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# Evaluation of the acute dermal exposure of the ethanolic and hexanic extracts from leaves of *Schinus molle* var. *areira* L. in rats

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ABSTRACT

*Ethnopharmacological relevance:* *Schinus molle* var. *areira* L. (Anacardiaceae) is employed in herbal medicine for many conditions, including respiratory, urinary and menstrual disorders, and as a digestive stimulant, diuretic, astringent and antidepressant. It is also known for its topical use as wound healer, antiseptic, for skin disorders and as repellent and insecticide. In the present work, the acute dermal exposure to ethanolic and hexanic extracts from leaves of *Schinus molle* var. *areira* was studied in rats.
*Materials and methods:* A single dose of 2000 mg/kg of body weight of ethanolic and hexanic extracts from leaves was applied on the shaved skin of male and female rats. After 24 h of exposure, the patch was removed and any sign of irritation was recorded. Behavioral and functional parameters in a functional observational battery and motor activity in an open field were assessed after the exposure to the extracts. Then, after 14 days of observation, animals were retested. Finally, histopathological studies were conducted on several organs.
*Results:* Slight signs of erythema and edema were observed in the skin site of exposure, but they disappeared after 48 h. The exposure to the hexanic extract produced an increase in parameters of activity, rearing and arousal assessed in the functional observational battery, which reversed after 14 days. On the other hand, the ethanolic extract caused an increase in locomotor activity, reflected in a higher number of rearings performed in the open field in the evaluation carried out on Day 14. No histopathological alterations were detected in the analyzed organs.
*Conclusions:* The results show that the acute dermal exposure of the ethanolic and hexanic extracts from leaves of *Schinus molle* var. *areira* only causes a slight and reversible skin irritation, and a mild stimulatory effect in rats. All these indicate that the topical use of these extracts would be safe.
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## 1. Introduction

*Schinus molle* var. *areira* L. (Anacardiaceae) is a tree native to the Andean regions of Peru, although at present it is widely distributed throughout Argentina, South Eastern Brazil, Peru, Colombia, Ecuador, Uruguay, Western Mexico and Guatemala (Heywood, 1993; Alonso and Desmarchelier, 2005). All parts of this species have been used medicinally by indigenous people and it is still employed in herbal medicine today in many countries. It is used for many conditions, including respiratory, urinary and menstrual disorders, and as a digestive stimulant, diuretic, astringent and antidepressant. It is also known for its topical use as wound healer, antiseptic and for skin disorders (Taylor, 2005; Martínez and Barboza, 2010). In recent years, numerous studies have confirmed many of these medicinal properties (Alonso and Desmarchelier, 2005; Schmidt et al., 2009). Abundant evidence also indicates that *Schinus molle* var. *areira* has insecticidal properties. In our laboratory, we observed that different extracts and essential oils from its leaves and fruits produced repellent and insecticidal effects on many insect pests (Chirino et al., 2001; Ferrero et al., 2006, 2007b; Descamps et al., 2008; Benzi et al., 2009).

Given that it is interesting to evaluate the potential of this plant as a therapeutic agent to treat various diseases and as an alternative for biological control of major pests, it is important to assess the harmful effects that such use could have on mammals. In recent works, we observed that the oral acute and subacute exposure to the ethanolic extract from fruits of *Schinus molle* var. *areira* in rats produced no toxicity in any of the tested animals; only a transitory stimulant effect was observed on the exposed rats (Ferrero et al., 2007a). When this extract was evaluated after oral subchronic exposure in mice, females showed leukocyte alterations and a lower total cholesterol level. On the other hand, the

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ethanolic extract from leaves exhibited a diuretic and a stimulant effect after oral subchronic treatment. In addition, a tendency to decrease blood glucose in males was observed in this assay (Bras et al., 2010).

This evidence suggests that *Schinus molle* var. *areira* could be very useful for the treatment of several pathologies and for pest control, as an alternative to the use of synthetic insecticides, without involving serious risk to human health. However, another potential route of exposure to this plant could be the dermal route, either for its topical use in medicine or for its usefulness as an insect repellent. For these reasons, in the present work we studied the acute dermal exposure to ethanolic and hexanic extracts from leaves of *Schinus molle* var. *areira* in rats. The nervous system functionality was analyzed by means of a Functional Observational Battery (FOB) and by assessing the motor activity in an open field. Also, histopathological examination was realized on several tissues.

## 2. Materials and methods

### 2.1. Plant material

Leaves from *Schinus molle* var. *areira* L. were collected in Bahía Blanca city, in the south of Argentina, in 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 and hexanic plant extracts

Fresh leaves (0.3 kg) from *Schinus molle* var. *areira* were macerated in 2 l of ethanol and 1.5 kg of the same material was macerated in 10 l of hexane at room temperature for 72–96 h. The solvents were completely evaporated at reduced pressure using a rotavap (LABOROTA 4000, Heidolph) at 180 rpm in water bath at 40 °C. The crude extracts obtained were kept at 4 °C until further use (yield: 11.9% and 1.8% from leaf material of ethanolic and hexanic extracts respectively).

### 2.3. Experimental animals

According to the OECD Guideline N° 402 (OECD, 1987), healthy young adult Wistar rats of both sexes were used. They were obtained from the animal colony of the Bioterio from the Biology, Biochemistry and Pharmacy Department and they 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 the experiment. According to the body weight (270–330 g for females and 420–480 g for males), they were randomly divided in groups of 8 animals which were housed in cages by sex 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, Eighth Edition (2010) as adopted and promulgated by the National Institute of Health.

### 2.4. Dermal acute exposure to the extracts

The experiment was conducted according to the protocols described by OECD Guideline N° 402 (OECD, 1987). The limit test was performed because no severe toxic effects we expected for exposure to these plant extracts.

The day before the test, the dorsal area of the trunk of all animals was carefully shaved. Eight rats of each sex were assigned to one of the three treatment groups: control (C), exposed to ethanolic extract (EE) and exposed to hexanic extract (HE). Each extract was applied uniformly over the clean skin of each rat in a dose of 2000 mg/kg of body weight and covered with a gauze patch and a plastic sheet which were held in place with non-irritating tape. The exposed surface area was approximately 10% of the total body surface area. Control animals were treated as the exposed ones, but without the application of the extract. During the exposure period of 24 h, animals were individually caged.

At the end of the exposure, the residual extract was carefully removed with water and any sign of local skin reaction, as erythema or edema, was recorded. Then, behavioral and functional parameters and motor activity were assessed in all the animals. Subsequently, animals were housed in their respective group cages and maintained there for 14 days. After that, the same parameters were evaluated to determine reversibility, persistence, or delayed occurrence of toxic effects. Finally, all the animals were euthanized and necropsy observations and histopathological examinations were carried out on several tissues.

### 2.5. Local skin reactions

At the end of the exposure, the test site of the animals was examined immediately after the patch was removed and a qualitative evaluation was made. Sings of local skin reactions were graded and recorded according to the following dermal irritation scoring system: 0 – no erythema or no edema, 1 – barely perceptible erythema or edema, 2 – well defined erythema or slight edema (edges of area well defined by definite raising), 3 – moderate to severe erythema or moderate edema (raised approximately 1 mm), 4 – severe erythema or edema (raised more than 1 mm and extending beyond the area of exposure) (Hayes et al., 2008). Any erythema or edema evidence was closely observed in subsequent days to determine its reversibility or irreversibility.

### 2.6. Functional observational battery (FOB)

After the day of exposure, behavioral and functional parameters of the animals were evaluated through a FOB. This included a thorough description of the animals' appearance, behavior, and functional integrity. Modeled on the clinical neurologic exam, this observational test assesses a variety of functional domains and is able to reliably detect nuances of behavioral change (US EPA, 1998; Markgraf et al., 2010). Procedural details and scoring criteria for the FOB protocol were performed according to Moser (2000) and were described by Ferrero et al. (2007a).

### 2.7. Open field observations

Motor activity is considered to be a test of nervous system function, and it reflects the integrated output of the sensory, motor and associative processes of the nervous system in case of the absence of systemic toxicity (Hübler et al., 2005). The locomotor activity of all animals was evaluated in an open field. Each rat was placed in an open arena 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 rat with all four paws, rearings (occasions on which the animals stood on their hind legs), groomings (face washing, forepaw licking and head stroking) and fecal boluses were scored each 5 min for 15 min. The number of squares crossed by rats and the rearings made by them were recorded as parameters of locomotor activity, whereas the number of groomings performed by rats and the number or fecal boluses deposited by them was considered as parameters of emotionality (Choleris et al., 2001; Maimanee

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et al., 2003). After each animal was removed, the open field was carefully cleaned with a damp cloth.

### 2.8. Histopathological examinations

All the animals were euthanized and necropsy observations and histopathological examinations were carried out on several tissues. Liver, kidneys, stomach and brain were weighed and intestine total length was recorded. Representative fragments of these organs were fixed in formol 10%, dehydrated by ethanolic solutions of increased graduation (70°, 80°, 96° and 100°) and xylene. These samples were included in paraffin in a stove at 58–60 °C. The histological sections of 5 μm thick were performed in Minot type microtome (Leica, RS 2165) and were stained with hematoxylin-eosin. Examination of histological sections was performed by light microscope (Olympus Bx51) and digital camera (Olympus C7070, Tokyo, Japan).

### 2.9. Statistical analysis

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

The data obtained in 15 total minutes of observation in the Open Field were analyzed using a two-way ANOVA followed by post hoc comparisons using Student *t*-test. For comparative analysis of the parameters evaluated every 5 min a repeated-measures ANOVA was used. Subsequently, to analyze the differences in each period of 5 min within each group, a *t*-test for paired samples was used, and to analyze the differences in each period of 5 min between groups, a two-way ANOVA was conducted.

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. Local skin reactions

Some of the animals exposed to ethanolic extract of leaves of *Schinus molle* var. *areira* showed slight to moderate erythema and no sign of edema after removing the patch of the test site. On the other hand, all animals exposed to the hexanic extract, exhibited slight to moderate erythema and slight edema, with well defined edges of the area. However, all these skin reactions disappeared in the subsequent 48 h.

### 3.2. Functional observational battery

Data obtained in the FOB carried out after the 24 h of exposure to extracts (Day 1) and after 14 days of observations (Day 14) are shown in Table 1.

Male rats exposed to hexanic extract from leaves of *Schinus molle* var. *areira* exhibited incremented levels of activity, rearing and arousal during the observations in the open arena on Day 1, compared to control male rats ($p < 0.05$ for all parameters). On Day 14, these effects were reverted (Fig. 1).

The other parameters evaluated in the FOB on Days 1 and 14, were not altered in either of the experimental groups compared to control groups.

### 3.3. Open field evaluations

During the 15 total minutes of observation in the open field carried out on Day 1, no significant differences between groups in the number of squares crossed and in the number of rearings done by rats were detected. However, on Day 14 the males exposed to ethanolic extract performed a higher number of rearings during the 15 min compared to the control ones ($p < 0.05$). Females exposed to this extract also showed similar behavior although the difference was not significant (Fig. 2).

To compare the activity in each period of 5 min separately, the data were analyzed using repeated measures ANOVA with Periods as within-subject factor and Group and Sex as between-subject factors. The analysis of data from Day 1 showed significant differences in the number of squares and in the number of rearings between the three periods of 5 min ($F_{(2;\,84)} = 129.72$; $p < 0.001$ and $F_{(2;\,84)} = 180.09$; $p < 0.001$, for each parameter respectively). By comparing the number of squares crossed and rearings performed by each group in each period of 5 min using paired samples *t*-test, all groups of rats showed greater locomotor activity during the first period of 5 min and declined in the second and in the third period ($p < 0.05$ for all comparisons). This gradual and significant decrease in their locomotion activity throughout the test session reflected that all animals have habituated to the OF. Fig. 3 illustrates the profiles of locomotor activity of all groups during sessions on Day 1 and Day 14.

When analyzing the data obtained on Day 14, the repeated measures ANOVA detected significant differences in activity parameters evaluated among periods of 5 min ($F_{(2;\,84)} = 121.20$; $p < 0.001$ for the number of squares, and $F_{(2;\,84)} = 158.73$; $p < 0.001$ for the number of rearings). In both cases, the paired samples *t*-test showed significant differences between the first period of 5 min and the second and third one within all groups ($p < 0.05$ for all comparisons), while differences between the second and the third period of 5 min were not significant.

On Day 14, repeated measures ANOVA also detected a significant interaction between the factors Period × Group in the number of rearings ($F_{(4;\,84)} = 3.22$; $p < 0.05$) and a significant difference between Groups ($F_{(2;\,42)} = 4.16$; $p < 0.05$). By analyzing each period of 5 min separately, it was observed that the number of rearings performed by both groups of females and males exposed to ethanol extract was higher than controls during the first 5 min ($p < 0.05$ for both cases).

In summary, on Day 1, the animals exposed to extracts showed the same activity levels to those of controls. However, on Day 14, males exposed to ethanol extract showed more rearings than controls during the first period of 5 min, and therefore, in the 15 total minutes of observation in the open field. Females exposed to the same extract also performed higher number of rearings than controls during the first 5 min, but this was not reflected in the 15 total minutes.

Besides, no significant differences between groups in the parameters of emotionality (grooming and number of fecal boluses deposited) assessed on Day 1 and on Day 14 were detected (data not shown).

### 3.4. Histopathological analysis

The histopathological examinations of liver, kidney, stomach, intestine and brain showed no morphological changes in rats exposed to both extracts, compared to control ones.

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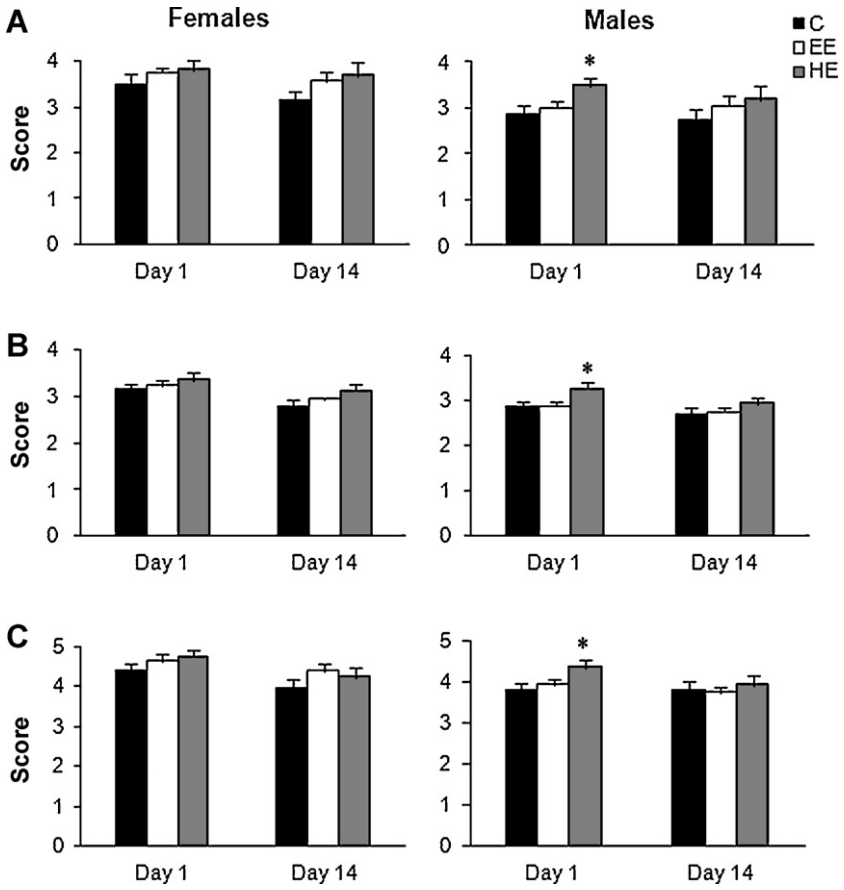


**Fig. 1.** Open field activity (panel A), rearing (panel B) and arousal (panel C) performed by female and male rats of control (C), exposed to ethanolic extract (EE) and exposed to hexanic extract (HE) groups during FOB evaluated on Day 1 and Day 14 after the dermal acute exposure to the extracts from leaves of *Schinus molle* var. *areira*. Data are expressed as the mean score of the scale used ± SEM, $n = 8$. $^{*}p < 0.05$ compared to control group.

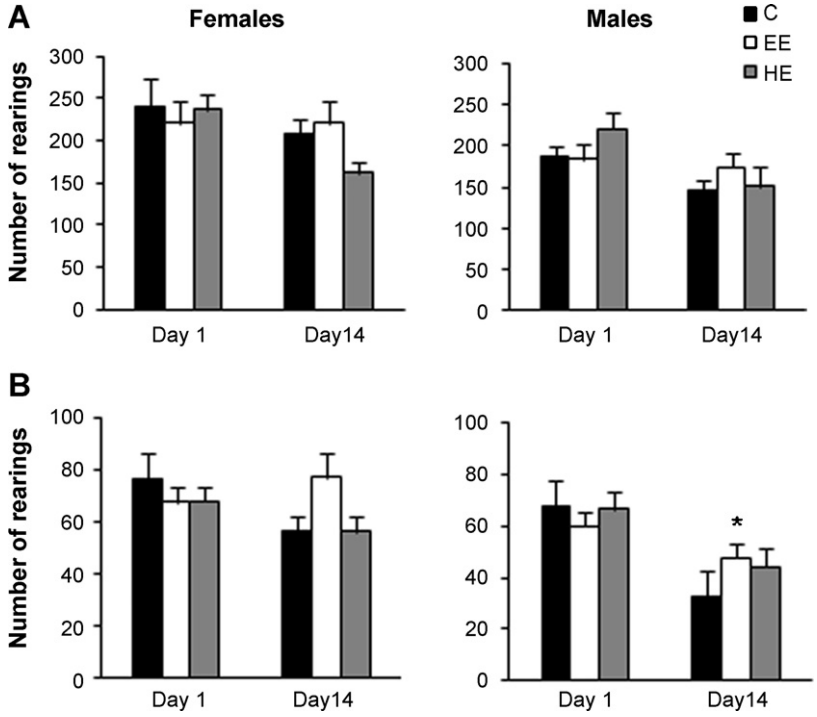


**Fig. 2.** Number of squares crossed (panel A) and number of rearings performed (panel B) by female (left side) and male (right side) rats of control (C), exposed to ethanolic extract (EE) and exposed to hexanic extract (HE) groups in the 15 total minutes of observation in the open field on Day 1 and Day 14 after the dermal acute exposure to the extracts from leaves of *Schinus molle* var. *areira*. Data are expressed as the Mean ± SEM, $n = 8$. $^{*}p < 0.05$ compared to control.

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**Table 1**
Parameters evaluated in the Functional Observational Battery after the dermal acute exposure to ethanolic and hexanic extracts from leaves of *Schinus molle* var. *areira* (Day 1) and after 14 days of the exposure (Day 14).

| | Day 1 | | | | | | Day 14 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Females | | | Males | | | Females | | | Males | | |
| | C | EE | HE | C | EE | HE | C | EE | HE | C | EE | HE |
| **Home cage observations** | | | | | | | | | | | | |
| Normal body posture (D) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Activity (R) | 1.50 | 1.63 | 1.38 | 1.38 | 1.38 | 1.00 | 2.50 | 3.00 | 2.25 | 2.75 | 2.00 | 1.75 |
| Palpebral closure (R) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Tremors (R) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Convulsions (D) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Biting (D) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vocalizations (B) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Hand-held observations** | | | | | | | | | | | | |
| Ease removal from cage (R) | 1.31 | 1.13 | 1.25 | 1.00 | 1.00 | 1.13 | 1.00 | 1.19 | 1.13 | 1.00 | 1.06 | 1.00 |
| Ease of handling (R) | 1.13 | 1.56 | 1.00 | 1.13 | 1.13 | 1.00 | 1.06 | 1.06 | 1.31 | 1.00 | 1.00 | 1.06 |
| Lacrimation (R) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Salivation (R) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Normal fur appearance (D) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Limb grasping (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| **Open field observations** | | | | | | | | | | | | |
| Activity level (R) | 3.50 | 3.75 | 3.81 | 2.88 | 3.00 | 3.50* | 3.13 | 3.56 | 3.69 | 2.75 | 3.06 | 3.19 |
| Rearing (R) | 3.19 | 3.25 | 3.38 | 2.88 | 2.88 | 3.25* | 2.81 | 2.94 | 3.13 | 2.69 | 2.75 | 2.94 |
| Arousal (R) | 4.38 | 4.63 | 4.75 | 3.81 | 3.94 | 4.38* | 3.94 | 4.38 | 4.25 | 3.81 | 3.75 | 3.94 |
| Normal gait (D) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Normal posture (D) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Unusual movements (D) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Stereotyped behaviors (D) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fecal boluses (C) | 0 | 0 | 0.38 | 0.50 | 0.13 | 0.13 | 0 | 0 | 0 | 0.63 | 0.38 | 0.88 |
| Urine pools (C) | 0.25 | 0 | 0.25 | 4.88 | 6.25 | 1.88 | 0 | 0 | 0 | 3.00 | 7.00 | 4.13 |
| Diarrhea (B) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Manipulative tests** | | | | | | | | | | | | |
| Click response (R) | 2.00 | 2.13 | 2.00 | 2.13 | 2.06 | 2.00 | 1.94 | 2.00 | 2.00 | 1.94 | 2.06 | 2.00 |
| Approach response (R) | 2.13 | 2.00 | 2.00 | 1.94 | 1.88 | 2.00 | 2.00 | 2.00 | 2.00 | 1.94 | 2.00 | 2.00 |
| Touch response (R) | 1.94 | 2.00 | 1.94 | 1.94 | 1.94 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Tail pinch response (R) | 1.88 | 2.19 | 2.25 | 2.25 | 2.06 | 1.88 | 2.00 | 1.56 | 1.63 | 2.06 | 1.94 | 2.00 |
| Pupil response (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Palpebral reflex (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Pinna reflex (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Flexor reflex (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Extensor reflex (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Forelimb hopping (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Proprioceptive reaction (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Forelimb extension (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Hindlimb extension (B) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Surface righting reflex (R) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Aerial righting reflex (R) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Landing foot splay (C) | 4.21 | 4.01 | 3.38 | 4.99 | 4.48 | 4.85 | 4.90 | 5.05 | 4.68 | 5.86 | 6.43 | 7.17 |

Descriptive (D) and binary (B) data expressed as percentage of incidence (analyzed by chi-square test).
Ranked (R) data expressed as the mean score of the scale used (analyzed by Kruskal–Wallis and Mann–Whitney tests).
Continuous (C) data expressed as mean value (analyzed by two-way ANOVA and *t*-test).
C, control group; EE, ethanolic extract group; HE, hexanic extract group.
* $p < 0.05$ compared to control group.

## 4. Discussion

Plants have been used throughout the ages for its various medicinal properties and also to combat microbes and insects. Currently, several studies have confirmed many of the biological activities attributed to them. However, it is also important to evaluate the toxic or deleterious effects that their use could cause in the short, medium or long term and also to take into account the possible routes of exposure for humans. For this reason, in this study we evaluated the effects of acute exposure of ethanol and hexanic extracts of leaves of *Schinus molle* var. *areira* in rats. Because its use may be topical, the dermal route was assessed.

On the one hand, observations were made locally at the site of skin contact with each of the extracts, to detect reactions to them. The results showed signs of mild transient irritation (erythema caused by the ethanol extract, and erythema and edema in the case of the hexanic extract) in some of the exposed animals. These signs reverted after 48 h. Since the dose tested was 2 g/kg of body weight (limit dose recommended for this type of study), it could be assumed that at therapeutic doses the effects would be imperceptible. In addition, it should be said that Alonso and Desmarchelier (2005), noted that the essential oil applied to skin of pigs and mice was found to be inactive as an irritant or vesicant substance, while in rabbits also was slightly irritating.

In this study, no significant alteration in FOB parameters evaluated in rats exposed to ethanol extract from leaves of *Schinus molle* var. *areira* was detected. This result agreed with those obtained in the FOB performed after subchronic oral exposure of this extract in mice (Bras et al., 2010). On the other hand, the hexanic extract from leaves of *Schinus molle* var. *areira* caused an increase in the parameters of activity, rearing and arousal in the open arena in males exposed to it. These stimulating effects reverted after 14 days, when the animals were retested. These results were also observed in the FOB performed after the oral acute and subacute exposure of the

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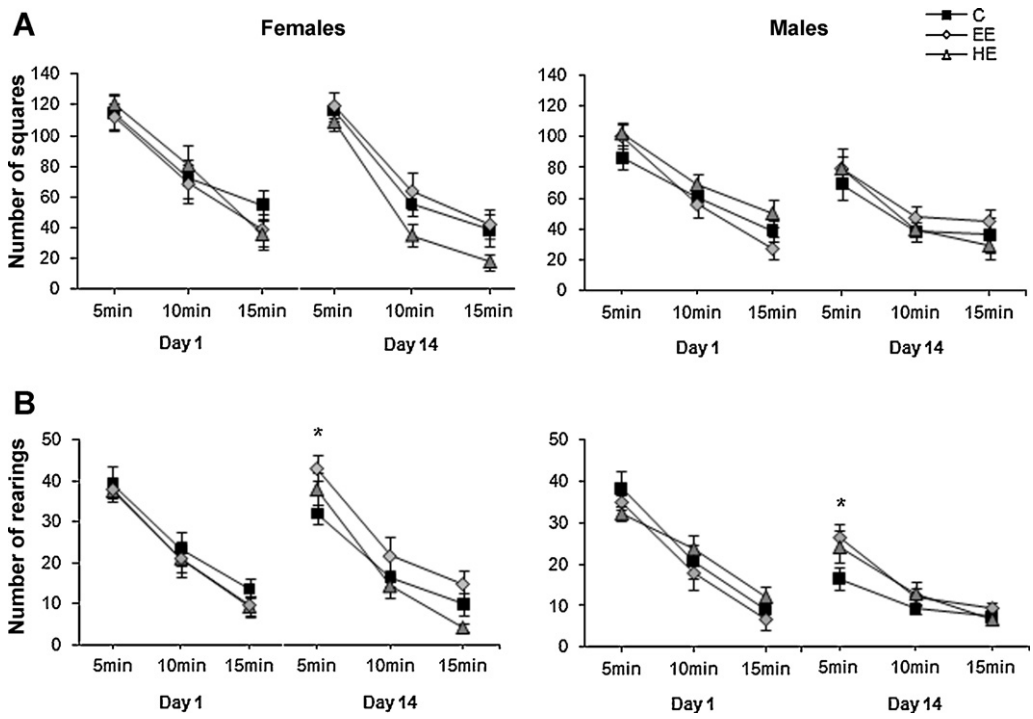


**Fig. 3.** Number of squares crossed (panel A) and number of rearings performed (panel B) by female (left side) and male (right side) rats of control (C), exposed to ethanolic extract (EE) and exposed to hexanic extract (HE) groups in the three periods of 5 min of observation in the open field on Day 1 and Day 14 after the dermal acute exposure to the extracts from leaves of *Schinus molle* var. *areira.* Data are expressed as the Mean ± SEM, $n = 8$. $^{*}p < 0.05$ significant difference of ethanolic extract group compared to control.

ethanolic extract from fruits of *Schinus molle* in rats (Ferrero et al., 2007a).

Furthermore, the evaluation in the open field showed a higher locomotor activity of animals exposed to ethanol extract from leaves of *Schinus molle* var. *areira* reflected by the increased number of rearings performed during the first 5 min of observation in the session carried out on Day 14. This same parameter was also altered after the subchronic oral exposure to this extract in mice (Bras et al., 2010), whereas, after subacute oral exposure to the ethanol extract from fruits, a hyperactive behavior in the OF was also observed (Ferrero et al., 2007a). All this evidence suggests that the different extracts of *Schinus molle* var. *areira* produce some kind of mild and transient stimulatory effect, both after oral and dermal exposure.

In recent studies, Machado et al. (2007, 2008) reported an antidepressant-like effect of the ethanolic and hexanic extracts from leaves of *Schinus molle* and showed that this effect depends on the interaction with the serotonergic, noradrenergic and dopaminergic systems. It is known that these neurotransmission pathways, as well as those related to glutaminergic and GABA systems, are involved in locomotor regulation (Vezina and Kim, 1999; Carey et al., 2004; Viggiano, 2008), so it would be possible to infer that some of the compounds present in the extracts of *Schinus molle* var. *areira* may produce an alteration in these systems, which is reflected in the stimulating effect observed.

The histopathological analysis of the studied organs did not detect abnormalities in them, so it could be assumed that dermal exposure to extracts produced no systemic toxicity.

Taking this into account and the fact that the dose used in this study was the limit dose recommended for this type of assays, it may be concluded that the acute dermal exposure of the ethanolic and hexanic extracts from leaves of *Schinus molle* var. *areira* only causes a slight and reversible skin irritation, and a mild stimulatory effect in rats. All these results indicate that the topical use of these extracts would be safe, either as a therapeutic agent or as an insect repellent.

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## References

Alonso, J., Desmarchelier, C., 2005. Plantas medicinales autóctonas de la Argentina. Bases científicas para su aplicación en atención primaria de la salud. Editorial L.O.L.A, Buenos Aires, pp. 26–33.

Benzi, V., Stefanazzi, N., Ferrero, A.A., 2009. Biological activities by essential oils from leaves and fruits of pepper tree (*Schinus molle* L.) to control rice weevil (*Sitophilus oryzae* L.). Chilean Journal of Agricultural Research 69, 154–159.

Bras, C., Domínguez, S., Codón, S., Minetti, A., Ferrero, A., 2010. Consequences of subchronic exposure to ethanolic extract from fruits and leaves of *Schinus molle* var. *areira* L. in mice. Journal of Ethnopharmacology 132, 321–327.

Carey, J.R., DePalma, G., Damianopoulos, E., Hopkins, A., Shanahan, A., Müller, P.C., Huston, J.H., 2004. Dopaminergic and serotonergic autoreceptor stimulation effects are equivalent and additive in the suppression of spontaneous and cocaine induced locomotor activity. Brain Research 1019, 134–143.

Chirino, M., Cariac, M., Ferrero, A.A., 2001. Actividad insecticida de extractos crudos de drupas de *Schinus molle* L. (Anacardiaceae) sobre larvas neonatas de *Cydia pomonella* L. (Lepidoptera: Torticidae). Boletín de Sanidad Vegetal. Plagas 27, 305–314.

Choleris, E., Thomas, A.W., Kavaliers, M., Prato, F.S., 2001. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neuroscience and Biobehavioral Reviews 25, 235–260.

Descamps, L.R., Stefanazzi, N., Sánchez Chopa, C., Ferrero, A.A., 2008. Actividad biológica de extractos vegetales de *Schinus molle* var. *areira* (Anacardiaceae) en *Tribolium castaneum* Herbst. (Insecta, Coleoptera, Tenebrionae), plaga de grano almacenado. Boletín de Sanidad Vegetal. Plagas 34, 595–606.

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Ferrero, A., Minetti, A., Bras, C., Zanetti, N., 2007a. Acute and subacute evaluation of ethanolic extract from fruits of *Schinus molle* in rats. Journal of Ethnopharmacology 113, 441–447.

Ferrero, A.A., Sánchez Chopa, C., Werdin González, J.O., Alzogaray, R.A., 2007b. Repellence and toxicity of *Schinus molle* extracts on *Blattella germanica*. Fitoterapia 78, 311–314.

Ferrero, A.A., Werdin Gonzalez, J.O., Sanchez Chopa, C., 2006. Biological activity of *Schinus molle* (Anacardiaceae) on *Triatoma infestans*, Klug 1834 (Hemiptera, Reduviidae). Fitoterapia 77, 381–383.

Hayes, B.B., Patrick, E., Maibach, H.J., 2008. Dermatotoxicology. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology. , fifth edition. CRC Press, Boca Raton, FL, p. 1383.

Heywood, V.H., 1993. Flowering Plants of the World. Update Oxford University Press, New York.

Hübler, N., Gottschling, B., Jacobs, M., von Landenberg, F., Hewicker-Trautwein, M., 2005. Functional observational battery and motor activity in rats after single administration of two NHE 1 inhibitors. Toxicology and Applied Pharmacology 208, 266–276.

Machado, D.G., Bettio, L.E.B., Cunha, M.P., Santos, A.R.S., Pizzolatti, M.G., Brighente, I.M.C., Rodrigues, A.L.S., 2008. Antidepressant-like effect of rutin isolated from the ethanolic extract from *Schinus molle* L. in mice: evidence for the involvement of the serotonergic and noradrenergic systems. European Journal of Pharmacology 587, 163–168.

Machado, D.G., Kaster, M.P., Binfaré, R.W., Dias, M., Santos, A.R.S., Pizzolatti, M.G., Brighente, I.M.C., Rodrigues, A.L.S., 2007. Antidepressant-like effect of the extract from leaves of *Schinus molle* L. in mice: evidence for the involvement of the monoaminergic system. Progress in Neuro-Psychopharmacology & Biological Psychiatry 31, 421–428.

Maimanee, T.A., Brain, P.F., Zari, T.A., 2003. Dietary fats influence "open-field" behavior of male and female laboratory mice. Laboratory Animals 37, 222–232.

Markgraf, C.G., Cirino, M., Meredith, J., 2010. Comparison of methods for analysis of functional observation battery (FOB) data. Journal of Pharmacological and Toxicological Methods 62, 89–94.

Martínez, G.J., Barboza, G.E., 2010. Natural pharmacopoeia used in traditional Toba medicine for the treatment of parasitosis and skin disorders (Central Chaco, Argentina). Journal of Ethnopharmacology 13, 86–100.

Moser, V.C., 2000. The functional observational battery in adult and developing rats. Neurotoxicology 21, 989–996.

OECD, 1987. Test No. 402: Acute Dermal Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. OECD Publishing.

Schmidt, C., Fronza, M., Goettert, M., Geller, F., Luik, S., Flores, E.M.M., Bittencourt, C.F., Zanetti, G.D., Heinzmann, B.M., Laufer, S., Merfort, I., 2009. Biological studies on Brazilian plants used in wound healing. Journal of Ethnopharmacology 122, 523–532.

Taylor, L., 2005. The Healing Power of Rainforest Herbs. Square One Publishers, Garden City Park, NY, p. 535.

United States Environmental Protection Agency (US EPA), 1998. Health Effects Test Guidelines, OPPTS 870.6200, Neurotoxicity Screening Battery.

Vezina, P., Kim, J., 1999. Metabotropic glutamate receptors and the generation of locomotor activity: interactions with midbrain dopamine. Neuroscience and Biobehavioral Reviews 23, 577–589.

Viggiano, D., 2008. The hyperactive syndrome: metanalysis of genetic alterations, pharmacological treatments and brain lesions which increase locomotor activity. Behavioural Brain Research 194, 1–14.