Investigation of Acute Toxicity, Anti-inflammatory, and Analgesic Effect of *Urtica dioica* L.

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Summary

In our study, we aimed to investigate the acute toxicity and analgesic effect of *Urtica dioica* L fixed oil (UD) in mice, and its anti-inflammatory effect in rats. The acute toxicity of UD was tested for the increasing doses between 0.2 and 12.8 mL/kg. The anti-inflammatory effect was studied in carrageenan induced tissue inflammation model and the effect of two different doses of UD was compared with that of isotonic saline, ethyl alcohol, and indomethacin. The analgesic effect was evaluated by tail-flick response and the effect of UD was compared with that of morphine hydrochloride and isotonic saline. It was found that the reduction in inflammation was 95.70% with indomethacin (3 mg/kg i.p.), 47.40 % with 0.05 mL/kg UD i.p. and 56.97% with 0.15 mL/kg UD i.p. Both UD doses showed statistically significant anti-inflammatory effect compared to the control groups but weaker than indomethacin. UD showed no significant analgesic effect compared to the control group. Fixed oil of UD was non-toxic. Our preliminary data show that UD fixed oil extract has a mild anti-inflammatory effect but it is not analgesic or toxic in the dose range examined.

Key words: *Urtica dioica* L, carrageenan, anti-inflammatory and analgesic effect, toxicity.

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Introduction

There has been growing interest in using plants in medicine and herbal therapy is becoming a common practice. There is also a tendency in Turkey towards pharmacological and toxicological studies of plants. In countries like Turkey, which has a wide flora, has a limited economical resources and limited technology for synthetic drug production, development of drugs obtained from natural sources and encouraging the use of herbal medicine could be a reasonable approach for the aim of supplying sufficient and cheap medicine (1). *Urtica dioica* L. (UD) is a perennial plant with stinging hairs belonging to the plant family Urticaceae with height of 30-100 cm. It is endemic in many parts of Turkey and seeds are widely used in folk medicine, particularly in the therapy of advanced cancer patients for a long time (2).

Uncini Manganelli et al (3) reported that UD had antiviral activity against feline immunodeficiency virus (FIV) infection. Daher et al (4) showed that UD decreased in total cholesterol, LDL cholesterol, LDL/HDL cholesterol ratio and plasma total apo B. Turkdogan MK et al (5) reported that UD fixed oil had significant hepatoprotective effect on carbon tetrachloride model in rats.

There has been no study related to acute toxicity, analgesic and anti-inflammatory effects of UD seeds fixed oil which is used in folk medicine to treat rheumatism (2,6). In the present study, we investigated acute toxicity and analgesic effects of UD in mice and its anti-inflammatory effects in rats.

Material and Methods

The seeds of UD were purchased from a local herbal store in Van, Turkey. The plant samples were kept at room temperature until they are finely ground. The seeds of the plant were ground in a mixer. Ground plant material was macerated with diethyl ether for 2 hours. The solvent was evaporated (Büchi RE 111 rotavapor and Büchi 461 water bath, Switzerland). The content of the fixed oil of the seeds was calculated. The yield of the fixed oil of UD was 24 %.

After obtaining the approval of the Ethical Committee of Animal Breeding and Research Yuzuncu Yil University, Faculty of Medicine (2005/06-03), male and female Sprague-Dawley rats (150-250 g) and male and female Swiss albino mice (15 weeks-old, 26-32 g) were obtained from the Animal House, Yuzuncu Yil University, Faculty of Medicine, Van, Turkey. All animals were housed in standard cages (48x35x22 cm) at room temperature (22 ± 2 °C), under artificial light from $7.^{\underline{00}}$ am to $7.^{\underline{00}}$ pm. Animals was deprived of food for 12 h but allowed free access to water before the experiments.

Indomethacin and lambda-carrageenan were obtained Sigma (Steinheim, Germany). Lambda-carrageenan (1%) was solved in isotonic saline solution (ISS) (0.9 % NaCl) and indomethacin was solved in ethyl alcohol (96 %).

For the acute toxicity test male and female Swiss albino mice were randomly assigned to eight groups with eight animals in each group. ISS was administered to the first group (control group) and UD solutions were administered to the other seven groups intraperitoneally (i.p) in increasing dosages of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mL/kg body weight. The percentage mortalities were converted to probits. Regression lines were fitted by the method of least squares and confidence limits for the LD_1, LD_{10}, LD_{50}, LD_{90} and LD_{99} values were calculated by the method of and Kouadio et al and Litchfield et al (7, 8).

The method of Winter et al (9) for anti-inflammatory effect was used with slight modification. Thirty male rats were divided into five groups of six animals each. The rats were fasted for 12 h and deprived of water only during the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in edematous response. Inflammation of the hind paw was induced by injecting 0.05 mL fresh lambda carrageenan (phlogistic agent) into the subplantar surface of the right hind paw. The control group-I was given ISS (0.1 mL) and the control group-II was given ethyl alcohol (0.1 mL). The third group (reference group) received indomethacin (3 mg/kg, ip), an anti-inflammatory agent (10) while the remaining two groups received UD at doses of 0.05 and 0.15 mL/kg, i.p by Hamilton injector.

The measurement of foot volume was accomplished by displacement technique using a plethysmometer (Ugo Basile 7140 plethysmometer, Italy), immediately before and three hours after the injection. The percentage inhibition of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the formula (7**)**:

$$I\ \% = [(1-(dt/dc)] \times 100$$

where *dt* is the difference in paw volume in the drug-treated group and *dc* is the difference in paw volume in the control group.

Analgesic response was assessed with a tail-flick apparatus (LSI Letica LE 7106, Barcelona-Spain) using a method initially described by D'Amour and Smith (11). Eighteen male mice were divided into three groups of six animals each. The animals were gently immobilized by using a glove, and the radiant heat was focused on a blackened spot 1-2 cm from the tip of the tail. Beam intensity was adjusted to give a tail flick latency of 5-8 sec in control animals. Measuring was terminated if the latency exceeded the end of time (15 sec) to avoid tissue damage. In all the experiments mice were tested twice 30 min before drug administration and the baseline latency was determined and the same tests were repeated 30, 90 and 150 min after drug administration. Morphine hydrochloride (10 mg/kg, subcutaneous) was used as reference standard (12). Only ISS (0.2 mL, i.p) was given to the control group. 0.1 mL/kg UD was given i.p to UD group.

The values were expressed as mean ± S.E.M (standard error of the mean). One-sample Kolmogorov-Smirnov test was applied for analysis of data distribution. Groups with normal distribution in this test were analyzed by one-way variance analysis (ANOVA). Statistically significant groups were analyzed for homogeneity of variances and homogenous groups ($p>0.05$ in Levene test) were tested with post-hoc Tukey LSD test (least significant difference test) (13, 14) $p<0.05$ were accepted as statistically significant.

Results

The fixed oil of UD was completely non-lethal even at doses reaching 12.8 mL/kg and considered non-toxic.

Table 1 shows the results of anti-inflammatory effects of i.p administered UD on carrageenan induced paw edema in rats. The reduction in lambda carrageenan induced inflammation was 95.70% with indomethacin, 47.40% with 0.05 mL/kg UD and 56.97% with 0.15 mL/kg UD. Anti-inflammatory effects of indomethacin and UD fixed oil extract were significant compared to the control groups ($p<0.05$), while 0.05 and 0.15 mL/kg UD were statistically less effective than indomethacin ($p<0.05$).

Table1. Effects of UD on rat paw edema

Groups (n=6)	**Dose**	**Paw edema (mean±SEM) (mL %)**	**Inhibition (%)**
Control (ISS)	0.1 mL	1.043 ± 0.127	-
Control (ethyl alcohol)	0.1 mL	0.988 ± 0.112	-
Indomethacin	3 mg/kg	[ab] 0.024 ± 0.006	95.70
UD	0.05 mL/kg	[abc] 0.549 ± 0.098	47.40
UD	0.15 mL/kg	[abc] 0.449 ± 0.101	56.97

Post-hoc LSD test:
a: $p<0.05$ compared to control (ISS) group,
b: $p<0.05$ compared to control (ethyl alcohol) group,
c: $p<0.05$ compared to indomethacin group.

Analgesic effect results are shown in Table 2. There was no significant difference between group ISS and group UD at baseline, 30th, 90th and 150th min of the study although in group morphine, analgesic effect was found significantly longer than group ISS and group UD at 30th and 90th min ($p<0.05$).

Table 2. Results of UD and the other groups on tail-flick test (mean ± SEM) (*n=6*).

Groups (n=6)	0th min (baseline)	30th min	90th min	150th min
ISS (control)	7.20±0.30	7.33±0.27	7.39±0.29	7.20±0.21
UD	6.70±0.64	7.82±0.72	7.42±0.44	7.58±0.68
Morphine	7.00±0.46	[a] 10.41±0.60	[a] 11.86±0.42	7.01±0.68

ISS: Isotonic saline solution
Post-hoc LSD (least significant difference) test:
a : $p<0.5$ compared to control-I (ISS) group,

Discussion

In the medical literature, there seems to be no investigation on the analgesic and anti-inflammatory effects of UD. This study showed that UD seeds fixed oil extract had an anti-inflammatory effect in lambda-carrageenan induced rat paw oedema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (15). Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents (16). Oedema formation due to carrageenan in the rat paw is a biphasic event (17). The initial phase is attributed to the release of histamine and serotonin (18). The second phase of oedema is due to release of prostaglandins, protease and lysosome (17,18). The second phase is sensitive to most clinically effective anti-inflammatory drugs (16,17). UD has been used in folk medicine as antipyretic, purgative, diuretic and to treat rheumatism (2,6). The therapeutic use of UD in rheumatism corresponds with the anti-inflammatory effect shown in this study.

Obertreis et al (19) showed that the antiphlogistic effects of the *UD folia* extract IDS 23 and the main phenolic ingredient caffeic malic acid were tested concerning the inhibitory potential on biosynthesis of arachidonic acid metabolites in vitro. The antiphlogistic effects observed in vitro might give an explanation for the pharmacological and clinical effects of IDS 23 in the therapy of rheumatoid diseases. Harput et al (20) found that aqueous UD extract stimulated the proliferation of T-lymphocytes and suppressed NO production in lipopolysaccharide-stimulated macrophages without affecting cell viability. Riehemann et al (21) showed that part of the anti-inflammatory effect of *Urtica extract* might be ascribed to its inhibitory effect on NF-kappa B activation.

The anti-inflammatory effect of UD shown in the present study in agreement with the literature cited above. In the acute toxicity experiment with UD there was no death within 72 h among the animals even 12.8 mL/kg high dose was used. These findings suggest that UD extract is almost non-toxic.

As a result, UD fixed oil extract had a weak anti-inflammatory effect in rats, UD had no analgesic effect in mice and did not have a toxic effect. Fixed oil extract of UD should be studied with various chromatographic methods to determine its constituents. The major components of UD could then be investigated in terms of their anti-inflammatory effects and the mechanisms of this effect.

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