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Original Research

Anti –Inflammatory Effect of Methanolic and Aqueous Extracts of *Urtica pilulifera* L. Seed in Rats

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Abstract

Wide range of acute and chronic inflammatory ailments, side effects of their available therapies and incomplete treatment of such patients push the researches to find new and more effective drugs. To reach this aim, in the current study, we evaluate *Urtica pilulifera* L. (family *Urticaceae*) as an introduced traditional herb for treatment of inflammation in Persian Medicine (PM). In an animal study, Anti-inflammatory effects of *U. pilulifera* were assessed in formalin-induced hind paw edema in rats. Sodium salicylate (300 mg/kg, i.p., SS) injection was used as a positive control drug and compared with methanolic extract of *U. pilulifera* (20 mg/kg; i.p.) (MUP), three different doses of aqueous extract of *U. pilulifera* (20, 40 and 80 mg/kg; i.p.) (AUP) and a group of distilled water (6 mL/kg; i.p.). As acute anti-inflammatory effect, AUP in doses 40 and 80 mg/kg decreased edema significantly ($p<0.05$). In chronic anti-inflammatory response, results indicated that all AUP doses had anti-inflammatory effects ($p <0.05$) with no significant difference with SS group. In conclusion, AUP had anti-inflammatory effects on both acute and chronic edema; while MUP was only effective in chronic inflammation.

Keywords: *Urtica pilulifera*; Inflammation; Rat; Persian medicine

Introduction

Anti-inflammatory drugs such as glucocorticoids and NSAID's (Non Steroid Anti-Inflammation Drugs) are the most frequently used drugs in prescriptions; however, they are not useful in all cases and have many reported side effects like gastrointestinal complications [1-4]. Considering the wide range of acute and chronic inflammatory disorders, as well as the side effects of common anti-inflammatory drugs and treatment failures of such patients with the current available drugs, development of new and more effective drugs is beneficial. Natural sources, traditional systems of medicine and medicinal plants are valuable treasures to find new drugs according to the ancient traditional medicine books [5].

WHO has requested member states to integrate their traditional medicines to main stream allopathic medicine [6]. Persian Medicine (PM), which originated from Iran, is one of the oldest traditional medicines with a great influence on the medicine during history [7].

The term of *Mohallel* in PM is too similar to anti-in-

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flammatory effect in current medicine because *Mohallel* drugs decrease volume of swelling and inflammations [8]. *Urtica pilulifera* L. (*UPL*) is one of the medicinal plants frequently cited as *Mohallel* in PM documents [9-13]. Also, the genus Urtica (Urticaeace) has a list of active agents like quercetin, isorhamnetin and kaempferol with remarkable anti-inflammatory activity [14]. In carrageenan-induced edema model in rats, petroleum ether extract of *UPL* seeds displayed a significant acute anti-inflammatory activity [15]. *UPL* and other species of the genus Urtica have been mentioned repeatedly in PM [8]. *UPL* extensively grows in the North regions of Iran. This species is named Nettle in English and *Anjereh* or *Gazaneh* in Persian [9].

In the present study, we aimed to study anti-inflammatory effects of methanolic and aqueous extracts of *U. pilulifera* (MUP and AUP, respectively) in an animal model of inflammation.

Materials and Methods

Plant material

U. pilulifera seeds were obtained from the traditional herbal market (Attari), authenticated by M. Kamalinejad and deposited in the Herbarium center of the School of Pharmacy, Shaheed Beheshti University of Medical Sciences (voucher No. 82). Then, the seeds were cleaned and powdered by mixer.

Preparation of methanolic extract

Firstly, 40 g of powdered seeds was mixed with 200 mL methanol (Merck, extra pure) in a magnet mixer for 5 minutes. Then, it was soaked for 3 days, filtered and dried at room temperature (25°C). Finally, 1.54 g dry extract was obtained. The dry extract was suspended in distilled water and administered intraperitoneally (i.p.) [16].

Preparation of aqueous extract

At first, 7.5 g of the seeds powder was boiled in 1500 mL of pure water for 5 minutes and then the solution was subjected to filtration. The filtrate was subsequently freeze dried via freeze-dryer apparatus (Eyela Rikakikai Co. LTD Freeze-dryer FD-1), giving 4 g dry extract which was stored at -18°C. The powdered extract was then used to make solutions of different concentration in distilled water [17].

Total phenolics measurement

The total phenolic content of AUP and MUP extracts was measured by Folin-Ciocalteu assay [18]. These extracts were added to 0.25 mL of Folin-Ciocalteu reagent and 0.5 mL of sodium carbonate solution (7.5%) and subsided in a dark place at normal room temperature for 30 minutes. Thereafter, by using the multimode plate reader, optical density (OD) was checked at 765 nm (Synergy H1, BioTek, USA). The reference standard was gallic acid (GA) which was used to draw calibration curve. The total phenolic content of AUP and MUP extracts was drawn from the calibration curve of GA, and the outcome was explained as mg of GA equivalents per g of AUP and MUP extracts (mg GAE/g).

Total flavonoids measurement

The measurement of total flavonoids content of AUP and MUP extracts was performed by the aluminum chloride colorimetric method [18]. Briefly, AUP and MUP extracts were added to 70 µL of sodium nitrite solution (5%) and subsided for 5 minutes before combination with pure water (1.3 mL), sodium hydroxide (1 M) (0.5 mL), and aluminum chloride (10%) (0.15 mL) and allowed to rest at room normal temperature for 5 minutes. Afterward, by using a multimode plate reader, OD was observed at 415 nm (Synergy H1, BioTek, USA). As reference standard, catechin (CC) was used to draw the calibration curve. The total flavonoids of AUP and MUP extracts were measured using the calibration curve of CC, and the final outcome was explained as mg of CC equivalents per g of AUP and MUP extracts (mg CCE/g).

Anti-inflammatory test

Male NMRI rats with weigh between 240 to 360 g (Pasteur Institute, Iran) were used in this study. They were kept in Plexiglas cages (7 rats per cage), with free access to water and food on a 12 h/12 h light and dark reels, normal room temperature (25°C ± 1) and relative humidity (55±5%). The ethics and method of the research were approved by the Tehran University of Medical Sciences as a thesis project (No. 1382/18811).

The formalin-induced paw inflammation model was used for anti-inflammatory test [19]. Formaldehyde solution (0.05 mL of 2.5 %) injected into right hind paw (sub-plantar region) of the rats. The negative control group was treated by distilled water (DW). Sodium salicylate (Merck, SS, 300 mg/kg, i.p.) was administered to the positive control group. Intervention groups received 20, 40, and 80 mg/kg i.p. doses of AUP and 20 mg/kg i.p. dose of MUP. The injection volume was 4 mL/kg for DW, SS, AUP and MUP treatments. Before and after formalin injection, the hind paw volume was determined and the difference between these two volumes was used as the degree of inflammation. Interventions were administered 35 minutes before formalin injection and the hind paw volume was observed just before administration of interventions and one hour after formalin injection (95 minutes after receiving the drug dose) in acute administration tests (day 0); and for seven days, in chron-

ic administration tests. In chronic inflammation, the rats treated by i.p. injection of drug (DW, SS, AUP or MUP) in days 1–7 just after hind paw volume measurement. In other words, only one paw injection of formalin was done on day 0; while the interventions were administered daily from day 1 to day 7 and paw volume measurement was done for estimating chronic effects. One hour after formalin injection on day 0, acute anti-inflammatory response was checked and one day to 7 days after that, chronic anti-inflammatory responses were checked by measuring hind paw edema using a mercury-balance plethysmometer set [20].

Statistical analysis

The outcomes of the study are explained as mean±SEM. The differences between AUP, MUP and SS groups in comparison with DW group were calculated by one-way ANOVA supported by LSD's test for the acute tests, and by means of Student's unpaired t-test for the chronic studies. When the p value was <0.05, the difference was accepted as significant.

Results

Total phenolic and flavonoid contents

Total phenolic and total flavonoid content of MUP extract were 153 mg GAE/g and 17.40 mg CC/g, respectively. While AUP extract contained 18.81 mg GAE/g total polyphenolic and 1.124 mg CC/g total flavonoid contents.

Anti-inflammatory tests

Table 1 demonstrates acute effects of AUP, MUP and SS. The numbers are mentioned as mean±SEM. AUP inhibited the paw edema with a dose related pattern but not dose dependent. SS (300 mg/kg) and AUP in doses 40 and 80 mg/kg, 1 h after formalin injection, decreased edema significantly in comparison with DW group ($p<0.05$). In MUP group no significant difference in acute anti- inflammatory study was observed.

In chronic anti-inflammatory effects (during 7 days) results state that MUP (20 mg/kg) and all AUP groups (20, 40 and 80 mg/kg) had anti-inflammatory effects in comparison with DW group ($p<0.05$) without any significant difference with the SS group.

Figure 1 demonstrates outcomes of chronic injection of DW, SS, MUP and AUP. In days 4, 5, 6, and 7, anti-inflammatory effect of SS (300 mg/kg) was significant, while MUP (20 mg/kg) and AUP (80 mg/kg) decreased inflammation in days 2, 3, 4, 5, 6 and 7. Recovery of half of the rats (in comparison with peak of paw edema in DW group) occurred around day one after formalin injection by MUP (20 mg/kg) and AUP (80 mg/kg), and around day 2.5 for SS (300 mg/kg), and in day 6 for the DW group.

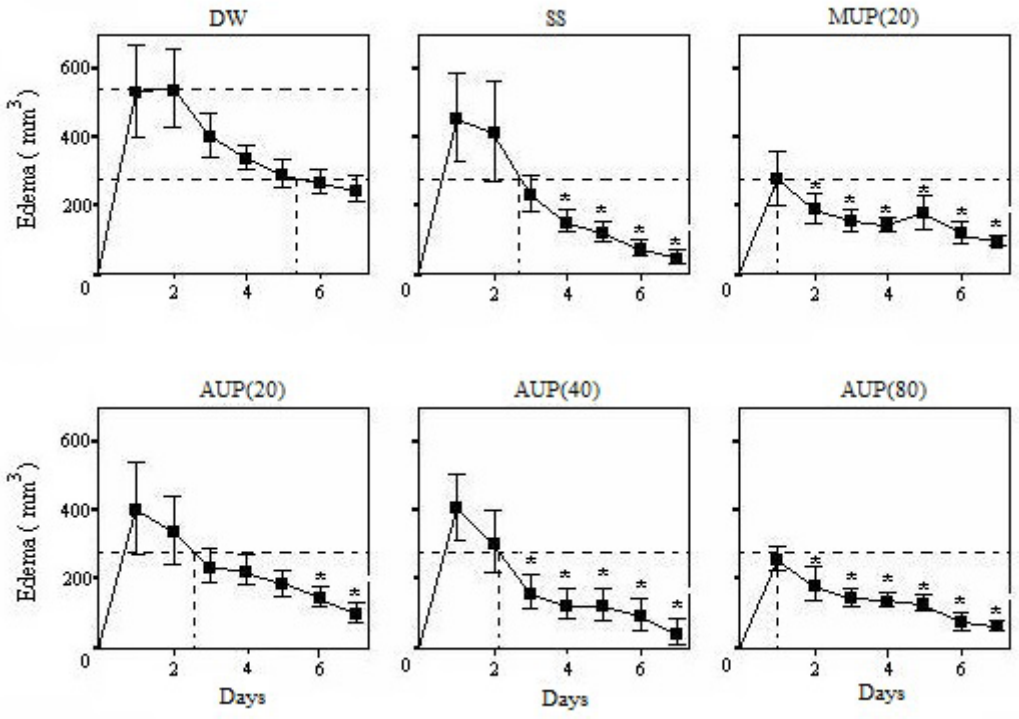

Figure 1. Effects of chronic administration of MUP (20 mg/kg, i.p) and AUP (20,40 and 80 mg/kg, i.p), SS(300 mg/kg, i.p.) and DW on formalin-induced paw edema during 7 days after formalin injection. Each point with vertical bar represent the mean±SEM. n=7 per group.
*P < 0.05 was considered statistically significant.

Table 1. Effects of acute administration of MUP, AUP and SS on formalin-induced paw edema, 1 h after formalin injection in rats

Sample	Dose (mg/kg)	*n*	Volume of edema (mm^3)	% inhibition
DW	-	7	303± 71	-
SS	300 (i.p.)	7	196± 37*	37.76
MUP	20 (i.p.)	7	285± 57	8.63
AUP	20 (i.p.)	7	259± 51	15.17
	40 (i.p.)	7	211± 29*	33.2
	80 (i.p.)	7	211± 66*	33.2

Percentage inhibition of inflammation= (1-(Vt/Vc)) X 100. Vt is the average paw thickness in treated groups (MUP, AUP and SS), Vc is the average paw thickness of the control group (DW).
* $P < 0.05$ was considered statistically significant

Discussion

A previously published study of *UPL* in Turkey has indicated anti-edema effect of the petroleum ether extract of *UPL* seeds in carrageenan-induced edema in rats [15]. Other studies have shown that hydroalcoholic extract of *Urtica dioica* L. and *Urtica urens* L. have anti-inflammatory effects [21-24]. In this study, our results support that the seeds extract of *UPL* has a significant anti-inflammatory effect that could suppress the rat paw edema created by formalin in chronic and acute administration. The effectiveness of 20 mg/kg dose of the MUP and 80 mg/kg dose of the AUP were significantly higher than SS in chronic administration. Also, 50% recovery time was shorter for the extracts, so these outcomes suggest that MUP and AUP extracts may be more effective in chronic administration. Furthermore, acute and chronic administration of the MUP and AUP extracts for 7 days did not develop any noticeable acute toxicity. Triterpenoids are as the main contents of the AUP extracts of *U. pilulifera* [8,19]. The anti-inflammatory activity of triterpenoids has been previously described [26-28]. Even more importantly, the suppression of adaptive nitric oxide synthesis (iNOS) and adaptive cyclooxygenase-2 (COX-2) enzymes has been confirmed for triterpenoids [25,26,28]. According to this fact, it seems that anti-inflammatory mechanism of AUP extract might be related to the triterpenoids present in the seed [15]. The process by which the MUP emerged anti-inflammatory effect could be the same of U. dioica, which was exhibited to inhibit the nuclear factor κB (NF-κB) activation [29]. NF-κB organizes immune mechanisms and plays an important role as a mediator of inflammatory responses, induces the expression of inflammatory genes, like cytokines and chemokines related genes. Also, NF-κB regulates differentiation, activation and survival of immune cells (like inflammatory T cells) and Urtica suppresses this nuclear factor [29,30].

On the other side, according to our analysis in this study, *UPL* is a good source of polyphenols and flavonoids which are well-known regulators of inflammation in the body [27,28].

On the basis of these outcomes, further studies on anti-inflammatory effects of *UPL* is recommended. Further projects should include in silico, in vivo and in vitro works on the different active ingredients of *UPL*.

Conflict of Interests

None.

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