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Gastroprotective effects of aqueous and hydroalcoholic extract of *Scrophularia striata* on ethanol-induced gastric ulcers in rats

Maryam Rezaei-Nasab¹, Gholamreza Komeili^{1*}, Bahman Fazeli-Nasab^{2,3}

¹Department of Physiology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

²Faculty scientific member, Agriculture research center, University of Zabol, Zabol, Iran

³Biotechnology research center, University of Zabol, Zabol, Iran

*Corresponding author: Gholamreza Komeili, Associate professor in Physiology, Department of Physiology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran. Tel: +985433235122, +989155412877, E-mail: rkomeili@gmail.com, Komeili@Zaums.ac.ir

ABSTRACT

Background and objectives: The *Scrophularia striata* plant has traditionally been used among people inhabited the Zagros as healing of wound and infection. Therefore, the aim of this study was to evaluate the wound healing effect of aqueous and hydroalcoholic extract of *Scrophularia striata* on the oxidative stress indices and ethanol-induced gastric ulcers in rats.

Material and methods: After collecting and drying the plants, hydroalcoholic and aqueous extracts were prepared by maceration method. 60 male rats were randomly divided into control, omeprazole, and four groups receiving aqueous and hydroalcoholic extracts (100 & 400 mg / kg) and were treated for two weeks. Then serumic levels of total antioxidant capacity (TAC), super oxide dismutase (SOD), and malondialdehyde (MDA) were measured as well as the stomach of rats was examined macroscopically.

Results: The results have demonstrated that administration of the extract was effective on the MAD, SOD, TAC, and the size and number of ulcers ($P < 0.01$), so that SOD and MAD has decreased with increasing dose of the extract but increased with TAC. Hydroalcoholic and aqueous extracts reduced the number and size of gastric ulcers

but there was no difference between them. In addition, by increasing the dose of extract, the number and size of ulcers decreases.

Discussion and conclusions: Administration of the *Scrophularia striata* extract could reduce indices of oxidative stress. Considering the macroscopic studies on the stomach, the herbal effective components probably stimulate collagen synthesis and faster wound contraction, angiogenesis, vascular dilatation, and also reduces inflammation, edema, bleeding and scarring. Further studies are needed to determine the mechanism of the therapeutic effect of the extract.

Key words: *Scrophularia striata*, gastric ulcer, oxidative stress, rat

INTRODUCTION

Gastric ulcers can be due to the increased acid secretion for various reasons such as non-steroidal anti-inflammatory drugs (NSAID), alcohol, long-term hunger, bad habits, and also intense and continuous stress[1]. According to the reports of World Health Institute, one out of every 10 Americans during their lives suffer from gastric ulcer disease and annually 15,000 deaths occur due to the consequences of this disease. The economic impacts of the disease are significant while they are more than 10 billion dollars annually in the United States[2]. NSAIDs are the most available drugs in all around the world and studies have shown that there is a relationship between consumption of these drugs and the incidence of gastric ulcers in western societies[3]. Bleeding and gastric mucosal lesions are the most common side effects of consuming these drugs, in a way that NSAIDs has been known as the second leading cause of peptic ulcer[4]. Treatment of gastric ulcer with omeprazole, metronidazole, and ranitidine is associated with side effects and problems such as autoimmune phenomena. There is the probability of relapse after treatment discontinuation, for this reason major efforts have been done for finding natural, effective ingredients in the treatment of stomach ulcers[5]. Wound healing is a remedial process which is created by skin and other tissues lesions[6]. One of the goals of medicine is healing in a shorter time and with fewer side effects. Shortening the healing time has great importance due to the minimization of the possibility of infection or wound complications and reduction of the cost[7].

Medicinal plants are valuable natural resources which are considered by the advanced countries of the world and are known as raw material for making safe drugs for humans. Iran is one of the richest plant sources of global pharmaceutical which has a high diversity of habitat conditions for a variety of plants[8]. *Scrophularia striata* is a medicinal, auto growth, perennial, and from Snapdragon family that is frequently found in temperate and tropical regions of Iran including Ilam, Khuzestan, Kermanshah, Kurdistan, Lorestan, South Khorasan, and Fars[9]. The chemical composition of this plant is not detected but it has been years that people living in the Ilam province that is used experimentally on different ways such as boiled food, incense and poultice in the treatment of different diseases, including inflammation and eye and ear infections, skin burns, wounds, episiotomy pain, and gastrointestinal disorders, colds, hemorrhoids, boils, etc. [9]. The plant has not only strong disinfection effect for urinary and digestive tracts but it has also multiple effects, including lowering blood pressure and treating depression. The branches of this plant are used as stomach tonic. The compounds such as alkaloids, resins, glycosides, Iridoids, Crypthophilic acid and flavonoids have been detected in Snapdragon family[10].

Shohani, in a study used hydro-alcoholic extract of *Scrophularia striata* to treat skin lesions in rabbits. In this study, the average time for complete healing in the groups without treatment and treated with Eucerin for 21 days, Phenytoin 1% for 16 days, and ointments 2%, 5%, and 10 % of this plant, respectively for 18, 17 and 16 days. In histological examinations also signs of improvement and the development of skin tissue in the treatment with *Scrophularia striata* extract have been more perfect[9].

Komeili et al assessed the effect of alcoholic extract of Ajowan seed on the wound healing of gastric ulcer created by ibuprofen in the rat and found that the extract of Ajowan in dose dependent manner improves the gastric ulcers in animals and its therapeutic effect was significant compared with omeprazole in doses of 125 and 250 mg/kg. Average number and area of gastric ulcer in the groups receiving the extract was significantly lower than the group receiving omeprazole[11].

Stomach ulcers in animals can be developed in animals through different ways such as the use of NSAIDs, alcohol, stress, cold water, etc. According to the approved disinfectants and antimicrobial properties of hydroalcoholic extract of *Scrophularia striata*[12] as well as a few reports about the healing effects of this plant on the open wounds of rabbit[9], anxiety and depression in adult male mice[13], oxidative stress caused by the nervous behavior[14], healing of grade 2 burn wounds in rats[15], and *Candida albicans* in laboratory conditions[16], the aim of this study was to examine the effects of aqueous and hydroalcoholic extract of *Scrophularia striata* stem on the healing of ethanol-induced gastric ulcer in rats.

MATERIALS AND METHODS

Preparation of extraction

Scrophularia plants collected from the mountains of Ilam (Zagros) and dried in the shade and then powdered. To extract the effective ingredients, maceration method[17] was used and for this purpose the two types of hydroalcoholic (70%) and aqueous extracts were used. 1000 ml of 70% ethanol and 1000 ml of distilled water were separately added to 200 grams of powdered plant and was held for 3 days, and then the extracts were isolated using filter paper and funnel and concentrated by vacuum distillation to the possible extent and were prepared at 100 and 400 mg concentration for use.

Population studied and creation of gastric ulcers

In this study, 60 adult male rats weighing between 200-250 grams were prepared from Laboratory Animal Research Center, Zahedan Medical Sciences University and were placed in individual cages for a week to adapt to new conditions. During the experiments, they were kept in 12:12h light/dark cycle, and 22-25 °C[17]. Then rats were randomly divided into 6 groups: control, the group receiving omeprazole and four groups receiving aqueous (100 & 400 mg/kg) and hydroalcoholic extracts (100 & 400 mg/kg). The protocol has been approved by the local Ethics Committee of Zahedan University of Medical Sciences.

To induce gastric ulcer, fasting rats were kept in specific cages with a mesh floor for 48 hours. In the meantime to prevent dehydration, animals were fed with water containing 0.2% NaCl and 2% sucrose[18]. After this period, each rats was administered by intragastrical 75% ethanol (4 ml/kg)[19]. Then animals were treated for two weeks with normal saline, omeprazole (200 mg/kg), aqueous extract (100 and 400 mg/kg) and hydroalcoholic (100 and 400 mg/kg) of *Scrophularia striata* respectively, with the volume of approximately 0.5 ml per rat.

Blood sampling and biochemical analysis

At the end of experiments, after 16 hours fasting, rats were anesthetized and blood samples were collected from the heart, the samples were centrifuged for ten minutes at 4500 rpm and serum was isolated. Biochemical parameters such as TAC, SOD and MDA were analyzed. The total antioxidant capacity was measured with ferric reduction ability of plasma (FRAP) method as described previously[20]. Serum malondialdehyde (MDA) and super oxide dismutase (SOD) levels were measured using the ZellBio kit (Zellbio GmbH, Deutschland) and by ELISA method.

Gastric ulcer examination assay

Stomach were cut in the large curved and then washed by normal saline, and was fixed described on the tray and was evaluated with a magnifying glass macroscopically and the photos were taken. Later photos were transferred to a PC and the level of stomach ulcers was calculated[21]. In this study, the loop (to count the number of wounds) and J. Score was used to determine the area of the wounds (Number of lesions smaller than a millimeter to a, number of lesions between 1 to 2 mm b and lesions larger than 2 mm c called and J to the following equation calculated $J = a + 2b + 3c$)[21]. The therapeutic index[18] was calculated from the following formula.

$$\text{Therapeutic index} = \frac{\text{number of control group wounds} - \text{number on wounds in test group}}{\text{number of control group wounds}} \times 100$$

Data analysis

The results were analyzed using SPSS version 17 (SPSS, Inc., Chicago, IL, USA). Comparison of the data between groups was performed using one-way analysis of variance (ANOVA) followed by LSD post hoc test. The results are presented as mean \pm SE and $P < 0.05$ was considered statistically significant.

RESULTS

Data analysis showed that the amount of MAD, SOD, and TAC was significantly different between groups ($P < 0.01$) (Table 1).

Table 1: Average of oxidative stress indices values in all groups

Variables	MDA ($\mu\text{mol/l}$)		SOD (U/l)		TAC ($\mu\text{mol/l}$)	
	Mean	SE	Mean	SE	Mean	SE
Control	53.1	2.8	48.6	1.9	300	25.8
Omeprazole	41.7*	0.6	43.2	2.8	331.9	22.6
Aqueous Extract 100	46.9	2.2	41.9	2.7	482.9*	24.5
Alcoholic Extract 100	47.7	2.3	40.2*	1.8	377	32.6
Aqueous Extract 400	42.2*	2.5	39.3*	0.8	874.5*	38.2
Alcoholic Extract 400	40.3*	1.1	37.1*	1.1	563.3*	36.7

* $P < 0.01$ in compare with control group (n=10).

LSD post hoc test showed that the administration of the extract could significantly reduce malondialdehyde in the groups receiving aqueous and hydro alcoholic extracts (at a dose of 400) compared with the control group that the effect is comparable with Omeprazole (Figure 1).

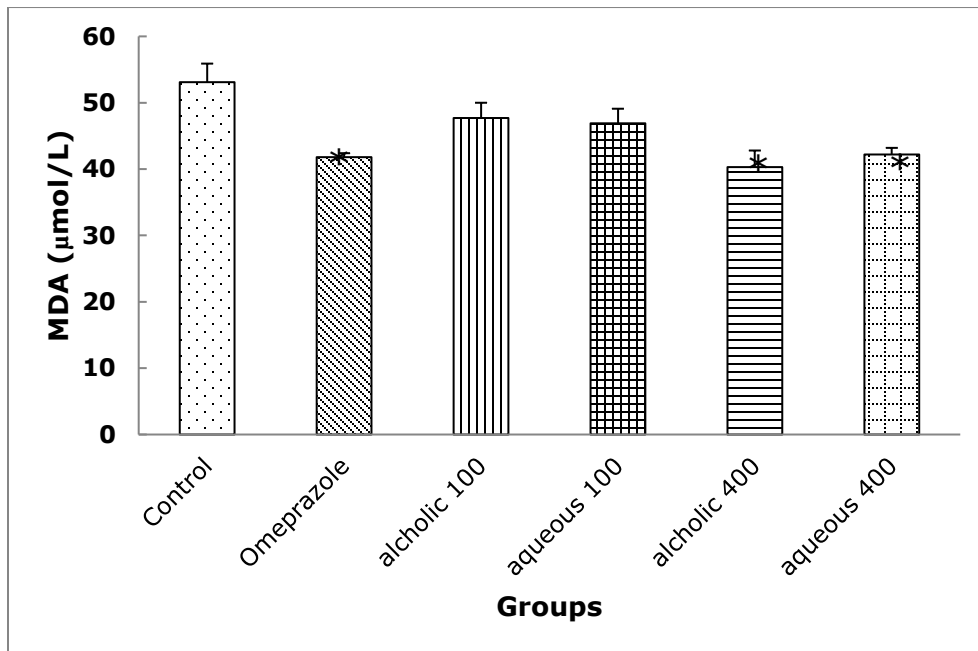


Figure-1: compare of MDA (µmol/L) shown as mean ± SEM in different groups (*P<0.01 in compare to control group, n = 10).

Additionally the amount of SOD had significantly reduced following by the administration of aqueous (400 doses) and hydro alcoholic (100mg and 400mg) extract compared with the control group (Figure 2).

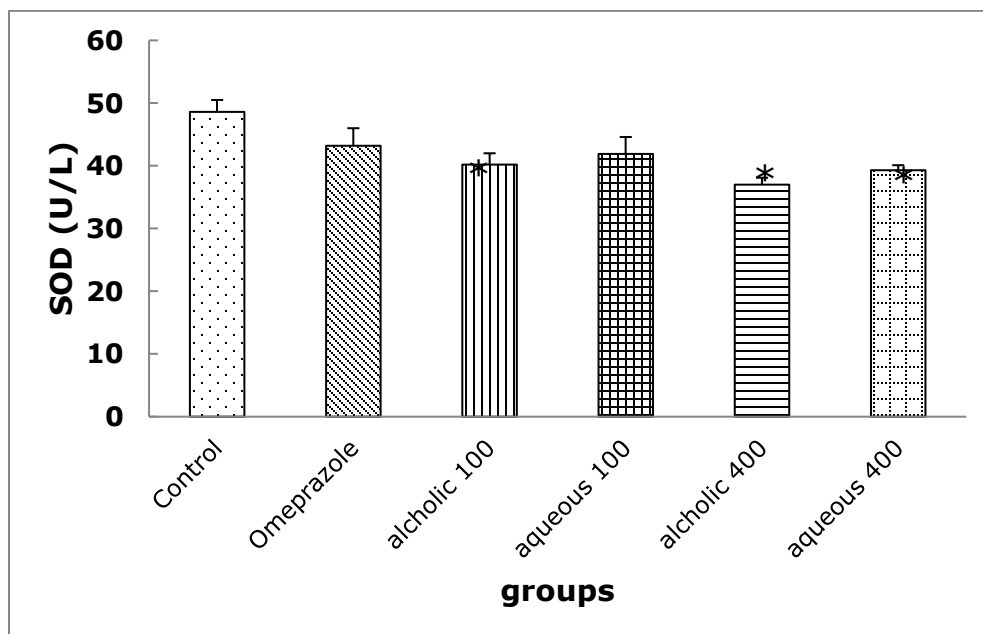


Figure-2: compare of SOD (IU/L) shown as mean ± SEM in different groups (*P<0.01 in compare to control group, n = 10).

Also, TAC values showed a significant increase compared to the control group through the administration of aqueous (100mg and 400mg) and hydro alcoholic (400 doses) extracts of the plant (Figure 3).

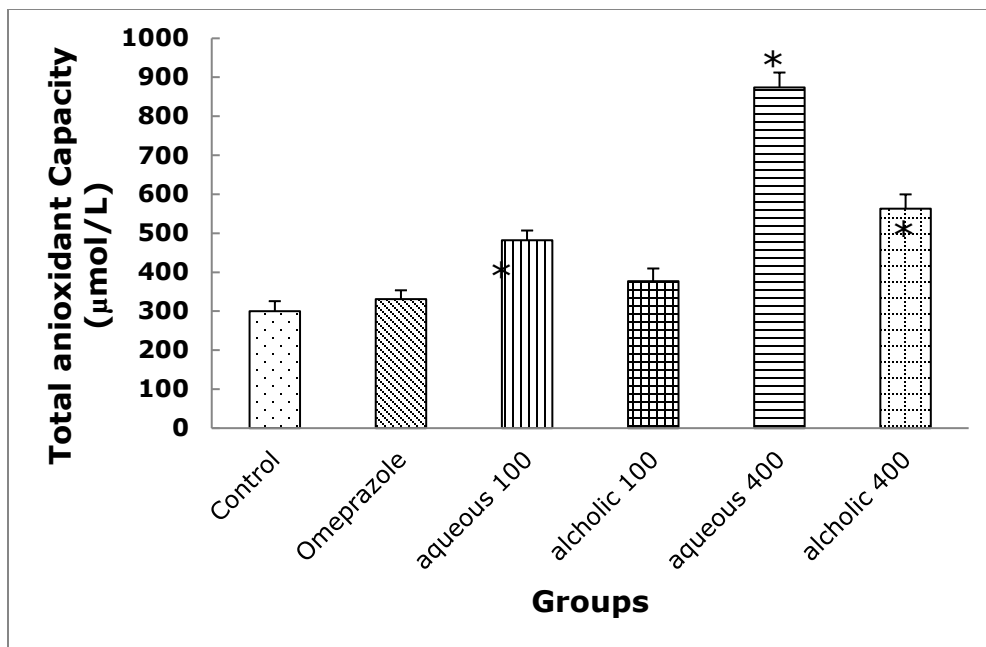


Figure-3: Compare of Total antioxidant Capacity (µmol/L) shown as mean ± SEM in different groups (*P<0.01 in compare to control group, n = 10).

It seems that the effect of plant extracts on the oxidative stress has been increased by increasing the dosage. The results of data showed that the type and dose of the extract on the number and dimension of ulcers was significant at the level of 1% (P <0.01). LSD post hoc test showed that the lowest number and size of ulcers was found in aqueous extract (dose 400) and the maximum number and size of lesions was seen in the control group (Table 2).

Table 2: Mean number and surface area of gastric ulcer (mm²) and therapeutic index in all groups

Groups	Number of ulcer	dimension (mm ²)	Therapeutic index (%)
	Mean ± SE	Mean ± SE	
Control	9.1 ± 0.23	43 ± 0.9	0
Omeprazole	3.1 ± 0.27*	12.4 ± 0.8*	65.9
Aqueous extract 100	1.9 ± 0.7*	6.6 ± 0.5*	79.1
Alcoholic extract 100	2.2 ± 0.4*	8.5 ± 0.8*	75.8

Aqueous extract 400	$1 \pm 0.4^*$	$1.7 \pm 0.3^*$	89
Alcoholic extract 400	$1.3 \pm 0.5^*$	$3.5 \pm 0.4^*$	85.7
*P<0.01 in comparing to control group, n = 10.			

Aqueous and hydroalcoholic extracts had a positive impact on reducing the number and dimensions of the stomach ulcer, but there was no significant difference between them; however, with increasing the dose, number and size of ulcers significantly reduced (Table 2). Both aqueous and hydroalcoholic extracts showed better therapeutic effects compared to omeprazole.

DISCUSSION

The findings of this study demonstrate that the amount of SOD and MAD have reduced with the increasing dose of extract while the TAC has increased. In various researches there has been a relationship between the reduction of MDA and TAC boost [22-25]; however, some studies have also reported reduction of MDA and no significant effect on TAC[26]. The increase in TAC was attributed to an increase in the amount of creatine kinase in the blood that is followed by the increase in GSH as one of the factors of TAC[27]. It also has been reported that an increase in plasma TAC due to opposition with the oxidative damage caused by activities and changes in platelet response to acute physical conditions[28] or returning antioxidants from tissues into plasma and the contrast between different antioxidants that improve plasma TAC[29].

The findings of this study also showed that the extract of *Scrophularia striata* had a significant positive impact on the size and number of gastric ulcers in dose-dependent manner, which is probably due to the anti-inflammatory, antibacterial, and antioxidant effects of extract components. Root and shoot of *Scrophularia striata* have antioxidant properties. Antioxidant and anti-bacterial properties of *Scrophularia striata* are related to flavonoids, monoterpenes and coumarin in the extract[30]. In some studies also the effect of *Scrophularia striata* has been evaluated on Staphylococcus aureus and Pseudomonas aspergillus bacteria in-vitro and the antibacterial effect has been found equal to Betadine[31].

Also shohani and colleagues concluded that the average time for complete healing using extract of *Scrophularia striata* in the groups including without treatment and treated with Eucerin 21 days, Phenytoin 1% for 16 days, ointments 2%, 5%, and 10%, respectively for 18, 17 and 16 days, and histology also reported that signs of improvement and the development of skin tissue in the treatment using *Scrophularia striata* extracts have been more perfect[9]. Azhdari Zarmehri et al also reported that the hydroalcoholic extract of *Scrophularia striata* on grade 2 burn wound healing was effective in rat[15]. Also, histological studies showed that the regeneration of tissue, blood vessels, and collagen have a significant increase in groups that treated with 2.5% and 5% of *Scrophularia striata* extract. Due to the antioxidant and anti-inflammatory properties of *Scrophularia striata* and its stimulant effect on the production of fibroblasts in the study, it can be a verification on the effect of plant extracts on alcohol-induced gastric ulcer.

Growth fibroblast stimulants help to wound healing; it has been found that increase in the number of fibroblasts in artificial skin lead to improve wound healing in-vitro[32]. This process may be disturbed due to the presence of oxygen radicals in bacterial infections[33]. Fibroblasts synthesize some primary components of the extracellular matrix of the wound bed such as fibronectin and proteoglycans that provide the perfect background for migration and cell proliferation[34]. Fibroblasts also synthesize the

collagen which causes tension in the wound bed[35]. Myofibroblasts, which are specialized fibroblasts involved in the wound-healing process through creating the contractile force of contraction[34].

Wound healing includes inflammation, proliferation and regeneration stages that each of them is composed of multiple stages that some of them are overlapping and not easily separable[36]. Hence the quality and quantity of each of the stages can lead to accelerated wound healing and reduce complications. On the other hand, effectiveness of *Scrophularia striata* in healing process can be due to the presence of the Iridoid glycoside compounds in different parts of the plant that can lead to reduce edema and cellular infiltration and proliferation of T- lymphocytes through the inhibition of prostaglandin E₂, and different interleukins (IL-1 α , IL2, IL-4), necrosis factor and interferon gamma[37, 38]. In addition, by increasing fibroblast growth, the field is provided for more secretion of collagen and faster wound healing[39]. Also, there are different glycoterpenoids in other species of *Scrophularia* that reduce edema and cellular infiltrates and have anti-inflammatory properties[40]. Of course the presence of glycosides and phenylpropanoids which are inhibitors of macrophages, thereby inhibitors of the production of chemical mediators of inflammation and ultimately reduce inflammation[41] and phenolic acids with antibacterial properties in some species of *Scrophularia* is another reason for being effective in healing gastric ulcers[42].

At the time of burn wound healing, increase in the number of blood vessels, fibroblasts, and epithelial density occurs in the affected areas[15]. Many studies have been conducted on the effect of different materials on the stages of tissue repair. Oil extracts of *Matricaria chamomilla L*[43], Alcoholic extract of green tea (*Camellia Sinensis*) [44], and *aloe vera* gel [45] with increasing numbers of fibroblasts accelerate the healing process in rat. A study on *Avana sativa L* shows that aqueous, methanol, and ethanol extracts on the wounds of rat reduce the number of treatment days [46]. *Jujube* fruit extract with improving the inflammation, also accelerates the burn wound healing in rat[47]. The extract of *Scrophularia* seed significantly stimulates fibroblasts[48] that this is consistent with this study to accelerate the healing of stomach ulcers caused by alcohol. *Scrophularia* has also compounds with antioxidant and anti-inflammatory properties[26] that approved the impact of this plant in the stomach restoration.

CONCLUSION

The study showed that the aqueous and hydroalcoholic extracts of the *Scrophularia striata* herb has substantial restorative effect on gastric ulcer caused by alcohol and complementary investigation to isolate and identify compounds of this extract and determination of the compound which played the most important role in wound healing in stomach and the exact mechanism of this effect needs further studies.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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