



**ORIGINAL ARTICLE**

## **Effect of Alcoholic Extract of Licorice (*Glycyrrhiza glabra* L.) Rhizome on Isolated Duodenum Motility in Male Rats and its Interference with Cholinergic, Nitroergic, and Adrenergic Systems**

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### **ABSTRACT**

*Licorice rhizome is used as a native herbal medicine in Iran. Considering its anti-spasmodic and anti-inflammatory effects, the present study was performed to investigate some mechanisms of licorice rhizome extract on duodenal motility in vitro. Fourteen adult male rats weighing 200 to 250g were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg) and their duodenum were isolated and divided into control and experimental groups. The mechanical activity of the duodenum was recorded in a tissue bath containing oxygenated Krebs solution using ADInstrument Power lab system. Mechanical activity in response to extract 43µg/ml (most effective concentration based on concentration/response experiments) in the presence of acetylcholine (10<sup>-5</sup> M) as the muscarinic receptor agonist, atropine (10<sup>-4</sup> M) as the muscarinic receptor antagonist, epinephrine (10<sup>-6</sup> M) as the β-adrenoceptor agonist, propranolol as β receptor antagonist, or L-NAME (10<sup>-4</sup> M) as the inhibitor of the NO synthase enzyme was measured. In the control group, the solvent of licorice rhizome extract was used along with one of the mentioned drugs. The results showed that contraction force exerted on the isolated duodenum pieces by acetylcholine in the presence of licorice rhizome extract remarkably reduced compared to that of the control group (p<0.05). However, this response in the presence of atropine, propranolol and N-w- nitro- L arginine methyl ester (L-NAME) was not changed significantly. According to the results of the study, alcoholic extract of licorice rhizome decreases bowel motility. This inhibitory effect is independent of cholinergic, β- adrenergic and nitroergic pathways.*

**Keywords:** licorice, motility; nitric oxide, acetylcholine, epinephrine

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### **INTRODUCTION**

Licorice is a native plant of the Mediterranean and certain regions of Asia such as Iran. Licorice is a leguminosae, whose scientific name is *Glycyrrhiza Glabra* L. The Greek word "glycyrrhiza" is composed of "glycys" meaning sweet and "rhiza" meaning root. From among different types of licorice, *Glycyrrhiza glabra* L. is considered as the most valuable type for medical purposes. Glyciritic acid which is an important pharmaceutical composition can be found in the root and stem of this plant, is 50 times sweeter than sugar. Depending on environmental conditions and plant type, the amount of glyciritic acid can range from 5 to 20 percent. The yellow color of licorice rhizome is due to flavonoids the most important of which is liquiritin [1]. The most important property of licorice rhizome has been reported to be its effect on gastrointestinal tract. This plant is beneficial in treatment of gastritis [2]. In licorice rhizome, there is a substance called chalcones liquiritigenin that can relieve muscle spasm. Licorice rhizome also has some other therapeutic properties: it is anti-allergic due to the presence of a composition called glycyrrhizin [3], antibacterial due to the presence of isoflavonoids [4], anti-inflammatory as a result of glycyrrhetic acid [5], and anti-viral [6]. It also decreases blood cholesterol [7]. In traditional medicine, licorice rhizome has been used to treat muscle spasm and swell, rheumatic arthritis, cough, asthma, and other thorax infections [1] and increase bile (8). The main neurotransmitter in the gastro-intestinal tract is acetylcholine, that increases contraction of intestinal smooth muscles by stimulating muscarinic receptors[9]. Five types of muscarinic receptors (M1-M5) have been identified in

the membrane of smooth muscles and cholinergic neurons of the gastrointestinal tract. Role of muscarinic receptor M3 in contractions is more than that of M2 [10]. Norepinephrine and epinephrine as inhibitory neurotransmitters play a role in regulating peristaltic motility [11]. Nitric oxide (NO), as intra- and intercellular messenger, plays an important role in intestinal motility [12].

Previous studies have shown that licorice extract can control contractions caused by acetylcholine in ileum of rabbits and guinea pigs [13]. Alcoholic extract of licorice leaves can reduce ileum contractions independent from  $\beta$ -adrenergic, opioid and nitric oxide receptors [14]. Moreover, there are evidences that show the isoliquiritigenin in licorice leaves has smooth muscle relaxant effects. Therefore, it can be a strong antispasmodic factor in the intestine [15]. In addition, there is also evidence that shows isoliquiritigenin has a dual effect on gastrointestinal motility: spasmogenic effect in isolated rat gastric fundus and spasmolytic effect in pig ileum and rabbit jejunum [16]. As the previous researches were performed on licorice leaf and some of its flavonoids on the stomach, ileum and jejunum with both spasmogenic and spasmolytic effects, the present study was aimed to investigate the effect of its rhizome extract on mechanical activity of duodenum and probable role of cholinergic, nitrergic, and adrenergic systems in this activity.

## METHOD AND MATERIALS

**Extraction:** Licorice rhizome was collected from Shiraz University farms and confirmed by a pharmacognosist. It was extracted through percolator method in the Faculty of Pharmacy, Shiraz.

Fourteen adult male Wister rats weighing 200-250 g were kept in an animal house for one week. The animal house had standard laboratory conditions with twelve-hour light and dark cycle, temperature of  $22\pm 2^\circ\text{C}$  and availability of enough food and water. The rats were deprived from food 12 hours before the experiment but they had access to water. The animals were anaesthetized with pentobarbital (50 mg/kg) and duodenums were isolated and transferred to a petri dish containing warm Krebs solution ( $37^\circ\text{C}$ ). One-cm-long duodenum pieces were provided without damaging the epithelium and duodenal muscle. Two duodenal pieces, a control one and an experimental one, were longitudinally placed and floated in an organ bath containing Krebs solution ( $37^\circ\text{C}$ ), PH: 7.4, oxygen 95%, and  $\text{CO}_2$  5%, connected to a force transducer and power lap system. Mechanical activity of the tissue was recorded with basal tension of 1 g [16]. Afterwards, each piece was exposed to an effective dose of acetylcholine ( $10^{-5}$  M) for two minutes in order to test their health. After the tissue was washed and its tension returned to its initial state, these activities were recorded in the presence of the extract with a concentration of  $43\mu\text{g/ml}$  and its solvent (normal saline) added with the same volume as extract. This concentration was determined by concentration/response experiments (see figure 1 in result section). Then, acetylcholine with a dose of  $10^{-5}$  M, atropine ( $10^{-4}$  M) [16], epinephrine ( $10^{-6}$  M), propranolol ( $10^{-4}$  M) [14], and L-NAME ( $10^{-4}$  M) were added. All of these activities were recorded in both groups in order to investigate their interference with cholinergic, nitrergic, and adrenergic systems.

The results were analyzed by SPSS version14 using ANOVA test. Post hoc Tukey's test was utilized to determine the difference between paired groups whenever the difference was found significant among groups. Level of significance was set at  $P\leq 0.05$ . The data were presented as Mean $\pm$ SEM in Table and figures.

## RESULTS

Fig 1 shows responses of the isolated duodenum to increasing concentrations of licorice rhizome extract. The highest percentage of relaxation occurred in concentration of  $43\mu\text{g/ml}$ .

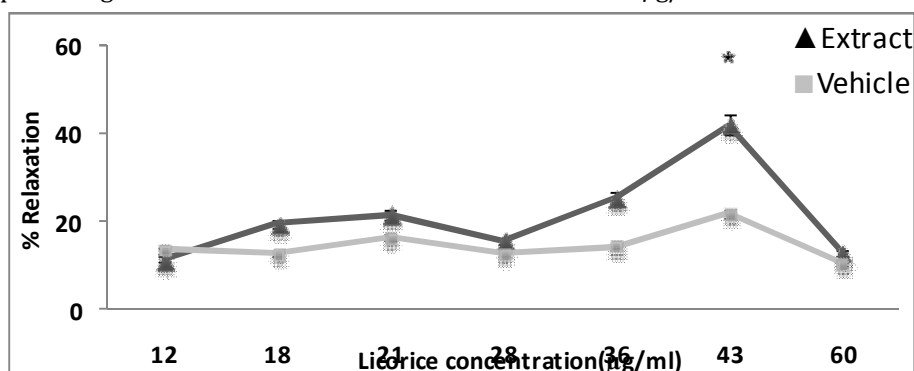


Fig 1. Relaxation of the isolated duodenum as a result of being exposed to different concentrations of licorice rhizome extract. solvent curve is in the absence of extract \* =  $P < 0.05$  compared to response to solvent.

Fig 2 illustrates the mechanical activity of duodenums in control state and after adding the effective concentration (43µg/ml) of the extract. Extract caused the contraction force to decrease with maximum effect at 25 minutes.

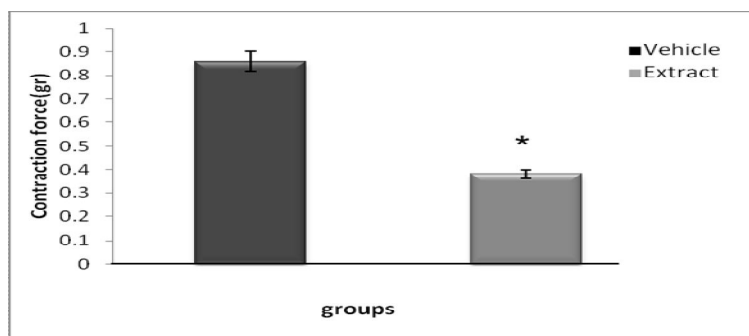


Fig 2. Isolated duodenum contraction in response to effective concentration of licorice rhizome extract (43µg/ml). \* =  $P \leq 0.05$ , \*\* =  $P < 0.01$  significant difference compared to solvent. Results are presented as mean  $\pm$  SEM

Fig 3 displays that adding the effective dose of extract (43 µg/ml) caused the contraction force to drop significantly after 25 minutes. Adding acetylcholine ( $10^{-5}$  M) raised the contraction force in the solvent group significantly while in the experimental (extract) group no change was observed. In addition, atropine did not inhibit the relaxation effect of extract.

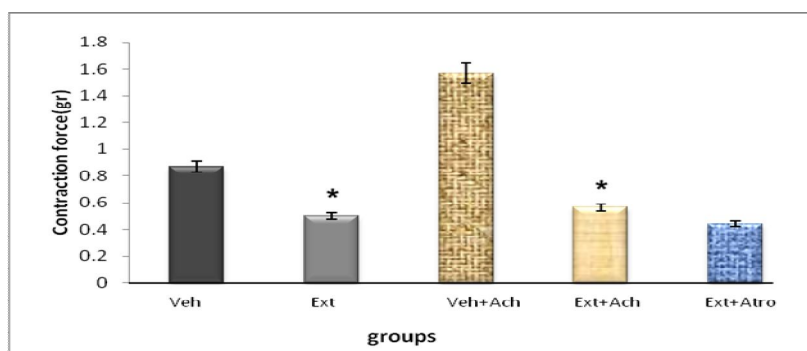


Fig 3. Isolated duodenum contraction force in response to effective concentration of licorice rhizome extract (Ext, 43 µg/ml) in the presence of acetylcholine (Ach) ( $10^{-5}$  M) and atropine (Atro) ( $10^{-4}$  M). \* =  $P < 0.05$  significant difference compared to solvent group. Results are presented as mean  $\pm$  SEM.

Fig 4 shows that adding extract (43µg/ml) caused the contraction force to drop after 25 minutes. Administrations of epinephrine ( $10^{-6}$  M) resulted in relaxation and decrease in contraction activity in both solvent and extract groups. Adding propranolol ( $10^{-4}$  M) did not inhibit relaxation effect of licorice rhizome extract.

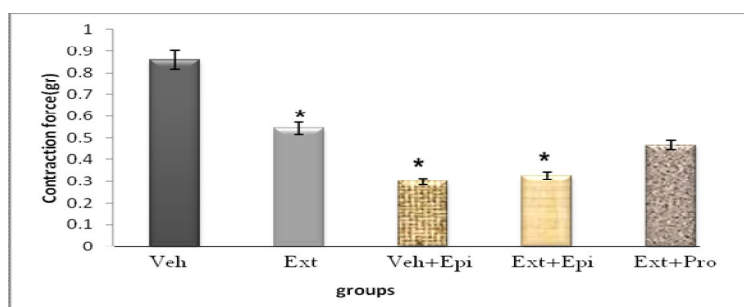


Fig 4. Comparing the level of contracting activity in isolated duodenum in response to epinephrine (Epi) ( $10^{-6}$  M) and propranolol (Pro) ( $10^{-4}$  M) in the presence of effective concentration of licorice rhizome extract (Ext) (43 µg/ml).  $P \leq 0.05$ \* significant difference compared to solvent group, (n=7 in each group). Results are presented as mean  $\pm$  SEM

Figure 5 shows that an effective dose of the extract (43 $\mu$ g/ml) could decrease the contraction force significantly after 25 minutes. Addition of L-NAME (10<sup>-4</sup> M) did not inhibit the relaxation effect of extract significantly in 35, 45, and 55 minutes in both groups.

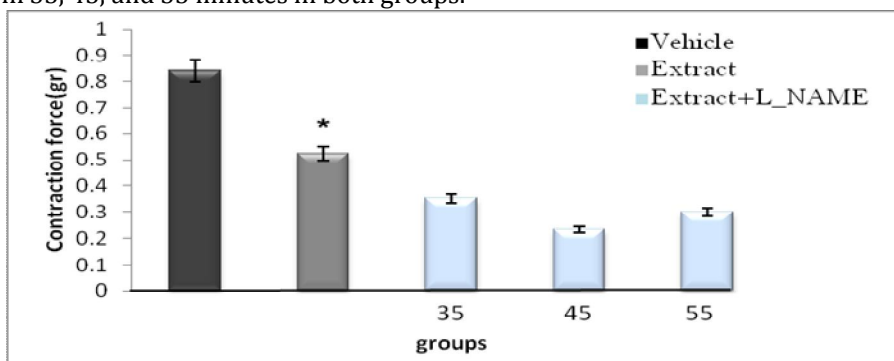


Fig 5. Contraction response of isolated duodenum to effective concentration of licorice rhizome extract (43  $\mu$ g/ml) in the presence of L-NAME (10<sup>-4</sup> M) in both groups. Extract and solvent. \* = P<0.05 significant difference compared to solvent group. Results are presented as mean  $\pm$  SEM

## DISCUSSION

The present study showed that contraction activity of the isolated duodenum in the presence of licorice rhizome extract, remarkably decreased. This response shows that the extract contains substances that have relaxation effect on intestinal smooth muscle.

The results of the study conducted by Sato *et al* have showed that isoliquiritigenin, a flavonoid available in licorice rhizome extract, has an antispasmodic effect in the rectum [15]. In a study conducted by Di Carlo it has been concluded that intra-peritoneal administration of some flavonoids such as apigenin, Kaempferol, and flavone could decrease motility of the small and large bowels [17]. The use of propranolol that caused the tension of duodenum to be decreased (see fig 4) shows that the beta adrenoceptors are present in this tissue, however beta antagonist propranolol did not affect the relaxing effect of extract implying that this effect is not through beta adrenoceptors. These results are in line with those reported by Liu *et al* who have concluded that isoliquiritigenin caused tracheal smooth muscles to relax but propranolol had no relaxant effect [18]. Capasso *et al* have also concluded that propranolol does not change the relaxant effect of quercetin (a licorice rhizome flavonoid) in isolated tracheal smooth muscles [19].

Moreover, no synthase inhibitor L-NAME also did not affect the relaxing effect of extract (see fig 5). This again implies that this relaxation is not through production of NO and therefore nitrergic system is not also involved. Chen *et al* reported that isoliquiritigenin a licorice rhizome flavonoid, reduced contractions caused by high concentration of potassium in ileum and jejunum, which has no interaction with NO system because applying L-NAME had no effect in preventing relaxation of ileum and jejunum [16]. In their study, Gharibnaseri *et al* have concluded that licorice extract antispasmodic effect on ileum is independent of nitric oxide synthase [14]. Liu *et al* have observed that isoliquiritigenin, can relax tracheal smooth muscles *in vitro* and *in vivo*. They have also concluded that L-NAME does not affect contraction created by acetylcholine in tracheal loops, which is in line with the results of the present study [18].

The other part of the results of the present study indicated that Ach significantly increased the basal tension of the duodenal pieces. This shows the tissue is intact and responsive. Administration of extract after Ach significantly reduced Ach-induced contractions. However non effectiveness of atropine to recover this effect (see fig 3) shows that Ach receptors are not involved in the effect of extract and means that cholinergic system is not also participate in this response. Huang *et al* have reported that quercetin has antispasmodic effect and influences the contraction caused by acetylcholine in isolated colon of guinea pig, which is in agreement with the results of the present study [20].

Regarding probable mechanism(s) of licorice rhizome alcoholic extract on duodenal specimens we may think about the role of calcium and potassium channels due to their contraction effect on smooth muscle. In a study conducted by Chen *et al*, the antispasmodic effect of isoliquiritigenin in rat ileum and rabbit jejunum has been reported to be due to the blockage of the calcium channels [16]. Moreover, Nagai *et al* have attributed the antispasmodic effect of isoliquiritigenin in pig ileum and rabbit jejunum to the blockage of the calcium channels [13]. In their study, Gharibnaseri *et al* have concluded that antispasmodic effect of licorice in the ileum is due to interference with calcium channels and activation of ATP sensitive potassium channels [14].

**CONCLUSION**

Overall, based on the results of the present study it can be stated that alcoholic extract of licorice rhizome can reduce bowel motility and have anti spasmodic effects. This inhibitory effect in duodenum is independent of cholinergic and  $\beta$ -adrenergic receptors and NO pathway. It is likely that this is due to activation of ATP-sensitive potassium channels or blocking calcium channels by some ingredients present in the extract. Further research is required to verify this hypothesis.

**Conflict of interest:** Authors confirm that there is no conflict of interest in this study

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