Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (4):33-40
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Anti-inflammatory and analgesic activities of methanolic extract from *Marrubium deserti* leaves and evaluation of their acute toxicity

Ghedadba Nabil* **and Hambaba Leila**

Laboratory of Chemistry of Materials and Living: Activity and Reactivity, Department of Biology, Faculty of Natural Sciences and Living, University Hadj Lakhdar, Batna, 05000, Algeria.

ABSTRACT

This work aims to investigate the acute toxicity of methanolic extract (MeOHE) from Marrubium deserti leaves and evaluate their in vivo anti-inflammatory and analgesic properties. Quantitative determinations of total polyphenols and flavonoïds revealed that MeOHE is rich in polyphenols (184 ± 0.78 mg GAE/mg of extract) and flavonoïds (28.48 ± 0.40 µg QE/mg of extract). In addition, the dosage of condensed tannins showed that MeOHE contains an amount of 5.75 ± 0.42 mg E-Catechin/mg of extract. The study of acute toxicity in Wister albino rats, at a dose of 2000 mg/kg and the dose of 5000 mg/kg shows that MeOHE did not produce any toxic signs or deaths in rats for all parameters studied. Assessment of anti-inflammatory activity in vivo by the paw edema assay induced by carrageenan showed that oral administration of MeOHE at a dose of 200 mg/kg in rats treated with carrageenan causes a significant decrease (86.4%) of inflammation compared with the control group and which is slightly greater than the effect of diclofenac (85.52%) that was used as a positive control. The analysis of C-reactive protein shows the absence of this protein in the plasma of rats treated with MeOHE of the plant. As regards the analgesic activity of the MeOHE has a very significant reduction in numbers of abdominal writhes (5.14 ± 0.23) at a dose of 400 mg/kg bw, these results are very similar to those obtained in the group treated with paracetamol (5 ± 1.58) at a dose of 400 mg / kg bw.

Keywords: *Marrubium deserti de Noé ex Coss*, Lamiaceae, Polyphenols, Acute toxicity, Anti-inflammatory activity, Analgesia activity.

INTRODUCTION

Inflammation is a response mode of the organism to a pathogen which aims to repair tissue damage. Sometimes the inflammation can be harmful because of the aggressiveness of the pathogen, its persistence, the seat of inflammation, by abnormal regulation of the inflammatory process, quantitative or qualitative abnormality of cells involved in the inflammation. The use of synthetic anti-inflammatory is always accompanied by undesirable side effects, whereas the use of phytochemicals is useful and without side effects [1]. Different species of the *Labiatae* family are used in traditional medicine in several countries. *Marrubium deserti* (Lamiaceae) known as ‘‘Djaada’’ in Algeria’s desert is considered a rare species. It never studied pharmacological side. However, the related species, *M. vulgare* is studied by a certain number of researchers to these anti-inflammatory properties.

M. deserti is endemic, occupies the entire central Sahara of Algeria [2, 3] and also pushes the Sahara in Morocco [4] and in arid regions in Tunisia. [5] This plant has several applications in traditional medicine. Bellakhdar [4] described the use of the leaves of *M. deserti* in many traditional recipes in Morocco to cure various diseases: colitis, colic, coughing and fever. Edziri *et al*. [5] reported that *M. deserti* leaves are used in traditional medicine in Tunisia as a remedy for asthma, diabetes and as a diuretic. This species also has antigenotoxic activity, antioxidant [6] and significant antiviral against Coxsackie B virus (CoxB-3) [5].

M. deserti is rich by flavonoïds. Zaabat et al. [6, 7] were able to isolate four glycosides of apigenin: apigenin-7-O-β-neohesperidoside; apigenin-7-O-glucoside; the terniflorin: apigenin-7-O-(6 "-E-*p*-coumaroyl)-glucoside and apigenin-7-O-glucuronide. From the dichloromethane extract of the aerial parts of *M. deserti*, [6] have been isolated for the first time two new labdane diterpene: the desertin ($C_{22}H_{36}O_8$) and marrulibacetal A ($C_{21}H_{32}O_7$). In addition, other diterpenes have already identified: cyllenin A and 15-epi-cyllenin A [6], marrubiin [8] and marrulactone [6, 8]. The aim of this study was to investigate the pharmacological activity of *M. deserti* species. Thus, the analysis focused on the search of main chemical drugs and the evaluation of anti-inflammatory and analgesic activities *in vivo* in rats and acute oral toxicity of the methanol extract of *M. deserti* leaves.

MATERIALS AND METHODS

Plant material

The leaves of *M. deserti* were collected from their natural habitat around Daya-Mogheul, Bechar. This plant was identified by Pr. Hocine Laouer, Department of Biology, Faculty of Science, University Ferhat-Abbas Setif, Algeria. The leaves were dried under shade for 25 days at room temperature, dried leaves parts were blended into fine powder and stored in the dark at a dry place.

Animals

Wistar albino rats for either sex (150-180 g) procured for Research institute of both sexes were housed in separately in plastic cage at temperature of (25) °C and 50-55 % relative humidity, with a 12 light/dark cycle respectively before and during the experiment. Animals were allowed the access to standard pellet diet and water *ad libitum.*

Chemicals and reagents

Standard phenolic acids “gallic acid”, flavonoïds “quercetin” and tannins “catechin” were obtained from "Sigma Aldrich". The Folin-Ciocalteu reagent and Aluminum Chloride ($AlCl_3$) were purchased from "Fluka Chemie". Carrageenan, Acetic acid puriss glacial, methanol and Formaldehyde (CH_2O) were purchased from “Sigma-Aldrich”, diclofenac (Dic) and Paracetamol were used in the present study also obtained from “Sigma-Aldrich”.

Preparation of plant extract

Amount of 500 mg of powdered leaves was extracted with petroleum ether three times 3 L for each time. Then, the marc was dried and extracted with dichloromethane three times 3 L for each time and with methanol three times 3 L for each time and the supernatants were filtered sequentially using cloth filter, cotton wool, and Whatman filter paper. The solvents were then evaporated under reduced pressure and controlled temperature (35 ° C) using a vacuum rotary evaporator “Buchi Rotavapor”.

Phytochemical screening

The phytochemical screening of MeOH extract was performed using standard method [9]. Phytochemical constituents such as phenolic compounds, steroids and terpenoïds (*Lieberman-Burchard's test*), saponins (*Frothing test*), flavonoïds (*Shinoda test*), alkaloïds (Mayer reagent) and tannins (*$FeCl_3$ test*) were qualitatively analyzed.

Polyphenols analysis

The total polyphenols were estimated by the method described by Stanković [10]. Ranslation of the Folin-Ciocalteu reagent (FCR) causes a reduction of its colorimetric properties, thus, the total polyphenol content is determined by extrapolation on a standard curve obtained from a serial dilution in distilled water gallic acid (125 mg/L). In each test tube was added an aliquot (0.25 mL) of the test sample (sample or gallic acid), 1.25 mL of FCR (diluted 1:10) and 1 mL (75 g/l) $NaHCO_3$. Blank was concomitantly prepared**,** containing 0.25 ml methanol, 1.25 ml 10% Folin-Ciocalteu’s reagent dissolved in water and 1 ml of 7.5% of $NaHCO_3$. After agitation, various solutions have been left to the dark place for 30 min at 40° C. Absorbance was then measured at 765 nm using spectrophotometer (UV/Visible). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The total phenolic content was expressed as mg of gallic acid equivalent per mg of extract (mg GAE/mg of extract).

Determination of total flavonoïds concentrations

Amount of 1 ml of each sample and standard (prepared in methanol) was added to 1 ml of the solution of $AlCl_3$ (2% dissolved in methanol). After 10 minutes, the absorbance was measured at λmax = 430 nanometers against the reagent blank prepared. The concentrations of flavonoïds have been deduced from the range of the calibration curve established with quercetin (0-35 mg/ml). The results were expressed as milligrams of quercetin equivalents per mg of extract (mg QE/mg of extract) [11].

Determination of tannins concentrations in the plant extracts

Condensed tannins were created by the vanillin method described by Julkumen-Titto [12]. Indeed, vanillin reacts with free flavan 3-ols and the terminal units of proanthocyanidins giving a red coloration whose intensity is proportional to the rate of flavanols present in the medium and which has an absorption maximum at 500 nm in length wave. Aliquots of 0.1 to 1 ml of the stock solution (0.5 mg/ml) catechin and extracts were added to a series of test tubes, the final volume in each tube was made up to 1ml by absolute methanol addition. 1.5 ml of 4% vanillin in methanol and dissolved in 750 µl of HCl (12M) at 37% were added and at one-minute intervals to each tube of the series and thereafter placed in a water bath set at 30° C for 20 minutes. The results were expressed as milligrams of catechin equivalents per mg of extract (mg CE/mg of extract).

Acute toxicity

Two doses of the methanol extract were tested on groups of five homogeneous albino rats of either sex (150-180 g body weight) previously fasted for 18 hours. Doses of 2000 to 5000 mg/kg are administered orally by means gastric intubation: the first day and the third day of treatment (72 hours). The control group received an aqueous physiological saline (NaCl 9 ‰) orally as vehicle at a dose volume of 10 ml/kg bw. The lots were quarantined for 14 days by regularly noting the various disturbances that is to say the change in behavior: comments focused on mobility, sensitivity to noise and pinching, diet, breathing, the appearance of feces [13] and death from the first day of treatment. At the end of treatment, animals were sacrificed by decapitation using chloroform for anesthesia. The blood of each animal was collected at a time into a tube containing anticoagulant (EDTA, heparin), for respectively metering the haematological and biochemical parameters.

Hematologic analysis

Blood samples collected in tubes containing anticoagulant (EDTA, heparin) were immediately used to determine levels of white blood cells, red blood cells, platelets and hematocrit according to standard methods [14].

Biochemical parameters

Blood collected in heparin tubes and centrifuged at 3000 rpm for 10 min. The serum was analyzed for various parameters such as Aspartase aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Cholesterol, Triglyceride, Urea, Blood glucose, Creatinine, Biliribin.

Anti-inflammatory Activity

Searching for anti-inflammatory properties was conducted on the model of plantar edema induced in the rat by injection of a 1% suspension (100 µl) of carrageenan in the right leg; technical based on those described by Winter et al. [15]. The tested products were administered orally 1 hour before the injection of carrageenan. The rats were fasted for 18 hours prior to treatment and divided into three groups of five rats each. Group A witness received 0.9% NaCl (10 ml/kg bw) only, group B was treated with 200 mg/kg bw of methanol extract, and rats of group C were treated with diclofenac (Dic), non-steroidal anti-inflammatory drug of reference at a dose of 100 mg / kg bw. Evaluation of the edema was followed by recording the diameter of the inflamed paw 0, 1, 2, 3, 4 and 5 hours after injection of the phlogogen agent. For each treatment group, average diameters obtained in these surveys (Dt) were compared to that obtained before treatment (D0) and for calculating the percentage of edema (inflammation percentage) from the formula (Dt - D0) / D0 * 100. While, the percentage inhibition of edema was calculated from the formula:

$$[(Dt - D0)_{witness} - (Dt - D0)_{traited}]/ (Dt - D0)_{witness} * 100$$ (Amezouar *et al*., 2013)

To determine exactly whether the MeOH extract plant has an anti-inflammatory effect, rats were anesthetized immediately after the last diameter measurement using chloroform and then blood was collected from the eye in tubes containing anticoagulant (EDTA) which was centrifuged at 3000 rpm for 10 min to determine the level of C-reactive protein (CRP). The sedimentation rate was also measured using pipettes VS.

Analgesic activity (*writhing Test*)

The analgesic effect of methanolic extract evaluated according to the number of abdominal writhing induced by the intra-peritoneal injection of acetic acid (1%) by the method described by [16]. Three groups of 5 rats of both sexes were formed. Lot white witness received distilled water; against the other lots were the methanolic extract of the plant (400 mg/kg) and the paracetamol (400 mg/kg). One hour after administration of the extract, the animals received by i.p. 1% acetic acid at a dose of 10 ml/kg. Five minutes after injection of acetic acid, writhing numbers were counted for each rat during 15 minutes. The analgesic effect was evaluated according to the following formula:

$$\% \text{ inhibition} = (1 - \frac{Wt}{Wb}) \times 100$$

$\mathbf{W_b}$: represents the average number of contortions of the control group rats and $\mathbf{W_t}$: is the average number of rats contortions of the Treaty lot.

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. *P* values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

Phytochemical screening

The characterization of chemical constituents of the methanolic extract revealed the presence of quinones, gallic and catechic tannins, alkaloids, sterols, polyterpenes, polyphenols, reducing compounds, flavonoïds and saponosides (Table **1**).

Table 1 Phytochemical constituents of methanolic extract from *M. deserti* leaves

Sample	Phytochemical constituents	Results
MeOH extract	Flavonoïds	+ ++
	Condensed Tannins	+ +
	Terpenoids	+ + +
	Steroid	+ +
	Alkaloid	++
	Saponin	++

For flavonoïds, tannins, terpenoïds and steroids, +: weak colour; ++: mild colour; +++: strong colour.
For alkaloids, +: negligible amount of precipitate; ++: weak precipitate; +++: strong precipitate.
For saponins, +: 1-2 cm froth; ++: 2-3 cm froth; +++ : >3 cm froth.

Polyphenols content, total flavonoïds and condensed tannins value

The results of quantitative analysis of phenolic compounds, total flavonoïds and tannins of the methanol extract of *M. deserti* leaves by UV-visible spectroscopy showed that it contained 184 ± 0.78 mg GAE/mg of extract, 28.48 ± 0.40 mg QE/mg of extract and 5.754 ± 0.42 CE/mg of extract respectively. Phenolic compounds are abundant in species belonging to the family Labiatae [17]. The levels of polyphenols and flavonoïds determined in this study are consistent with those reported by previous studies carried out on the same species (Table **2**).

Table 2 Polyphenols content, total flavonoïds and condensed tannins of methanolic extract from *M. deserti* leaves

Sample	Bioactifs Compounds	Results
MeOH extract	Polyphenols content [(a)]	184 ± 0.78 *
	Total flavonoïds [(b)]	28.48 ± 0.40***
	Condensed tannins [(c)]	5.75 ± 0.42 ***

[(a)] Gallic acid equivalent (mg GAE /mg extract), [(b)] Quercetin equivalent (mg QE/mg extract); [(c)] mg Catechin E per mg of extract.
: highly significant value (P < 0.0001), *: significant value (P < 0.05).*

Acute oral toxicity

In this study, the oral administration of the methanolic extract at all given doses (2000 mg/kg and 5g per Kg) did not produce any visible sign of acute toxicity or instant death in rats tested during the period of observation. The results of hematologic parameters and biochemical profile of the treated and control groups are presented in Table 3 and 4.

Table 3 Effect of methanolic extract from *M. deserti* leaves on hematologic parameters of rats in acute toxicity (mean ± SEM, n= 5)

Parameters	Methanolic extract from *M. deserti* treated group		
	Control group	2000 mg/Kg	5000 mg/Kg
Red Blood ($\times 10^{12}$/L)	4.9 ± 0.6	4.68 ± 0.25	4.62 ± 0.81
White blood cells ($\times 10^{9}$/L)	8.6 ± 0.5	8.9 ± 2 .07	9.14 ± 1.16
Platelets ($\times 10^{9}$/L)	669.8 ± 139.7	681.6 ± 128.3	649 ± 49.05
Hemoglobin (g/dL)	14.3 ± 1.0	13.9 ± 0.67	13.7 ± 0.08

Values expressed as mean ±STD; each batch comprising 5 animals (n=5/lot).

Table 4 Effect of methanolic extract from M. deserti leaves on biochemical parameters of rats in acute toxicity (mean ± SEM, n= 5)

Parameters	Methanolic extract from *M. deserti* treated group		
	Control group	**2000** mg/Kg	**5000** mg/Kg
Glucose (g/l)	1.18 ± 0.24	1.12 ± 0.02	1.01 ± 0.08
Urea (g/l)	0.39 ± 0.10	0.38 ± 0.04	0.37 ± 0.05
Cholesterol (g/l)	0.42 ± 0.09	0.37 ± 0.02	0.41 ± 0.11
Triglycerides (g/l)	0.28 ± 0.07	0.24 ± 0.03	0.31 ± 0.04
HDL-Cholesterol (g/l)	0.20 ± 0.05	0.16 ± 0.01	0.18 ± 0.07
LDL-Cholesterol (g/l)	0.23 ± 0.05	0.21 ± 0.07	0.23 ± 0.06
ALT (UL)	8.34 ± 0.95	5.26 ± 0.97*	5.46 ± 0.64*
AST (UL)	7.34 ± 2.27	5.85 ± 0.70	7.23 ± 0.73
Bilirubin (mg/l)	0.52 ± 0.17	0.46 ± 0.08	0.50 ± 0.09

*Values expressed as mean ±STD; Significance with Tukey's test following one way ANOVA is evaluated as *P < 0.05. ALT : Alanine aminotransferase and AST : Aspartate aminotransferase.*

No statistically significant differences ($P > 0.05$) were recorded in the most biochemical parameters analyzed after 14 days except for the ALT ($*P < 0.05$) raised with groups treated with the (2 and 5 g/Kg). Moreover, there was no effect on the levels of indicators of liver and kidney functions such as alanine amino-transferase (ALT), aspartate amino-transferase (AST) and Bilirubin. This result demonstrated that "*M. deserti*" did not induce any damage to the liver.

These results of acute toxicity are consistent with those reported previously by [18] that dealt five female rats in an acute oral study with a single dose of 2000 mg / kg of methanol extract of *M. vulgare*. Over a period of 14 days the animals were observed. No changes could be detected in the skin, fur, eyes, mucous membrane (nasal), the central nervous system and the autonomic nervous system. The data suggest that the toxic dose of the methanolic extract of *M. vulgare* is greater than 2000 mg /kg witch consistent with our data.

In toxicology, it is known that pharmacodynamic substance, and the LD_{50} is less than 5 mg/kg bw is super toxic. That having a LD_{50} between 5 and 50 mg / kg bw is an extremely toxic substance. One whose LD_{50} belongs to the interval 50 to 500 mg/kg bw is considered highly toxic. One whose LD_{50} is in the range 500 to 5000 mg/kg bw is moderately toxic. The substance having an LD_{50} of between 5000 and 15000 mg/kg bw is slightly toxic and finally one whose LD_{50} is greater than 15,000 mg / kg bw is said to be non-toxic [19]. According to this classification, MeOH extract of *M. deserti* administered orally is slightly toxic or nontoxic. The difference in toxicity depending on the mode of administration, was observed by [19] with capsaicin and manganese. This difference was also observed with the decoction of leaves *Pilostigma reticulatum* and *Ziziphus mauritiana* crude extract [19]. Transaminases (AST and ALT) are good indicators of liver function and biomarkers to predict the possible toxicity of drugs [20] Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury [21]. Any elevation pertaining to these enzymes indicate their out flow into the blood stream due to damage in liver parenchymal cells. Furthermore, the enzyme (ALT) decreased significantly in animals treated with the dose of 2000 mg/Kg. Our results show that the MeOHE could have a hepato-protective effect in the animals which according to the work of Elberry *et al*. [**22**].

Anti-inflammatory Activity

The results of the anti-inflammatory activity obtained were compared to those of Diclofenac (group 3) and those of the control which received saline (Group 1). The evolution of inflammation for different groups is shown in Table 5.

Table 5 Effect of methanolic extract (200 mg/Kg) and diclofenac (200 mg/Kg) on the plantar edema induced by carrageenan in rats

Groups and doses (mg/Kg)	Time (H) and plantar diameter (mm)				
	1h	**2h**	**3h**	**4h**	**5h**
Control group	6.4 ± 0.5	6.87 ± 0.7	6.99 ± 0.6	6.87 ± 0.4	5.62 ± 0.7
Extract (200mg/Kg)	6.40 ± 1.0	5.94 ± 0.4	4.79 ± 0.2***	4.49 ± 0.5**	4.41 ± 0.2**
Diclofenac (200 mg/Kg)	5.37 ± 1.0	5.95 ± 0.5	4.73 ± 0.5***	4.71 ± 0.6**	4.38 ± 0.3**

*Values represented as mean ±standard deviation (n = 5). * P <0.05. ** P <0.01. *** P <0.001 is considered significant compared to the control.*

According to the results of the table it is noted that the inflammation caused by carrageenan increases with time and reaches a maximum of 6.99 ± 0.6 mm for three hours. From these results, it appears that the methanol extract of the plant inhibits significantly the inflammatory response. This inhibition is a function of time, or at equal time 1h the diameter of the paw is measured 6.40 ± 1.0 mm, and the time equal to 2 hours the inhibition was 5.94 ± 0.4 mm and showed no significant difference compared to control. At time equal to 3 hours the extract represents a highly significant inhibition of 4.79 ± 0.2 mm and which is very close to the effect of diclofenac 4.73 ± 0.5 mm. Then, this inhibition is continuous over time, one can deduce that the extract has an anti-inflammatory effect similar to the effect of diclofenac. The administration of DIC (200 mg / kg bw) significantly prevented the development of the

inflammation at the level of the plantar paw of the rats at the fourth and the fifth hour after the administration of carrageenan (4.71 ± 0.6 mm and 0.3 ± 4.38 mm) respectively. Fig. 1 shows the percentage inhibition of edema induced by carrageenan.

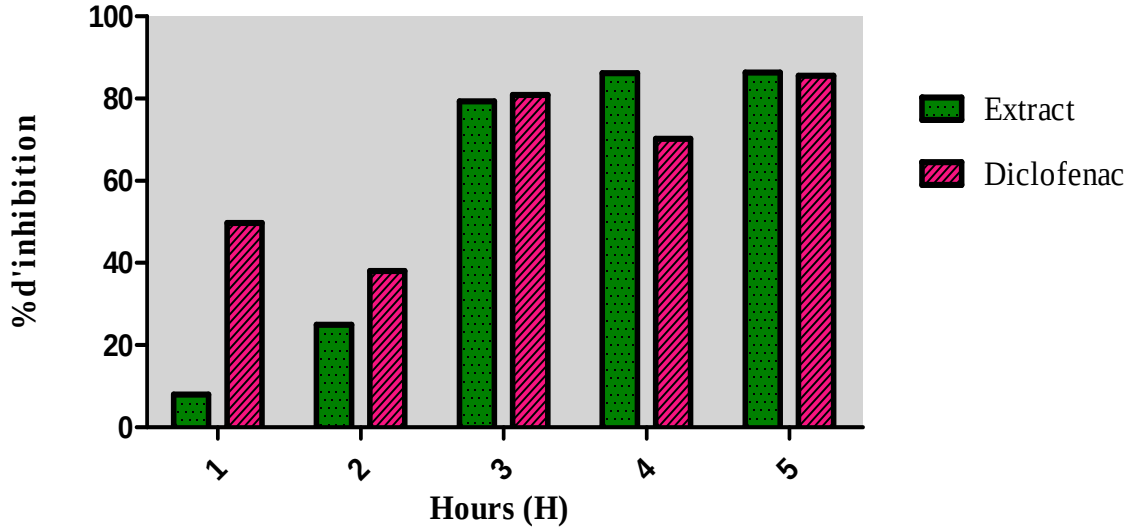

Fig. 1 Histogram percentage inhibition of paw edema after the treatment in the test of the anti-inflammatory activity

Assessment of percentage inhibition shown that *M. deserti* methanolic extract has anti-inflammatory activity. In the 1st hour the MeOH extract at a dose of 200 mg/kg shows a percent inhibition of 8. 86% lower than that obtained with diclofenac at a dose of 200 mg/kg (49.68%). At the third time the effect of the extract (79.35%) is almost similar to that of diclofenac (80.86%). Thereafter and the fourth and fifth time it appears that the effect of the extract is higher slightly than that of the positive control (diclofenac) and represents 86.2% inhibition values, 86.40% respectively. The results of the analysis show the presence of CRP in serum of treated rats carrageenan which shows the role of carrageenan in the induction of inflammation, whereas this protein is absent in the serum of other rats treated by the extract or diclofenac standard.

The administration of *M. deserti* methanol extract at a dose of 200 mg/kg bw prevents significantly (P <0.05) the plantar edema in rats from the third hour of treatment. This suggests the significant anti-inflammatory effect of the extract of the plant, it could be due to the richness of the methanol extract of *M. deserti* in bioactifs compounds, mostly polyphenols, flavonoïds and phenylpropanoïds glycosilated. Flavonoïds have been reported to exhibit antioxidant [23, 24]**,** anti-inflammatory [25] and hepatoprotective [22] activities. Furthermore, condensed tannins have been suggested to possess free radical scavenging and antioxidant, anti-inflammatory and hepatoprotective activities. Taking all these reports into consideration, it is plausible to suggest that the anti-inflammatory activity of MeOH extract of *M. deserti* involved, partly, synergistic action of alkaloids, flavonoïds, condensed tannins, and saponins. However, the work performed by Sahpaz and colleagues [26] demonstrated the potent anti-inflammatory effect of these compounds cause the inhibition of cyclo-oxygenase 2. Flavonoïds are also inhibitors of the C3 convertase of the alternative pathway and complement activation by inhibiting the binding between factor B and C3. More, recently Ghedadba *et al*. [27] have identified four groups of these compounds in methanolic extract of the plant *M. vulgare*: verbascoside, arenarioside, ballotétroside and forsythoside B. The results obtained in this study are consistent with those found by Kanyonga et al. [8, 28]. Also, other studies have shown that many species of the family Lamiaceae such as *Thymus vulgaris* L., *Rosmarinus officinalis* develop an anti-inflammatory activity *in vivo* [8].

The cellular and molecular mechanism by which the λ-carrageenan induced inflammatory process is known. It stimulates the release of histamine and serotonin from mast cells, starting it with a cascade of events that produce other mediators that contribute to the establishment of the acute inflammatory response [29]. Indeed, the carrageenan induced during the early phase (1-2 h) of the inflammatory response, the production of pro-inflammatory factors such as histamine, serotonin, leukotrienes, PAF and prostanoids. These factors cause vascular changes leading to plasma exudation. In addition to their inhibition of the production of pro-inflammatory mediators, secondary metabolites inhibit neutrophil recruitment to the pleural cavity through the inhibition of the

expression of adhesion molecules on the endothelial cell wall veins [30]. Flavonoïds block the migration of leukocytes to the inflammatory site by inhibiting adhesion molecules ICAM-1 and VCAM-1, and this regulation by TNF-α. Tsuda et al [31] report that the administration of cyanidin 3-O-β-glucoside inhibits inflammation induced by zymosan. Also, treatment with cyanidin 3-O-β-glucoside, reduced the increase in concentrations of NO, TNF-α, IL-1ß, and CINC-1 (cytokine-indudec neutrophil chemoattractant 1). Moreover, cyanidin 3-O-β-glucoside normalises levels of several acute phase proteins including α2 -macroglobulin, albumin, and transferrin [32].

Flavonoïds inhibit leukocyte migration by blocking their adhesion to the vascular wall. This effect is due to inhibition of the synthesis of IL-1 and TNF-α, the major inducers of the expression of adhesive molecules on the vascular wall [33]. It has been reported indeed that quercetin blocks adhesion of leukocytes to the endothelial wall of the umbilical veins by inhibiting the expression of ICAM-1 [34]. Gallic acid in turn inhibits leukocyte migration by inhibiting the molecules VCAM-1 adhesion, *ICAM-1* and *E*-selectin in vascular endothelial cells, this inhibition is due to inhibition of IL-1 , TNF-α and NF-*k*B [35]. In meningitis cytokines such as TNF-α and IL1 cause accumulation of leukocytes in the liquid serebro-spinale which may cause neurological damage. Taking these data together, the methanol extract of the leaves of *M. deserti* likely exerts its anti-pleuritic effect by reducing the production of inflammatory mediators involved in the development stage of the acute inflammatory response induced by the λ-carrageenan and by the inhibition of leukocyte recruitment to the pleural cavity by exerting anti-chemoattractant effects.

Analgesic activity (writhing Test)

This test consists of checking the inhibitory action of the MeOH extract of plant on pain induced in rats by intra-peritoneal (ip) injection of a dilute solution of acetic acid (writhing test). Abdominal contractions induced by injection of acetic acid were used to evaluate the analgesic effect of the plant. Table 6 shows the effects of the paracetamol, and the MeOH extract on the number of writhes induced by the injection of acetic acid (1%).

Table 6 Analgesic effect of the methanol extract on the abdominal contractions induced in rats by injection of acetic acid

Groups	Dose (mg/Kg)	Contractions number	Inhibition percentage
Witness	/	23.4 ± 2.88	-
Extract	**400**	5.14 ± 0.23***	73. 29%
Paracetamol	**400**	5 ± 1.58 ***	79%

Values represented as mean ± standard deviation (n = 5).
**: P < 0.05, **: P < 0.01. ***: P < 0.001 were considered significant compared to the control (n = 5).*

From these results, the MeOH extract has a very significant reduction in numbers of abdominal writhes (5.14 ± 0.232) at a dose of 400 mg/kg bw, these results are very similar to those obtained in the group treated with paracetamol (5 ± 1.58) at a dose of 400 mg/Kg bw. Compared with the results of other authors, it appears that the percentage of inhibition of abdominal contortions in the group treated by MeOHE plant (73.29%) is higher in the present study compared to results of [28] who found a percent inhibition (35.3%). Moreover, these results are very similar to those obtained in the group treated with paracetamol, which present a percentage inhibition (79%).

The contractions induced by i.p. of acetic acid are a method used to study peripheral analgesic effect of a substance. The pain caused by the injection of acetic acid is due to the release of serotonin, histamine, bradykinin, substance P and prostaglandins (PGE2α, PGF-2α). These chemical mediators stimulate peripheral nociceptive neurons induce and increased vascular permeability [1, 18]. The analgesic activity of *M. deserti* methanol extract may be due to the presence of marrubiin and its derivatives [6]. Thus the work of Stulzer *et al*. [8] on the plant *M. vulgare* "Antioedematogenic effect of marrubiin obtained from *Marrubium vulgare*" have shown the beneficial action of marrubiin against these chemical mediators which leads to an inhibition of pain.

CONCLUSION

The data obtained show that methanolic extract of *M. deserti* leaves has both anti-inflammatory and analgesic activities which may be produced by the plant inhibiting various chemical mediators including prostaglandins and bradykinins. The relatively high LD_{50} value of 5000 mg/kg bw obtained for the plant shows that it may be safe in or non toxic to rat. The result obtained justifies the use of the plant species by traditional medicine practitioners in Algeria. However, more studies are needed to further elucidate the mechanism of the anti-inflammatory and analgesic actions of *Marrubium deserti*. It is important to note that these results were obtained in rats. It is therefore essential to carry out experiments at first in another animal model, and then in a second time in humans, to obtain confirmation of the potential of this plant.

REFERENCES

[1] Y Chen; H Tsai; T Wu. *Planta Medica*, **1995**, 61(1), 2–8.
[2] F Chebrouk; M Hadj-Mahammed. *Annales de la Faculté des Sciences et Sciences de l'Ingénieur*, **2009**, 1, 75-81.
[3] H Dendougui; S Seghir; Z Belloum; F Benayache; F Leon; I Brouard; J Bermejo; Benayache S. *Rec. Nat. Prod*, **201**1, 5(4), 300-304.
[4] J Bellakhdar. La pharmacopée marocaine traditionnelle: Médecine arabe ancienne et savoirs populaires. *Ibis Press*, 1997; p 764.
[5] H Edziri; M Mastouri; M Aouni; Verschaeve L. *South African Journal of Botany*, **2012**, 80, 104-109.
[6] N Zaabat; AE Hay; S Michalet; N Darbour; C Bayet; I Skandrani; L Chekir-Ghedira; S Akkal; Dijoux-Franca, MG. *Food and Chemical Toxicology*, **2011**, doi:10.1016/j.fct.2011.08.026.
[7] N Zaabat; N Darbour; C Bayet; S Michalet; A Doléans-Jordhem; L Chekir-Ghedira; S Akkal; Dijoux-Franca; MG. *Phytothérapie*, **2010**; DOI 10.1007/s10298-010-0589-y.
[8] HK Stulzer; MP Tagliari; JA Zampirolo; V Cechinel-Filho; Schlemper V. *Journal of Ethnopharmacology*, **2006**, 108(3), 379-84.
[9] AB Aliyu; AM Musa; MS Abdullahi; H Ibrahim; Oyewale AO. *Acta Poloniae Pharmaceutica Drug Research*, **2011**, 68, 67-73.
[10] M S Stanković. *Kragujevac J. Sci*, **2011**, 33, 63-72.
[11] T Bahorun; B Gressier; F Trotin; C Brunete; T Dine; T Vasseur; TC Gazin; M Pinkas; M Luycky; Gazin M. *Arznein Forsch/Drug Res*, **1996**, 46, 1-6.
[12] R Julkumen-Titto. *Journal of Agriculture and Food chemistry*, **1985**, 33, 213-217.
[13] TC Lienou; FX Etoa; B Nkegoum; CA Pieme; Dzeufiet DPD. *Afr. J. Trad. Cam*, **2007**, 4(2), 127-134.
[14] MB Gome; K Kouakou; A Toure; Traore F. *Int. J. Biol. Chem. Sci*, **2011**, 5(5), 1777-1789.
[15] F Amezouar; W Badri; M Hsaine; N Bourhim; Fougrach H. *Pathologie Biologie*, **2013**, http://dx.doi.org/10.1016/j.patbio.03.005.
[16] S Hunskaar; O Fasmer; Hole K. *Journal of Neuroscience Methods*, **1985**, 14 (1), 69–76.
[17] A Wojdylo; J Oszmianski; Czemerys R. *Food Chem*, **2007**, 105, 940–949.
18 A Tjolsen; OG Berge; S Hunskaar; JH Rosland; Hole K. *Pain*, **1992**, 51(1), 5–17.
[19] KP Nirmal; G Subhasish; Indrani C. *Int J Pharm Bio Sci*, **2013**, 4(2), 131 –143.
[20] J Hilaly; Z Israili; Lyouss B. *J Ethnopharmacol*, **2004**, 91, 43–50.
[21] J Ozer; M Ratnerb; M Shawc; W Baileya; Schomaker S. *Toxicology*, **2008**, 245, 194-205. DOI: 10.1016/J.TOX.2007.11.021
[22] AA Elberry; FM Harraz; SA Ghareib; AA Nagy; SA Gabr; MI Suliaman; Abdel-Sattar E. *Journal of Basic and Clinical Pharmacy*, **2010**, 001, 247-254.
[23] J Ferreira; D Luthria; T Sasaki; Heyerick A. *Molecules*, **2010**, 15(5), 3135–3170.
[24] A Tapas; D Sakarkar; Kakde R. *Tropical Journal of Pharmaceutical Research*, **2008**, 7 (3), 1089–1099.
[25] H Sandhar; B Kumar; S Prasher; P Tiwari; M Salhan; Sharma P. *Internationale Pharmaceutica Sciencia*, **2011**, 1, 24–41.
[26] S Sahpaz; N Garbacki; M Tits; Bailleul F. *Journal of Ethnopharmacology*, **2002**, 79(3), 389–392.
[27] N Ghedadba; H Bousselsela; L Hambaba; S Benbia; Mouloud Y. *Phytothérapie*, **2014**, 12, 15-24.
[28] PM Kanyonga; MA Faouzi; B Meddah; M Mpona; EM Essassi; Cherrah Y. *Journal of Chemical and Pharmaceutical Research*, **2011**, 3(1), 199-204.
[29] S Cuzzocrea; B Zingarelli; P Hake; AL Salzman; Szabo C. *Free Radic Biol Med*, **1998**, 24(3), 450-459.
[30] EJR Middleton; C Kandaswami; Heoradies TC. *Pharmacol Rev*, **2000**, 52, 673-751.
[31] T Tsuda; F Horio; Osawa T. *Journal of Nutrition Science Vitaminol*, **2002**, 48, 305-310.
[32] JB Calixto; MM Campos; MF Otuki; Santos AR. *Planta Med*, **2004**, 70(2), 93-103.
[33] Kj Cho; CH Yun; DY Yoon; YS Cho; G Rimbach; L Packer; Chung AS. *Toxicol Appl Pharmacol*, **2000**, 168, 64-71.
[34] S Anné; M Agarwal; MP Nair; SA Schwartz; M Ballow; C Kandaswami; Middleton E. *J Allergy Clin Immunol*, **1994**, 93, 276.
[35] M Takatoshi; K Noriaki; H Tadashi; S Yusuke; N Yoshinori; Ichiro TTK. *Arterioscler Thromb Vasc Biol*, **1999**, 19, 1412-1420.