

Analgesic effect of the methanol extract of *Erica arborea* (L.) in mice using formalin test

*^{1,2}Mohajjel Nayebi A., ³Nazemiyeh H., ^{1,2} Omidbakhsh R., ³Çobanoglu S.

¹Department of Pharmacology and Toxicology, ²Drug Applied Research Center, ³Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Background and the purpose of the study: *Erica arborea* L. (Ericaceae) has been used in Turkey folk medicine as a diuretic, urinary antiseptic and laxative. However, its other pharmacological effects have not been yet elucidated clearly. The aim of this study was to investigate analgesic effects of its methanolic (MeOH) extract in mice using formalin test, as a model of tonic inflammatory pain.

Methods: The MeOH extract of aerial parts and its fractions (20, 40, 60, 80 and 100% MeOH in water) were prepared by maceration and solid phase extraction method respectively. Effects of the MeOH extract (10, 20 and 30 mg/kg, i.p.) and different fractions (5 mg/kg, i.p.) were compared with analgesic effects of the morphine (10 mg/kg, i.p.) and indomethacine (5 mg/kg, i.p.) as standard analgesic drugs.

Results and major conclusion: Results showed that the MeOH extract of *E. arborea* (10 mg/kg, i.p.) similar to the morphine (10 mg/kg, i.p.) and indomethacine (5 mg/kg, i.p.) decreased formalin-induced paw licking time. Among the prepared-fractions of the MeOH extract, only fraction of 20% (5 mg/kg, i.p.) caused significant decrease in paw licking behavior. Moreover, the MeOH extract (10 mg/kg, i.p.) did not produce any motor deficit effects in rotarod test. From the results it may be concluded that the MeOH extract and fraction of 20% of *E. arborea* have a good analgesic effects in formalin test.

Keywords: *Erica arborea*, MeOH extract, Analgesia, Formalin test

INTRODUCTION

The *Erica arborea* L. (Ericaceae) is a Turkish endemic species which is distributed in the Mediterranean and Anatolian regions and is also native to a number of other countries in Africa, Temperate Asia and Europe (1). This evergreen shrub or small tree is known as funda, tree heath or briar root in Turkey, and its leaves and flowers have been used as diuretic, urinary antiseptic, diet tea and laxative (2-3). It also has been shown that different extracts of leaves and flowers of *E. arborea* have considerable antioxidant effects (4). Also isolation of some new flavonoid and phenylethanoid glycosides and their antioxidant properties have been reported (5). There are reports about the anti-inflammatory and antinociceptive activity of the ethyl acetate extract of *E. arborea* in PGE₂-induced hind paw edema model and *p*-benzoquinone-induced abdominal constriction test (6). Analgesic effects of the MeOH extract of this plant in hot plate test has also been reported (7). In the present study, analgesic effects of the MeOH extract and fractions

obtained from *E. arborea* aerial parts were investigated in mice by using formalin test, as a model of chronic inflammatory pain.

MATERIALS AND METHODS

Chemicals

The dimethyl sulfoxide (DMSO), dichloromethane and methanol were purchased from Merck (Germany); indomethacine was from Roche (Germany) and morphine sulfate was from Temad (Iran-Tehran). The MeOH extract, indomethacine and morphine were dissolved in normal saline; and fractions were dissolved in 2%(W/W) DMSO. Solutions for injections were prepared freshly on the days of experimentation and injected intraperitoneally (i.p.) 30 min before initiation of the formalin test.

Animals

The experiments were carried out on male Swiss albino mice weighing 30-35 g. Animals were housed in standard polypropylene cages, eight per

cage, under a 12:12 h light/dark schedule at an ambient temperature of 25 ± 2 °C and had access to food and water freely. All experiments were carried out under the ethical guidelines of the Tabriz University of Medical Sciences, for the care and use of laboratory animals (National Institutes of Health Publication No 85-23, revised 1985).

Plant

The aerial parts of *E. arborea* were collected from the forest around Çanakkale-Turkey (western Anatolian) during flowering period and their identities was confirmed by Dardanel Herbarium of Department of Biology, Faculty of Sciences and Arts, Çanakkale Onsekiz Mart University, Turkey.

Extraction

The dried leaves and flowers of *E. arborea* (300g) were extracted with MeOH-H₂O mixture (70:30) by maceration for 24h (800ml×3). The extracts were combined and concentrated using a rotary evaporator at a maximum temperature of 45°C. The MeOH extract (2g×3) was subjected to SPE fractionation (Sep-Pak, C₁₈ cartridge, 10 g) using a step gradient of MeOH-water mixtures (20:80, 40:60, 60:40, 80:20, 100:0). The resulting fractions were dried under reduced pressure at 40°C and residues were kept at 4°C until being used.

Behavioral study

The rats were placed in a quiet room during the light phase of the light–dark cycle. The formalin test was carried out in a transparent plastic chamber (40×25×20 cm) with a mirror placed under the floor at angle of 45° to allow an unobstructed view of the paws. Each animal was placed in the chamber for a 30 min habituation period. Thereafter, 25 µl of diluted 2.5% formalin was injected subcutaneously into the plantar region of the hind paw for noxious stimulation. Then, the mice were returned to the chamber; and recording of the behavior started immediately which lasted for 60 min. The duration of licking behavior (8-10) as a formalin-evoked response was recorded during 0-5 min (early phase) and 20-60 min (late phase) by an observer blind to treatment.

The rotarod test was used to assess the ability of the animal to “log roll” and to maintain balance on a 1-inch diameter rod revolving at a constant rate of 6 revolutions per minute (11-12). This test requires a high degree of sensorimotor coordination and is therefore used to test more subtle neurological deficits. The ability of all animals to pass the rotarod test was checked regularly prior and after drug administration.

Statistics

Descriptive statistics and comparisons of differences between each data set were calculated by the use of SigmaStat software. The data were expressed as Mean±SEM, and analyzed by one-way ANOVA in each experiment. Statistical significance was accepted at the level of $P<0.05$. In the case of significant variation ($P<0.05$), the values were compared by Tukey test. The results of rotarod test were analyzed by student t-test.

RESULTS AND DISCUSSION

Results of this study shows that the extract of *Erica Arborea* produces analgesia ($P<0.05$) in both phases of the formalin test (8,13) only at the dose of 10 mg/kg, i.p. (Figure 1). This is in accordance with our previous report on the analgesic effects of the MeOH extract of *E. arborea* in hot plate test, as a model of acute thermal pain (7). Doses of 20 and 30 mg/kg of the extract decreased licking time, but this effect was not statistically significant ($P>0.05$). It seems that some compounds of the extract may have proalgesic effect so that increasing the dose results in decrease of analgesic effect. Furthermore, the fraction of 40% MeOH extract not only had no analgesic effect but also decreased ($P<0.05$) pain threshold (Figure 2). Thus it may be suggested that some chemicals of this plant may have proalgesic effect.

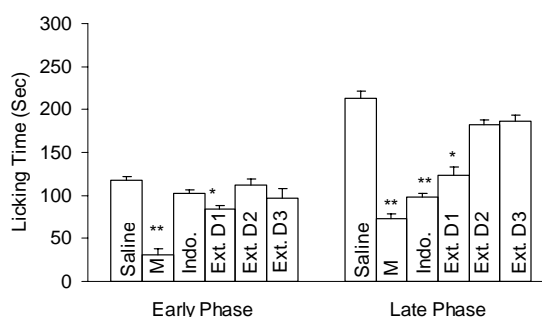


Figure 1. The effect of morphine (10 mg/kg, i.p.), indomethacen (5 mg/kg, .ip.) and three i.p. doses of *E. arborea* extract (Ext. D1= 10 mg/kg; Ext. D2= 20 mg/kg; Ext.D3=30 mg/kg) on formalin test. N= 8 mice per each group, one-way ANOVA followed by Tukey test. * $P<0.05$ and ** $P<0.01$ when compared with saline group M=Morphine; Indo.=Indomethacen

The analgesic effects of the extract and fraction of 20% were compared with standard analgesic drugs such as morphine and indomethacen (Figures 1 and 3). These drugs induce distinctive analgesic effect on biphasic responses elicited by intraplantar injection of formalin, in a way that morphine has a marked analgesic effects in both early and late phases (14,15) while indomethacen produces analgesic effects only in the late phase

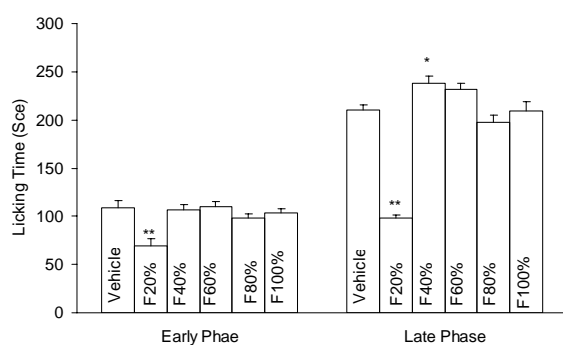


Figure 2. The effect of the MeOH fractions (5 mg/kg, i.p.) on formalin test. N= 8 mice per each group, one-way ANOVA followed by Tukey test. * P<0.05 and **P<0.01 when compared with vehicle (DMSO 2%)-treated group. F=Fraction

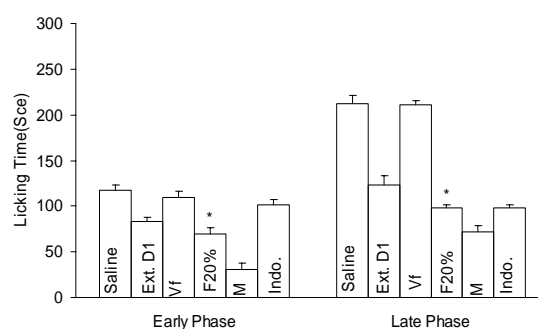


Figure 3. Effect of the Extract (Ext.D1=10 mg/kg, i.p.), fraction 20% (5 mg/kg, i.p.), morphine (10 mg/kg, i.p.) and indomethacen (5 mg/kg, i.p.) on formalin test. N= 8 mice per each group, one-way ANOVA followed by Tukey test. *P<0.05 when compared with Ext.D1 group, Vf= Vehicle of fractions; F= Fraction; M= Morphine; Indo= Indomethacen

Table 1. The results of rotarod test in Control (Saline) and the MeOH extract-administered (10mg/kg, i.p.) mice.

	Control (Saline)	Extract (10 mg/kg, i.p.)
Time (Sec)	41.6±3.8	39.8±4.2

Mean ±SEM, n=8 mice per each group, Student t-test.

(16). Results of this study show that the MeOH extract and fraction of 20% induce analgesia both in early and late phases (Figures 1 and 2). Thus, a possible involvement of opioidergic mechanism in the observed effects may be suggested.

Since fractionation of the MeOH extract results in separation and concentration of closely related compounds in individual fractions, therefore, the analgesic effect of the different fractions at half dose of the extract (5 mg/kg, i.p.) was investigated. Results show that only fraction of 20% of the MeOH extract had a good analgesic effect (Figure2) and this analgesic effect was

significantly more than MeOH extract (10 mg/kg, i.p.) (Figure 3) . It seems that concentrations of chemical(s) with analgesic effect in the fraction of 20% are higher than other fractions.

Rotarod test was used to overrule any possible sedative or motor deficit effects of the MeOH extract at the analgesic dose (10 mg/kg, i.p.). The extract did not produce any significant motor deficiency (Table 1). Thus, it may be concluded that the observed analgesic effects are not due to any sedative or motor deficit effects. On the basis of the results of the present study, the extract of *E. arborea* and its constituents may have a good pain relief effects.

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