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Effect of Aqueous Extract of *Glycyrrhiza glabra* on rabbit ileum motility in comparison with Acetylcholine, Atropine and Ondansetron

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ABSTRACT

Objectives: Gastrointestinal disorders such as irritable bowel syndrome can affect the quality of life and increase the risk of psychological problems such as depression. Drugs such as hyoscyamine and metoclopramide and 5-HT_3 receptor antagonists such as Alosteron are used nowadays for symptomatic management, but their use is associated with adverse effects leading to decreased patient compliance. Nowadays, natural plant-based medicine is preferred by people due to its fewer adverse effects; therefore, the current study was planned to evaluate the effect of *Glycyrrhiza glabra* on the intestinal smooth muscle of rabbits. Our study aims to define the mechanism of action of *G. glabra* in promoting or inhibiting gut motility.

Materials and Methods: An experimental *in vitro* study was conducted in the Pharmacology Department of Services Institute of Medical Sciences Lahore in January 2023. Rabbit ileal tissue was used. PowerLab (ADInstruments) was used to record the contractions of ileal smooth muscles. After mounting, the tissue was given to rest for 30 min, after which baseline contractions were recorded. Then, 0.8 mL of acetylcholine (10^{-5}) was added, and contractions were recorded for 30 s. Freshly prepared Tyrode's solution was used to rinse the tissue three and then given rest for 3 min. Then, ileal tissue was treated with *G. glabra* 5%, 15% and 20% and their effect was recorded. Acetylcholine served as a positive control, and the action of *G. glabra* 5%, 15% and 20% solution was compared with it. After that, *G. glabra* was used in the presence of drugs that inhibit intestinal motility, atropine and ondansetron 0.0036 µM and 0.036 µM, respectively.

Results: Results showed that *G. glabra* 5%, 15% and 20% increased intestinal motility significantly ($P < 0.0001$) in comparison with acetylcholine. However, when *G. glabra* was used in the presence of antagonists, atropine and ondansetron, then, it reduced intestinal motility significantly ($P < 0.0001$).

Conclusion: Aqueous extract of *G. glabra* has a dual effect on gut motility that is a direct muscarinic receptor agonist and indirect modulator of enteric vagus nerve terminal through serotonin 5-HT_3 receptors.

Keywords: *Glycyrrhiza glabra*, Acetylcholine, Atropine, Ondansetron

INTRODUCTION

The second brain of our body has been attributed to the enteric nervous system (ENS) due to numerous reasons. The ENS is independent in its functionality. It is connected to the central nervous system (CNS) through the sympathetic and parasympathetic nervous systems through

the vagus nerve and prevertebral ganglia, respectively.[1] The normal functioning of ENS is dependent upon numerous neurotransmitters. Many of them are also found in CNS. The ENS also makes use of more than 30 neurotransmitters, most of which are identical to the CNS neurotransmitters, such as acetylcholine, dopamine and serotonin. An interesting fact is that approximately 90% of the serotonin in our body is present in our gut, along with 50% of dopamine.[2] Defects in the normal functioning of the gastrointestinal (GI) tract lead to functional GI disorders. These are mainly due to an imbalance of neurotransmitters, but there are no structural abnormalities. The main domain of research in neuro-gastroenterology is common functional GI disorders such as irritable bowel syndrome (IBS), which is very common.[3] GI disorders such as IBS can affect the quality of life and increase the risk of psychological problems such as depression.[4] The most common symptom of IBS is recurrent abdominal pain, which is usually relieved by defecation, and sometimes, there are alterations in bowel habits.[5] Altered intestinal movements in IBS can cause diarrhoea, constipation or both, along with abdominal pain.[6] Currently, available drugs for symptomatic management of IBS include anticholinergics such as hyoscyamine and metoclopramide and 5-HT_3 receptor antagonists such as ondansetron. Long-term use of these medications is associated with serious adverse effects. Hyoscyamine can cause urinary retention, dry mouth and other anticholinergic symptoms. Chronic use of metoclopramide is associated with a risk of Tardive dyskinesia, and serious cardiovascular complications can occur with ondansetron.[7] Due to these adverse effects, patient's compliance with the treatment remains the main hindrance in the management of GI disorders such as IBS. Nowadays, people have started using herbal medicines. The World Health Organization estimated that nowadays, nearly 60% of the population worldwide and 80% of the population in developing countries consider natural medicine for their primary healthcare needs.[8] *Glycyrrhiza glabra*, a herb commonly called licorice, belongs to the Leguminosae family. Its multiple species are distributed in diverse geographical areas around the world. Its therapeutic potential in numerous health issues is mainly due to various phytomolecules present in its extract.[9] *G. glabra,* due to its extraordinary potential, is listed in Chinese Pharmacopeia officially, and it is also used as a flavouring agent in commercial products of foods and beverages for decades.[10] Its roots and stem are rich in glycyrrhizic acid which is an essential pharmaceutical ingredient and is proven to be 50 times sweeter than sugar.[11] Licorice rhizome is reportedly having significant effects on the GI tract, making it popular for its use in gastritis and many other functional GI disorders.[12] Based on these findings of literature and medicinal usage of *G. glabra*, we proposed to observe the possible mechanism of action of *G. glabra* on rabbit ileal tissue.

MATERIALS AND METHODS

Animals and housing conditions

This was an *in vitro* experimental study. The study was approved by Institutional Review Board letter no. IRB/2023/1135/SMS. A healthy New Zealand white rabbit weighing approximately 2–3 kg was obtained from the local market. Animals were kept in the animal house of the Pharmacology Department Services Institute of Medical Sciences, Lahore, at controlled room temperature (25–27°C) and humidity (45–65%). Animals were kept on a standard diet that consisted of carrots and tap water *ad libitum*.

Plant used and extract preparation

Roots of *G. glabra,* which belongs to the family *Fabaceae* in powdered form, were purchased from the local market. To prepare its aqueous extract, it was kept soaked in distilled water overnight and then filtered with muslin cloth twice. The following three strengths of *G. glabra* were prepared using distilled water:

i. 5% solution
ii. 15% solution
iii. 20% solution.

Chemicals for study

Acetylcholine chloride (Sigma Chemical Co., USA), Atropine (Sigma Chemical Co., USA) and ondansetron hydrochloride (Werrick Pharmaceuticals Pakistan) were purchased. Distilled water was used to prepare all the solutions and their dilutions freshly at the time of the experiment. The following molar solutions were prepared:

i. Acetylcholine (10^{-5})
ii. Atropine (10^{-5})
iii. Ondansetron (0.0036 μM and 0.36 μM).

Preparation of tissue

A rabbit ileum strip of 2–3 cm in size was dissected from an overnight fasting rabbit.[13] Before mounting in the organ bath, intestinal tissue was rinsed extensively with normal saline. Then, it was mounted in an isolated organ bath of 50-mL capacity, filled with Tyrode's solution and had a continuous supply of oxygen. Tyrode's solution was freshly prepared using sodium chloride (8.0 g), potassium chloride (0.28 g), magnesium chloride (0.1 g), sodium bicarbonate (1.0 g), sodium dihydrogen phosphate (0.05 g), calcium chloride (0.2 g) and anhydrous glucose (1.0 g) dissolved in 1.0 L of deionised water. PowerLab (ADInstrument) was used for plotting the cumulative dose response curve. One side of the ileal tissue was connected to the base of the oxygen tube inside the organ bath, and the other side was attached with a research-grade force displacement transducer

DT-475 (USA) with the help of a thread. The computer with the software was connected to IWorx/214 (USA). After that, the DIN connector of the DT-475 displacement transducer was plugged into channel 3 of the iWorx/214 unit. Then, the tissue was left for equilibration for approximately 15 min, during which Tyrode's solution was changed twice. The displacement transducer recorded the resting ileal smooth muscle contractions.[14]

Preparation of dose-response curves

After mounting, the tissue was left for equilibration for an interval of 30 min. Then, 0.8 mL of Ach (10^{-5}) was administered in the organ bath. After obtaining maximum contraction of Ach, the tissue was rinsed with Tyrode's solution 3 times to wash out the agonist. The same procedure was followed for different concentration solutions of *G. glabra,* i.e., 5%, 15% and 20%, to record the force of contraction of rabbit ileum. In the next step of the experiment, *G. glabra* 5% was used in the presence of inhibitors of intestinal motility, atropine (10^{-5}) and ondansetron 0.0036 μM and 0.36 μM, respectively. This was done to elucidate the underlying mechanism of action of *G. glabra*. *G. glabra* 5% was used only to compare with antagonists because it showed a maximum increase in the force of contraction.

Statistical analysis

Results were analysed using GraphPad Prism version 6, which was represented as means ± standard deviation. One-way analysis of variance was used to check variation in response amongst different study groups. *Post hoc* Tukey's test was used to check significant differences in response amongst various groups. For all the results, a $P < 0.005$ was considered statistically significant.

RESULTS

Effect of acetylcholine and *G. glabra* (5%, 15% and 20%) on rabbit ileum strip

The baseline force of contractions was 1.080 mN ± 0.0189. There is an increase in the force of contraction in ileum tissue after treatment with acetylcholine that is 2.163 mN ± 0.052. 5%, 15% and 20% aqueous extract of *G. glabra* also increased the force of contraction to 4.002 mN ± 0.522, 1.678 mN ± 0.116 and 2.336 mN ± 0.011, respectively. 5% and 15% solutions have significantly increased force of contraction as compared to normal and Ach-induced contractions. Its value is mentioned which is $P \leq 0.0001$, which is statistically significant, whereas 20% solution was found to have a statically significant difference from normal, but its response is statistically insignificantly different from Ach [Figure 1].

Effect of atropine and *G. glabra* 5% on rabbit ileum strip

The baseline force of contraction was 1.045 mN ± 0.057. Atropine reduced the baseline force of contraction to 0.194 mN ± 0.067. This difference in the force of contraction is statistically significant $P \leq 0.0001$. When *G. glabra* 5% solution was added in the presence of atropine, the force of contraction further reduced to 0.075 mN ± 0.059, which was also statistically significant $P \leq 0.0001$ [Figure 2].

Effect of ondansetron 0.0036 μM and *G. glabra* 5% on rabbit ileum strip

A low dose of ondansetron 0.0036 μM reduced the force of contraction as compared to normal baseline gut activity significantly (0.1263 mN ± 0.05048). It might have acted as a spasmolytic as it has blocked the stimulatory effect of serotonin on the vagus nerve terminal. When ileal tissue was treated with 5% *G. glabra* in the presence of a low dose of ondansetron, it significantly reduced the force of contraction (0.0200 mN ± 0.01462). The difference between ondansetron 0.0036 μM and *G. glabra* 5% was statistically significant [Figure 3].

Effect of ondansetron 0.36 μM and *G. glabra* 5% on rabbit ileum strip

The baseline force of contraction was 0.1707 mN ± 0.02638. The increase in the force of contraction in ileum tissue after treatment with a high dose of 5-HT_3 receptor antagonist, ondansetron 0.36 μM (1.019 mN ± 0.05643) in comparison to *G. glabra* 5% solution (0.3047 mN ± 0.05144) showed spasmogenic activity. It might have produced this response by acting on some other receptors in the GI tract. The difference between ondansetron 0.36 μM and *G. glabra, 5% solution,* was statistically significant, $P \leq 0.0001$ [Figure 4].

DISCUSSION

Functional GI disorders are a diverse group of disorders in which the ability of the GI tract to coordinate muscular activity is impaired.[15] Recently, the use of natural compounds having therapeutic potential is gaining popularity due to their availability, safety and cost effectivity. *G. glabra* is famous for its ethnopharmacological values from ancient times. Numerous Pharmacological studies in the literature have shown that multiple extracts of this compound have diverse biological effects such as antioxidant, antibacterial, antiviral, anti-inflammatory and, most importantly, antispasmodic effects on intestinal motility.[16] Rabbit ileal tissue was selected for this experiment because it is found to be more suitable for checking the response of agonist in the presence of antagonists.[17] ENS is mainly responsible for controlling intestinal motility through multiple mediators, mainly

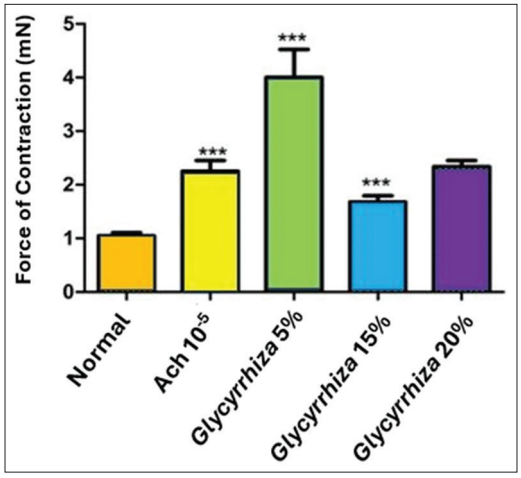

Figure 1: Effect of acetylcholine and *Glycyrrhiza glabra* (5%, 15%, 20%) on rabbit ileum in comparison with baseline contraction. *** indicate $P \leq 0.0001$.

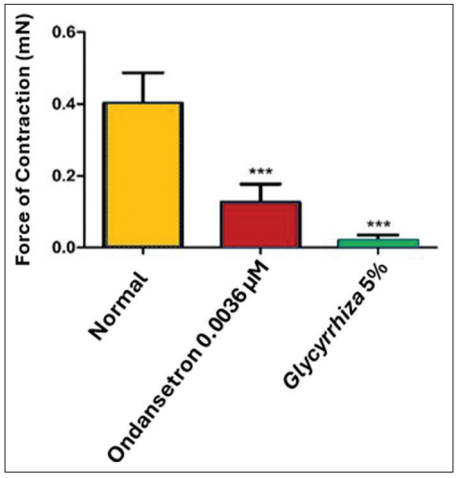

Figure 3: Graphical representation of ileum contraction in normal, ondansetron 0.0036 µM and *Glycyrrhiza glabra* 5% group. *** indicate $P \leq 0.0001$.

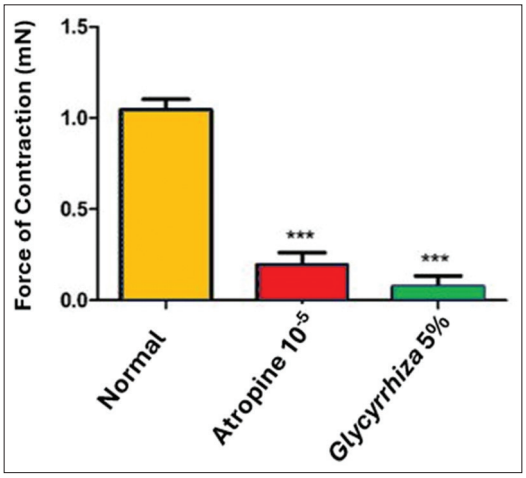

Figure 2: Graphical representation of ileal contraction in normal, atropine and *Glycyrrhiza glabra* 5% group. *** indicate $P \leq 0.0001$.

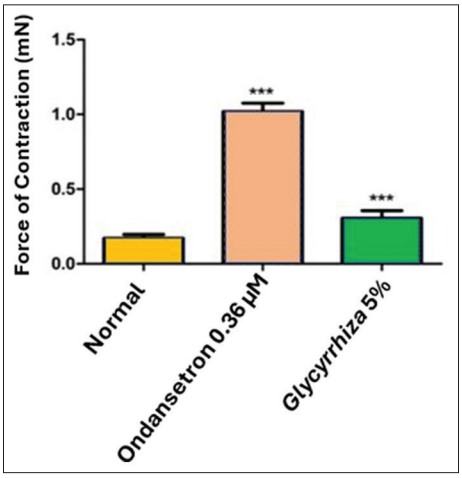

Figure 4: Graphical representation of ileal force of contraction in normal, ondansetron 0.36 µM and *Glycyrrhiza glabra* 5% group. *** indicate $P \leq 0.0001$.

acetylcholine, histamine, serotonin, bradykinin, dopamine, etc. Acetylcholine is a prokinetic by acts on muscarinic receptors, while serotonin receptor stimulation leads to nausea, vomiting and abdominal pain. Atropine is an antagonist of muscarinic receptors, and ondansetron is a 5-HT_3 receptor antagonist which inhibits colonic motility, especially left colon increasing colonic transit time.[18] It has been previously demonstrated that isoliquiritigenin, which is obtained from *G. glabra,* reduces intestinal motility in the lower part of the intestine.[19] In this study, to find the underlying mechanism of aqueous extract of *G. glabra,* we used isolated rabbit ileal tissue. Acetylcholine served as a gold standard muscarinic agonist that showed a significant ($P < 0.0001$) spasmogenic effect, which was mediated through muscarinic receptors. *G. glabra* 5% solution showed a significant spasmogenic effect, which was even significantly greater than acetylcholine ($P < 0.0001$). A similar significant spasmogenic effect was seen with *G. glabra* 15% and 20% solution. These results are in accordance with a previous study that showed its spasmogenic effect in rat stomach fundus which was also because of its ability to stimulate muscarinic receptors.[20] *G. glabra* is found to be a muscarinic agonist as it has increased the ileal force of contraction which is even significantly greater than that produced by prototype muscarinic agonist, i.e. acetylcholine. This higher spasmogenic effect as compared to Ach may also be due to its indirect stimulating effect on the vagus nerve terminal through serotonergic modulation. Enterochromaffin cells release serotonin in response to multiple chemical stimuli, which activate 5-HT_3 receptors on vagal afferents.[21] 5-HT_3 receptors are heteroreceptors that regulate vagal output in ileal smooth muscle cells. They might be activated by *G. glabra,* which explains its additive spasmogenic effect in our

study. This indirect effect is further supported by the results of our study when *G. glabra* significantly reduced gut motility in the presence of a low dose of ondansetron, i.e. 5-HT_3 receptor antagonist as serotonin receptors were blocked, and it was unable to indirectly stimulate gut motility. When *G. glabra* is given in the presence of atropine, the ileal force of contraction is markedly inhibited, which may be because atropine has already blocked muscarinic receptors through which *G. glabra* was directly acting to stimulate the ileal force of contraction. This is also in accordance with a previous study which showed similar results, that is spasmogenic in the presence of acetylcholine and spasmolytic in the presence of atropine. Atropine can antagonise cholinergic transmission in ileal smooth muscle cells by attenuating serotonergic modulation (5-HT_3) of vagal outflow.[22] The spasmolytic action of *G. glabra* can also be attributed to the blockade of voltage-gated calcium channels.[20] In our experiment, ondansetron, a 5-HT_3 antagonist, showed a dual response: increased intestinal force of contraction at high doses and decreased force of contractions at low doses. This is in accordance with a previous study that showed a similar dual response and attributed it to the partial agonistic activity of ondansetron on peripheral 5-HT_3 receptors, or it might increase the ileal smooth muscle motility by its interaction with other receptors.[23] Response of *G. glabra* in the presence of a low dose of 0.0036 µM of ondansetron was inhibition of intestinal motility due to blockage of serotonin receptors. When the ileal tissue treated with a high dose of 0.36 µM ondansetron was given *G. glabra* 5%, it decreased the exaggerated spasmogenic effect of a high dose of ondansetron. This can be explained on the basis of *G. glabra*'s ability to modulate serotonergic pathway, antagonising ondansetron-induced changes in ileal motility at high doses. It also shows that *G. glabra* might keep the ileal contractions within physiological limits, as also evident from the results of our study. Hence, our study shows that when *G. glabra* was used in the presence of spasmolytic agents, it may act as an effective spasmogenic drug.

CONCLUSION

From the results of our study, it can be concluded that an aqueous extract of *G. glabra* has a dual effect on gut motility that is a direct muscarinic receptor agonist and indirect modulator of enteric vagus nerve terminal through serotonin 5-HT_3 receptors. Clinical application of these drugs can be in the management of IBS, where altered intestinal motility decreases patients' quality of life. Further, clinical trials are recommended in this domain.

Ethical approval

The research/study was approved by the Institutional Review Board at Services Institute of Medical Sciences, Lahore, Pakistan, number IRB/2023/1135/SIMS, dated 25th July 2023.

Declaration of patient consent

Patient's consent is not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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