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ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF HYDROETHANOLIC EXTRACT OF *URTICA DIOICA*

MOHAMMAD REZA FARAHPOUR[1*] AND LIDA KHOSHGOZARAN[2]

1: Department of Clinical Sciences, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran

2: Medical Student, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

***Corresponding Author: E Mail: E Mail: mrf78s@gmail.com: Tel: +98 4414373676; Fax: +98 441 3460980**

ABSTRACT

Urtica *dioica* (nettle) is a medicinal plant commonly has been used to treat rheumatic pain, colds, cough and against liver insufficiency in traditional medicine. This study was performed to evaluate anti-nociceptive and anti-inflammatory effects of hydroalcoholic extract of *Urtica dioica*. Plant extract was prepared in hydroethanolic solution by maceration at room temperature (24-26ºC) for 3 days, then the extract filtered and concentrated in administration. Adult albino mice (25-30g) and Wistar rats (200-220g) of both sexes were used in this experiment. For Assessment of thermal and pain sensitivity, Anti-nociceptive actions of *Urtica dioica* were assessed in mice using the tail-immersion (flicking response) test, and acetic acid-induced writhing test. Anti-inflammatory activities of extract, were assessed by the formalin induced inflammation test. According to the results hydroalcoholic extract of Urtica produced significant inhibition on nociception induced by acetic acid and formalin administered, compared to control group ($P<0.05$).

In conclusion, this study demonstrated that the hydroalcoholic extract of *Urtica dioica*, had dose dependent, antinociceptive and anti-inflammatory effects in experimental models.

Keywords: Antinociceptive, Anti-inflammatory, Hydroethanolic extract, Urtica dioica

INTRODUCTION

Recently, opioids or non-narcotics drugs, like salicylates and corticosteroids hydrocortisone are widely used to treat pain and inflammation. On the other hand, synthetic drugs are very expensive and their side effects is not deniable. On the contrary, in folk medicine, many medicinal herbs have been used for pain and inflammation relief with negligible adverse effects **[1, 2]**. In recent years efforts have been made to introduce new medicinal plants to develop cheaper, safer and more effective drugs.

Urtica is a wide group of plants which are distributed in subtropical regions. Several species of *Urtica* are used in Chinese folk medicine as antipyretic, anti-inflammatory and anti-nociceptive **[3]**. It is reported that *Urtica dioica* anti-inflammatory and antiproliferative effects act by inhibiting of NF-kappa B **[4]** on human prostate cancer cells **[5]**. It is reported that dried or fresh leaves or flowering aerial parts of *Urtica dioica L.*, *Urtica urens L.* and/or mixtures of these are recommended for symptomatic treatment of pain **[6]**, antihyperglycemic **[7]**, antioxidant, antimicrobial, antiulcer and analgesic activities **[8]**. Other studies showed that *Urtica dioica* has a bradycardial effect **[9, 10]**. In Turkish folk medicine, *Urtica dioica* has been used to treat rheumatic pain, colds and cough **[11]** and against liver insufficiency **[12]**. Based on the literature the aim of the current study was to evaluate antinociceptive and anti-inflammatory activities of hydroalcoholic extract of *Urtica dioica* leaves, in experimental animals.

METHODOLOGY

Preparation of Extracts

Urtica dioica leaves, was collected from the central district of the region of Hamedan, (Hamadan province, Iran) in July 2013. The plant was authenticated in Department of Botany Sciences, Agriculture and Natural Resources Research Center, Hamadan, Iran. Around 400 g of fresh plant material (leaves) was dried naturally on laboratory benches at room temperature (23-24ºC) for seven days until crisp and powdered in an electric blender. Then, 100 g of the plant powder was suspended in 400 ml of hydroethanolic solution for 96 h at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No 1). The filtrate was placed in an oven to dry at 40 ºC. The clear residue obtained was used for the study. The obtained extracts were kept at -15 °C until further use.

Animals & Treatment

Adult albino mice (25-30g) and Wistar rats (200-220g) in both sexes were used in this study based on taking into account international principles and local regulations concerning the care and use of laboratory animals **[13]**. Animals provided *ad libitum* standard food and water and kept in

rooms maintained at 22 ± 1° c with a 12-h light/dark cycle. Each animal was used once in each experiment.

Acute Oral Toxicity Study

The Acute oral toxicity test was conducted according to the method of Gorzalczany [14]. Oral The control group received only water, and the remaining groups received increasing doses up to 300 mg/kg of the hydroethanolic extract, orally, by means of a gastric catheter. Animals were maintained in a cage with free access to a standard diet and water ad libitum and them were observed twice a day, for up to 15 consecutive days. Besides the number of deaths, other parameters such as weight loss, movement, lethargy, ataxia, convulsions, diarrhoea and presence of secretions were observed.

Antinociceptive Study

Hot Water Tail Immersion

For Assessment of thermal sensitivity and measuring pain sensitivity, heat stimulus Tail-immersion (warm water) test was used. In tail - immersion tests, middle part of the tail of rats was immersed in a warm water bath ($55^{\circ C}\pm0.5^{\circ C}$) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off time 20s). The tail flick latency in seconds constituted the animals' reaction time of the heat stimulus. Shortening of the tail withdrawal time indicated sensitivity, which is attributed to central mechanism(s) (15). Mice were divided into 5 groups (n=6 in each). Group 1 received intraperitoneal injections of normal saline (10 ml/kg, i.p.). Group 2 received intraperitoneal injections of morphine (10 mg/kg, i.p.) as a reference drug. Animals in groups 3, 4 and 5 received an intraperitoneal injection of hydroethanolic extract of *Urtica dioica* (25, 50, 100 mg/kg, i.p.), respectively. The time interval between the onset of tail heating and the withdrawal response was measured manually (in seconds) using a stopwatch at 0, 30, 60 and 90 min after extracts administration. In the absence of a response, tail heating was stopped after 20 s (cut off time) to prevent tissue damage. 'Tail-flick' latency for each rat was calculated as the average of two consecutive measurements **[16-19]**.

Writhing Test

The abdominal writhes were induced by intraperitoneal (i.p.) injection of acetic acid solution (0.8%, 0.1 mL/10g) in mice. One hour after the administration of the saline (p.o.), were given different doses of hydroethanolic extract (25, 50, 100 mg/kg, i.p.), and acetic acid solution. Mice were placed individually into glass beakers and allowed to elapse for 5 min. The number of writhes produced in these animals was counted for 30 min. For scoring purposes, a writhes is indicated by stretching of the abdomen with simultaneous stretching of one hind limb. The control group and a reference analgesic drug

group (groups 1 and 2) received normal saline (10 ml/kg, i.p.) and Diclofenac (10 mg/kg, i.p.), respectively **[20]**.

Anti-inflammatory Study

Formalin Induced Inflammation

The formalin test was conducted according to the method of Saxena **[21]**. Rats were divided into 5 groups (6 mice per group). Inflammation was produced by subaponeurotic injection of 0.1 ml of 2% formaldehyde in the right hind paw on the first and third days. Animals were treated daily with the different doses of hydroethanolic extract, Diclofenac and saline for 10 days. The daily changes in paw size were measured by wrapping a piece of thread round the paw and circumference with a meter rule.

Statistical Analysis

Experimental results were expressed as Mean ± S.E.M and Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using one way ANOVA. P-values less than 0.05 were considered significant differences.

RESULTS

Acute Toxicity Test

Acute toxicity results of the oral administration of *Urtica dioica* hydroethanolic extract are presented in **Table 1**. Administration of the standardized Urtica dioica extract (at doses up to 50% concentration) showed no mortality in mice. No significant differences in body weight between the control and any of the treated groups were noted at any time. There were no toxicity signs observed on the skin, fur or eyes of the animals. There were no noticeable behavioral changes in salivation, sleeping pattern, diarrhea or lethargy in treating animals. The LD_{50} level of the hydroethanolic extract was found to be 5770 mg/kg in mice.

Antinociceptive Activity

Hot water result showed significant ($P<0.05$) reduction of pain at 30, 60 and 90 min following extracts medication. Morphine, a positive reference showed a significant analgesic effect in the hot-water test beginning 30 min after treatment among the treated groups ($P<0.05$) and the highest dose (100 mg/kg) showed better results during the whole experiment **(Table 1)**.

Different doses of extract were subjected to testing their analgesic activity using the acetic acid-induced writhing method. Significant protection against writhing was observed in animals treated with all doses of the hydroethanolic extract of urtica. The extract produced a significant and dose-dependent inhibition of acetic acid induced writhing **(Table 2)**. The analgesic activities induced by the highest dose (100 mg/kg) of the hydroethanolic extracts showed better inhibition of writhe numbers (52.49%), although other doses (2.5 and

5 g/kg) had significant inhibition percentage (33.95and 35.83%). Standard drug (Diclofenac) showed the best inhibition compared to the control group.

Anti-inflammatory Activity

Anti-inflammatory activity of the test extracts was measured against acute paw edema induced by formalin. Formalin-induced inflammation in the mouse's paw represents a classical model of acute inflammation that used for evaluation of anti-inflammatory activity of drugs or plant extracts. The extract produced a significant and dose-dependent inhibition of formalin induced inflammation **(Table 3)**. Hydroethanolic extract of Urtica in 100 mg/kg showed very good anti-inflammatory activity compared to other doses. However, 25 and 50 (mg/kg) and standard drugs showed significant reduction of inflammation compared to control group.

Table 1: Effects of the hydroethanolic extract from *Urtica dioica* (UD) on the reaction time (s) of mice exposed to the hot water test in mice.

Group	Dose	Reaction time (s)			
		0 min	30 min	60 min	90 min
Control	10 (ml/kg)	5.51±0.09	9.24±0.02^{a}	9.53±0.03^{a}	6.79±0.04^{a}
Morphine	5 (mg/kg, i.p.)	5.62±0.08	28.38±0.1^{c}	29.17±0.07^{c}	27.81±0.04^{c}
UD-treated	25 (mg/kg, i.p.)	5.58±0.06	13.28±0.04^{a}	14.22±0.08^{b}	11.31±0.06^{b}
UD-treated	50 (mg/kg, i.p.)	5.59±0.05	14.92±0.04^{a}	15.65±0.08^{b}	12.01±0.01^{b}
UD-treated	100 (mg/kg, i.p.)	5.61±0.07	18.15±0.04^{b}	20.06±0.08^{b}	15.99±0.06^{b}

Each value is presented as Mean S.D. Note: ^{a,b,c} are presenting significant (P<0.05) differences between marked groups. n= 6.

Table 2. The effects of the different doses hydroethanolic extract of *Urtica dioica* (UD) and Diclofenac on acetic acid-induced writhing model in mice

Group	Dose	Number of writhes	Inhibition (%)
Control	10 (ml/kg)	28.8±1.095^{a}	-
Diclofenac	10 (mg/kg, i.p.)	11.8±0.83^{d}	63.31
UD-treated	25 (mg/kg, i.p.)	24±0.70^{b}	33.95
UD-treated	50 (mg/kg, i.p.)	21.4±0.89^{b}	35.83
UD-treated	100 (mg/kg, i.p.)	15±0.75^{c}	52.49

Each value is presented as Mean ± S.D. Note: ^{a,b,c} are presenting significant (P<0.05) differences between marked groups. n = 6

Table 3: Effects of the Hydroethanolic extract from *Urtica dioica* (UD) on acute paw edema induced by formalin in rats

Group	Dose	Days				
		1	2	3	4	5
		Change of pow size (cm)				
Control	10 ml/kg	0.73±0.05[a]	0.42±0.05[a]	0.32±0.04[a]	0.66±0.03[a]	0.38±0.01[a]
Diclofenac	5 (mg/kg)	0.41±0.02[e]	0.19±0.02[d]	0.09±0.01[d]	0.42±0.02[d]	0.24±0.02[d]
UD-treated	25 (mg/kg)	0.65±0.03[b]	0.37±0.01[b]	0.24±0.02[b]	0.56±0.05[b]	0.36±0.02[ab]
UD-treated	50 (mg/kg)	0.60±0.02[c]	0.34±0.01[b]	0.23±0.02[b]	0.53±0.05[bc]	0.33±0.02[b]
UD-treated	100 (mg/kg)	0.52±0.18[d]	0.27±0.02[c]	0.14±0.02[c]	0.51±0.05[c]	0.29±0.03[c]
		Days				
		6	7	8	9	10
		Change of pow size (cm)				
Control	10 ml/kg	0.28±0.02[a]	0.27±0.02[a]	0.28±0.02[a]	0.33±0.04[a]	0.39±0.03[a]
Diclofenac	5 (mg/kg)	0.11±0.01[c]	0.06±0.02[d]	0.04±0.03[c]	0.03±0.01[d]	0.02±0.02[d]
UD-treated	25 (mg/kg)	0.32±0.02[a]	0.22±0.04[b]	0.25±0.03[a]	0.24±0.02[b]	0.27±0.04[b]
UD-treated	50 (mg/kg)	0.30±0.02[a]	0.21±0.04[b]	0.23±0.02[a]	0.21±0.01[b]	0.25±0.04[b]
UD-treated	100 (mg/kg)	0.18±0.04[b]	0.14±0.02[c]	0.12±0.02[b]	0.11±0.02[c]	0.15±0.03[c]

Each value is presented as Mean S.D. Note: [a,b,c] are presenting significant (P<0.05) differences between marked groups. n = 6

DISCUSSION

Pain is a condition which is regularly dealt with in daily clinical practice. Hence, any attempt to contribute an easily available analgesic drug from the available flora is always accepted without any reluctance which has no much cost and the least side effects have been reported for them **[22]**. Formalin-induced inflammation in the rat paw represents a classical model of acute inflammation that used for evaluation of anti-inflammatory activity of drugs or plant extracts. The purpose of this paper was to establish a scientific basis for one of the traditional uses of *Urtica dioica* as a painkiller and anti-inflammatory plant in mice. The results indicate that administration of *Urtica dioica* in a dose dependent manner had antinociceptive activity. Randall *et al.*, **[6]** based on historical evidence, reported since roman times nettle (*Urtica dioica*) has been used for the treatment of joint/muscle pains. These results support the historical evidence that the sting of nettles is an effective and safe treatment for musculo-skeletal pain.

Urtica dioica has anti-inflammatory effect by inhibiting NF-kappa B **[4]** which might one of the mechanisms which impress its antinociceptive activity. In our study, higher doses of hydroethanolic extract showed better inhibition of NF-Kappa B. In the previous studies, using the hotplate test in mice, aqueous nettle (Urtica dioica) extract (1200 mg/kg, i.p) showed much greater resistance to thermal stimulation in mice **[23]**. In our study, hot water test showed that hydroethanolic extract significantly increased pain threshold from 30 min posttest initiation. In the acetic acid-induced

writhing test in mice, hydroethanolic extract in a dose of 25, 50 and 100 mg/kg, i.p produced a dose-dependent inhibition in writhing which was more pronounced than that of diclofenac. The Antinociceptive activity of opioid and non-steroidal anti-inflammatory agents (NSAIDs) can be determined by the writhing test **[24]**. The association between antinociceptive activity and moderate anti-inflammatory effect observed with these extracts and NSAIDs. It is a well-documented that NSAIDs exert analgesic and anti-inflammatory properties via blockade of cyclooxygenase (COX) activity **[25]**. Intraperitoneal or oral ethanolic Nettle extract was ineffective in the hot plate test in rodents **[26]** but in our experiment hydroethanolic extract of Urtica showed significant effect in hot water tail immersion test. Local application to the tail of a lyophilized hydroethanolic Urtica leaf extract (100 mg/ml) was associated with an increase in thermal threshold in the tail-flick test. Based on our data, we think it might relate to inhibition of prostaglandins' (PGEs) in the pain area. Intraperitoneal injection of acetic acid up-regulates PGEs like PGE-2 and PGF-2α in the peritoneal fluid of acetic acid-induced mice **[27]**. Thus, the antinociceptive effect of the hydroethanolic extract might mediate by peripheral effects, including the PGE synthesis inhibition. The central action confirmed in the hot water test (25, 50 and 100 mg/kg) showed that the maximum effect was reached at 90 min. This test is considered to be sensitive to drugs which act at the supra-spinal modulation level of the pain response **[28]**.

Gulcin *et al.*, **[8]** showed that pretreatment with nettle and metamizol inhibit the acetic Acid-induced writhing in mice. The inhibitory effects of nettle were in a dose-dependent manner which 50, 100 and 200 mg/kg showed 62.1, 70.4 and 89.2% decrease in inflammation, respectively. It is suggested that the antinociceptive and anti-inflammatory effects of the hydroethanolic extracts might relate to their flavonoids, tannins and anthocyanins contents. Numerous flavonoids include rutin, quercetin, luteolin, hesperidin and bioflavonoids produced antinociceptive and anti-inflammatory activities **[29, 30]**. There are few reports on the role of tannins in antinociceptive and anti-inflammatory activities **[31]**. Recently, it has been shown that crocins and crocus glycosides exhibited an anti-inflammatory effect on some models of inflammation **[32]**. In the current study, anti-inflammatory activity was confirmed by paw edema induced by formalin in rats. Carrageenan encourages paw edema releases histamine, serotonin, bradykinin, substance P, platelet activating factor and PGEs **[33, 34]**. In addition, treatment with the *Urtica dioica* extract significantly inhibited the paw edema. This evidence suggests that the anti-inflammatory actions of the hydroethanol extract

are related to inhibition of one or more signal intracellular pathways involved with these mediators effects. Based on Gulcin *et al.,* **[8]**, nettle exhibits, free radical inhibitor or scavenger activity as well as a primary antioxidant that reacts with free radicals, which may limit free radical damages occurring in the human body **[35]**. We believe that it can be causes for nettle's long-term analgesic activity, especially in hot water test. Also, other substances, e.g. flavonoids, caffeoyl malic acid, caffeic acid and caffeoyl-esters potentially have the capacity to diminish pain via different mechanisms but merit needs more studies to identify their theraputic properties on pain and inflammation **[36]**.

CONCLUSION

Several studies have been carried out on the different extracts of Urtica dioica (Nettls), antinociceptive effect using different methods. In the present study, we have been using a tail flick test using thermal simulation, writhing test and formalin induced inflammation test to evaluation antinociceptive and anti-inflammatory effects of hydroethanolic extract of *Urtica dioica*. The results clearly showed that hydroethanolic extract of *Urtica dioica* has a dose-related antinociceptive action in treated groups, especially at high levels. Also, we suggesting that the extract might represent potential therapeutic options for the treatment of pain related diseases but we think further studies are necessary to assess the potential clinical use of this plant or its extract as an analgesic drug.

Conflict of Interest

The authors declare that they have no conflict of interest.

Informed Consent

This manuscript does not contain any studies with human subjects performed by any of the authors.

Human and Animal Rights

All experimental executed in according to the Guide for the Care and Use of Laboratory Animals. In this study, protocols for animal experiment were approved by the institutional animal ethical committee.

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