# Anti-inflammatory effect of alcoholic *Urtica dioica* extract in male NMRI rats

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## A B S T R A C T

**Background and Objective**: Regarding side effects of acute and especially chronic inflammation and incomplete treatment of patients suffering from these side effects, the new and effective strategies are needed. For this purpose, in the present study, we scientifically evaluate an introduced folk herb *Urtica dioica*, for treatment of inflammation.

**Materials and Methods**: The Sham, control and treatment groups were subjected to four methods in order to measurement the inflammation: 1) Formalin-induced hind paw inflammation. In 2nd and 3rd methods, respectively, inflammation was induced by xylene and acetic acid application to ear and peritoneum. In the last method (chronic pain) the weight difference of cottons implanted in groin border of rats, before and after 7 days were compared in control and treatment groups.

**Results**: Statistical analysis has shown a significant difference between rate of inflammation in control and treatment groups. The extract in doses of 50 and 100 mg/kg could reduce inflammation produced by formalin 24.52 ± 2.2 and 22.71 ± 2.1 % respectively. However, three doses of the extract (20, 50 and 100 mg/kg) have significantly reduced the acetic acid produced peritonitis, 21.45 ± 2.4, 18.55 ± 2.2 and 27.49 ± 1.8 % respectively. Results in chronic inflammation examination showed that the extract in doses over 400 mg/kg could have diminished inflammation 24.08 ± 2.1 %.

**Conclusion**: This study shows that alcoholic *Urtica dioica* extract could markedly reduce the chronic and acute inflammation.

## 1. Introduction

Inflammations, especially chronic inflammations are prevalent side effects of many diseases, weakening the immune system. In addition to infectious disorders, this process delays the treatment of the main disease. Although using frequent chemical drugs such as corticosteroids have been effective in reducing inflammation, side effects of these drugs are completely recognized and inevitable. Therefore, new study introduces supplementary treatments, especially herbal medicine at low costs with minimum side effects (1).

*Utrica dioica*, U.d, is one of the medical herbs, especially in Iran, which has been used as anti turgid treatment in ancient Persian medicine (2, 3). Also it has been known to have anti oxidant

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(4) and blood fat decreasing effects (5). Although there are some reports showing that it contains allergen ingredients (6), many studies have reported anti inflammatory effects of this herb, of which antibacterial effects (1), prostatitis treatment, colon infection treatment, reduction of alcohol induced toxicity in liver, and treatment of many inflammations, especially knee and femoral bone arthritis can be mentioned (7-10). Of course, the pain-relieving effect of this herb has to be mentioned. It is noteworthy to mention many reports about this herb have been clinical and case reports. Based on this, in the present study, experimental-laboratorial model has been decided for the herb. According to the above-mentioned, multiple models of acute and chronic inflammations have been adopted. Acute inflammation models are done by injecting formalin, xylene, and acetic acid, which are standard forms of acute inflammation (11). Besides, regarding the significance of studying chronic inflammation, groin plate implantation model, which is one of the best inflammation evaluation models, has been used (12).

Regarding the inflammation treatment problems, especially chronic form of that, based on anti-turgid reports of *Utrica dioica*, mostly in Persian herbal medicine books, in this study the anti-inflammatory (acute and chronic) effects of alcoholic *Utrica dioica* extract in male rats with multiple methods of evaluating the inflammation have been studied, in order to form an experimental and scientific model.

## 2. Materials and Methods

### 2.1. Animals under experiment

In this study, male NMRI rats weighing 300-350 g were used. The animals were stored in equal temperature and daytime situations with unrestrained access to one type of nutrition and water. It is notable that each animal was used only in one experiment.

### 2.2. Materials

- Evans blue color substance: Used with concentration of 30 mg/kg to measure the inflammation
- Formalin 2.5%:to form hind palm inflammation in the animal (50 µl)
- Ketamine: to anesthetize to the animal
- Xylene: to form ear inflammation in the animal (0.03 ml)
- Acetic acid: to form peritonitis
- Acetone solution (3/5) + Sodium sulphate (1/5) 1%: to extract evance blue color substance from foot and ear parts of the animals
- Utrica dioica extract (in variable doses)

### 2.3. *Utrica dioica* alcoholic extract producing method

After obtaining *Utrica dioica* from local stores, and precise recognition of it, probable impurities were separated. The dried herb was completely grin

ded and turned into powder. Then, was stored in laboratory with a 1 to 4 ratio to methanol 70% for 24 hours, and then was filtered using big and small leach papers. Pure extract was stored in 50°C tissue bath in order to evaporate the alcohol. Dry extract was powdered and variable concentrations in milligrams to each kilogram of animal weight were produced using normal saline (12).

### 2.4. Administered inflammatory tests

In this study, multiple methods were used to form acute and chronic inflammation.

### 2.5. Inflammation test with formalin:

This test was used in order to study acute inflammation. At first, 50µl of formalin 2.5% was injected to hind palm of the rats. Then the animal was anesthetized by specific doses of ketamine + rompuan, and after that, trachea was cannulated and the animal breathed using pulmotor. After that, the animal was operated and by opening the chest and revealing the heart, evans blue (30 mg/kg) was injected by left ventricle (the substance, in proportion with inflammation value, passes trough vessels of the inflamed location and enters interstitium). About 30 minutes after injecting evance blue, the foot was dissected from the wrist, and cut into smaller pieces using scissors, and stored in acetone + sodium sulphate (1%) with a ratio of 3/5 to 1/5, respectively. The foot containing receptacle with respective solutions was stored on horizontal shaker for 24 hours (meanwhile, evance blue moves to acetone + sodium sulphate from the foot). Receptacles

containing foot and respective solutions were centrifuged for 5 minutes on 2000 rounds, and finally, light absorption of the centrifuged liquid was read in 620 nm using Spectronic 20 Genesys spectrophotometer. Light absorption, regarding the value of color substance, shows the inflammation value. In treatment group, *Utrica dioica* extract was interperitoneally injected to the animal about 20 minutes before injecting evance blue in doses of 20, 50, 100, and 200 mg/kg.

### 2.6. Acute ear inflammation test:

Xylene (0.03 ml) was used to form acute ear inflammation in the animals. Following anesthesia and administration of artificial breathing using a chip, xylene was subcutaneously injected on auricle. About one hour after injection (maximum eruption of the inflammation), evance blue was injected, using the method administered in formalin test. As described before, ears were dissected from inflamed location in a crescent form, cut into pieces, stored in acetone + sodium sulphate solution, and finally the light absorption was measured. In treatment group, Utrica dioica extract was injected to the animals with doses previously described, about 20 minutes before injecting the color substance.

### 2.7. Peritonitis test

10 ml/kg of acetic acid 0.07% was interperitoneally injected, in order to form peritonitis. After about 20 minutes, exactly as described previously, evance blue color substance was injected, then peritoneal fluid was collected, and light absorption was read. Also in this test, previously mentioned extract doses were injected to the animals in variable groups.

### 2.8. Chronic groin inflammation test by cotton implantation

For this test, the rats were anesthetized using ketamine. Then, a small incision was made in the groin (on both sides), and a 30 mg cotton roll (used in dentistry) smeared by ampicillin, was implanted inside the incision. After suturing the incision, we waited for the animal to regain consciousness. The animal was stored in animal room for 7 days, and then again anesthetized, and with a groin incision, the cotton rolls were ejected. After drying in 60°C for 24 hours, each cotton roll was weighed, and the difference of the weight before and after implantation was measured as the inflammation. in treatment group, herbal effective dose (50 mg/kg) was injected to the animal every 2 days.

### 2.9. Administered groups

In this study, 3 groups were used: 1- control group (didn't receive the extract) 2- control Sham group 3- treatment group (received the extract), and each group was tested by the four methods. Therefore, 12 sub-groups, each one containing 9 - 12 animals, were studied. According to administration of different doses in treatment group (15 doses, each one with n=8), a total of 250 animals were experimented.

### 2.10. Statistical studies

Results of each group were described using Mean SEM. Then comparison between each two groups were made using varience analyze test, and comparison between groups was made using post hoc Tukey test. $P<0.05$ was indicated as data punctuality level.

## 3. Results

### 3.1. Effect of *Utrica dioica* on inflammation due to formalin injection

A comparison of foot extracted solution light absorption data between control group and extract-receiving groups shows that the injection of Utrica dioica in doses of 50 and 100 mg/kg about 20 minutes before injecting formalin, has been able to reduce the inflammation in the injected foot, by the meaningful value of 24.522.20 and 22.712.1 percent with probability of $P<0.05$ (Fig. 1).

### 3.2. Effect of *Utrica dioica* on ear inflammation due to xylene injection

As shown in Fig. 2, a comparison of ear extracted solution light absorption between control group and extract-receiving groups, shows no punctual difference between control and treatment groups at none of the used doses. Therefore, it can be resulted that Utrica dioica extract is not effective in reducing the inflammation due to xylene injection to ear.

### 3.3. Effect of *Utrica dioica* on peritonitis

Statistical analysis of the data of peritoneal fluid light absorption after inflammation due to acetic acid injection in this area, shows that Utrica dioica has been capable of reducing peritonitis in all of the three administered doses (Fig. 3). As seen in the figure, the herbal extract in three doses of 20, 50, 100 mg/kg has been able to reduce peritonitis by 21.452.1, 18.552.2, and 27.491.8 percent, respectively, and with probability of $P<0.05$.

### 3.4. Effect of *Utrica dioica* on chronic inflammation

Statistical comparison between weights of planted plates in groin of extract-receiving groups and control group showed that there was no significant change in the mean weight of these plates in extract-receiving rats in comparison with control group in doses of 50, 100, and 200 mg/kg, but in high doses (400 mg/kg), a significant reduction of about 24.172.4 percent, with a punctuality probability of $P < 0.05$ occurred.

## 4. Discussion

Inflammation, especially chronic state of it, has caused many clinical problems for patients. Therefore, despite the new and variable methods of inflammation treatment, a vast and intricate research range for that has been created (13). Studies completed or in progress, try to create new, better, more effective treatments with fewer side effects. Because of that, despite advanced pharmacy and variable chemical drugs to cure inflammation and the inability to successfully treat inflammation especially chronic inflammation and vast side effects of artificial drugs which occur to patients (12), in this study we tried to use herbal medicine due to lower cost, fewer side effects, and easier accessibility (13). As shown in our experiment with formalin, the herbal extract is capable of significantly reducing the inflammation due to formalin injection in hind palm. Regarding that inflammation due to formalin (in the inflammatory phase of formalin injection)is mostly because of releasing peripheral inflammatory intermediates (11), and these intermediates are released following the first or acute phase of formalin in which pain receptors are stimulated (14), and according to pain relieving effects of Utrica dioica (15), it can be argued that probably Utrica dioica extract has reduced the inflammation by inhibiting release of peripheral inflammatory intermediates. Reports showing the capability of Utrica dioica in inhibiting the release of cytokines, TNF, and VIP which are the most important inflammatory mediators, support this idea (3, 16). Also, clinical report on the basis of lower use of non-steroidal drugs in patients who have used the herbal extract, can be deducing that the herb contains substances with anti inflammatory effect similar to non-steroidal drugs (17, 18) or it has reinforced the drugs.

About significant effect of the extract in reducing the peritonitis due to acetic acid injection, regarding that in inflammation due to acetic acid injection, the acid increases capillary membrane permeability in peritoneum to increase capillary permeability, therefore it can be deduced that the herbal extract has been able to significantly reduce peritoneum capillary permeability increased due to acetic acid injection. Comparing the formalin data and above mentioned data, it can be resulted that decreasing capillary permeability can be one of the inflammation reducing mechanism of Utrica dioica. About chronic inflammation in groin, Utrica dioica extract in high doses has been capable of significantly reducing the inflammation. Regarding the release of inflammatory intermediates in inflammation especially chronic form of that (12), it can be deduced that the herb has meaningfully inhibited release of these intermediates. According to effect of the extract in high doses, it can be deduced that in chronic inflammation due to high level of inflammatory intermediates supporting each other, probably high value of effective compound of the herb in high doses of usage has been able to deactivate some of these inflammatory intermediates. About non-effectiveness of the herbal extract on acute ear inflammation, it can be said that according to low effect of Utrica dioica extract on acute inflammation due to xylene injection to the ear (in other tests of this study, acute inflammation has been reduced), but perhaps the ear inflammation due to xylene has been low for the extract to affect on. However, lower blood flow in the ear cannot be disregarded. In conclusion, the data of this study show that alcoholic Utrica dioica extract is able to reduce variable types of acute and chronic inflammation. Probably, regarding reduce of peritonitis, decrease of capillary permeability can be one of the important reasons of reduce of in-

flammation by the herbal extract. About value and level of releasing inflammatory intermediates and the effect of the herb on them, further studies (especially immunological studies) are needed.

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