ANTI-INFLAMMATORY AND ACUTE TOXICITY EVALUATION OF AQUEOUS INFUSION EXTRACT OBTAINED FROM AERIAL PARTS OF MARRUBIUM DESERTI DE NOÉ GROWING IN ALGERIA

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Abstract

Background: *Marrubium deserti* de Noé, which is locally known as "Merriouet saharaui", is widely used in Algeria as a traditional treatment of many ailments. In this study, the anti-inflammatory and acute toxicity of the aqueous infusion extract from aerial parts of *Marrubium deserti* were investigated. Meanwhile, acute oral toxicity of *M. deserti*, as well as its anti-inflammatory activity is reported for the first time.
Materials and Method: The anti-inflammatory activity was evaluated using carrageenin-induced paw oedema in mice at three different doses (250, 500 and 1000mg/kg body weight. Data were analyzed by one-way ANOVA followed by Dunnett's t-test.
Results: The aqueous infusion extract (250, 500 and 1000mg/kg body weight, orally administered, n=6) showed a significant ($P<0.05$) inhibition of carrageenin-induced mice paw oedema by 11.22, 20.73 and 44.03% respectively in the third hour when compared to the control group. Acetylsalicylic acid (ASA, 50mg/kg) as the positive control showed 32.08% inhibition. The oral LD_{50} values in mice were found to be greater than 2000mg/kg. The relatively high oral median lethal dose (>2000mg/kg) suggests that the aqueous infusion extract has a relatively low acute toxicity when taken orally for a single dose.
Conclusions: The present study indicates that *M. deserti* has a significant anti-inflammatory effect and confirms its traditional use as a treatment of pain, yet it suggests further investigations to be carried out to determine the active chemical constituents.

Keywords: *Marrubium deserti*, aqueous infusion extract, acute toxicity, anti-inflammatory activity.

Introduction

Inflammation is clinically defined as a pathophysiological process characterized by redness, oedema, fever, pain, and loss of function (Kim et al., 2004; Hunskaar et Hole, 1987).

Although, the currently used steroidal anti-inflammatory drugs (SAID) and nonsteroidal anti-inflammatory drugs (NSAID) in the relief of inflammatory disorders (Li et al., 2003; Dugowson et Gnanashanmugam, 2006), these conventional drugs have various and severe adverse effects (Perini et al., 2004). Therefore, there is a need to develop new anti-inflammatory agents with minimum side effects (Raquibul Hasan et al., 2009).

Plants are an important source of new chemical substances with potential therapeutic use of inflammation. As a result, more people are turning to herbal medicine as an alternative treatment (Rashid et al., 2014; Calixto et al., 2000). The genus *Marrubium* consists of about 97 species of Lamiaceae (Popoola et al., 2013).

Most of them grow in the Mediterranean region, moderate zones of the Eurasian continent and some countries of Latin America (Rigano et al., 2007; Meyre-Silva et al., 2005).

In Algeria, there are seven species of *Marrubium* mentioned by Quezel and Santa (1963): *M. vulgare* L., *M. supinum* L., *M. peregrinum* L., *M. alysson* L., *M. alyssoides* Pomel, *M. willkommii* Magn. and *M. deserti* de Noé, but Ozenda (1991) cited only one species which is *M. deserti* de Noé. This plant is an Algerian endemic species popularly known as "Merriouet saharaui" which grows spontaneously in sandy and stony valleys. *M. deserti* is used in traditional medicine to treat respiratory diseases, fever (Hammiche et Maiza, 2006), digestive disorders, colic, intestinal parasitosis, helminthiasis, dysmenorrhoea, cough (Ould El Hadj et al., 2003) and scorpion stings (Zaabat et al., 2011).

Bibliographical surveys showed that a few phytochemical and biological investigations so far have been carried out on this species. Recently, pharmacological studies have indicated that *M. deserti* has antioxidant, antiviral, antigenotoxic and anti-microbial activities (Laouer et al., 2009; Edziri et al., 2007; Edziri et al., 2012).

Moreover, Phytochemical studies with *M. deserti* have also revealed the presence of diterpene, sterols, flavonoids and phenylpropanoids (Dendougui et al., 2011; Zaabat et al., 2010). Also, the analysis of the essential oils of *M. deserti* by GC-MS showed that the major constituents were δ-cadinene, germacrene D, trimethylpentadeca-2-one and 9-methyl-undecene (Chebrouk et al., 2011).

In spite of the various use of *M. deserti* in a large number of Algerian traditional preparations, there has been no published work regarding its acute toxicity and anti-inflammatory activity. The aim of this study was to investigate the anti-inflammatory activity and the acute toxicity (LD_{50}) of aqueous infusion extract of *M. deserti* aerial parts using the carrageenin-induced mice paw oedema model.

An attempt to determine the chemical constituents responsible for this anti-inflammatory activity was made.

Materials and Methods
Plant Material

Marrubium deserti de Noé was collected in the flowering stage on April 2012 in Ain Zaatout which is located in the north of Biskra (northeast of Algeria; longitude between 4°15' and 6°45' East, and latitude North between 35°15' and 33°30'). Plant samples were identified by

the national institute of the agronomic research of Algeria (INRAA), Tamanrasset, Algeria. The aerial parts of the plants were dried in the shade at room temperature and then preserved in paper bags in order to avoid possible fungal contamination. Plants were cut to small pieces, then crushed into a fine powder by a mechanical grinder and used for further studies. The aqueous infusion extract was prepared according to the traditional method, the fine powder of *M. deserti* was dissolved in boiling normal saline water (100ml); the hot infusion was then left to reach room temperature and filtered. The filtrate was used for biological tests on animals at different doses.

Animals

Albino mice (*Mus musculus*) weighing 20±5g, were kindly provided by the laboratory of microbiology of centre of research and development (CRD-Saidal), Algeria. The animals were fed pellet diet from the national office of animal feed, Bejaia (ONAB) and water *ad libitum*. They were also kept and maintained under standardized laboratory conditions of temperature and light (24±1°C and 12h light/dark cycle), respectively with constant humidity of 50%.

Acute toxicity assessment

Acute oral toxicity of the aqueous infusion extract of *M. deserti* was evaluated in both sexes albino mice, as per OECD guideline (organization for economic co-operation and development, Guideline-423, adopted on 17th December 2001), with slight modifications. Thirty animals were equally divided into three groups (n=10) as per sex (5 males and 5 females). They were fasted for 18h prior to experiments. The aqueous infusion extract was administered with a dose of 300 and 2000mg/kg body weight whereas the control group received normal saline water only. Food and water was withheld for a further 3-4 hours after drug administration. Mice were closely observed for 4 hours after the initial administration and then once daily for the following days. The behavioral changes closely observed for were: hyperactivity, ataxia, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Total observation period for eventual mortality was 14 days (Sim et al., 2010).

Anti-inflammatory activity

Albino mice were fasted for 16 hours before the experiments and then divided into five groups each contains six mice. The aqueous infusion extract of aerial parts of *M. deserti* was administered to three different groups at different doses (250, 500 and 1000mg/kg body weight), while the other two groups served as negative and positive controls and received normal saline water and standard drug; acetylsalicylic acid (ASA, 50mg/kg), respectively. After one hour, acute inflammation was produced by injecting 0.05ml of carrageenin (prepared as 1% suspension in normal saline water with the addition of 1 to 2 drops of Tween-80) into the plantar aponeurosis of the left hind paw of mice. Then, after 3 hours from the injection of carrageenin suspension, the animals are quickly sacrificed by using the diethyl ether. The two posterior legs were very quickly excised at the tarso-articulation level and then weighed using an analytical balance (Levy, 1969). For each group, we calculated, the increase in the weight of the swollen leg (left posterior leg), which received the carrageenin suspension compared to the weight of the healthy leg (right posterior leg) according to the formula:
Left Leg Weight (LLW) - Right Leg Weight (RLW). We also calculated the mean of weight (M) for each group. The evolution of the oedema (% oedema) is given by measuring the mean of both legs for each group according to the formula:

$$\% \text{ Oedema} = \frac{M(LLW) - M(RLW)}{M(RLW)} X100$$

The determination of the percentage of oedema inhibition allowed us to evaluate the anti-inflammatory potential of the aqueous infusion extract and to compare it with the reference drug (ASA). The percentage of oedema inhibition between both tested and control group is calculated as follows:

$$\% \text{ Oedema inhibition} = \frac{\% \text{ of the control oedema} - \% \text{ of the test oedema}}{\% \text{ of the control oedema}} X100$$

HPLC analysis

High-performance liquid chromatography (HPLC) was performed using a Waters Alliance 2695 chromatography system and UV detector with bars of diodes surveyor. Chromatographic runs were performed using C_{18} column (20X4.6 mm, 5µm). The chromatographic data system was controlled by the Empower Software from Waters. The extract was analyzed using solvent system B (methanol) in solvent system A (0.1% phosphoric acid in water) with the following gradient: 1-10min (30% B); 1-20min (30 to 40% B); 20-60min (40 to 100% B). The solvent flow rate was 1ml/min.

Statistical analysis

The data were presented as Mean±S.D. Significance between control and treated groups was tested by one-way ANOVA followed by Dunnett's t-test. P<0.05 was considered to be significant.

Results and Discussion
Acute toxicity effect

Herbal medicine is gaining popularity in developing countries. Such remedies are often believed to be harmless; hence these treatments are natural and commonly used for self-medication without supervision (Rosidah et al., 2009). Experimental screening methods are, therefore, important in order to ascertain the safety and efficiency of herbal products as well as to establish its active components (Ogbonnia et al., 2008). The investigation of acute toxicity is the first step in the toxicological analysis of herbal drugs (Déciga-Campos et al., 2007). Overall, animal models have a good predictability for human toxicities of around 70-80% (Kola et Landis, 2004). Since no reports on toxicity and safety of the aqueous infusion extract of *M. deserti* are available, it was necessary to study the toxicity of the extract tested to maximize their benefits. The results of this study showed that the aqueous infusion extract of *M. deserti* given in a single dose of 300 and 2000mg/kg body weight had no effect and did not produce any abnormal behavior during 4 hours after drugs administration. Throughout the 14 days observation period, there were no significant changes in behavior and no mortality in both sexes of mice, therefore, the medium lethal dose (LD_{50}) value of orally administered *M. deserti* aqueous infusion extract is greater than 2000mg/kg. This dose is considered moderately toxic according to the classification of Hodge et Sterner (1949).

Anti-inflammatory activity

The carrageenin-induced inflammatory response was described by Winter et al. (1962) in the rats and in 1969 in mice (Levy, 1969). We determined the anti-inflammatory activity by using inhibition of carrageenin-induced paw oedema which is the standard experimental model for acute inflammation (Magaji et al., 2008; Patel et al., 2010). The results are presented in Table 1, and were comparable to those obtained from the negative control group.

Table 1: The oedema percentage on the carrageenin-induced paw oedema of mice				
Treatment	Dose (mg/kg)	M (LLW) (g)	M (RLW)(g)	% Oedema
Control	-	0,1096±0,129	0,087±0,005	26%
ASA	50mg/kg	0,1059±0,012	0,0904±0,01	17,66%
Aqueous infusion Extract of M. deserti	250mg/kg	0,1381±0,03[*]	0,1122±0,021[**]	23,08%
	500mg/kg	0,1656±0,01[***]	0,1373±0,009[***]	20,61%
	1000mg/kg	0,1527±0,001[***]	0,1333±0,013[***]	14,55%
One way ANOVA	F 15,98		13,82	
P < 0,05	< 0,05			df 2,758

Values represent Mean±S.D (n=6 in each group), [*]$P<0.05$, [**]$P<0.01$, [***]$P< 0.001$ as compared to control was considered significant. Percentage of the oedema is calculated as: %Oedema= [M (LLW)-M (RLW)/M (RLW)] X100. ASA: Acetylsalicylic acid; M (RLW): Mean of the weights of the right legs; M (LLW): Mean of the weights of the left legs.

There was a gradual increase in paw oedema weight of mice in the negative control group. The aqueous infusion extract of *M. deserti* orally administered at 250 and 500mg/kg body weight in mice significantly ($P<0.05$) showed 11.22% and 20.73% inhibition of oedema respectively (Figure 1) compared to the negative control group, while at higher dose (1000mg/kg), this extract showed greater activity than the ASA (50mg/kg) with 44.03% and 32.08% inhibition of oedema respectively. These results indicated that the effect of aqueous infusion extract of *M. deserti* is reflecting in dose dependent manner.
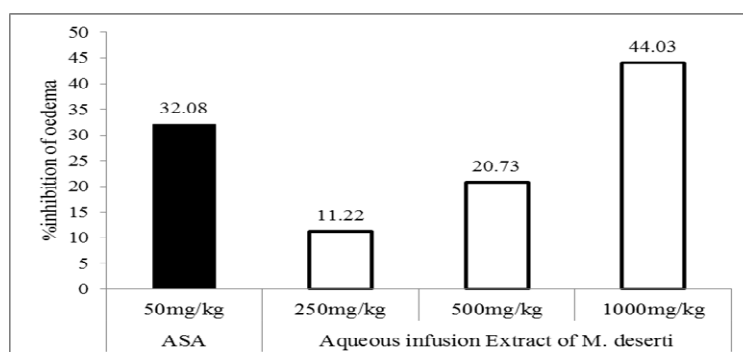

Figure1. The inhibitory effect of the aqueous infusion extract of aerial parts of *M. deserti* compared with ASA on carrageenin-induced paw oedema of mice.
(n= 6 in each group), P<0, 05. %Inhibition of Oedema is calculated as: %Oedema inhibition= [%control oedema - %test oedema / %control oedema] X100.

In this study, all doses of the aqueous infusion extract showed a significant inhibitory effect on the oedema formation in the third hour after carrageenin injection, thus exhibiting anti-inflammatory effect against acute inflammation. The development of carrageenin-induced oedema is biphasic; the first phase is attributed to the release of histamine, serotonin and kinins in the first hour; while the second phase is attributed to the release of prostaglandins and lysosomal enzymes from the second to the third hour (Morris, 2003; Brooks et Day, 1991). The results of the anti-inflammatory effect suggested that the main mechanism of action of the aqueous infusion extract of *M. deserti* may involve prostaglandin biosynthesis pathway and also may influence other mediators of inflammation. The ability of the aqueous infusion extract to inhibit carrageenin-induced paw oedema suggested that it possesses a significant effect against acute inflammation. This anti-inflammatory effect may be due to the presence of flavonoids, which many studies reported their in vitro and in vivo anti-inflammatory properties (Kim et al., 2004; Narayana et al., 2001). Flavonoids are known to inhibit the prostaglandin enzyme synthase and to produce anti-inflammatory effects (Oweyele et al., 2005). Indeed, the HPLC profile obtained from the aqueous extract of *M. deserti* (**Figure 2**), showed the apigenin as a free

flavone, which may explain the high anti-inflammatory effect of the aqueous extract of aerial parts of *M. deserti*. Works of Mafuvadze et al. (2011) suggested that apigenin has important chemopreventive properties for breast cancer, through its anti-inflammatory activity.
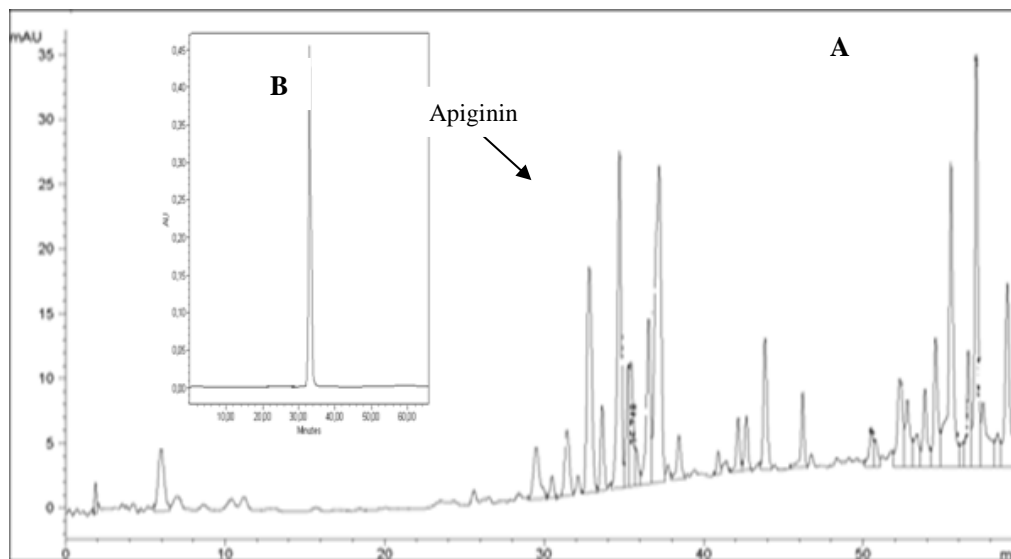

Figure 2: A: HPLC profile of the aqueous extract of aerial parts of M. deserti, visualized at λ=365nm. B: Chromatogram HPLC of apigenin standard injected in the same conditions as the extract of *M. deserti*

In conclusion, this study showed that the aqueous infusion extract of *M. deserti* possess a good anti-inflammatory activity which is confirmed by the inhibition of carrageenin-induced paw oedema. Thus, this plant may be used as an alternative herbal remedy for treating inflammatory disorders. Further investigations are needed to identify the possible mechanism of action and the active constituents responsible for the anti-inflammatory properties of the aqueous infusion extract of *M. deserti*.

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