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Consequences of subchronic exposure to ethanolic extract from fruits and leaves of *Schinus molle* var. *areira* L. in mice

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ABSTRACT

Aim of the study: Several extracts of *Schinus molle* var. *areira* L. plant proved to be useful for the treatment of different pathologies and for the control of insect pest. Due to these potential uses, it is necessary to study their safety. In this work, we evaluated the effects of subchronic exposure to ethanolic extracts from leaves and fruits of *Schinus molle* var. *areira* in mice.
Materials and methods: The plant extract was added to the diet at 1 g/kg body weight/day for 90 days. At the end of the exposure, behavioral and functional parameters in a functional observational battery and motor activity in an open field were assessed. Finally, several biochemical and histopathological studies were realized.
Results: The exposure to extract from leaves produced an increase in the number of rearings in the open field and of urine pools in the functional observational battery. On the other hand, the exposure to extract from fruits produced an increase in the neutrophil count and a decrease in the lymphocyte count and in the total cholesterol levels. None of the exposures affected the different organs evaluated.
Conclusions: Our results suggest that subchronic exposure to ethanolic extracts from leaves and fruits of *Schinus molle* var. *areira* should be potentially useful in the treatment of lipid pathologies and safe to use.

1. Introduction

It is known that *Schinus molle* var. *areira* (Anacardiaceae), whose vulgar names are "aguaribay" or "molle", is an American species native of South America (Heywood, 1993). All parts of the plant have been used in traditional medicine throughout the regions where it can be found. In Peru, the infusion and decoction of its leaves it is used as digestive and wound healer, and the oleoresin is employed to calm toothache. In Argentina and Uruguay, the leaf tea is used to treat menstrual disorders and for respiratory and urinary infections. In Chile, the bark tea is taken as stimulant, antidepressant and astringent, and in cases of rheumatism. In Mexico, its leaves are employed as diuretic and in eyes diseases. In Brazil, it is used in cases of bronchitis, cough, fever and flu (Duke, 1985; Alonso and Desmarchelier, 2005; Taylor, 2005). In the last years, many studies have been performed in this plant and interesting results have been obtained about its biological effects. It is reported that it exert several activities, such as: antibacterial, antiviral, antiseptic, diuretic (Duke, 1985), hypotensive (Bello et al., 1996), antitumoral (Ruffa et al., 2002), analgesic (Barrachina et al., 1997) and anti-inflammatory (Yueqin et al., 2003). Another more recent study demonstrated that the leaves of this plant produce an antidepressant-like effect (Machado et al., 2007).

Abundant evidence also indicated that *Schinus molle* var. *areira* has insecticidal properties. In our laboratory, we observed that different extracts and essential oils from leaves and fruits of *Schinus molle* produced repellent effects on neonate larvae of *Cydia pomonella* (Chirino et al., 2001), ovicidal activity and repellent effect on first nymphs of *Triatoma infestans* (Ferrero et al., 2006), and repellent and adulticidal activity on *Blatella germanica* (Ferrero et al., 2007b) and *Tribolium castaneum* (Descamps et al., 2008). Other authors also demonstrated that essential oils from leaves of this plant showed repellent activity on *Musca domestica* Linnaeus (Wimalaratne et al., 1996).

This evidence suggests that *Schinus molle* var. *areira* plant could be very useful for the treatment of several pathologies and for some pest control. Due to their potential therapeutic and insecticide uses, we considered that it is necessary to investigate its safety. In a recent work, we observed that the acute and subacute exposure to ethanolic extracts from fruits of *Schinus molle* var. *areira* in rats did not produce toxicity in any of the animals evaluated (Ferrero et al.,

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2007a). This experiment only demonstrated a transitory stimulant effect in the exposed rats.

Considering that the potential chronic toxicity associated with this extract needs to be evaluated, in the present work we studied the subchronic exposure to ethanolic extract from fruits and leaves of *Schinus molle* var. *areira* in mice. The nervous system functionality was analyzed by means of a functional observational battery (FOB) and by assessing the motor activity in an open field. The FOB is a series of observational and manipulative tests designed to assess the neurological integrity of the test subject (Moser et al., 1988), whereas motor activity is an apical measure of neurobehavioral function (MacPhail, 1987). Finally, several hematological and biochemical parameters were determined. Also, histopathological examination was realized on several tissues.

2. Materials and methods

2.1. Plant material

Fruits and leaves from *Schinus molle* var. *areira* L. were collected in Bahía Blanca city, in the south of Argentina, during summer. Botanical identification was performed at the herbarium of Departamento de Biología, Bioquímica y Farmacia of Universidad Nacional del Sur (voucher herbarium specimen number: BBB 10444).

2.2. Preparation of ethanolic plant extracts

Fresh fruits and leaves (1 kg and 1.5 kg, respectively) from *Schinus molle* var. *areira* were macerated in ethanol (3×2 l) at room temperature for 72 h. The solvent was completely evaporated at reduced pressure using a rotavap (LABOROTA 4000, Heidolph) at 180 rpm in water bath at 40 °C. The crude extracts from both vegetal organs were kept at 4 °C until further use (yields: 10.82% and 6.44% from fruit and leaf material, respectively).

2.3. Experimental animals

Healthy CF1 mice of both sexes 8 weeks old were used. They were obtained from the colony of the animal facility from the Biology, Biochemistry and Pharmacy Department which were maintained under constant conditions of temperature (22 ± 1 °C) and humidity (70%), in a 12 h light:12 h dark cycle (lights on at 6:00 h) during all the experiment. According to the body weight (27.4 ± 2 g for females and 35.6 ± 2 g for males), they were randomly divided into three groups of 20 animals each (10 female and 10 male) which were housed in pairs of the same sex in each cage and acclimatized for a week before starting the experiment.

All animals had free access to tap water and standard diet (Ganave®, Ratas y ratones, Alimentos Pilar S.A., Argentina) throughout the experiment.

The care and the handling of the animals were in accordance with the internationally accepted standard Guide for the Care and Use of Laboratory Animals (1996) as adopted and promulgated by the National Institute of Health.

2.4. Subchronic exposure to the extracts

The experiment was conducted according to the protocols described by OECD Guideline No. 408 (OECD, 1998).

The plant extracts were incorporated into the diet and fed daily to rodents over a period of 90 days at a dose of 1000 mg/kg body weight/day. For this reason, the amount of food consumed and the body weight of each mouse were weekly measured in order to deliver a constant dosing throughout the study. The selected dose is the maximum dose indicated for subchronic assays of toxicity (OECD, 1998). The limit test was performed because no severe toxic effects we expected for exposure to this plant extracts.

One group of mice was exposed to the ethanolic extract from fruits of *Schinus molle* var. *areira*, the other group received the ethanolic extract from leaves and the third one was used as control group. Control mice were fed only with the standard diet. During the exposure, all the animals were observed for signs of toxicity. At the end of the exposure, behavioral and functional parameters and motor activity were assessed in all the animals. Subsequently, blood samples were obtained for hematological analysis by retro-orbital bleeding under light ether anaesthesia (Fukuta, 2004). After that, mice were euthanized and blood samples were taken by cardiac puncture for biochemical analysis. Finally, necropsy observations and histopathological examinations were realized on several tissues.

2.5. Functional observational battery (FOB)

On the 90th day of exposure, behavioral and functional parameters of the animals were evaluated through a FOB. It included a thorough description of the animals' appearance, behavior, and functional integrity (US EPA, 1998). Procedural details and scoring criteria for the FOB protocol were performed according to Moser and Ross (1996) and modified for mice (Youssef and Santi, 1997; Ingman et al., 2004).

Briefly, measurements were first carried out in the home cage. The observer recorded each animal's posture, activity and palpebral closure. The presence or absence of clonic or tonic movements, spontaneous vocalizations and biting were also noted. Then the animal was removed from its cage, rating the ease of removal and handling. All signs of lacrimation, salivation and piloerection were rated. Other abnormal clinical signs were also recorded. The animal was next placed in an open field arena having a piece of clean absorbent paper on the surface, and allowed to freely explore for 3 min. During that time, the observer ranked the mouse's arousal, gait score, activity level and rears as well as any abnormal postures, unusual movements, stereotyped behaviors, pelvis elevation and tail position. At the end of the 3 min, the number of fecal boluses and urine pools, and presence or absence of diarrhea were recorded. Next, sensorial responses were ranked according to a variety of stimuli (click stimulus using a metal clicker, approach and touch rump with a blunt object, pinch of the tail using forceps, and touch of the corner of the eye and the inside of the ear with a fine object). Also, surface righting reflex was evaluated. In landing foot splay, the tarsal joint pad of each hindfoot was marked with ink and the animal was then dropped from a height of 15 cm onto a recording sheet. Finally, the wire maneuver was carried out. The animal was suspended from a horizontal wire by forelimbs and released.

2.6. Motor activity

Each mouse was placed in an open field of 50 cm × 50 cm × 60 cm whose floor was divided into 12 cm × 12 cm squares by black lines. The number of squares entered by each mouse with all four paws, rearings, groomings and fecal boluses were scored for 15 min. After each animal was removed, the open field was carefully cleaned with a damp cloth.

2.7. Hematological and biochemical parameters

A volume of 0.4 ml of blood was obtained from each mouse by retro-orbital bleeding under light ether anaesthesia with ethylendiamine tetraacetate (EDTA) as anticoagulant (25 μl/ml of blood). These samples were used immediately for the determination of hematological parameters, employing an automatic analyser (Coulter T890). The parameters measured were total erythrocytes,

Table 1
Parameters evaluated in the functional observational battery after the subchronic exposure to ethanolic extracts from fruits and leaves of *Schinus molle* var. *areira*.

Endpoints	Control		Fruits		Leaves	
	♀	♂	♀	♂	♀	♂
Home cage observations						
Normal body posture (D)	100	100	100	100	100	100
Activity (R)	3.30	2.80	3.40	2.80	3.00	2.90
Palpebral closure (R)	1.00	1.00	1.00	1.00	1.00	1.00
Clonic movements (D)	0	0	0	0	0	0
Tonic movements (D)	0	0	0	0	0	0
Biting (D)	0	0	0	0	0	0
Vocalizations (B)	0	0	0	0	0	0
Hand-held observations						
Ease of removal from cage (R)	2.00	2.00	2.00	2.00	2.00	2.00
Ease of handling (R)	2.00	2.00	2.00	2.00	2.00	2.00
Salivation (R)	1.00	1.00	1.00	1.00	1.00	1.00
Lacrimation (R)	1.00	1.00	1.00	1.00	1.00	1.00
Piloerection (B)	0	0	0	0	0	0
Normal fur appearance (D)	100	100	100	100	100	100
Normal respiration (D)	100	100	100	100	100	100
Normal cardiovascular signs (D)	100	100	100	100	100	100
Normal limb tone (D)	100	100	100	100	100	100
Normal abdominal tone (D)	100	100	100	100	100	100
Limb grasping (B)	100	100	100	100	100	100
Open field observations						
Activity level (R)	3.05	2.95	3.10	3.25	3.25	3.05
Rearing (R)	1.10	1.10	1.00	1.10	1.00	1.10
Arousal (R)	3.35	3.25	3.55	3.55	3.55	3.40
Normal gait (D)	100	100	100	100	100	100
Stereotyped behaviors (D)	0	0	0	0	0	0
Pelvic elevation (R)	3.00	3.00	3.00	3.00	3.00	3.00
Normal tail position (D)	100	100	100	100	100	100
Fecal boluses (C)	1.60	1.60	1.80	1.60	1.80	2.70
Urine pools (C)	0.10	0.90	0.20	0.50	0.7	2.80**
Diarrhea (B)	0	0	0	0	0	0
Manipulative tests						
Approach response (R)	2.00	2.00	2.00	2.00	2.00	2.00
Touch response (R)	2.00	2.00	2.00	2.00	2.00	2.00
Click response (R)	1.95	1.90	1.95	1.90	1.75	1.95
Tail pinch response (R)	2.00	2.00	2.00	2.00	2.00	1.95
Palpebral reflex (B)	100	100	100	100	100	100
Pinna reflex (B)	100	100	100	100	100	100
Flexor reflex (B)	100	100	100	100	100	100
Extensor reflex (B)	100	100	100	100	100	100
Righting reflex (R)	1.00	1.00	1.00	1.00	1.00	1.00
Landing foot splay (C)	4.22	4.00	4.22	3.78	4.47	3.96
Wire maneuver (R)	1.20	1.40	1.10	1.35	1.10	1.40

Descriptive (D) and binary (B) data expressed as percentage of incidence (chi-square test); Ranked (R) data expressed as the mean score of the scale used (Kruskal–Wallis test); Continuous (C) data expressed as mean value (two-way ANOVA test); ♀, female mice; ♂, male mice.
** $p < 0.01$ compared to control group.

leukocytes and platelet counts, hematocrit and hemoglobin levels, leukocyte differential counts and erythrocyte indices.

Blood samples for biochemical analysis were obtained by cardiac puncture as a terminal procedure. Heparin was used as anticoagulant (30 UI/ml of blood). The samples were centrifuged at $1500 \times g$ for 10 min (Rolco CM 36R centrifuge) to obtain plasma. For the hepatic function evaluation, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined, while for the renal function, urea and creatinine were evaluated. Glucose and total cholesterol were assessed for carbohydrate and lipid metabolism study, respectively. Biochemical determinations were made using specific kits (Wiener Lab, Argentina) by measurement of the optical density of the reaction products at the corresponding wavelength with a spectrophotometer (Shimadzu UV-1203, UV-VIS spectrophotometer).

2.8. Histopathological examinations

Euthanized animals were macroscopically examined. Liver, kidneys, stomach and brain were weighed and intestine total length was recorded. Representative fragments of these organs were fixed in Bouin's solution, dehydrated by serial ethanol solution and enclosed with paraffin. Sections 5 μm thick were stained with hematoxylin–eosin and examined under a light microscope (Olympus Bx51).

2.9. Statistical analysis

Behavioral test measures in FOB were continuous (providing interval data), ranked (ranks based on a defined scale), descriptive or binary (presence or absence of a sign). Continuous data were tested using a two-way ANOVA followed by *t*-Student test when differences between groups were detected. The ranked data were analyzed using the Kruskal–Wallis nonparametric test. For descriptive and binary data, each experimental group was compared to the control group using a chi-square test.

The open field data, as well as hematological and biochemical data, were submitted to a two-way ANOVA followed by post hoc comparisons using *t*-Student test. Hematological data presented as percentages were previously transformed to $p' = \arcsin\sqrt{p}$ (Zar, 1999).

Probability values less than 0.05 were considered to be significant.

All statistical analysis was made using software SPSS 7.5 for Windows.

3. Results

3.1. Functional observational battery

Data obtained in the FOB are shown in Table 1. Neither group of mice exposed to the ethanolic extract from fruits of *Schinus molle* var. *areira* nor the other one exposed to its leaves produced alterations in the parameters evaluated in the home cage, in hand-held observations or during the manipulative tests. However, in the open field arena, the group of males exposed to the ethanolic extract from leaves exhibited a significant increment in the number of urine pools deposited ($p < 0.01$) compared to the control males. The other parameters evaluated in the open field arena were not altered in either of the experimental groups.

3.2. Motor activity

Evaluations in the square open field indicated that the exposure to the extracts did not modify the number of the squares crossed by the animals during a total of 15 min (Fig. 1). However, female mice exposed to the ethanolic extract from leaves showed a significant increment in the number of rearings measured during the period of observation ($p < 0.05$) compared to control group (Fig. 2).

When the number of groomings and fecal boluses were analyzed as emotionality parameters, none of the measures demonstrated any significant differences between control and experimental groups (data not shown).

3.3. Hematological and biochemical analysis

Data from the hematology and the clinical biochemistry are respectively shown in Tables 2 and 3.

About females exposed to the fruit extract, a significant increment in the percentage of segmented neutrophils and a significant decrease in the percentage of lymphocytes were detected ($p < 0.01$ in both cases compared to control group). The males exposed to the fruit extract did not show any difference in the parameters evaluated compared to control animals.

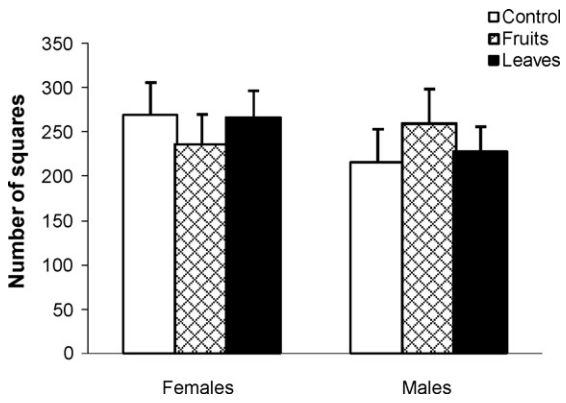

Fig. 1. Motor activity evaluated in the open field after the subchronic exposure to the extracts. Data are expressed as the mean ± SE of the number of squares crossed by the mice during a period of 15 min. *$p < 0.05$ compared to control (two-way ANOVA followed by *t*-Student test).

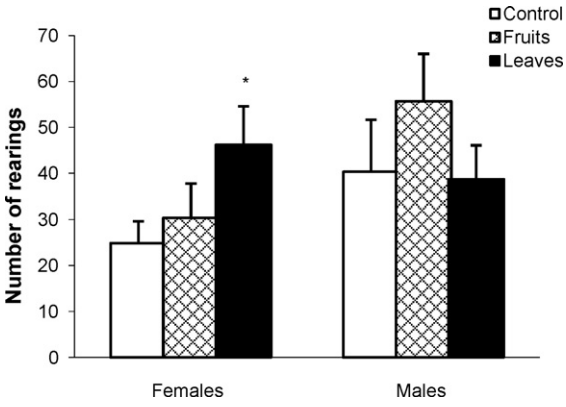

Fig. 2. Motor activity evaluated in the open field after the subchronic exposure to the extracts. Data are expressed as the mean ± SE of the number of rearings recorded during a period of 15 min. *$p < 0.05$ compared to control (two-way ANOVA followed by *t*-Student test).

Animals exposed to the leaf extract did not present any difference in the hematological parameters evaluated, compared to control, except the males for which a significant decrease in the mean corpuscular volume (MCV) was detected ($p < 0.05$). However, the males from this exposed group had MCV values within the refer-

Table 2
Hematological data from mice subchronically exposed to ethanolic extracts from fruits and leaves of *Schinus molle* var. *areira*.

Parameters	Control		Fruits		Leaves	
	♀	♂	♀	♂	♀	♂
Erythrocytes (10^6/mm^3)	9.63 ± 0.1	9.3 ± 0.1	9.8 ± 0.1	9.3 ± 0.1	9.7 ± 0.2	9.2 ± 0.1
Leucocytes (10^3/mm^3)	9.3 ± 1.8	8.7 ± 1.9	7.4 ± 0.6	6.3 ± 1.0	9.5 ± 4.4	6.8 ± 0.8
Hematocrit (%)	44.7 ± 0.6	43.4 ± 0.3	44.8 ± 0.3	43.3 ± 0.7	44.7 ± 1.1	41.9 ± 0.4
Hemoglobin (g/dl)	14.1 ± 0.5	13.6 ± 0.2	14.5 ± 0.1	13.7 ± 0.3	14.5 ± 0.4	13.7 ± 0.2
MCV[a] (fl)	46.3 ± 0.4	46.6 ± 0.4	46.0 ± 0.4	46.3 ± 0.4	46.1 ± 0.2	45.5 ± 0.3*
MCH[b] (pg)	14.7 ± 0.6	15.0 ± 0.3	15.0 ± 0.2	14.8 ± 0.3	14.9 ± 0.1	15.0 ± 0.0
MCHC[c] (%)	31.3 ± 1.0	31.6 ± 0.4	32.4 ± 0.2	31.6 ± 0.5	32.5 ± 0.2	32.4 ± 0.2
Neutrophils (%)	8.3 ± 1.0	19.6 ± 2.4	14.6 ± 1.6**	16.0 ± 1.7	11.2 ± 1.1	21.0 ± 3.5
Eosinophils (%)	0	0.8 ± 0.5	0.3 ± 0.2	0.5 ± 0.3	0.1 ± 0.1	1.0 ± 0.5
Basophils (%)	0	0	0	0	0	0
Lymphocytes (%)	88.2 ± 0.8	75.4 ± 3.5	81.9 ± 1.9**	80.5 ± 1.6	85.8 ± 1.3	75.3 ± 3.4
Monocytes (%)	3.5 ± 0.7	4.2 ± 1.0	3.2 ± 0.6	3.0 ± 0.6	2.9 ± 0.6	2.8 ± 0.5
Platelets (10^3/mm^3)	1159 ± 79.5	992 ± 40.5	987 ± 38.0	993 ± 47.2	1023 ± 51.6	992 ± 83.4

Data expressed as mean ± SE. ♀, female mice; ♂, male mice.

[a] MCV, mean corpuscular volume.

[b] MCH, mean corpuscular hemoglobin.

[c] MCHC, mean corpuscular hemoglobin concentration.

* $p < 0.05$ compared to control group (two-way ANOVA followed by *t*-Student test).

** $p < 0.01$ compared to control group (two-way ANOVA followed by *t*-Student test).

Table 3
Plasma biochemical data from mice subchronically exposed to ethanolic extracts from fruits and leaves of *Schinus molle* var. *areira*.

Parameters	Control		Fruits		Leaves	
	♀	♂	♀	♂	♀	♂
Glucose (mg/dl)	303.3 ± 17.0	403.0 ± 34.5	319.0 ± 17.2	409.2 ± 21.9	310.4 ± 9.2	354.7 ± 15.0
Creatinine (mg/dl)	0.45 ± 0.02	0.40 ± 0	0.45 ± 0.02	0.41 ± 0.03	0.41 ± 0.04	0.38 ± 0.01
AST[a] (IU/l)	76.2 ± 9.3	70.5 ± 6.0	66.8 ± 4.6	73.2 ± 10.3	66.1 ± 5.7	63.4 ± 5.0
ALT[b] (IU/l)	15.7 ± 2.3	18.2 ± 3.2	15.9 ± 1.8	19.2 ± 2.8	16.3 ± 2.2	18.2 ± 3.0
Urea (mg/dl)	62.3 ± 2.7	51.8 ± 2.7	55.8 ± 3.8	55.9 ± 3.3	61.2 ± 4.2	57.0 ± 2.6
Cholesterol (mg/dl)	79.5 ± 2.5	122.7 ± 6.0	68.3 ± 2.6*	119.5 ± 3.5	75.4 ± 4.7	117.6 ± 4.5

Data expressed as mean ± SE. ♀, female mice; ♂, male mice.
[a] AST, aspartate aminotransferase.
[b] ALT, alanine aminotransferase.
* $p < 0.05$ compared to control group (two-way ANOVA followed by *t*-Student test).

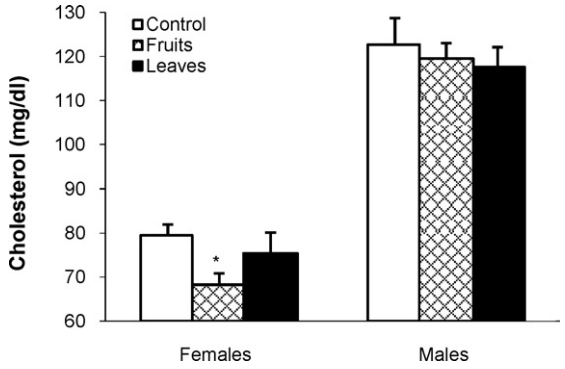

Fig. 3. Plasma cholesterol levels measured in mice after subchronic exposure to ethanolic extracts from fruits and leaves of *Schinus molle* var. *areira*. Data expressed as mean ± SE. *$p < 0.05$ compared to control (two-way ANOVA followed by *t*-Student test).

ence range for mice, which is determined approximately in 40–55 fl (Everds, 2004).

When biochemical data were analyzed, a significant decrease of the cholesterol level from females exposed to the fruits was detected compared to control females ($p < 0.05$) (Fig. 3). The former showed a 14.1% cholesterol mean level lower than the latter.

No other difference was detected by statistical analysis in the rest of the biochemical parameters evaluated in plasma, but in Fig. 4 it can be seen that the glycemia from males exposed to the leaf extract has a mean value clearly inferior to the control male group. Although this difference was not statistically significant, it is important to note the tendency of the leaf extract to lower the glucose mean level in the mice exposed.

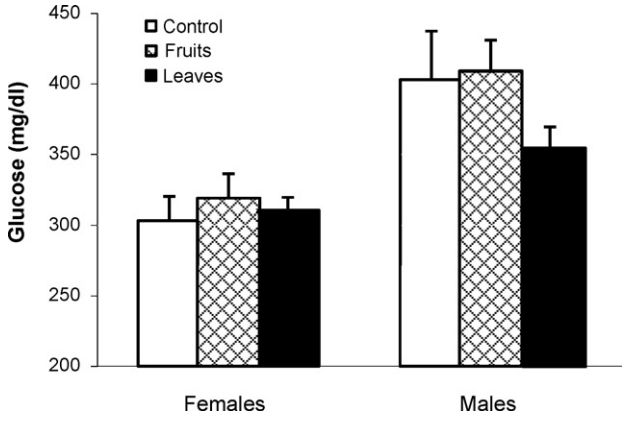

Fig. 4. Plasma glucose levels measured in mice after subchronic exposure to ethanolic extracts from fruits and leaves of *Schinus molle* var. *areira*. Data expressed as mean ± SE. *$p < 0.05$ compared to control (two-way ANOVA followed by *t*-Student test).

3.4. Histopathological analysis

The histopathological examinations of liver, kidney, stomach, intestine and brain showed no changes at the end of the subchronic exposure to the extracts in any of the mice.

4. Discussion

The subchronic exposure to ethanolic extract from leaves of *Schinus molle* var. *areira* produce an increase in the number of rearings in the open field in female mice, while in males caused an elevated number of urine pools in the functional observational battery. Moreover, a smaller mean corpuscular volume value was observed in males exposed compared to control animals. Furthermore, the subchronic exposure of females to ethanolic extract from fruits of *Schinus molle* var. *areira* produced an increase in the neutrophil count, a decrease in the lymphocyte count and a decrease in the total cholesterol levels.

The increase in the number of rearings in the open field observed in the females exposed to the ethanolic extract from leaves reflect a stimulant effect produced by this extract, because the rearing is a parameter of motor activity. We obtained the same results when we evaluated the effect of subacute exposure to ethanolic extracts from fruits (Ferrero et al., 2007a). Considering that the activation of mesolimbic dopamine pathways stimulates the locomotor activity (Vecina and Kim, 1999) and that other neurotransmission pathways, such as serotonergic and glutaminergic, are also involved in locomotor regulation (Carey et al., 2004), the ethanolic extract from leaves of *Schinus molle* var. *areira* may cause functional alterations in some of these neurotransmission systems.

With regard to the elevated number of urine pools produced by the males exposed to ethanolic extract from leaves, this effect could reflect a diuretic action of the extract. The diuretic effect of *Schinus molle* var. *areira* was already described by Duke in 1985. Also, the hipotensive effect produced by this plant (Bello et al., 1996) could be attributed to its diuretic activity.

Even when in the hematological study we obtained a smaller mean corpuscular volume value in the males exposed to ethanolic extract from leaves, we considered this result not relevant since this value is within reference values.

When the mice were subchronically exposed to ethanolic extract from fruits of *Schinus molle* var. *areira*, only the females showed leukocyte alterations and a lower total cholesterol level. These changes may be indirect effects of the action of this extract on hormonal regulation. Several studies have demonstrated estrogen receptor expression in primary lymphoid organs and mature peripheral B and T cells (Nilsson et al., 1986; Bellido et al., 1993). Other works have also established that estrogen is a potent inhibitor of stromal cell-dependent B cell lymphopoiesis in vitro. In bone marrow, all the precursors beyond the early pro-B cell stage are affected by estrogen (Smithson et al., 1995; Kincade et al.,

2000). Furthermore, chronic estrogen treatment diminished lymphocyte numbers in both developmental (thymus, bone marrow) and mature (spleen, lymph nodes) lymphoid organs (Ansar Ahmed et al., 1999). With respect to neutrophils, they increased during pregnancy, suggesting that progesterone and estrogen have a role in increasing neutrophil count (Bouman et al., 2005).

On the other hand, estrogens also modify the lipid and lipoprotein levels. Several studies have demonstrated that in different hormone replacement therapy regimens, all estrogen alone regimens raised high density lipoprotein (HDL) cholesterol and lowered low density lipoprotein (LDL) cholesterol and total cholesterol, while progestagens had little effect on estrogen-induced reductions in LDL and total cholesterol (Godsland, 2001).

The phytochemical analysis reveals that *Schinus molle* var. *areira* contains flavonoids, steroidal saponins and sterols (Pozzo-Balbi et al., 1978; Yueqin et al., 2003). Several studies have proved that a group of flavonoids have estrogenic activity because they are structurally similar to steroid hormones, particularly estrogens, and they can bind to estrogens receptors (Harborne and Williams, 2000; Rosenberg Zand et al., 2002). Moreover, these compounds have been studied for the effects they exert on lipid metabolism, especially for their cholesterol-lowering properties (Nijveldt et al., 2001; Plat and Mensink, 2005; Peluso, 2006; Brufau et al., 2008).

This evidence suggests that such compounds present in the ethanolic extract from fruits of *Schinus molle* var. *areira* could increase estrogenic activity in the females exposed and this effect could be responsible for the leukocyte alterations and the lower total cholesterol levels observed in this animal group. These speculations could be confirmed in future works.

On the other hand, males exposed to ethanolic extract from leaves of *Schinus molle* var. *areira* also showed a tendency to diminish their glucose levels. This effect could be related to the presence of flavonoids in the extract, which have beneficial effects on glucose homeostasis as it has been demonstrated in many studies (Hanhineva et al., 2010).

The decrease in glucose levels in males exposed to ethanolic extract from leaves and the lower total cholesterol levels observed in the females exposed to ethanolic extract from fruits, effects which have not been previously described, are very important actions of the mentioned extracts on the metabolic regulation. The confirmation of these effects could be very useful for the consideration of *Schinus molle* var. *areira* as a valuable tool in the treatment of diabetes and lipid pathologies, taking into account that the subchronic exposure to the extracts did not produce toxicity in mice.

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