicados en el COL o herbario del Instituto de Ciencias Naturales, de la Universidad Nacional de Colombia con sede en Bogotá y el Centro Nacional para la Agro-industrialización de Especies Aromáticas y Medicinales (CENIVAM). Para la obtención de los extractos se partió de 200 g de material limpio, secado en horno con aire circulante a 40 °C, molido, pesado y etiquetado. Se maceró en frío con etanol al 96% por un periodo de 3 días, realizando concentraciones sucesivas a presión reducida en un rotaevaporador R-124, vacuum controller V-800, marca Buchi. El extracto etanólico obtenido se envasó en frascos con tapa rosca y se almacenaron a 4 °C hasta la realización de los ensayos, los cuales se llevan a cabo mediante el método de Dilución en agar descrito por Mitcher et al. (1971) para determinar la actividad de diferentes dosis (40, 20 y 10 mg/mL) diluidos en 400, 200 y 100 µL de dimetilsulfóxido, respectivamente. El control positivo es sulfato de estreptomicina 10 µg/mL y el control negativo agar Mueller Hinton; las pruebas se realizan en tres tratamientos por triplicado cada uno para cada extracto probado, con lecturas a las 24 horas.

Cepas de microorganismos

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtillis* ATCC 6633, *Klebsiella pneumoniae* ATCC 70063, *Pseudomona aeruginosa* ATCC 13076, *Salmonella tiphy* CMDM-UJ 045.

RESULTADOS Y DISCUSIÓN

Los resultados muestran 27 especies agrupadas en 4 familias botánicas (Gesneriaceae 9 spp, Melastomataceae 3 spp, Piperaceae 9 spp, y Rubiaceae 6 spp, de las cuales 14 (52%) presentan inhibición total contra *S. aureus* y 10 (37%) presentan inhibición contra más de una bacteria, (Tabla 1).

Las publicaciones sobre actividad antimicrobiana siempre están aumentando, pero a partir del año 2000 se han incrementado considerablemente de acuerdo con los indicadores cenciométricos en el periodo de 1975 a 2007 realizados por CENIVAM-PNP (2007) (Fig. 1), no obstante son pocos o casi nulos los reportes de estudios en ese sentido para la biodiversidad de la región (bases de datos de Scopus, Science Direct y Pubmed); solo aparecen registros farmacológicos y antimicrobianos a partir del año 1992 (Otero et al., 1992; Otero et al., 2000a,b,c; Pino-

Benítez et al., 2006; Ledesma y Pino-Benítez, 2006; Pino-Benítez y Córdoba, 2007; Mesa–Arango et al., 2007; Pino-Benítez, 2008; Pino-Benítez et al., 2008a,b). En este trabajo la bacteria *Staphylococcus aureus* fue sensible a la mayoría de los extractos etanólicos probados (52%), esta bacteria ofrece muestras evolutivas de resistencia a antibióticos reportada por varios autores (Cantón et al., 1999, Bamber y Neal 1999, Suller & Russell 2000, Gobernado 2002, Levy 2004, Degrossi & Wachsman 2005, Rodríguez y Vesga 2005, Cabrera et al., 2007, Fica et al., 2007, Reina-Figueroa et al., 2007). Lo anterior no solo valida el conocimiento popular si no que da valor agregado a la riqueza florística regional.

Tabla 1. Evaluación antibacteriana a 27 spp. del Pacífico colombiano.

Extracto/ Cepa	*Staphylococcus aureus*			*Escherichia coli*			*Klebsiella pneumoniae*			*Bacillus subtilis*			*Pseudomonas aeruginosa*			*Salmonella tiphy*		
mg/mL	40	20	10	40	20	10	40	20	10	40	20	10	40	20	10	40	20	10
Besleria barclayi	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Columnea consanguinea	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Columnea parviflora	+	ND	ND	-	-	-	-	-	-	ND	ND	ND	-	-	-	-	-	-
Columnea picta	+++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Columnea rubricuata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Drymonia killipi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Napheanthus apodemus	+++	-	-	+++	-	-	-	-	-	+++	+++	-	-	-	-	-	-	-
Paradrymonia darienensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paradrymonia sericea	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bellucia pentamera	+++	+++	+++	-	-	-	-	-	-	-	-	-	+++	+++	+++	-	-	-
Clidemia dentada	+++	+++	++	+++	+++	+++	ND	ND	ND	+++	+++	+++	+++	+++	++	+++	+++	++
Miconia reducens	+++	+++	++	+++	+++	+++	ND	ND	ND	+++	+++	+++	+++	+++	++	+++	+++	++
Manekia sidowii	+++	+++	+	++	-	-	-	-	-	++	++	-	-	-	-	-	-	-
Piper aff arboreum	+++	+++	-	++	-	-	-	-	-	+++	+++	-	-	-	-	-	-	-
Piper gorgonillense	+++	+++	-	+++	-	-	-	-	-	+++	+++	+++	-	-	-	-	-	-

Tabla 1. Evaluación antibacteriana a 27 spp. del Pacífico colombiano (continuación…).

Extracto/ Cepa	*Staphylococcus aureus*			*Escherichia coli*			*Klebsiella pneumoniae*			*Bacillus subtilis*			*Pseudomonas aeruginosa*			*Salmonella tiphy*		
mg/mL	40	20	10	40	20	10	40	20	10	40	20	10	40	20	10	40	20	10
Piper tricuspe	+++	+++	+++	++	++	-	-	-	-	+++	+++	+++	-	-	-	-	-	-
Piper tuberculatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piper multiplinervium	+++	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-
Piper peltatum	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piper hispidum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piper sanctifelicis	+++	+++	++	-	-	-	-	-	-	+++	+++	+++	-	-	-	-	-	-
Coccocipselum lanceolatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isertia laevis	+++	+++	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palicourea guianensis	+++	+++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Psychotria cooperi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Psychotria poeppigiana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sabicea colombiana	+++	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control +	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control -	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: La inhibición bacteriana es insipiente.

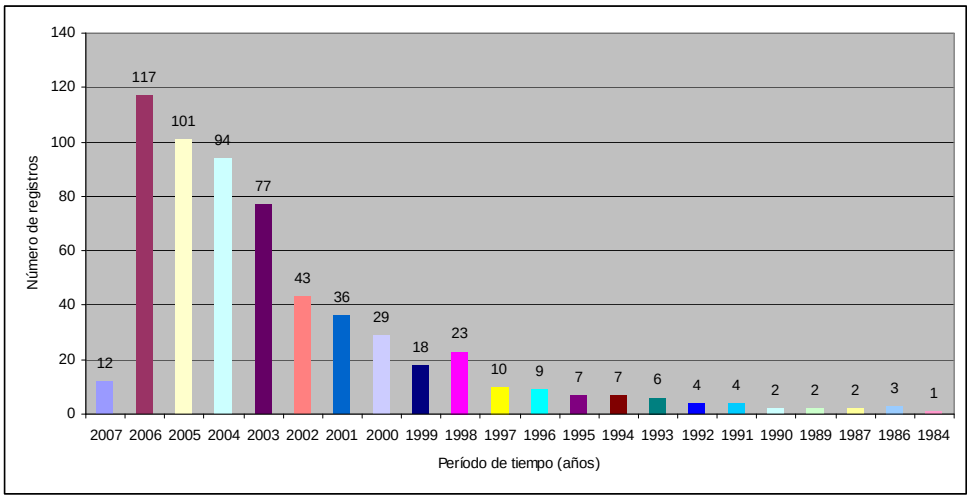
++: Hay sensibilidad parcial, o sea que la inhibición bacteriana no se realiza de forma total.

+++ La inhibición es total, o sea que el crecimiento bacteriano es afectado en su totalidad.

-: Hay resistencia o sea que el crecimiento bacteriano no es afectado en su totalidad.

Control +: Streptomicina 10 ug/mL Control -: Agar Mueller Hinton ND: No determinado

Figura 1: Publicaciones por año sobre Antimicrobial activity of essential oils and plants extract.



Fuente: CENIVAM-PNP. 2007.

CONCLUSION

La familia Gesneriaceae mostró pocas especies bioactivas (2/9), mientras que la familia Piperaceae mostró mayor número de especies con bioactividad (7/9). Las Melastomataceae fueron las más bioactivas frente a número de cepas probadas.

REFERENCIAS

Bamber AI, Neal TJ. 1999. An assessment of triclosan susceptibility in methicillin resistant and methicillin sensitive *S. aureus*. J Hosp Infect 41:107-109.

Cabrera C.E., Gómez RF, Zúñiga AE. 2007. La resistencia de bacterias a antibióticos, antisépticos y desinfectantes una manifestación de los mecanismos de supervivencia y adaptación. Colomb Med 38(2):149-158.

Cantón R, Mir N, Martinez-Ferrer M, Sanchez B, Soler I., Baquero F. 1999. Estudio prospectivo de *S. aureus* con sensibilidad disminuida a los glucopeptidos. Rev Esp Quimioter 12(1):48-53.

CENIVAM-PNP. 2007. Cálculos basados en información de la base de datos *ISI Wos*, procesados con MS-Excel. [Fecha de consulta 20/04/2007].

Degrossi M, Wachsman M. 2005. Problemas en el desarrollo de antimicrobianos: emergencia de mecanismos de resistencia. Infectología 6(3):119-132

Fica A, Jemenao M, Bilbao P, Ruiz G, Sakurada A., Pérez –Zuñiga I., Gompertz M. 2007. Emergencia de infecciones por *Enterococcus* sp resistente a vancomicina. Rev Chilena Infectol 24(6):462-471

Forero E & Gentry A. 1989. Lista anotada de las plantas del Departamento del Chocó Ed 1, pp 09-16.

Gobernado M. 2002. Resistencia en *S. aureus* ahora a la vancomicina. Rev Esp Quimioter 15(3):211-214.

Instituto Geográfico Agustín Codazzi (IGAC). 2006. Chocó características Geográficas Ed I Imprenta del Instituto, geográfico Agustín Codazzi, Bogotá D. C., pp. 18-24.

Ledesma E, Pino Benítez N. 2006. Estudio Fitoquímico y Actividad Antibacteriana de *Drymonia killipi* Wieahl. y *Paradrymonia darienensis* Seem. en el Municipio de Quibdó. Rev Inst Univ Tecn del Chocó 24:5-10.

Levy SB. 2004. Antibacterial resistance: Worldwide, causes, chalenges and responses. Nature Med 10:122-129

Mesa-Arango C, Martínez C, Zapata B, Pino N, Bueno JG, Stashenko E. 2007. Actividad *in Vitro* anti *Candida* y anti *Aspergillus* de extractos y aceites esenciales de plantas de la familia Piperaceae. Sci Tech 33:247-249.

Mitcher L.A., Leu R.P, Bathala M.S, Wu WN, Beal J.L. 1971. Antibiotic agents from higer plants, I Introduction rationale and methodology. Lloydia 35(2): 157-166.

Otero P. R, Tobón G., Gómez L. F., Osorio R., Valderrama R., Hoyos D., Evans N., J. Barona. 1992. Accidente ofídico en Antioquia y Chocó. Aspectos clínicos y epidemiológicos. Acta Med Colomb 17: 229-249.

Otero P. R., Fonnegra R., Jiménez S.L., Núñez V., Evans N., Alzate S. P., García M. E., M. Saldarriaga, A. Díaz, Osorio G., R. Valderrama, Duque A., Vélez H. N. 2000a. Snakebite and ethnobotany in the northwest region of Colombia. Part I: Traditional use of plants. J Ethnophrmacol 71: 493-504.

Otero P. R., V. Núñez, J. Barona, R. Fonnegra, S.L. Jiménez, R.G. Osorio, M. E. García, A. Díaz. 2000b. Snakebite and ethnobotany in the northwest region of Colombia. Part III: Neutralization of the hemorrhagic effect of bothrops atrox venom. J Ethnopharmacol 73:233-241.

Otero P. R., Fonnegra R, Jiménez S. L. 2000c. Plantas utilizadas contra mordeduras de serpientes en Antioquia y Chocó, Colombia. Ed. I Grandacolor, medellín, Colombia, pp. 78-299.

Pino-Benítez N, Escobar P, Leal S, Zorro M. 2006. Actividad antibacteriana, antiparasitária y citotoxicidad en células Vero de extractos naturales derivados de Piperáceas del Chocó, Colombia. Fitoterapia 6(1):101.

Pino-Benítez N., Escobar P., Bueno G., Mesa A. 2007. Evaluación de la citotoxicidad y de la actividad antimicrobiana y antiparasitaria del extracto etanólico de las hojas de *Piper tricuspe* C. DC. Actualidades Biológicas 29 (1): 237-238

Pino-Benítez N, Córdoba Y. 2007. Actividad antimicrobiana y fitoquímica preliminar de plantas usadas como colorantes en el municipio de Quibdó-Chocó. Sci Tech 33: 387-390.

Pino-Benítez N, Ledesma E, Martínez L. 2008a. Actividad antibacteriana de extractos de siete especies de la familia Gesneriaceae. Salud UIS 40:137-139.

Pino-Benítez N, Martínez L, Stashenko E. 2008b. Actividad antibacteriana del aceite esencial de *Siparuna conica* y *Siparuna guianensis* de la familia Monimiaceae. Salud UIS 40:140-142.

Pino-Benítez N. 2008. Actividad antibacteriana a partir de extractos de hojas de seis especies del género *Piper L*. (Piperaceae). Rev Inst Univ Tecn del Chocó 27(1):67-75.

Rangel ChO, Rivera-Diaz. 2004. Diversidad y Riqueza de Espermatófitos en el Chocó Biogeográfico. In Rangel, O (Ed) Colombia Diversidad Biótica IV, El Chocó Biogeográfico / Costa Pacífica. Ed 1, pp 83-104

Rangel ChO, Lowy PDO. 1993. Tipos de vegetación y rasgos fitogeográficos. In P. Leyva (Ed) Colombia Pacífico Tomo I Ed 1, pp 182-198.

Reina-Figueroa J, Ramirez LA, Villeda G, Ortiz- Ibarra F. 2007. Primer informe sobre *S. haemolyticus* con susceptibilidad reducida a vancomicina de un paciente con neuroinfección neonatal en una institución mexicana. Gac Med Mex 143(2):163-165.

Rodríguez CA, Vesga O. 2005. *S. aureus* resistente a vancomicina. Biomédica 25(4):575-587.

Suller MT, Russell AD. 2000. Triclosan and antibiotic resistance in *S. aureus*. J. Antimicrob Chemother 46:11-18.

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Comunicación | Communication

Comparación del efecto insecticida de dos especies de *Aloysia* (Verbenaceae) sobre *Rhizopertha dominica* (Insecta, Coleoptera, Bostrichidae)

[Comparation of the insecticidal effect of two species of *Aloysia* (Verbanaceae) against *Rhizopertha dominica* (Insecta, Coleoptera, Bostrichidae)]

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Abstract

Rhizopertha dominica is an important pest that affects grains in the post harvest period**.** Insects control has been based on the use of synthetic insecticides. Plants have been studied to obtain essential oils with toxic effects as an alternative. Essential oils from leaves of *A. polystachya* and *A. citriodora* were extracted using a Clevenger-type apparatus. Fumigant toxicity was tested using glass flasks and glass bells. Filter papers treated with hexane solutions and hexane as control, were placed inside the bells. Concentrations evaluated were**:** 0.16, 0.24, 0.32 and 0.48 (mg/cm^2). Ten adults were introduced on each flask. 5 replicates were done. Lethal concentration 50 (LC50) was calculated . The results showed that *A. citriodora* and *A. polystachya* have the same effect against *R. dominica.*

Keywords: Aloysia polystachya; Aloysia citriodora;*Llesser grain borer; Fumigant toxicity.*

Resumen

Rhizopertha dominica es una plaga importante que afecta a los granos en la etapa de poscosecha. El control de este tipo de insectos plaga se ha basado en el uso irracional de insecticidas sintéticos. La búsqueda de productos alternativos ha derivado en el estudio de plantas, con el fin de obtener aceites esenciales con efectos tóxicos. Los aceites esenciales de *A. polystachya* y *A. citriodora* se obtuvieron por destilación por arrastre de vapor de agua con un aparato tipo Clevenger. Para evaluar la actividad fumigante se utilizaron frascos y campanas de vidrio, dentro de las últimas se colocaron papeles de filtro tratados con las soluciones hexánicas y hexano como control. Las concentraciones utilizadas fueron: 0,16; 0,24; 0,32 y 0,48 (mg/cm^2). Se introdujeron 10 insectos en cada uno de los frascos. Se realizaron 5 réplicas. Se calculó la concentración letal 50 (LC50). Los aceites de *A. citriodora* y *A. polystachya* fueron igualmente efectivos.

Palabras Clave: Aloysia polystachya; Aloysia citriodora; *Barrenador del grano; Toxicidad fumigante.*

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INTRODUCCIÓN

Rhizopertha dominica es una de las plagas más importantes que afectan a los granos en la etapa de poscosecha, causando pérdidas millonarias por la reducción en la calidad y cantidad de los mismos. El control de estas plagas se ha basado principalmente en el uso de insecticidas sintéticos, lo cual ha desarrollado resistencia en estos insectos, además de dejar residuos sobre los granos (Rajendran y Sriranjini, 2008). Los aceites esenciales extraídos de plantas aromáticas se presentan como formas alternativas en el control de estas plagas. Estos productos están compuestos por una mezcla compleja de componentes principales que presentan múltiples propiedades, incluyendo efectos tóxicos, repelentes, ovicídas, anti-alimentarios, etc. (Wang et al., 2006). Prakash y Rao (1997) describieron 866 especies de plantas que sintetizan productos químicos efectivos contra insectos *Aloysia polystachya* y *Aloysia citriodora* son dos especies de Verbenáceas que se distribuyen por América Central y Sudamérica y son ampliamente utilizadas por sus propiedades curativas (Pascual et al., 2001). En el presente trabajo se evaluó el efecto insecticida de los aceites esenciales de *A. polyastachya* y *A. citriodora*.

MATERIALES Y MÉTODOS

Extracción de los aceites esenciales

Las hojas de *A. polystachya* y *A. citriodora* se recolectaron en verano en la ciudad de Lamarque, Río Negro (39º 24' lat. 65º 42' long.) y en la provincia de Salta (24º 47' lat. 65º 24' long.), respectivamente. Los aceites esenciales se aislaron a partir de hojas frescas, por destilación por arrastre de vapor de agua en un aparato tipo Clevenger durante 3 a 4 horas. El rendimiento de los aceites fue de 0.538% (p/v) y 0,34% (p/v), para *A. polystachya* y *A. citriodora*, respectivamente. El cálculo del rendimiento se realizó en base al peso de material vegetal fresco.

Evaluación de la actividad insecticida

Los adultos de *Rhizopertha dominica* provinieron de una colonia susceptible que se criaron en recipientes de vidrio cerrados con tela de malla fina y se mantuvieron en condiciones controladas de temperatura (28 ± 1° C), HR (70%) y fotoperíodo (12L:12O) utilizando como alimento trigo entero. Para evaluar el efecto fumigante se utilizaron frascos de vidrio de 40 mL con tapa a rosca, en el interior de los mismos se introdujo una campana de vidrio de 0,8 cm de diámetro y 2,8 cm de alto cubierta con una malla fina para evitar el contacto entre los insectos y la solución. Dentro de las campanas se colocó un papel de filtro de 5cm^2 tratado con 40 µL de las soluciones hexánicas de aceite de hojas de *A. polystachya* y *A. citriodora*, a las concentraciones de 0,16; 0,24; 0,32 y 0,48 (mg/cm^2). Papeles de filtro rociados con 40 µL de hexano se utilizaron como control. Se colocaron 10 insectos adultos de 3 a 4 días de edad en cada uno de los frascos de 40 mL (Pascual Villalobos et al., 2004). Se realizaron 5 réplicas. Para evaluar la respuesta de los insectos a los aceites se calculó la concentración letal 50 mediante el Micro Probit 3.0.

RESULTADOS Y DISCUSIÓN

Los aceites esenciales de *A. polystachya* y *A. citriodora* produjeron mortalidad en adultos de *R. dominica*. El aceite de *A. citriodora* fue tan efectivo como el de *A. polystachya*, ya que las diferencias en la CL50 no fueron significativas (Tabla 1).

La mortalidad fue concentración-dependiente tanto para el aceite de *A. polystachya* como para el de *A. citridora* (Fig. 1).

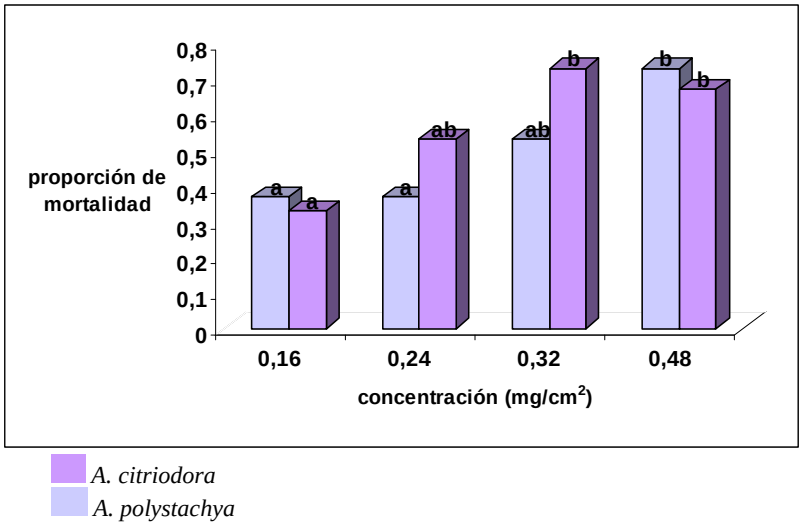
Los aceites esenciales de *A. polystachya* y *A. citriodora* produjeron efecto fumigante en adultos de *R. dominica*. Una situación similar fue observada al evaluar esta actividad con alcaravea, coriandro (Umbelliferae) y albahaca (Labiateae) sobre *Rhizopertha dominica* (Pascual Villalobos et. al., 2004). La dependencia entre la concentración y la efectividad de los aceites también fue informada por Ogendo et al. 2008 con aceites esenciales de *Ocimum gratissimum* (Labiateae) en *Rhizopertha dominica*.

Tabla 1. Actividad fumigante de los aceites esenciales de *A. polystachya* y *A. citriodora* sobre adultos de *R. dominica.*

Planta	CL 50 (mg/cm^2)	95% IC	CL 95 (mg/cm^2)	95% IC	Pendiente ± Es	X^2
A. citriodora	0,223 a	(0,13- 0,28)	1,33 a	(0,68- 30,19)	2,11± 0,70	3,1
A. polystachya	0,277 a	(0,2- 0,376)	1,61 a	(0,8-31)	2,15± 0,68	1,59

CL50: Concentración letal 50; CL95: concentración letal 95; IC 95%: intervalo de confianza del 95%; ES: error estándar de la pendiente. Los valores de las columnas seguidos por la misma letra indican que no se han hallado diferencias significativas (p< 0,05).

Figura 1: Incidencia de la concentración (mg/cm^2) de los aceites de *A. citriodora* y *A. polystachya* sobre la proporción de mortalidad de *R. dominica.*



CONCLUSION

Los aceites esenciales de *A. citiodora* y *A. polystachya* podrían ser utilizados en el manejo integrado de las plagas de grano almacenado.

REFERENCIAS

Ogendo JO, Kostyukovsky M, Ravid U, Matasyoh JC, Deng AL, Omolo EO, Kariuki ST, Shaaya E. 2008. Bioactivity of Ocimum gratissimum L. Oil and two of its constituents against five insect pests attacking stored food products. J Stored Prod Res 44:328-334.

Pascual ME, Slowing K, Carretero E, Sánchez Mata D, Villar A. 2001. Lippia: traditional uses, chemistry and pharmacology: a review. J Ethnopharmacol 76:201-214.

Pascual-Villalobos MJ, Ballesta-Acosta MC, Soler A. 2004. Toxicidad y repelencia de aceites esenciales en plagas de almacén del arroz. Bol San Veg (Plagas) 30:279-286.

Prakash A, Rao J. 1997. Botanical pesticides in agriculture. Division of entomology, Central Rice Research Institute. CRC. 461p.

Rajendran S, Sriranjini V. 2008. Plant products as fumigants for stored-product insect control. J Stored Prod Res 44: 126-135.

Wang J, Zhu F, Zhou XM, Niu CY, Lai CL. 2006. Repellent and fumigant activity of essential oil from *Artemisia vulgaris* to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J Stored Prod Res 42:339-347.

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Biomarcadores para evaluación de genotoxicidad potencial*

[Biomarkers for evaluation of potencial genotoxicity]

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Abstract

In order to evaluate the potential mutagenic effects and/or the interaction that different xenobiotics can exert over DNA, different toxico-genetic assays were developed. We evaluated the aqueous extracts of (*Aloysia citriodora* Palau-Verbenaceae) "Cedrón" widely used in folk medicine through: a) Micronucleus Test; b) Comet Assay; c) Mitotic Index; d) Cellular Proliferation Kinetics; e) Sister Chromatid Exchanges. Human peripheral blood lymphocytes were treated with different concentrations of infusion and freeze-drying infusion (100 and 1000 µg/mL) and the statistical analysis was developed using ANOVA test. When the analysis of different biomarkers between control and exposed cultures was performed, no statistical differences were founded ($p>0.05$). These results suggest that aqueous extracts neither induce cytotoxic/genotoxic effect nor double strand breaks in DNA under our experimental conditions. Therefore, the extracts could be of benefit for human consumption.

Keywords: *Biomarkers; Genotoxicity; Verbenaceae;* Aloysia citriodora*; Cedrón.*

Resumen

Con el objeto de evaluar la interacción y/o el efecto mutagénico potencial sobre el ADN que distintos xenobióticos pueden ejercer, se utilizan diferentes ensayos de toxicidad genética. Se evaluó el extracto acuoso de (*Aloysia citriodora* Palau-Verbenaceae) ¨Cedrón¨, ampliamente utilizado en medicina folclórica, mediante: a) Ensayo del micronúcleo; b) Ensayo del Cometa; c) Índice Mitótico; d) Cinética de proliferación celular; e) Intercambio de Cromátidas Hermanas. Se utilizaron linfocitos humanos de sangre periférica expuestos a concentraciones de 100 y 1000 µg/mL (infusión y liofilizado de infusión). El análisis estadístico se efectuó mediante la prueba de ANOVA. Los resultados obtenidos no muestran diferencias estadísticamente significativas respecto de los valores control para los biomarcadores evaluados ($p>0.05$), indicando que no inducirían efecto cito-genotóxico ni daño a la doble hélice del ADN en las condiciones de este diseño experimental. Los extractos evaluados como infusión y/o liofilizado podrían ser utilizados benéficamente para el consumo masivo.

Palabras Clave: *Biomarcadores; Genotoxicidad; Verbenaceae;* Aloysia citriodora*; Cedrón.*

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INTRODUCCIÓN

Verbenaceae es una familia de plantas con alta diversidad biológica, amplia distribución geográfica y usos variados (farmacéuticos, alimentarios, textiles, cosméticos y en perfumería). De ellas, *Aloysia citriodora* Palaú -Verbenaceae, vulgarmente conocida como "Cedrón", tiene históricamente en la Argentina y otros países de Sudamérica un lugar en la medicina tradicional, la que reporta beneficios de su consumo como digestiva, antiespasmódica, antipirética, calmante ante dolores estomacales, para el tratamiento de la dispepsia, asma, diarrea, indigestión y además un efecto sedante leve (Carnat et al., 1999; Pascual et al., 2001). Es una Droga Oficial monografiada desde la 5a edición de la Farmacopea Nacional Argentina (1966). En la actualidad se la comercializa en farmacias y herboristerías y se la encuentra integrada a los ¨mix¨ de hierbas para consumo en infusiones.

Si bien es de uso masivo, esto no implica que se conozca en aspectos como su genotoxicidad, su actividad antioxidante y su composición química. El presente trabajo forma parte de un estudio más amplio y multidisciplinario donde se encaran los aspectos mencionados, avocándonos aquí y proponiendo como objetivo una evaluación del riesgo/beneficio de su consumo desde la óptica de la genética toxicológica a fines de determinar posibles efectos beneficiosos del mismo. Para ello se estudiaron plantas, recolectadas en el Departamento de Calingasta, región de Cuyo, Provincia de San Juan y en dos formas de preparación, infusión y liofilizado de la infusión (ambas según FNA), mediante el uso de biomarcadores clásicos de la genética toxicológica como lo son el Ensayo de Micronúcleo con Bloqueo de la Citocinesis (CBMN) para la detección de agentes que actúen como clastógenos o aneunógenos; el Ensayo del Cometa (*Comet Assay*) para detectar agentes que interactúen con la doble hélice de ADN; Índice Mitótico (IM) para evaluar citotoxicidad; Cinética de Proliferación celular (CPC) para evaluar la citostaticidad de un agente y el intercambio de Cromátides Hermanas (ICH) para detectar inestabilidad cromosómica.

MATERIALES Y MÉTODOS

Material vegetal

Como fuente para la preparación de la infusión se utilizaron plantas de cultivos (cosecha 2006-2007) localizados en Barreal (31° 38´ 0´´ S, 68° 28´ 0´´ W) (6.626 msnm), Departamento de Calingasta, región de Cuyo, Provincia de San Juan. Las hojas fueron la parte utilizada.

Preparación de la infusión y dosis a evaluar

La preparación de la infusión se realizó según Farmacopea Nacional Argentina (5ª edición) al 5% p/v. Se esterilizó por filtración (filtro 0,22 µm). A partir de la misma se preparó el liofilizado que se reconstituyó en agua destilada y se esterilizó por filtración (filtro 0,22 µm).

Las concentraciones seleccionadas para la evaluación de cito y genotoxicidad fueron 100 µg/mL y 1000 µg/mL, tomando las alícuotas correspondientes de cada una de las preparaciones a estudiar.

Muestras de sangre

Se empleó sangre entera heparinizada de dos dadores sanos.

Ensayo del Micronúcleo con Bloqueo de la Citocinesis (CBMN)

A partir de linfocitos aislados por gradiente de Ficoll (Bøyun, 1964) se estableció un cultivo en multiplaca de 24 wells según la técnica descripta por Kirsch Volders, et al. (2003) con modificaciones (1.10^6 células/mL en medio RPMI 1640, 15% de suero fetal bovino (SFB), 10 µg/mL fitohemaglutinina (PHA). Se incubó a 37 °C en atmósfera de CO_2 (5%) y a las 24 h se adicionó el extracto en las dos concentraciones de trabajo y Mitomicina C (0,025 µM) como control positivo. A las 44 h de cultivo se agregó Citocalasina B (4,5 µg/mL) y a las 72 h se realizó la cosecha. Los extendidos se realizaron por citocentrifugación y luego de secados se fijaron con Metanol-Acético (6:1) y se colorearon con Giemsa 10%.

Se contabilizaron células mono, bi, tri y tetranucleadas así como el número de micronúcleos cada 2000 células binucleadas. De este modo se estableció el Índice de División Nuclear (IDN) = (mononucleadas + 2 x binucleadas + 3 x trinucleadas + 4 x tetranucleadas)/N total de células y la frecuencia de micronúcleos (‰) (Fenech, 2000).

Electroforesis en gel de una célula

Viabilidad celular: Mediante una coloración fluorescente con bromuro de etidio (BE) y naranja de acridina (NA) (100 µg/mL BE y 100 µg/mL NA) (Mc Gahon, 1995), se contabilizaron 200 células y se estableció el porcentaje de células viables y células no viables (Mercille y Massie, 1994).

Ensayo Cometa alcalino

Se utilizó la técnica descripta por Singh et al. (1988) con modificaciones. Cada muestra se procesó por duplicado incluidos los controles negativos y positivos (H_2O_2 50 µM). El extracto de *A. citriodora* (100mg/mL y 1000 mg/mL) se incubó con 50 µL de sangre y 950 µL de RPMI 1640 durante 2 h a 37 ºC. El pellet se mezcló con agarosa de bajo punto de fusión al 1% a 37 ºC. La suspensión celular se distribuyó sobre portaobjetos previamente cubiertos con una capa de agarosa de punto de fusión normal al 1% y se adicionó una tercera capa de agarosa de bajo punto de fusión al 1%. Finalizada la preparación de los extendidos, se los sometió a la acción de una solución de lisis (NaCl 2,5 M; Na_2EDTA 100mM; Tris 10mM; 1% Triton X-100 y DMSO 10%, pH 10) durante 1 h a 4 ºC. Se realizó la electroforesis con buffer de corrida (200 mM Na_2EDTA; 10N NaOH, pH > 13) 20 min a 24 V y 300 mA (0,75 V/cm) a 4 ºC. Se realizaron lavados con buffer de neutralización (Tris 0,4M, pH 7,5) y se coloreó con bromuro de etidio (0,02 mg/mL). Se observó al microscopio de fluorescencia, realizando la cuantificación del daño por medio de un ocular graduado. Se calculó el Índice de Daño mediante la siguiente fórmula: ID = Nº cél. Cat. I +2 x Nº cél. Cat. II +3x Nº cél. Cat. III + 4 x Nº cél. Cat. VI (Tice et al., 200).

Biomarcadores Citogenéticos

Cultivo de linfocitos de Sangre periférica (LSP)

Se llevó a cabo de acuerdo a la técnica descripta por Carballo et al. (2006). Para ello se incubó durante 72 h a 37 ºC sangre entera heparinizada en medio de cultivo RPMI 1640 suplementado con SFB (15 %), PHA, Bromo deoxiuridina (BrDU, 32 µM) y la alícuota correspondiente del extracto a evaluar. Se trabajó por duplicado y se realizaron controles negativos y positivos (Mitomicina C). Antes de la finalización del cultivo se adicionó Colchicina (0,1 µg/mL) como veneno mitótico. Posteriormente se realizó un tratamiento hipotónico con KCL 0,075 M seguido de una fijación con metanol-ácido acético glacial (3:1) frío. Luego de sucesivos lavados se confeccionaron los extendidos cromosómicos mediante la técnica de Splash.

La coloración diferencial de cromátides o fluorescencia plus Giemsa (FPG) se llevó a cabo con solución de Hoescht 33258 (1 mg/mL) y posterior exposición a la luz ultravioleta (Perry y Wolf, 1974). Luego los preparados se incubaron en solución salina citratada doble concentración (2XSSC) a 60 ºC durante 2 h y se coloreó con solución de Giemsa al 3% en buffer fosfato.

Evaluación microscópica

Índice Mitótico (IM)

Se contabilizaron 1000 núcleos totales por individuo, por concentración y por preparación ensayada. El valor del IM se calculó mediante la siguiente fórmula: IM= Nº de células en metafase/ Nº total de células (Rojas *et al.*, 1993).

Cinética de proliferación celular (CPC)

Se estimó mediante el porcentaje de células en primera (M1), segunda (M2) o tercera (M3) división mitótica y se expresa por medio del Índice de Replicación (IR) a partir de la fórmula matemática: IR= Nº células M1 +2 x Nº células en M2 + 3x Nº de células en M3 / 100. (Gonsebatt y Mutchinik, 1990).

Intercambio de Cromátidas Hermanas (ICH)

La frecuencia de ICH se estableció contabilizando el número de intercambios de material genético por metafase, sobre un número total de 30 células (2n=46±2) para cada individuo, concentración y preparación a ensayar. Se utilizaron las metafases en las que la tinción diferencial permite ver una cromátida clara y otra oscura (segunda división). Los resultados se informan como ICH/ célula.

Evaluación estadística

El tratamiento estadístico de los ensayos antes mencionados se realizó mediante Test de Análisis de la Varianza de dos colas (ANOVA) con diseño de bloques al azar (ANOVA no paramétrico) (Graphpad Instat).

RESULTADOS Y DISCUSIÓN

Se analizaron extractos de *A. citriodora* en 2 formas de preparación (infusión y liofilizado de la infusión), mediante biomarcadores clásicos de la genética toxicológica. Los resultados muestran que las distintas concentraciones de la infusión y del liofilizado no inducen un aumento estadísticamente significativo (ANOVA p>0,05) de la frecuencia de micronúcleos (Tabla 1) ni del Índice de División Nuclear (IDN) en linfocitos de sangre periférica, así como tampoco muestran diferencias las dos formas de preparación evaluadas (infusión y liofilizado).

El individuo que presenta un nivel basal de MN notablemente más alto (individuo 2, Tabla 1) exhibe una disminución estadísticamente significativa del número de micronúcleos frente a la exposición a la infusión así como al liofilizado.

En el estudio de Viabilidad Celular se determinó que el porcentaje de células muertas no excede el 20% en ninguno de los tratamientos. Esto indicaría que cualquier efecto genotóxico potencial es debido al agente en estudio y no a falsos positivos generados por procesos de muerte celular producto de la metodología.

En la evaluación genotóxica mediante el Ensayo del Cometa (Fig. 1) no se observan diferencias significativas entre las células tratadas con los extractos analizados y sus controles negativos correspondientes.

Los biomarcadores evaluados: IM (Tabla 2), CPC expresado mediante el IR (Tabla 3) e ICH (Tabla 4) no muestran diferencias estadísticamente significativas respecto de los controles para ninguna de las preparaciones ensayadas (ANOVA $p>0.05$).

Tabla 1. Frecuencia de Micronúcleos (‰) inducida en cultivo de linfocitos de sangre periférica expuestos a extractos acuosos de *Aloysia citriodora.*

Tratamiento (µg/mL)	MN (‰) (X ± DS)			
	Infusión		Liofilizado	
	Individuo 1	Individuo 2	Individuo 1	Individuo 2
Control negativo	2,31 ± 0,78	14,23 ± 1,17	3,74 ± 1,74	24,2 ± 1,69
100	2,49 ± 2,27	13,6 ± 2,01	4,40 ± 0,71	21,8 ± 0,7
1000	3,60 ± 0,53	6,5 ± 1,5	3,80 ± 1,41	13,22 ± 2,2

Tabla 2. Efecto de los extractos acuosos de *Aloysia ctriodora* sobre el Índice de Replicación en cultivo de linfocitos de sangre periférica.

Tratamiento (µg/mL)	IR (X ± DS)			
	Infusión		Liofilizado	
	Individuo 1	Individuo 2	Individuo 1	Individuo 2
Control negativo	1,47 ± 0,05	1,43 ± 0,18	1,50 ± 0,02	1,57 ± 0,11
100	1,53 ± 0,08	1,38 ± 0,08	1,59 ± 0,04	1,50 ± 0,07
1000	1,51 ± 0,01	1,29 ± 0,06	1,52 ± 0,00	1,57 ± 0,06

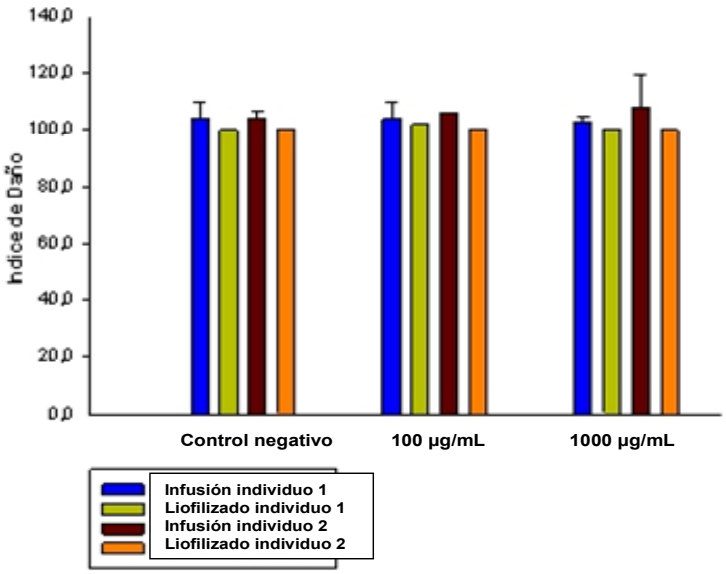
Tabla 3. Variación del Índice Mitótico en cultivo de linfocitos por exposición a extractos acuosos de *Aloysia citriodora*

	IM (X ± DS)			
Tratamiento (µg/mL)	Infusión		Liofilizado	
	Individuo 1	Individuo 2	Individuo 1	Individuo 2
Control negativo	3,35 ± 1,20	2,33 ± 0,06	3,25 ± 0,49	2,47 ± 1,26
100	2,39 ± 0,00	3,08 ± 0,31	3,08 ± 0,61	2,15 ± 0,49
1000	2,35 ± 0,21	2,18 ± 0,15	2,45 ± 0,21	3,00 ± 0,28

Tabla 4. Frecuencia de Intercambio de Cromátidas Hermanas (ICH/célula) observada en linfocitos de sangre periférica expuestos a extractos acuosos de *Aloysia citriodora*.

	ICH (ICH/célula) (X ± DS)			
Tratamiento (µg/mL)	Infusión		Liofilizado	
	Individuo 1	Individuo 2	Individuo 1	Individuo 2
Control negativo	3,28 ± 1,75	4,83 ± 3,00	5,15 ± 2,81	4,35 ± 1,83
100	3,83 ± 1,45	6,55 ± 3,09	5,86 ± 1,51	4,72 ± 1,96
1000	3,88 ± 1,40	5,92 ± 1,88	5,52 ± 1,33	5,25 ± 2,08

Figura 1: Evaluación del Índice de Daño (X ± DS) inducido por extractos acuosos de *Aloysia citriodora* en linfocitos humanos.



CONCLUSIÓN

Los resultados obtenidos a partir de los biomarcadores de efecto seleccionados (Ensayo del Micronúcleo, Ensayo del Cometa, IM, IR, ICH) sugieren que el extracto de *A. citriodora* analizado bajo las formas de infusión y liofilizado no induciría citotoxicidad ni genotoxicidad. Por lo tanto no tendrían principios activos que interactúen con el ADN, en forma perjudicial, generando algún tipo de daño de simple o doble cadena. Esto se deduce a partir de los resultados obtenidos los cuales no muestran diferencias estadísticamente significativas respecto de los controles para ninguno de los biomarcadores evaluados.

Al mismo tiempo, se pone en evidencia la susceptibilidad frente a los distintos biomarcadores cuando se observan las diferencias interindividuales. Los efectos observados podrían deberse a la presencia de polifenoles en los extractos acuosos, de los cuales la literatura ha reportado propiedades antioxidantes. A partir de estos hallazgos se buscará profundizar en la caracterización de esta planta medicinal, focalizando los estudios en sus potenciales efectos benéficos.

REFERENCIAS

Bøyum A. 1964. Separation of white blood cells. Nature 204:793-794.

Carballo MA, Hick A, Larramendy M, Solonesky S, Mudry MD. 2006. Genotoxic bioassays of thiabendazole (TBZ). J Appl Toxicol 26:293-300.

Carnat A, Carnat AP, Fraiss D, Lamaison JL. 1999. The aromatic and polyphenolic composition of lemon verbena tea. Fitoterapia 70(1):44-49.

Farmacopea Nacional Argentina. 5a Ed. 1966.

Fenech M. 2000. The *in vitro* micronucleus technique. Mutat Res 455:81-95.

Gonsebatt ME y Mutchinick O. 1990. Human lymphocyte proliferation kinetics in Hanks' BSS supplemented with autologous plasma and in synthetic medium. Mutat Res 243(4):255-258.

Kirsch-Volders M, Sofuni T, Aardema M, Albertini S, Eastmond D, Fenech M, Ishidate M, Kirchner S, Lorge E, Morita T, Norppa H, Surrallés J, Vanhauwaert A, Wakata A. 2003. Report from the *in vitro* micronuleus assay working group. Mutat Res 540:153-163.

Mc Gahon A, Martín S, Bissonnette R, Mahboubi A, Shi Y, Mogil R, Nishioka W, Gree D. 1995. The end of the (cell) line: methods for the study of apoptosis *in vitro*. Method Cell Biol 46:153-185.

Mercille S y Massie B. 1994. Induction of apoptosis in nutrient-deprived cultures of hydridoma and myeloma cells. Biotechnol Bioeng 44:1140-1154.

Pascual ME, Slowing K, Carretero E, Sánchez Mata D, Villar A. 2001. *Lippia*: traditional uses, chemistry and pharmacology: a review. J Ethnopharmacol 76:201-214.

Perry P y Wolff S. 1974. New Giemsa method for the differential staining of sister chromatid. Nature (London) 261:156-161.

Rojas E, Herrera LA, Sordo M, Gonsebatt ME, Montero R, Rodríguez R, Ostrosky-Wegman P. 1993. Mitotic index and cell proliferation kinetics for the identification of antineoplastic activity. Anti-Cancer Drugs 4:637-640.

Singh NP, McCoy MT, Tice RR, Schneider EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184-191

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. 2000. The single cell gel/Comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environm Mol Mutagen 35:206–221.

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