

## EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SOME LIBYAN MEDICINAL PLANTS IN EXPERIMENTAL ANIMALS

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**Abstract** – *Ballota pseudodictamnus* (L.) Benth. (Lamiaceae), *Salvia fruticosa* Mill. (Lamiaceae) and *Thapsia garganica* L. (Apiaceae) are three well-known medicinal plants from the Libyan flora, which have long been used for the treatment of inflammations. The aim of the present study was to investigate, for the first time, the anti-inflammatory property of the methanol (MeOH) extracts of the aerial parts of these plants. Shade-dried and ground aerial parts of *B. pseudodictamnus*, *S. fruticosa* and *T. garganica* were Soxhlet-extracted with MeOH. The extracts were concentrated by evaporation under reduced pressure at 40°C. The anti-inflammatory activity of the extracts was evaluated using the carrageenan-induced mice paw edema model. The administration of the extracts at a dose of 500 mg/kg body weight produced statistically significant inhibition ( $p < 0.05$ ) of edema within 3 h of carrageenan administration. The results demonstrated significant anti-inflammatory properties of the test extracts. Among the extracts, the *S. fruticosa* extract exhibited the most significant inhibition of inflammation after 3 h (62.1%). Thus, *S. fruticosa* could be a potential source for the discovery and development of newer anti-inflammatory 'leads' for drug development. The anti-inflammatory activity of *B. pseudodictamnus* and *S. fruticosa* could be assumed to be related to high levels of phenolic compounds, e.g., flavonoids, present in these plants.

**Key words:** *Ballota pseudodictamnus*; *Salvia fruticosa*; *Thapsia garganica*; carrageenan

### INTRODUCTION

A number of medicinal plants growing in Libya have traditionally been used for the treatment of inflammation: *Ballota pseudodictamnus* (L.) Benth. (Lamiaceae), *Salvia fruticosa* Mill. (Lamiaceae) and *Thapsia garganica* L. (Apiaceae) are three of such Libyan species (El-Gady and El-Mograby, 1999). These plants also possess analgesic and wound-healing properties. Previous bioactivity studies on *Ballota pseudodictamnus* (Couladis et al., 2001;

Erdogan-Orhan et al., 2010) and *Salvia fruticosa* (Sarac and Ugur, 2009; Pasiadis et al., 2010) revealed predominantly their antioxidant and antimicrobial properties. *Salvia fruticosa* was also reported to possess larvicidal, acetylcholinesterase inhibitory, hypoglycemic, antispasmodic activities, and anti-proliferative properties against colon cancer cell lines (Moharram et al., 2006; Xavier et al., 2008; Koliopoulos et al., 2010; Senol et al., 2010). *Thapsia garganica* exhibited cytotoxicity, particularly against prostate cancer cell lines (Appendino et al.,

2005; Liu et al., 2006). As part of our recent initiative for ethnopharmacological screening of Libyan medicinal plants, particularly, for anti-inflammatory properties, we now report on the anti-inflammatory activity of the extracts of *B. pseudodictamnus*, *S. fruticososa* and *T. garganica* using the carrageenan-induced mice paw edema model.

## MATERIALS AND METHODS

### *Plant materials*

The aerial parts of *Ballota pseudodictamnus*, *Salvia fruticososa* and *Thapsia garganica* were collected from the Aljabal Alakdar area, Libya, in April 2010. Plants were identified by Dr Mohamad Abouhadra, Faculty of Science, Tripoli University, Tripoli, Libya. Voucher specimens, respectively, J.122/2010, J.123/2010 and J.124/2010, were deposited in the herbarium of the Faculty of Pharmacy, Tripoli University, Tripoli, Libya.

### *Extraction of plant material*

Shade-dried and ground aerial parts (195, 230, and 96 g) of *B. pseudodictamnus*, *S. fruticososa* and *T. garganica*, respectively, were Soxhlet-extracted with 4, 4.5, and 2.5 l of methanol (MeOH). The extracts were concentrated by evaporation under reduced pressure at 40°C to yield 43.5, 47.5, and 55 g of *B. pseudodictamnus*, *S. fruticososa* and *T. garganica* extracts, respectively. The extracts were resuspended in water to achieve the concentration of 50 mg/ml.

### *Qualitative tests for flavonoids and tannins*

The tests for flavonoids and tannins were carried out as follows (Onwukaeme et al., 2007). An aliquot of the resuspended plant extract in water was reduced to dryness in a boiling water bath. The residue was treated with dilute NaOH, followed by the addition of dilute HCl. A yellow solution with NaOH, which turned colorless with dilute HCl, would confirm the presence of flavonoids. This test was also performed with the positive control, quercetin.

To an aliquot (1 ml) of the resuspended plant extract in water in a test tube, one drop of sulfuric acid was added, followed by 2-3 drops of 15% ferric chloride test solution. A blue or violet color would indicate the presence of tannins. This test was also performed with the positive control, gallic acid.

### *Animals*

Swiss albino female mice (3-4 weeks old, 20-25 g) were used in this study. The mice were obtained from the Animal House of the National Centre for Medical Research in Libya. Animals were maintained under standard environmental conditions (temperature at  $25 \pm 0.5^\circ\text{C}$  and humidity at  $55 \pm 0.5\%$ ) and had free access to feed and water *ad libitum*. Experiments on animals were performed strictly in accordance with the guidelines provided by the Institutional Animal Ethics Committee as well as the EU regulations 86/609, and were approved by the Ethical Review Committee, Tripoli University, Libya.

### *Assessment of anti-inflammatory activity: the carrageenan-induced mice paw edema model*

Anti-inflammatory activity of the extracts was evaluated by the carrageenan-induced edema model in mice (Williamson et al., 1996; Morris, 2003; Ahmed et al., 2005). The albino mice were divided into ten groups (N = 5). Acute inflammation was induced by an intradermal injection of 0.02  $\mu\text{L}$  of 2% of carrageenan in water (w/v) (Sigma-Aldrich) in the right hind paw of mice. Group 1 served as a control, and received water intraperitoneally (i.p., 200  $\mu\text{L}$ ). Groups 2-9 were injected (i.p.) with the MeOH extracts (500 mg/kg of body weight) 30 min before the injection of carrageenan solution. Group 10 was the positive control group, and received aspirin (100 mg/kg of body weight, i.p.). The edema volume was measured 3 h after the injection of carrageenan solution using a plethysmometer (Ugo, Basile). The difference in volume between the injected and uninjected paw was calculated and taken as the edema volume for all groups. The edema volume of the groups treated with the MeOH extracts of plants or aspirin was compared with the control group and the percent of

**Table 1.** Effect of the MeOH extracts of *Ballota pseudodictamnus*, *Salvia fruticosa* and *Thapsia garganica* in mice observed in the carrageenan-induced edema test.

Plant extracts/controls	Average edema volume in $\mu\text{L}$	% inhibition after 3 h
Control (water, 200 $\mu\text{L}$ )	$82.0 \pm 1.6$	-
<i>Salvia fruticosa</i> (500 mg/kg body weight, i. p.)	$31.0 \pm 1.1$	$62.1 \pm 3.5$
<i>Ballota pseudodictamnus</i> (500 mg/kg body weight, i. p.)	$40.0 \pm 2.1$	$51.2 \pm 2.9$
<i>Thapsia garganica</i> (500 mg/kg body weight, i. p.)	$42.0 \pm 1.5$	$48.7 \pm 2.5$
Positive control (aspirin, 100 mg/kg of body weight, i. p.)	$37.0 \pm 1.9$	$54.8 \pm 3.06$

The initial hind paw volume of the mice was determined volumetrically. Each point represents the mean  $\pm$  S.E.M of 5 mice.  $p < 0.05$ , compared with the control group (Student's *t*-test).

**Table 2.** Qualitative tests for flavonoids and tannins in the extracts of *Ballota pseudodictamnus*, *Salvia fruticosa* and *Thapsia garganica*.

Plant extracts/controls	Test for flavonoids	Test for tannins
<i>Salvia fruticosa</i>	++	++
<i>Ballota pseudodictamnus</i>	++	++
<i>Thapsia garganica</i>	-	+
Positive control (quercetin)	+++	NP
Positive control (gallic acid)	NP	+++

+++ = Highly intense and conclusive color reaction; ++ = Moderately intense and conclusive color reaction; + = Weak and inconclusive color reaction; N/D = Not performed.

inhibition was calculated using the equation  $\text{Do}-\text{Dt}/\text{Do} \times 100$ , where Do was the average inflammation (hind paw edema) of the control group of mice at 3 h and Dt was the average inflammation of the drug-treated (extracts or reference drug) mice at the same time.

#### Statistical analysis

Data were analyzed using the Student's *t*-test and the experimental values were expressed as mean  $\pm$  SEM. Statistical significance was considered to be a P value  $< 0.05$  in all cases.

## RESULTS AND DISCUSSION

Anti-inflammatory activity of the MeOH extracts of the aerial parts of the extracts of *B. pseudodictamnus*, *S. fruticosa* and *T. garganica* was assessed by the carrageenan-induced mice paw edema model (Ahmed et al., 2005). Administration of the extracts at a dose of 500 mg/kg (10 mL/kg) of body weight produced statistically significant inhibition ( $p < 0.05$ ) of edema

within 3 h of carrageenan administration (Table 1). Among the plant extracts, *S. fruticosa* extract exhibited the most significant % of inhibition of inflammation after 3 h (62.1%), and this effect was apparently better than that of aspirin (54.8%). Although the dose of aspirin was one fifth of that of the crude extract, it is common practice to administer much higher doses of crude extracts in such assays as several compounds are generally present in these extract. The extracts of *B. pseudodictamnus* and *T. garganica* inhibited paw edema volume by 51.2 and 48.7%, respectively.

In the qualitative tests for flavonoids and tannins, *B. pseudodictamnus* and *S. fruticosa* extracts showed the presence of these classes of compounds. The test for flavonoids was negative with the *T. garganica* extract, and the positive response in the test for tannins was extremely weak and inconclusive (Table 2).

Edema is a pathophysiological inflammatory condition characterized by swelling, redness, ele-

vated temperature and pain. The reduction of carrageenan-induced edema could be successfully used as a measure of the anti-inflammatory property of test samples in animals. The carrageenan-induced inflammation model is considered an excellent predictive test for anti-inflammatory agents acting by the mediators of acute inflammation (Mossa et al., 1995).

All three extracts showed considerable anti-inflammatory activity, evident from the significant reduction of carrageenan-induced paw edema volume in mice (Table 1). The effect of the extracts in the inflammation process induced by carrageenan suggested that the extracts might have affected the time-delayed system in a similar fashion to glucocorticoids (Ahamed et al., 2005; Silva et al., 2005).

*Ballota pseudodictamnus* and *S. fruticosa* are well known for producing a variety of polyphenolic compounds including flavonoids (Moharram et al., 2006; Hennebelle et al., 2008; Erdogan-Orhan et al., 2010), whereas *T. garganica* has been reported to biosynthesize simple phenylpropanoids and thapsigargin-type sesquiterpene lactones (Liu et al., 2006). Also, in the qualitative tests for flavonoids and tannins, the presence of these classes of compounds was confirmed in *B. pseudodictamnus* and *S. fruticosa*. Because the anti-inflammatory activity of *B. pseudodictamnus* and *S. fruticosa*, as observed in the present study, was more prominent than that of *T. garganica*, it is reasonable to infer that the anti-inflammatory property of the first two species might be due to the presence of polyphenolic compounds, e.g., flavonoids and tannins. In fact, it has previously been shown that the anti-inflammatory activity of plant extracts could be linked to their polyphenol content, particularly to flavonoids, which are known to possess anti-inflammatory properties (Ahmad et al., 2000; Kiessoun et al., 2010). It can be assumed that there is a link between the reported high level of antioxidant activity of these species and their anti-inflammatory activity, and again, probably because of the high levels of polyphenols present in the extracts.

The results demonstrated considerable anti-inflammatory properties of the test extracts; the extract of *S. fruticosa* was the most potent of the three. Thus, *S. fruticosa* could be a potential source for the discovery and development of new anti-inflammatory leads for drug development.

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