RESEARCH ARTICLE

Evaluation of traditionally claimed anti-ulcerant potential of poly-herbal preparation using rat model

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ABSTRACT:

Background: Despite the proven effectiveness of many traditional polyherbal medicines, their scientific mechanisms of action often remain unclear. Qarahine is one such compound preparation, and its purported therapeutic benefits for ulcer treatment require empirical validation to understand its rationale. **Objective:** This study was conducted to validate the anti-ulcer effect of the polyherbal preparation Qarahine through integrated in-vivo and in-vitro studies, specifically measuring its ulcer-curative potential and assessing its effectiveness against duodenal ulcers. **Methods:** The anti-ulcer activity was evaluated in vivo using rodent models of ethanol-induced, NSAID-induced, and stress-induced gastric ulcers at two dose levels (50 and 100 mg/kg). Histopathological examination of stomach tissues provided supporting evidence. The in-vitro spasmolytic effect was assessed on isolated rabbit jejunum preparations, testing the crude extract on spontaneous, high K+ (80 mM)-induced, and carbachol (1 µM)-induced contractions, with verapamil used as a standard reference. **Main Outcome Measures:** The primary outcomes were the percentage cure of induced ulcers in the different models and the concentration-dependent spasmolytic response in the isolated tissue experiments. **Results:** Qarahine demonstrated significant, dose-dependent anti-ulcer activity. It cured 31.1% and 60% of ethanol-induced ulcers, 46.7% and 86.7% of NSAID-induced ulcers, and 33.3% and 76.3% of stress-induced ulcers at doses of 50 and 100 mg/kg, respectively. Histopathological analysis strongly corroborated these findings. In vitro, the extract exhibited a concentration-dependent spasmolytic effect, inhibiting contractions induced by high K+ and carbachol, similar to the calcium channel blocker verapamil, suggesting a potential mechanism involving calcium channel blockade. **Conclusion:** The results of the present study provide robust scientific evidence for the anti-ulcer efficacy of Qarahine. Based on these findings, Qarahine can be recommended as an effective and alternative remedy for the treatment of gastric ulcers.

Key Words: *antispasmodic; calcium channel blockade; histopathology.*

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Introduction

Peptic ulcer is a common global problem that affects millions of people worldwide characterized by lesions in the gastric and duodenal epithelium associated with acute or chronic inflammation [1]. Due to relapses and recurrences after the cessation of treatment, a search for safer potential drugs is being carried out [2]. According to world health organization, most population rely on traditional medicines for their health requirements [3]. Since these medicines are relatively safer and cheaper than synthetic or modern medicine [4]. The gastric mucosa is continuously exposed to potentially injurious agents such as acids, pepsin, bile acids, food ingredients, bacterial products and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [5].

However, scientific rationales behind these medicines are not understood despite the effectiveness is known from personal experiences if used in therapeutic doses. Symptoms of ulcer include epi-gastric pain of a burning (postprandial pain and pain relieved by food or antacids), nausea, vomiting, belching and bloating. Complications of protracted untreated cases include anemia caused by gastro-intestinal blood loss, weight loss attributed to a reduced appetite caused by fear of pain and vomiting associated with a gastric ulcer or pyloric stenosis and mucosal perforation [6] High cost of treatment and unbearable side effects of the conventional anti-ulcer drugs leads to poor compliance and resultant treatment failures. There is, Therefore there is need to develop safe, effective and affordable alternatives in the symptomatic management of peptic ulcer disease [7] Various synthetic anti-ulcer drugs are presently available, and some of these like Misoprostol are specifically used to prevent or treat the NSAID induced gastric ulcer [8]. However, each of these drugs confers simpler to severe side effects such as diarrhea, itching, skin rash, dizziness, and inactivation of some antifungal drugs (proton pump inhibitors), confusion in elderly patients, headache and anti androgenic effect (H2 receptor blockers), constipation, vomiting, indigestion, back pain, and dizziness (Sucralfate), bleeding diathesis and abortion for pregnant women (Misoprostol) [9]. Thus, there is a growing interest on nontoxic, antiulcer formulations from medicinal plants, and many taxa of medicinal plants have been assessed worldwide for their anti ulcerogenic effects. In the developing nations, this turn of events has also been prompted, in part, by the high cost of the modern antiulcer medication [10]. Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world. Some of the causes of these disorders are: stress, smoking, nutritional deficiencies and ingestion of Nonsteroidal anti-inflammatory drugs [11].

Materials and Methods

Plant material (Qarahine: a poly herbal preparation)

Per 3 g of Qarahine contains: *Cochlospermum gossypium* (0.615 g), *Glycyrrhiza glabra* (1.845 g), *Koalinum ponderosum* (0.102 g), *Lapis lazuli* (0.039 g), *Magnesium silicate* (0.051 g), *Pistacia terebinthus* (0.309 g), *Silicate of Magnesia & Ferrum* (0.039 g).

Crude extract preparation

The product was purchased from local herbalist in Lahore, Pakistan. Crude extract was prepared

By macerating powdered drug formulation in 70% hydro-alcoholic solution at room temperature for three days and shacked intermittently as previously followed by [12]. Macerate was filtered from double layered muslin cloth, then from the filter paper. This process was practiced again with residue to obtain maximum extract yield form soaked material. Then vaporized the filtrate in rotator evaporator at reduced pressure (-760 mmHg) and temperature (40-50ºC) to convert into a viscous mass [13]. The semi-solid extract was stored in refrigerator until used.

Drugs and chemicals

Commercially available drugs sucralfate (Pacific Pharmaceuticals Pvt. Ltd), ranitidine (GSK), misoprostol (Atco Laboratories Pvt. Ltd) and aspirin (Reckitt Benckiser) used for the study were purchased from local pharmacy.

Animals. Locally breed male rabbits (1.0-1.5 kg) and albino rats (200-250 g) of either sex were accommodated in the animal house of Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan. Guidelines of National Research Council [14] were followed to perform experiments, as instructed by the Animal Ethics Committee of Faculty of Pharmacy, The University of Lahore.

In vivo experiments

Induction of ulcer

Rats were assigned in five groups with five rats in each. Method for induction of ethanol, NSAIDs and the stress induced ulcer was adopted with little modification, formerly used by [15-17], respectively. Animals were given the exposure of ulcerogen (ethanol, NSAIDs and stress) for 3 days. On 4th day group I (normal) and group II (diseased) was slaughtered and stomach was dissected out. Stomach was cut opened along the greater curvature for gross examination of ulcerative lesions. Other groups were treated with 50 and 100 mg/kg dose of extract or with the standard drug, 12 hourly for next 10 days. On 11th day animals were slaughtered and stomach was examined for ulcerative lesions. The exposed inner surface was photographed and tissues were preserved in 10% formalin for histo-pathological evaluation.

Calculation of ulcer index

Ulcer index and percentage curative was calculated by using formula described previously by [18]

$$\text{Ulcer index (UI)} = \left(\frac{\text{arithmetic mean of Intensity in a group}}{\text{total number of animals}} + \frac{\text{number of ulcer positive animals}}{\text{total number of animals}}\right) \times 2$$

$$\text{Percentage curative} = \frac{\text{UI control} - \text{UI Treated}}{\text{UI Control}} \times 100.$$

0 = No ulcer,

1 = Mild redness

2 = Superficial lesions

3 = Deep lesions

4 = Penetrated ulcers/ Bleeding[19].

In vitro experiment

In vitro experiments were performed on isolated rabbit jejunum by following the method previously described by [20]. Rabbit jejunum was removed out after surgical opening of abdominal cavity. The jejunum was kept in Tyrode's solution. Approximately 2-3 cm length of jejunum was mounted in tissue organ bath filled with 20 ml Tyrode's solution, supplied with oxygen and warmed at 37 C. Tyrode's solution composition in mM was: KCl 2.68, NaCl 136.9, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaHPO 40.42 and glucose 5.55 (pH 7.4). Each tissue was left untreated for 30 min to equilibrate before the addition of any drug and then stabilized with repetitive (3-5 times) exposure of 0.3 µM acetylcholine and subsequent washing with Tyrode's solution. These experimental conditions allow testing of drugs effect on spontaneous rhythmic contractions of jejunum. Changes in isotonic responses of intestine were recorded through Bioscience transducer, connected to a computer through a data acquisition system: power Lab (AD Instruments).

Results

In vivo experiments

Ethanol induced ulceration

Ulcer score in the diseased group was found at 4.0. While in Qrh 50 and 100 mg/kg (body weight) treated group, the ulcer score was significantly ($P<0.001$) reduced to 1.2 and 0.6, respectively, similar to sucralfate (100 mg/kg) 0.6.ulcer index of diseased group was found to be 3.6 and of the Qrh 50 and100 mg/kg it was 2.48 and 1.44, respectively,

similar to the ulcer index of sucralfate1.44.Percentage cure with Qrh 50 and 100 mg/kg was found to be31.1% and 60%, respectively, similar to sucralfate 60% (Figure 1B).

Histo-pathological evaluation of stomach tissue showed mild, superficial gastritis with mild chronic inflammatory cell infiltrate in Qrh 50 mg/kg and mild congestion in gastric sub epithelial tissues was seen in 100 mg/kg, similar to sucralfate treated group (Figure 1A).

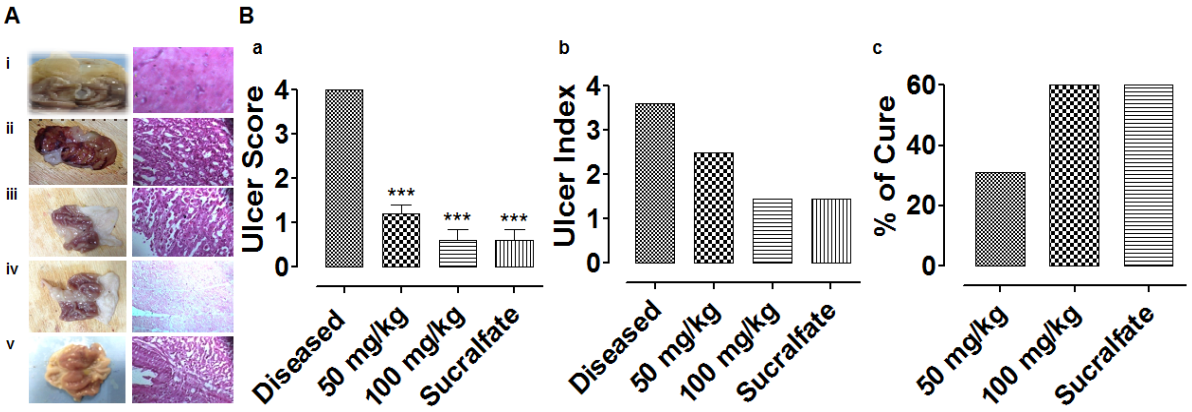

Figure 1: (A) Showing gross view photographic and histopathological images of rat's stomach in**(i)**control group, **(ii)** diseased group, **(iii)**Qarahine (Qrh) 50 mg/kg **(iv)**Qrh 100 mg/kg and **(v)** sucralfate 100 mg/kg treated groups and **(B)** bar chart showing the **(a)** ulcer score, **(b)** ulcer index and **(c)** percentage cure of different treatment groups in ethanol (1 ml/

NSAIDs induced ulceration

Ulcer score in the diseased group was found at 4.0. While in Qrh 50 and 100 mg/kg (body weight) treated group, the ulcer score was significantly (P<0.001) reduced to 1.0 and 0.5, respectively, similar to misoprostol (100 mg/kg) 0.6. Ulcer index of diseased group was found to be 3.6, of the Qrh 50 and100 mg/kg was 1.92 and 0.48, similar to ulcer index of misoprostol 1.44. Percentage cure with Qrh 50 and 100 mg/kg was found to be 46.67% and 86.67%, respectively, similar to sucralfate 60% (Figure 2B).

Histo-pathological evaluation of gastric tissue showed mild superficial gastritis with mild chronic inflammatory cell infiltrate in Qrh 50 mg/kg and mild congestion in gastric sub epithelial tissues was seen in 100 mg/kg, similar to ranitidine treated group (Figure 2A).

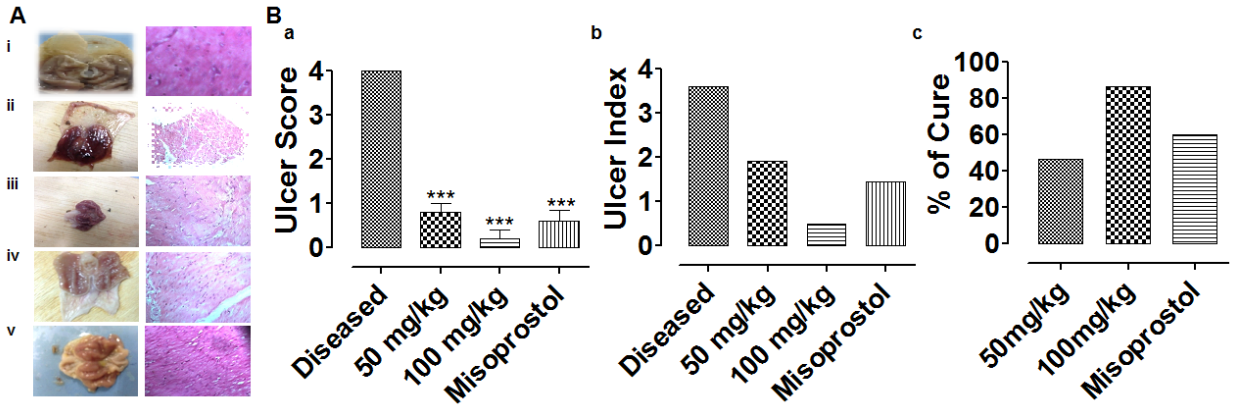

Figure 2: (A) Showing gross view photographic and histopathological images of rat's stomach in**(i)**control group, **(ii)** diseased group, **(iii)** Qarahine (Qrh) 50 mg/kg **(iv)** Qrh 100 mg/kg and **(v)**misoprostol 100 mg/kg treated groups and **(B)** bar chart showing the **(a)** ulcer score, **(b)** ulcer index and **(c)** percentage cure of different treatment groups in aspirin (200 mg/kg) induced ulcerative rats.Values are shown as mean ± S.E.M., n=5, ***p<0.001.

Stress induced ulceration

Ulcer score in the diseased group was found at 4.0. While in Qrh 50 and 100 mg/kg (body weight) treated group, the ulcer score was significantly (P<0.001) reduced to 1.0 and 0.5, respectively, similar to ranitidine (50 mg/kg) 0.6. Ulcer index of diseased group was found to be 3.6, of the Qrh 50 and100 mg/kg was 2.4 and 0.96, similar to ulcer index of misoprostol 1.44. Percentage cure with Qrh 50 and 100 mg/kg was found to be 33.3% and 76.3%, respectively, similar to sucralfate 60% (Figure 3B).

Histo-pathological evaluation of gastric tissue showed mild superficial gastritis with mild chronic inflammatory cell infiltrate in 50 mg/kg and mild congestion in gastric sub epithelial tissues was seen in 100 mg/kg, similar to misoprostol treated group (Figure 3A).

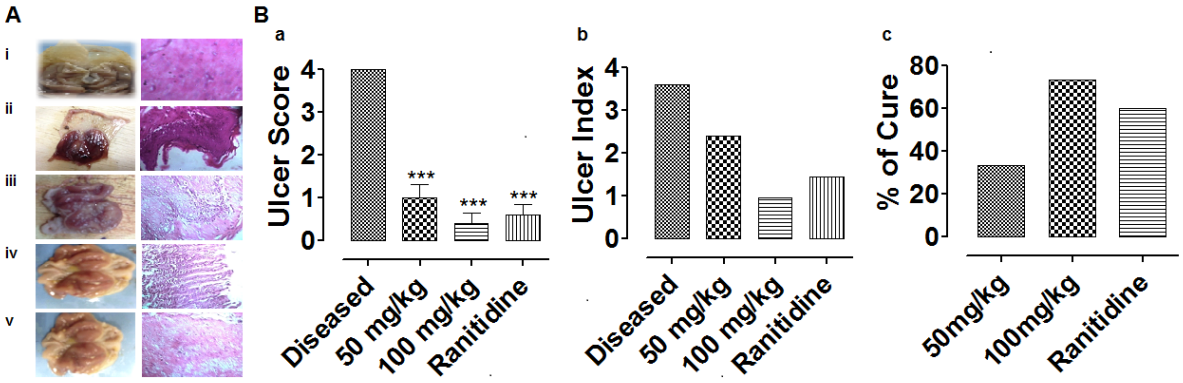

Figure 3: (A) Showing gross view photographic and histopathological images of rat's stomach in **(i)** control group, **(ii)** diseased group, **(iii)** Qarahine (Qrh) 50 mg/kg **(iv)** Qrh 100 mg/kg and **(v)**ranitidine50 mg/kg treated groups and **(B)** bar chart showing the **(a)** ulcer score, **(b)** ulcer index and **(c)** percentage cure of different treatment groups in stress induced ulcerative rats. Values are shown as mean ± S.E.M., n=5, ***p<0.001.

***In vitro* studies**

Spasmolytic effect of Qrh.Cr was observed on spontaneous contractions of isolated rabbit jejunum, when given in cumulative concentrations (0.01-10 mg/ml) with EC50 value of 3.84 (95% CI, 2.22-3.39, n=5) (Figure 4A), similar to verapamil (0.01-10 µM) (Figure 4B), with EC50 value of 2.89 (2.43-3.43, n=5). A complete relaxation with an ascending concentration of Qrh.Cr was also observed at 1 mg/ml, in high K+ (80 mM) and carbachol (1 µM) mediated jejunum contraction with EC50 value of 0.177 (0.13-0.236, n=5), 0.197 (0.12-0.32, n=5) (Figure4A), similar to verapamil, with EC_{50} value of 0.039 (0.030-0.052, n=5), 0.24 (0.123-0.324, n=5), respectively (Figure 4B).

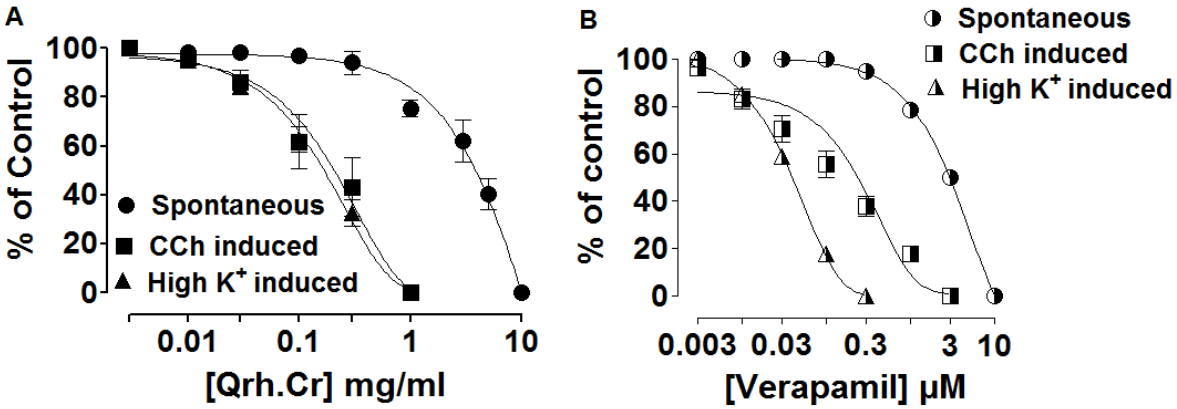

Figure 4: Showing relaxant effect of **(A)** Qrh.Cr (crude extract of Qarahine) and **(B)** verapamil on spontaneously contracting, high K^+ [80 mM] and carbachol (CCh; 1 µM) mediated contraction of rabbit jejunum preparations. Values expressed with mean ± S.E.M. n = 5.

Discussion

Ethanol is known as one of the principle risk factor for the development of gastric ulcers [21]. It reduces the secretion of bicarbonates and depletes gastric mucus production [22] that destroy the protective mucous layer of stomach and expose the stomach lining cells to proteolytic and hydrolytic actions of pepsin and gastric acid (HCl), causing damage to the membrane [21]. Since the Qrh (50 and 100 mg/kg) significantly ($p<0.001$) reverse the ethanol induced damage to the gastric mucosa, suggests that anti-ulcer activity associated with Qrhis due to its capability to produce cyto-protective action, as done by the sucralfate (100 mg/kg).

The role of PGs in the stomach is the protection of epithelium by regulation of mucosal protective layer [23] and decreasing the gastric acid secretions [24]. NSAIDs cause gastric ulceration by inhibition of cyclooxygenase enzymes, which leads to decrease mucus and bicarbonate discharge. It also decreases gastric blood flow, disturbs the aggregation

of platelets and changing in micro vascular structures, results in epithelial injury and internal bleeding [25]. Thus, NSAIDs inhibits the prostaglandin synthesis leading to increased gastric mucosal injury ultimately causes the gastric ulceration. The results obtained from the study shows that the ulcer surface area and mean ulcer score were not only significantly ($p<0.001$) reversed in groups treated with Qrh (50 and 100 mg/kg), as compared to their respective control group but also found more effective even than misoprostol (100 mg/kg). Therefore, it can be believed that the anti-ulcer activity of the Qrh by increasing the synthesis of endogenous prostaglandins, which in turn promotes mucus secretion and enhances the mucosal barrier against the actions of various damaging agents.

Histamine releases in stress, results in reduction of mucus production and increase in acid secretion, leads to gastric ulceration [26]. Stress enhances vagal activity that results an increase in gastrointestinal motility and has also been reported to be one of the factors, involved in stress induced ulcers [27]. It has been suggested that in conditions of emotional tension, there is greater destruction of mucus and decrease in synthesis of its components [28]. Significant ($p<0.001$) reversal of stress induced ulcer, indicates the anti-ulcerogenic ability of Qrh (50 and 100 mg/kg) that is possibly mediated by blocking the H2 receptors similar to ranitidine (50 mg/kg) and also indicate the presence of anti-cholinergic property that was also confirmed when Qrh.Cr was tested on carbachol (1 μM) induced contraction on isolated rabbit jejunum.

Effectiveness in the duodenal ulcer of anti-ulcerogenic agent is very much dependent on its gastric motility [29]. It would perform better healing effect, if the intestinal transit time of the drug is increased and the involvement of calcium channel blocking activity has been observed previously as underlying cause of spasmolytic effect [30]. K^+ at higher conc. > 30 mM is well known to cause smooth muscle contraction mediated through L-type calcium channels by heavy influx of calcium into the cell [31]. Calcium channel blocker is that agent that inhibits high K+ induced contraction [32]. Presence of spasmolytic effect in Qrh.Cr was possibly mediated through calcium channel blocking activity, similar to verapamil, indicates effectiveness of Qrh in duodenal ulcer.

Conclusion

Qarahine; being the polyherbal formulation contains multiple constituents, involved in ulcer healing through various mechanisms, but significant healing effect against aspirin induced ulcer model indicates that major role is mediated by prostaglandin mechanism. Presence of spasmolytic effect possibly due to calcium channels blockage activity supports its effectiveness against duodenal ulcer. Keeping in view the results of study, it can be perceived that Qarahine possesses anti-ulcerogenic activity that validates the use of Qarahine as a natural remedy in the treatment of gastric and duodenal ulcer.

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Conflict of interest

Authors declares no conflict of interest.

Ethical statement

All the research protocol are in accordance with the Animal ethics committee

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