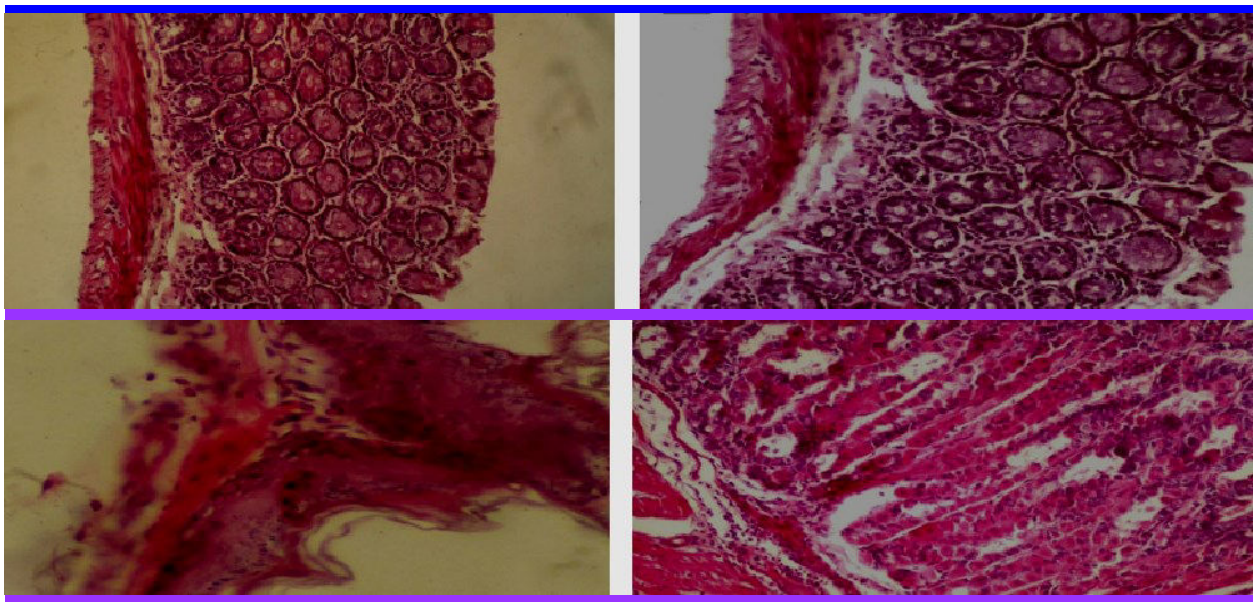

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Gastro-protective effect of ethanolic extract of *Mentha longifolia* in alcohol- and aspirin-induced gastric ulcer models

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Abstract

The objective of the study was to evaluate anti-ulcer potential of *Mentha longifolia*. The rats (four groups) were given orally the vehicle (normal saline, 2 mL/kg) as control group, ethanol extracts of the plant at dose of 100 and 200 mg/kg of body weight and ranitidine (20 mg/kg) as standard drug (positive control). Ulcer was induced by oral administration of alcohol (80%) and aspirin (200 mg/kg). The gastric tissues were examined to determine ulcer index and percentage protection. Histopathological evaluation was also performed to support the results. Ethanol extract of *M. longifolia* showed reduction in ulcer index ($p<0.05$). Treatment with ethanolic extract of *M. longifolia* at dose of 100 and 200 mg/kg of body weight gave ulcer protection (30.5 ± 4.5%) and (47.1 ± 5.9%) respectively. In conclusion, ethanolic extract of *M. longifolia* has anti-ulcer activity.

Introduction

The problem of resistance and tolerance to the existing drugs has created a decreased efficacy of these drugs in use. This problem has been tried to be overcome by increasing the drug delivery to the target site by the use of polymers (Khalid et al., 2009; Hussain et al., 2011) or through nanotechnology (Naz et al., 2012; Ehsan et al., 2012), synthesis of new drugs, either by the use of proteomics (Qadir, 2011), or synthesis from lactic acid bacteria (Masood et al., 2011), or marine microorganisms (Javed et al., 2011). However, now-a-days, the trend is also being changed to the use of herbal products or extracts to control the diseases. The plant kingdom still holds many species containing substances of medicinal value which have yet to be discovered: large numbers of plants are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ali et al., 2013; Mallhi et al., 2014; Saleem et al., 2014a; Qadir et al., 2014), hypoglycemic, amebicidal, anti-fertility, cytotoxic, antibiotic (Amin et al., 2012), Spasmolytic, bronchodilator, anti-oxidant (Janbaz et al., 2012) and anti-parkinsonism properties.

Mentha longifolia with common name of "Jungli podina" or "Wild mint" and in English "Horse mint" belongs to the family Lamiaceae. It is a perennial essential oil bearing plant that grows wild. *M. longifolia* is widely distributed rhizomatous herb native to Europe, Western and Central Asia generally known as "Puneh" in Persian in Iran. It is used as carminative, digestive, tonic, antispasmodic stomachic, anti-oxidant (Nickavar et al., 2008) anti-inflammatory agent and as antimicrobial. It contains important phytochemicals such as flavonoids, tannins and saponins (Razavi et al., 2012).

The extract of leaves is used against dysentery and vomiting. Dry powder of leaves is used as condiment and in asthma (Hazrat et al., 2011). Leaves of the plant are used as carminative and in diarrhea (Haq et al., 2011). As a cooling medicine, the leaves are used when soaked in water and taken as infusion (Kumar et al., 2009).

The present work was conducted to evaluate anti-ulcer

effect of *M. longifolia* on alcohol and aspirin ulcerated Wistar albino rats.

Material and Methods

Laboratory animals

Wistar albino rats weighing between 150-200 g were purchased from National Institute of Health (NIH), at Islamabad and accommodated at animal house, in the College of Pharmacy, Government College University Faisalabad, Pakistan. Rats were placed at room temperature 22 ± 2°C and 12/12 period of light and dark with proper ventilation facility. The relative humidity of 44 to 56% was maintained. Rats of different groups were placed in different cages to facilitate accurate evaluation. In order to avoid the coprophagy, rats were kept in cages having wire bottom. Proceeding to the experimentation, for one week rats were acclimatized and given the standard rodent pellet diet and water *ad libitum*. The experiments were performed by the consent of the Directorate of Research and Advanced Studies with the approval of the Ethical committee of Government College University Faisalabad, Pakistan. The rats were sacrificed according to the rules by the same committee.

Collection and identification of plant material

Fresh plant of *M. longifolia* was collected from different areas of Punjab. The plants were identified for the authentication purpose by the taxonomist at the Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Preparation of extract

Fresh plants were collected, washed with distilled water, shade dried and then powdered with the commercial grinder. The dried powder was extracted by cold maceration method. 1.5 kg powder of each plant was macerated with ethanol for 5 days on room temperature. "Whatman filter paper" Number 4 was used to filter the sample and then evaporated with rotary evaporator. The extract yield obtained was 9.9% for *M. longifolia*.

Alcohol-induced gastric ulcer model

The rats were separated into four groups, each of six animals and kept on fasting for 24 hours with water *ad libitum*. Animals were given orally the vehicle (normal saline) as control group, ranitidine (20 mg/kg) as standard drug (positive control), ethanol extracts of the plants at dose of 100 mg/kg and 200 mg/kg (Group 3 A and B). After one hour, ulcer was induced by administering 1 mL of 80% alcohol orally to all animals. Animals were sacrificed by cervical dislocation, an hour after the administration of alcohol. Stomachs were removed, opened along the greater curvature, pinned on the soft board and stomach lesions were evaluated to determine the ulcer index and ulcer protection (Dhasan and Jagadeesan, 2010).

Aspirin-induced gastric ulcer model

The rats were separated into four groups, each of six animals. Control animals were administered normal saline (2 mL/kg) for 8 days and then fasted for 24 hours. The second group was treated with ranitidine (20 mg/kg) as standard drug. Third and fourth groups (labeled as Group 4A and B) were administered orally ethanol extract at doses of 100 and 200 mg/kg for 8 days. Ulcer was induced by administering aqueous suspension of aspirin (200 mg/kg) orally on the day of sacrifice. The rats were sacrificed 4 hours later and their stomachs were opened to determine the ulcer index and ulcer protection (Dhasan and Jagadeesan, 2010).

Ulcer index

The number of ulcers was counted by using the magnifying glass. Severity scores: Normal coloration as 0, red coloration 0.5, spot ulcer 1.0, hemorrhagic stress 1.5, deep ulcer 2.0 and perforations as 3.0.

Ulcer index = $(UN + US + UP) \times 10^{-1}$

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

Percentage of ulcer protection

Percentage protection was calculated by using the following formula:

$$\% \text{ Protection} = \frac{UI_{\text{Ulcer control}} - UI_{\text{Treated}}}{UI_{\text{Ulcer control}}} \times 100$$

Histopathological evaluation of gastric tissues

Specimens of the stomach walls from each rat were fixed in formalin and further processed through graded alcohol concentration and were graded in blocks of paraffin. Stomach sections were cut at thickness of 5 mm and stained with hematoxylin and eosin. Histopathological evaluations were carried out on the collected gastric tissue samples according to the method as reported by Bancroft and Gamble (2002).

Statistical analysis

One-way ANOVA (analysis of variance) was used for statistical analysis. Results were represented by mean ± SEM.

Results

Ulcer index and % ulcer protection for different groups

Table I

Ulcer index and % ulcer protection

Groups		Treatment	Dose (mg/kg) PO	Ulcer index	% of ulcer protection
Group 1 (Control)		Normal saline	2 mL/kg	3.7 ± 0.1	-
Group 2 (Standard)		Ranitidine	20 mg/kg	0.8 ± 0.0[b]	78.2 ± 1.4[b]
Group 3 (Alcohol-induced ulcer)	A	*Mentha longifolia* extract	100 mg/kg	2.6 ± 0.1[a]	30.5 ± 4.5[a]
	B		200 mg/kg	1.9 ± 0.2[a]	47.1 ±5.9[a]
Group 4 (Aspirin-induced ulcer)	A		100 mg/kg	1.6 ± 0.3[a]	57.6 ± 8.0[a]
	B		200 mg/kg	1.4 ± 0.2[a]	63.1 ± 3.8[b]

Data are mean ± SEM; [a]p<0.05, [b]p<0.01

are given in Table I. It was very interesting that control group and standard group gave the same results for both alcohol-induced ulcer and aspirin-induced ulcer. Ulcer index for control group was 3.7 ± 0.1. Ranitidine caused significant reduction in ulcer index (0.8 ± 0.0) in comparison with the control. Treatment with *M. longifolia* in alcohol-induced ulcer at doses of 100 and 200 mg/kg (Group 3) decreased the ulcer index to 2.6 ± 0.1 and 1.9 ± 0.2, and gave ulcer protection 30.5 ± 4.5% and 47.1 ± 5.9% respectively. Treatment with *M. longifolia* in aspirin induced ulcer at dose of 100 and 200 mg/kg (Group 4) decreased the ulcer index to 1.6 ± 0.3 and 1.4 ± 0.2, and gave ulcer protection 57.6 ± 8.0% and 63.1 ± 3.8% respectively.

The histopathological evaluation of the sections of gastric mucosa in different groups pretreated with ethanolic extracts of *M. longifolia* supported the results by showing their anti-ulcer potential. Gastric mucosa of alcohol (1 mL of 80%)-induced ulcer have shown intramucosal hemorrhages and aspirin (200 mg/kg) induced ulcer has shown striking necrosis of mucosal cells with associated hemorrhages (Figure 1). No histological changes have been observed in the stomach pretreated with the ethanolic extract of 200 mg/kg of *M. longifolia* in comparison with atrophy of stomach associated with edema in rat stomach administered with 100 mg/kg of ethanolic extract of the same plant. Anti-ulcer potential at both the doses was significant.

Discussion

The ulcer induced models in present study characterize some of the most common factors causing stomach ulcer in humans. Various factors and means are involved in the ulcer induction and stomach mucosal injury induced by different animal models. These include weakening of stomach wall, mucosal injury as incorporated by NSAID's and production of free radicals. NSAID's including aspirin cause damage to the stomach mucosa by causing a decrease in the level of prostaglandins by inhibiting the synthesis of prostaglandins (Dhashan and Jagadeesan, 2010).

Induction of stomach ulcer by ethanol is assumed to be due to the stasis in the stomach blood flow which is a contributing factor in the development of the gastric hemorrhages and also the tissue injury due to necrosis. Alcohol has the ability of rapidly penetrating into the mucus lining of the stomach thus causing the enhanced permeability of intracellular membrane for water and sodium and ultimately causing cell and plasma membrane injury (Gupta et al., 2012). The damage induced in stomach due to ethanol is accompanied with oxygen free radicals which are significantly produced and ultimately they cause the effective lipid peroxidation. Overall effect is the cell and cell membrane damage (Vinothapooshan and Sundar, 2010). The necrosis of the stomach mucosa by the ethanol takes place in multifactorial manner. Ethanol cause the rupturing of the cells in the wall of blood vessels and by disrupting the barrier of mucus bicarbonate, it can easily reach the mucosal lining of the stomach. These changes are because of the biological factors, such as peroxidation of lipids, generation of free radicals, stress due to intracellular oxidation, permeability variations and depolarization of the mitochondrial membrane preceding to cell loss (Alrashdi et al., 2012).

The ethanolic extract of the *M. longifolia* has significantly provided the fortification of stomach mucosa against ethanol produced ulcer in rats as it is shown by decreased values of ulcer index in comparison with the control group. It suggests its potent cytoprotective effect. Similarly, in case of "aspirin induced ulcer model" of albino rats, ethanolic extract of *M. longifolia* elucidated in the results, a reduced ulcer index and increased percentage protection.

Histopathological assessment of the tissues of gastric mucosa of rats has also established the fact that the ethanolic extract of *M. longifolia* has shown significant anti-ulcer effect in dose-dependent manner.

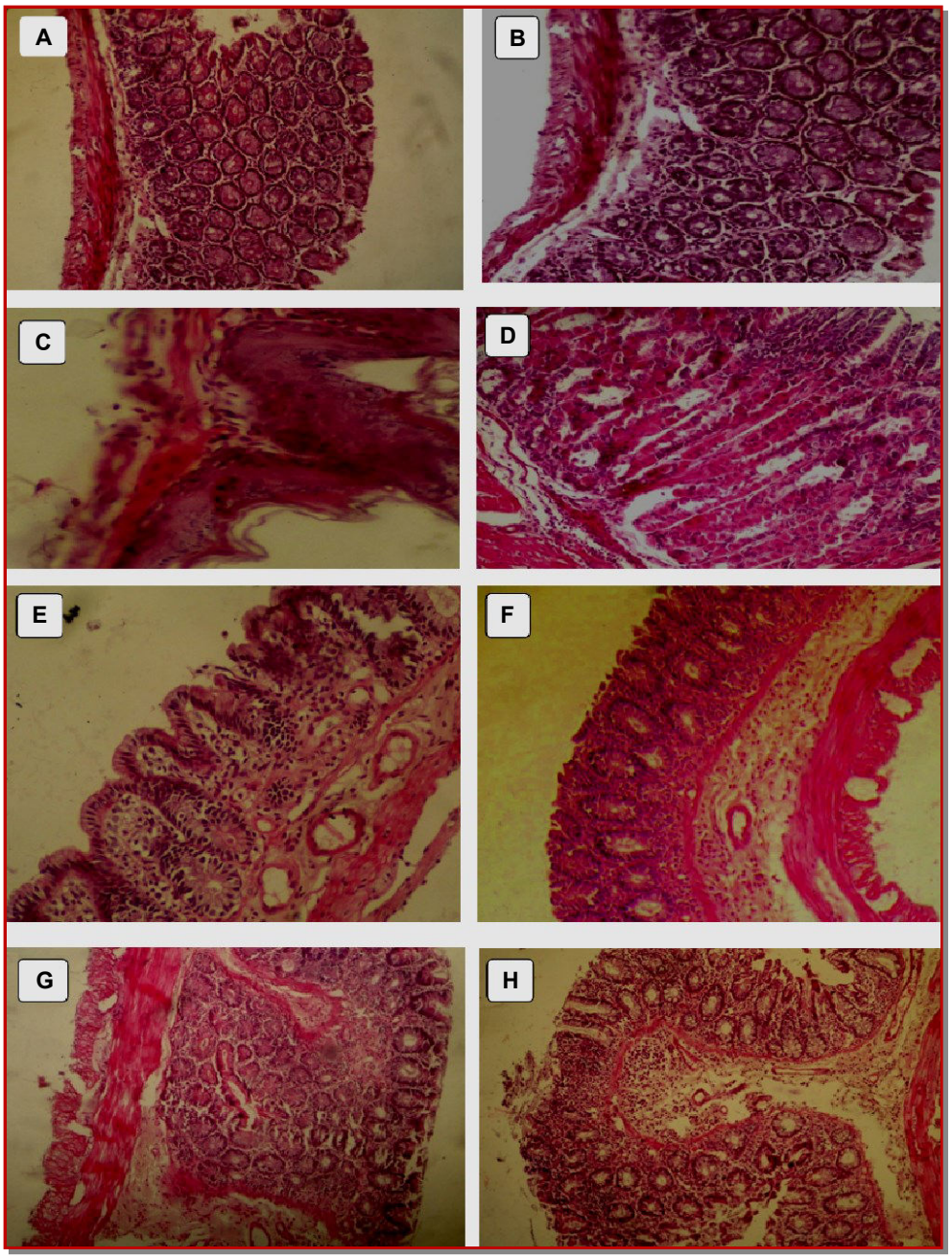

Figure 1: Histological examination of gastric mucosa of normal group (A); pretreated with the ranitidine 20 mg/kg (B); alcohol - induced ulcer (C); showing mucosal ulceration along with severe intramucosal hemorrhages; aspirin-induced ulcer (D); showing striking necrosis of gastric mucosa associated with hemorrhage; pretreated with ethanolic extract of 100 mg/kg of *M. longifolia* in alcohol-induced ulcer (E); showing mucosal congestion; pretreated with ethanolic extract of 200 mg/kg of *M. longifolia* (F); showing no histological changes in alcohol-induced ulcer; pretreated with ethanolic extract of 100 mg/kg of *M. longifolia* in aspirin-induced ulcer (G); congestion of submucosa associated with edema; pretreated with ethanolic extract of 200 mg/kg of *M. longifolia* (H); showing no histological changes in aspirin-induced ulcer

The substance to act as an efficient drug in the disease of peptic ulcer is supposed to be acting either by reduction of the aggressive factors on gastrointestinal mucosa or by increase in mucosal integrity against them (Khatib et al., 2010).

Many products are available in the market place for the treatment of stomach ulcer, including PPI's, antacids, anticholinergic drugs and H_2-receptor antagonists. Several adverse reactions are produced by most of these drugs. In 1980, the World Health Organization has recommended the evaluation of the effectiveness of the plants in those conditions where there is unavailability of safe synthetic

drugs. Thus, it creates a need for less toxic, cheap and more effective, anti-ulcer agent. The medicinal plants are among the most striking source of new therapeutic agents, and they have shown significant results in the management of stomach ulcers.

Although etiology of ulcer is unidentified in the majority of the cases so it is by and large, established that gastric ulcers occur due to the loss of balance between offensive factors and the preservation of the integrity of mucosa through defensive factors (Abdulla et al., 2012).

Ranitidine is having both the anti ulcer and protective activity and pretreatment with the ethanolic extracts of *M. longifolia* can partially decrease the ulcer area and gastric ulceration. The plant has shown their dose-dependent protective activity against alcohol and aspirin induced ulcer significantly and it is comparable with the protection provided by the standard drug ranitidine as shown in the results.

It was concluded from this study that ethanolic extract of *M. longifolia* has anti-ulcer activity against alcohol and aspirin-induced ulcers.

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