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Assessment of methanolic extract of *Marrubium vulgare for* anti-inflammatory, analgesic and anti-microbiologic activities

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ABSTRACT

This paper describes the antioedematogenic, analgesic and antimicrobiologic profile of a methanolic extract of Marrubium vulgare. The results of evaluation of the anti- inflammatory activities against inflammation induced by carrageenen and PGE-2, and analgesic activity in the p-benzoquinone-induced abdominal constriction test showed that the activity of the methanolic extract at a 200 mg/Kg dose was similar to that of the reference drugs. The extract also showed a significant anti-microbial activity at 200, 400, and 600 mg/mL. Our results support the use of M. vulgare in traditional medicine in Mediterranean region for its anti-inflammatory, analgesic and anti-microbiologic properties

Keywords: *Marrubium vulgare*; methanolic extract; anti-inflammatory; analgesic; anti-microbial activity.

INTRODUCTION

Marrubium vulgare L. (Lamiaceae) commonly known as “horehound” in Europe, or “Marute” in the Mediterranean region, is naturalized the latter and Western Asia and America. In the Mediterranean, *M. vulgare* is frequently used in folk medicine to cure a variety of diseases. The plant is reported to possess hypoglycemic [1,2], vasorelaxant [3], antihypertensive [4], analgesic [5–8], anti-inflammatory [9], antioxidant activity [10], antiedematogenic activity [11], and many other biological activities. In this study, the anti-inflammatory, analgesic and antimicrobial properties of *M. vulgare* have been evaluated using rodent models.

EXPERIMENTAL SECTION

2.1. Plant Material and Preparation of Extracts

M. vulgare plant samples were collected from the Tamara district of Morocco during the month of May, 2007. The plant was authenticated at the Department of Biology, Faculty of Science-Rabat. A specimen of the original collection was placed in the herbarium of the Faculty of Medicine and Pharmacy of Rabat. Whole plant of *M. vulgare* was dried in the shade and crushed to a fine powder. The dried powder of the plant (200 g) was extracted in a Soxhlet apparatus with methanol. The extract was evaporated to dryness *in vacuo* using a rotary evaporator at 70 °C to give a yield of 39.2%.

2.2. Drugs and Chemicals

The following drugs and chemicalks wer used in the studies: carrageenan (Sigma, St. Louis, Missouri, USA), PGE2 (Fluka Chemie AG), *p*-benzoquinone (Merck). Indomethacin (Bayer AG) and acetylsalicylic acid (Bayer AG), The plant extracts were suspended in a mixture of arabic gum 5% and given to the test animals by mouth. The control group received the same treatment as the test groups except that the drug was replaced with an appropriate volume of vehicle. Indomethacin (10 mg/kg) and acetylsalicylic acid (ASA; 100 mg/kg) in 5% of gum arabic were used as reference drugs.

2.3. Animals

The study was performed on adult male Swiss mice (20–30 g), bred at the Laboratory of Pharmacology, Faculty of Medicine and Pharmacy of Rabat. The food was withdrawn on the day before the experiment; however, they were allowed free access to water. Throughout the experiments, the animals were handled according to the prescribed ethical guidelines for laboratory animals.

2.4. Anti-inflammatory Tests

2.4.1. Carrageenan-induced Hind Paw Oedema Model

The carrageenan-induced hind paw oedema model was used for the determination of anti-inflammatory activity [16–18]. Six animals were used for each extract dose, as well as the control and reference groups. For extract, 100 and 200 mg/kg doses were administered. One hour after oral administration of the extract, drug or vehicle, the subplantar tissue of right hind paw of each mouse was injected with 25 μL of 20 mg/mL of freshly prepared carrageenan in physiological saline (0.9% NaCl). For control purposes, 25 μL of saline was injected into subplantar tissue of left hind paw. Thereafter, paw oedema was measured at 1.5 h intervals for 6 h. The difference of thickness in footpad was measured with calipers. Indomethacin (10 mg/kg) was used as the reference drug.

2.4.2. PGE-2-induced Hind Paw Oedema Model

PGE-2-induced hind paw oedema model was also used for the determination of anti-*inflammatory* activity following the method described by Kasahara *et al.* [19]. Six animals per group were given either an extract, control and reference drug (indomethacin, 10 mg/kg). The extract dose was 100 and 200 mg/kg for the plant extract. One hour after oral administration of extract, drug or vehicle (control), each mouse received 5 μL of freshly prepared suspension of PGE-2 (1 mg/mL) in tyrode's solution by injection into the subplantar tissue of the right hind paw except that for control, 5 μL of tyrode's solution was injected into the left hind paw. Thereafter, paw oedema was measured at 15 min interval for 75 min. The difference of thickness in footpad was measured with calipers.

2.5. Analgesic Test

The *p*-benzoquinone-induced abdominal constriction test [16,17,20] was performed on mice in order to determine analgesic activity. Six animals were used for each of the following groups: extract, control (distilled water) and reference drug (ASA, 100 mg/kg). For the extract, the doses administered were 100 and 200 mg/kg. One hour after oral administration of extract, drug, or reference drug, the mice were given intraperitoneal injections of 0.1 mL/10 g body weight of 2.5% w/v of solution of *p*-benzoquinone (PBQ) in distilled water. The mice were then observed individually 5 min after PBQ injection for the number of abdominal contractions (writhing movements) for a period of 15 min.

2.6. Anti-microbial Activity

The methanolic extract of *M. vulgare* obtained was tested for the anti-microbial activity against various bacterial strains. Sterile nutrient agar plates and Sabouraud agar were prepared and incubated at 37°C for 24 h to check for any contamination. Sterile filter paper discs (Whatman No.1) of 5 mm diameter were soaked in five different dilutions of the methanolic extract and placed in appropriate position on the surface of the plate. The *in vitro* antibacterial activity of different extract of *M. vulgare* at 50, 100, 200, 400 and 600 mg/mL was studied by disc diffusion method against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Candida albicans*. The Petri dishes were incubated at 37 °C for 18 h and the diameter of the zone of inhibition measured in mm. The activity of the methanolic extract was compared with ciprofloxacin (10 µg/mL) and nystatin. The zone of inhibition was calculated by measuring the minimum dimensions of the zone of no microbial growth around the disc and minimum inhibitory concentrations were determined.

RESULTS AND DISCUSSION

3.1. Analgesic Activity

Table 1 shows the dose - response data of the methanol extract of *M. vulgare* with regard to *p*-benzoquinone-induced abdominal constriction. The extract (200 mg/kg dose) significantly inhibited (35.3%) abdominal constriction in the mice, but no effect was observed at 100 mg/kg dose. Consequently, 200 mg/kg dose was selected for further experiments.

Table 1. Effect of the methanolic extract of *M. vulgare* on *p*-benzoquinone-induced writhings in mice

Extract/Drug	Dose (mg/Kg)	Number of writhings ± SEM	Inhibition ratio (%)
Control		42.2 ± 4.8	
***M. vulgare* extract**	100	39.3 ± 2.7	6.9
	200	27.3 ± 2.4**	35.3
ASA	100	21.3 ± 2.4***	54.3

**: p < 0.05. **: p < 0.01. ***: p < 0.001 (compared to control, n = 6)*

Table 2. Effect of the methanolic extract of *M. vulgare* on carrageenan-induced paw edema in mice

Extract/Drug	Dose (mg/Kg)	90 min		180 min		270 min		360 min	
		Swell. thick. ± SEM	Inh. (%)	Swell. thick ± SEM	Inh. (%)	Swell. thick ± SEM	Inh. (%)	Swell. thick ± SEM	Inh. (%)
Control		43.2 ± 3.9	0	55.2 ± 3.5		60.5 ± 2.3		64.3 ± 4.5	
Extract M. vulgare	100	41.7 ± 3.7	3.5	51.6 ± 4.9	6.5	59.1 ± 3.6	2.3	61.2 ± 4.7	4.8
	200	34.7 ± 3.2	19.7	41.5 ± 2.1	24.8*	39.9 ± 4.1	34.0**	44.2 ± 2.2	31.3**
Indomethacin	10	34.0 ± 3.1	21.3	35.3 ± 1.9	36.1**	37.1 ± 2.1	38.7***	39.9 ± 1.8	37.9***

Swell. thick. = Swelling thickness (x10-2 mm); SEM = standard error mean; Inh. = Inhibiton.
**: p < 0.05. **: p < 0.01. ***: p < 0.001 (compared to control, n = 6)*

3.2. Anti-inflammatory Activity

The extract also showed significant inhibition (34.0%) at a dose of 200 mg/kg in the carrageenan-induced hind paw edema test compared at the 100 mg/Kg dose (Table 2). In this regard the inhibitory effect of the extract (34%) was close to that of indomethacin (38.7%) after 270 min.

The anti-inflammatory effects of the extracts on hind-paw oedema induced by prostaglandin E2 are indicated in Table 3. This time measurements were kept between 0–60 min, with 15 min intervals. The extract at the 200 mg/Kg dose showed considerable inhibition (23.2–27.2 %) towards hind-paw oedema while the 100 mg/Kg extract did not demonstrate any noticable activity. Maximum inhibition by the extract (27.2%) was observed after 45 min, after which it decreased. The reference drug (indomethacin) presented a similar trend, with a peak at 38% inhibition after 45 min.

Table 3. Effect of the methanolic extract of *M. vulgare on* PGE-2-induced paw edema in mice

Extract/ Drug	Dose (mg/ Kg)	0 min		15 min		30 min		45 min		60 min		75 min	
		Swell. thick ± SEM	Inh. (%)	Swell. thick ± SEM	Inh. (%)	Swell. thick ± SEM	Inh. (%)	Swell. thick ± SEM	Inh. (%)	Swell. thick ± SEM	Inh. (%)	Swell. thick ± SEM	Inh. (%)
Control	-	1.5 ± 0.9		11.4 ± 1.5		20.3 ± 1.9		27.6 ± 2.3		25.9 ± 2.5		19.7 ± 1.6	
***M. vulgare* extract**	100	1.6 ± 0.7		15.8 ± 1.1		24.7 ± 1.4		29.5 ± 1.7		31.7 ± 2.4		27.6 ± 1.8	
	200	1.5 ± 0.8		13.1 ± 1.3		15.6 ± 1.1	23.2[a]	20.1 ± 1.4	27.2[b]	20.1 ± 1.2	19.7	17.6 ± 1.4	10.7
Indomethacin	10	1.5 ± 0.6		9.8 ± 1.3	14.0	13.9 ± 1.4	31.5[b]	17.1 ± 1.3	38.0[c]	20.4 ± 1.1	21.2[a]	16.8 ± 1.5	14.7

Swell. thick = Swelling thickness (x10-2 mm); SEM = standard error mean, Inh.= Inhibition.
[a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$ (compared to control, n = 6)

3.3. Antimicrobial Activity

The methanolic extract of *M. vulgare* exhibited moderate to significant antibacterial activity against five out of six tested bacterial organisms, as compared to the standard ciprofloxacin (10 µg/mL). The study revealed that methanolic extract of the crude drug was very effective against *B. subtilis, S. epidermidis, S. aureus* (Gram positive bacteria) and *C. albicans* and moderately effective against *P. vulgaris* and *E. coli* while ineffective in the case of *P. aeruginosa* (Gram negative bacteria).

Table 4. Antibacterial and antifungal activity of methanolic extract of *M. Vulgare*

Germs	Diameter of inhibition (mm) (mg/mL)					Ciprofloxacin 10 µg/mL*	Nystatin*	MIC mg/mL
	50	100	200	400	600			
B. subtillis	0	14	16	20	26	32	-	100
E. coli	0	0	0	13	17	25	-	400
S. aureus	0	15	17	20	25	30	-	100
S. epidermidis	0	10	13	19	23	28	-	100
P. vulgaris	0	0	0	9	11	25	-	400
P. aeruginosa	0	0	0	0	0	24	-	0
C. albicans	0	10	16	21	27	-	30	100

**: (6 mm) diameter disc; 0: Resistant; -: Not used*

DISCUSSION

According to our findings, the methanolic extract of *M. vulgare* produced potential antinociceptive and anti-inflammatory effects in experimental mice when assessed by chemical

methods of nociception including *p*-benzoquinone-induced writhings, carrageenan induced paw edema and PGE-2-induced paw edema. In the *p*-benzoquinone writhings tests a dose related antinociceptive effect of the extract was observed. The methanolic extract *M. vulgare* significantly ($p < 0.05$) inhibited the abdominal constrictions in tested experimental mice as compared to control group (Table 1). Comparing these results with those of the reference group, we find that the dose of 200 mg/kg of the extract has a moderate activity compared to the reference product (35.3% for extract to 54.3% for the reference). Moreover, these results are in good agreement with another study [8,9]. As shown in Table 1, the abdominal constrictions produced after administration of p-benzoquinone might be related to sensitization of nociceptive receptors to prostaglandins. It is, therefore possible that the methanolic extract of *M. vulgare* exerted their analgesic effect probably by inhibiting the synthesis or action of prostaglandins.

The methanolic extract (100 and 200 mg/kg) administered orally on inflamed or non inflamed paws, significantly reduced the increase in carrageenan-induced (Table 2) and the PGE-2 induced (Table 3)paw edema. The methanolic extract of *M. vulgare* showed significant anti-inflammatory activity at 200 mg/Kg, as compared with control and reference groups. These results suggest that the plant extract attenuated the pain by inhibition of cycloxygenase and lipoxygenase. Moreover, these results are in good agreement with another study by Stulzer *et al*. [11]. In the present study, the methanolic extract of *M. vulgare* exhibited significant anti-inflammatory effects. As inflammation is a peripheral process, therefore, it is suggested that the extract also exerted peripheral effects. Further, recent research on *M. vulgare* has revealed the presence of phytochemicals such as flavonoids [7], which have potential antinociceptive and anti-inflammatory effects. In addition, several studies have demonstrated that bioactive flavonoids, as well as biflavonoids, produce significant antinociceptive and/or anti-inflammatory activities [12–14].

The methanolic extract of *M. vulgare* exhibited moderate to significant antibacterial activity (Table 4) against five out of six tested bacterial organisms as compared to the standard ciprofloxacin (10 µg/mL). The study revealed that methanolic extract of the crude drug was very much effective against B. subtilis, S. epidermidis and S. aureus (Gram positive bacteria) and moderately effective against P. vulgaris and E. coli while ineffective in case of P. aeruginosa (Gram negative bacteria). The M.I.C was 100 mg/mL for the most sensitive bacteria and *Candida*, but was 400 mg/mL for *E. coli* and *P. vulgaris* which showed moderate sensitivity. These results confirm the observations of other authors on the antimicrobial activity of *M. vulagre* [15]. Thus on the basis of the results it is inferred that the methanolic extract of M. vulgare had *in-vitro* antibacterial properties. Further studies will be necessary to understand the mechanisms of action underlying the effects of the extract and their active compounds.

CONCLUSION

Thus on the basis of the results it is inferred that the methanolic extract of *M. vulgare* showed a significant biological activities at a dose of 200 mg/kg body weight for analgesic and anti-inflammatory *activity*, and a antibacterial activity at a dose of 200, 400 and 600 mg/mL. These anti-inflammatory, analgesic and antibacterial effects support the use of *Marrubium* in folk medicine to cure a variety of diseases. However, it will be important in future studies to identify the active constituents responsible for the observed activities of *M. vulgare.*

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