

Effect of hydroalcoholic leaf extract of *Ziziphora tenuior* L. on pain in male rats

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ABSTRACT

Background and Objectives: *Ziziphora tenuior* is one of the traditional medicinal herbs; the main active chemical composition of this herb is an ingredient named as Pulegone which is known-well for its anti-inflammatory and analgesic effects. Considering the side effects of synthetic drugs, researchers' attention has been attracted to the use of herbal medicines. The aim of the present study was to compare the anti-inflammatory effect of hydroalcoholic extract of *Ziziphora tenuior* L. and dexamethasone in adult male rats.

Materials and Methods: In this study, after inducing ear edema via xylol, the anti-inflammatory activity of hydroalcoholic extracts on vascular permeability in NMRI rats was studied. Sixty rats were divided into 6 groups of 10 as follows: control (no treatment), sham (receiving physiological saline), groups 3, 4, and 5 treated with *Ziziphora tenuior* extracts which received doses of 20, 50 and 100 mg/kg, respectively, and groups 6 through 10 which received dexamethasone at a dose of 10 mg/kg. Thereafter, the data were collected and analyzed using SPSS 17 statistical software and one-way ANOVA test.

Results: The results of the present study showed that intraperitoneal injection of *Ziziphora tenuior* extracts at doses of 50 and 100 mg/kg, results in a significant decrease in inflammation induced via xylol in male rats ($p < 0.05$), that was similar to dexamethasone at a dose of 10 mg/kg. Furthermore, the group receiving 100 mg/kg of *Ziziphora tenuior* extract showed less inflammation as compared to the group receiving dexamethasone. However, no significant difference was seen.

Conclusion: *Ziziphora tenuior* extract dose-dependently and significantly decreases inflammation of ear edema test induced via xylol. Probably, the presence of flavonoids found in the herb causes its anti-inflammatory effects. However, more research is required to achieve the therapeutic effects of the herb.

1. Introduction

Inflammation is a pathophysiological response to tissue damage from healthy to injured tissue, which in turn leads to localized accumulation of plasma fluid, and blood cells. In this defense mechanism, a series of complex events and various mediators are involved to induce, maintain or exacerbate inflammatory

reactions. Therefore, anti-inflammatory factors are effective in the treatment of pathological reactions (1). Considering the adverse effects of chemical substances, there has been a strong trend toward a return to herbal medicines and natural substances as important sources of therapeutic regimens in recent years.

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There has also been novel viewpoints regarding herbs. Studies and investigations in the past decades have been initiated on herbal medicines and their physiological and pharmacological effects. Furthermore, herbs are robust resources of new chemical substances with strong therapeutic effects. In this regard, the *Ziziphora tenuior* medicinal herb can be named, which belongs to the Lamiaceae family. This herb comes from a thick, shaggy bush and the height of the plant is 20 to 50 cm. The plant has small, cross, and more or less lance-shaped leaves, without petiole. It is also full of small, white, pink, and purple flowers. The medicinal properties of this herb are namely the treatment of gastrointestinal disorders such as diarrhea and cramping (2). In addition *Ziziphora tenuior* has anti-bacterial (3, 4), antioxidant (5, 6), and sterilizing effects for the intestine (2, 3, 7). It also replenishes sputum or phlegm and it is also utilized as an anti-cold medicine (6). Meanwhile, the main active chemical composition of this herb is known as pulegone, which its anti-inflammatory and analgesic effects are vividly clear (8, 9). Thus, it can be utilized in the treatment of fever, menstrual cramps and stomach tones (10). Some reports also suggest that the analgesic effects of Lamiaceae family plants are due to compounds such as carvacrol, flavonoids, and steroids (11). Therefore, considering the therapeutic effects of this herb in the treatment of fever, menstrual cycle symptoms, and stomach tones in traditional medicine and since its anti-inflammatory effect has not been reported, this study aimed to investigate the anti-inflammatory effect of *Ziziphora tenuior* and dexamethasone in adult male rats.

2. Materials and Methods

2.1. Collection and identification of plants

In August 2015, *Ziziphora tenuior* leaves and head branches were collected from Sirjan, Pariz. Afterwards, they were dried at 25°C under shade, thereafter they were powdered by mechanical grinding. Subsequently, dry powdered *Ziziphora tenuior* were kept frozen in plastic bags in the freezer until testing date.

2.2. Extraction method

Twenty grams of powdered leaves and head branches of *Ziziphora tenuior* were poured into a

capped flask. Afterwards, 200 ml of 70% ethanol was added to the mixture. Next, the flask was capped, and the solution was kept for 48 hours. Meanwhile, the contents of the flask were given a shake every 12 hours. After 48 hours, the contents of the flask were filtered via filtering paper and funnel glass inside the beaker. Consequently, the filtered solution was poured into a balloon and rotated with average rounds per minute and at 75 degrees celsius. Consequently, after separating the upper undissolved solution from the concentrated precipitation, the precipitation extract was spread on a glass until dried. After the extracted powder has dried, which is to note that it is actually made up of 58.6% concentrated liquid herb, thereafter, it is collected, and the resulting powder was utilized for production of 50, 100, and 150 mg/kg doses, also it is to note that in producing the solution, physiological saline was used.

2.3. Determining the acute lethal dose (LD50) of hydroalcoholic extract of the plant

Subsequent to intraperitoneal injection of the extract to various groups, the animals were under care and scrutiny for 48 hours and after 48 hours, animal death rate was specified. Furthermore, in this study, to investigate the acute lethal dose of the extract, high doses of the extract (200, 400 and 800 mg/kg) were also utilized.

2.4. The method of inducing ear edema inflammation via utilizing edema-ear test

For this purpose, 0.2 ml of *Ziziphora tenuior* hydroalcoholic extract dissolved in sterile saline with values of 20, 50, and 100 mg/kg was injected intraperitoneally. 15 minutes later, 0.05 ml of ketamine was injected intraperitoneally. Then after, the rats became unconscious. Next, 0.03 ml of pure Xylol was injected into the dorsal surface of the right ear and the left ear was kept intact as a control. Two hours after xylol injection, the rats were sacrificed, then a piece of ear about 7 mm in diameters were taken from each ear via Cork borer instrument. The ear components were weighed and the weight difference between right and left ears were used as a measuring scale of the rate of inflammation (12). Control groups received physiological saline.

2.5. Experimental groups

The study was registered in Ethics Committee of Kazerun University of Medical Sciences as Ku.REC.2014.062. Group 1 (control): This group is composed of animals that received no kind of drugs. Group 2 (Sham), animals in this group received physiological saline as the solvent of the extract. Groups 3, 4, and 5 received a *Ziziphora tenuior* extract at doses of 20, 50 and 100 mg/kg, respectively. Group 6 received dexamethasone at a dose of 10 mg/kg. All materials were injected intraperitoneally. Next, after a 15-minute lapse, 0.05 ml of ketamine was intraperitoneally injected, then xylol was injected on the dorsal surface of the right ear, two hours later, pieces were taken.

2.6. Statistical analysis of data

The collected data were analysed via one way ANOVA and Tukey tests using SPSS17 statistical software. Significance level was 0.05. All results were presented as mean \pm SE.

3. Results

Results of the present study displayed that intraperitoneal injection of 50, and 100 mg/kg of hydroalcoholic extract of *Ziziphora tenuior* causes significant reduction in xylol-induced inflammation in adult male rats, which is analogous to 10 mg/kg of concentrated dexamethasone (Figure 1). Furthermore, it was shown that the recipients of 100 mg/kg of *Ziziphora tenuior* extract had the greatest anti-inflammatory effect as compared to the other groups ($p < 0.05$). In addition, the anti-inflammatory impact of 100 mg/kg of *Ziziphora tenuior* extract was much greater than dexamethasone, however, the difference was not statistically significant (Figure 2).

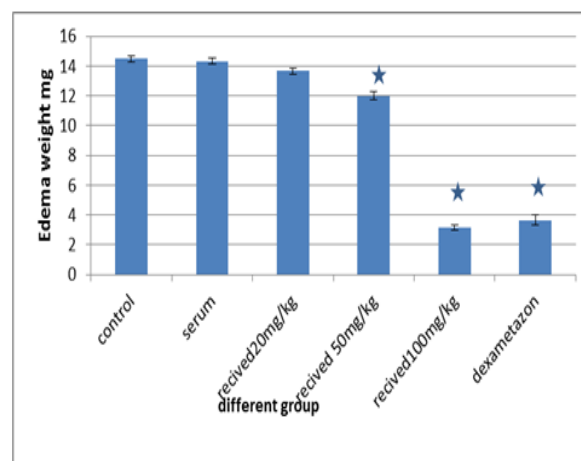


Figure 1. The effect of intraperitoneal injection of *Ziziphora tenuior* extract at doses of 50 and 100 mg/kg in reducing inflammation induced by Xylol in male rats.

Untreated control group (Co), Sham group (Se) receiving physiological saline, and the group receiving 10 mg/kg of dexamethasone (Dex). * Indicates a significant difference at the level of $p < 0.05$ as compared to the control group. Values are based on the average \pm standard error.

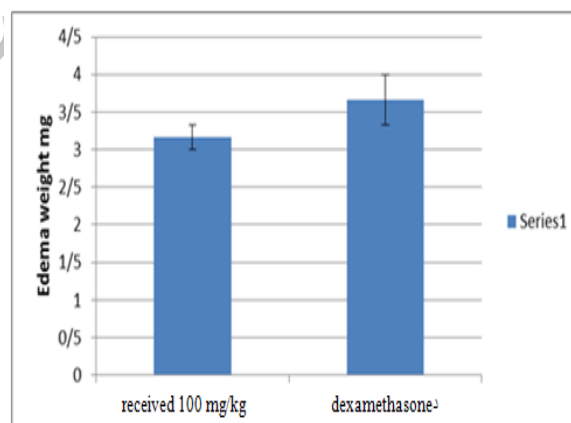


Figure 2. The effect of intraperitoneal injection of 100 mg/kg of *Ziziphora tenuior* extract and concentrated dexamethasone (10 mg/kg) on lessening the inflammation which was induced by xylol in adult male rats.

4. Discussion

The results of the present study showed that *Ziziphora tenuior* hydroalcoholic extract has standard anti-inflammatory effects and it is analogous to anti-inflammatory drugs such as dexamethasone. Furthermore, it acts against edemas induced in adult male rats via xylol. In ear edema induced tests, inflammatory markers and mediators are released following stimulation. This affair leads to dilation of the arteries, and veins followed by increase of vascular permeability (13). Local inflammatory response to tissue injury or infection, leads to increase of blood flow in the injured area, and also the migration of leukocytes and plasma cell molecules to protect the damaged tissue. In addition, local anti-inflammatory response increases microvascular permeability, and leakage of plasma proteins (including antibodies) complements into tissue (14, 15). In inflammatory response, lymphocytes and macrophages attack sites of inflammation, and cause local reactions such as fibrosis processes to occur. In the time sequence of these reactions, different cytokines are involved in the pathogenesis of inflammatory reactions. A number of cytokines are involved in the initiation and development of hypersensitivity. However, the function of cytokines is very complicated (16). Inflammatory pain leads to the release of hyperallergic mediators and markers (e.g., prostaglandins, and catecholamines) that adjust sensitivity of pain receptors (17). The anti-inflammatory function of plants is attributed to the presence of a great amount of sterols, Triterpenes, and flavonoids (18). Other researches have demonstrated that many flavonoids such as rutin, quercetin, luteolin, hesperidin, and B-flavonoids produce analgesic anti-inflammatory activity (19). Geographic diversity in Iran has resulted in a rich resource of plant species which one of these herbal plants belongs to the Lamiaceae family termed *Ziziphora tenuior*. *Ziziphora tenuior* is found in different regions of Iran, especially in the South East, North and North-West, and has numerous health benefits. Of the natural compounds found in Lamiaceae plant family, are limonene, carvacrol, gamma terpene, cineol, beta-carotene, niacin, and thymol (1, 3, 4, 7). Flavonoids act as

anti-inflammatory agents by inhibiting the chemical mediators of the inflammatory response markers, such as nonsteroidal anti-inflammatory drugs. These anti-inflammatory drugs act via inhibiting the enzyme activity of prostaglandin synthesis pathway (20). Several researches have announced that the anti-inflammatory effects of flavonoids may be due to anti-histamine, anti-bradykinin, and anti-serotonin or via inhibition of enzymes such as 12-lipoxygenase and 5-lipoxygenase. Certainly, the chemical components of *Ziziphora tenuior* as an enforcer of pharmacological activity is still unknown (21).

Conclusion

It is concluded from this study that *Ziziphora tenuior* extract dose-dependently exerts anti-inflammatory effect as shown in xylol-induced inflammation of ear edema test. Probably, flavonoids present in this plant have anti-inflammatory effects. Further researches in this field are demanding.

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