ORIGINAL ARTICLE

Antiulcer activity of *SafoofeVaj*, a polyherbal Unani formulation, in Indomethacin induced gastric ulcer in rat

Nadir Shahzad[1], Abdul Wadud[1]*, Shaista Perveen[1], Shariq Shamsi[2]

[1]Department of Ilmul Advia, National Institute of Unani Medicine, Bengaluru, Karnataka, India, [2]Department of Ilmul Saidla, National Institute of Unani Medicine, Bengaluru, Karnataka, India

Abstract

Aim: *SafoofeVaj* is recommended in flatulence, dyspepsia, gastritis, burning sensation in the stomach, and associated disorders in Unani literature. The aim of this study was to evaluate its antiulcer activity in Indomethacin induced gastric ulcer owing its related use. **Materials and Methods:** Wistar rats of either sex were used in this study. Ulcer was induced by administration of Indomethacin. Animals were divided into 10 groups of 6 animals each. Groups were designated as negative control, positive control, two standard control as pre-treated, and post-treated with ranitidine, three test groups as pre-treated with 400 mg/kg and 600 mg/kg 1000 mg/kg, and three curative groups as post-treated with 400 mg/kg, 600 mg/kg, and 1000 mg/kg. Animals were treated for 5 days before ulcer induction in preventive groups and after the ulcer induction in curative groups. The stomach of animal was observed under lens for any lesions. The scoring was done to calculate the ulcer index and ulcer score. Histopathology was carried out for microscopic lesion. **Results and Discussion:** A reduction in the ulcer score was obverted more in pre-treated groups ($P < 0.05$) indicating the preventive effect of the test drug. The test drug showed a dose-dependent effect. Histological findings supported other findings. **Conclusions:** The study demonstrated that test drug possessed significant anti-ulcer effect.

Key words: Indomethacin, Ranitidine, *SafoofeVaj*, Ulcer, Unani medicine

INTRODUCTION

Peptic ulcer is a lifestyle disease having profound negative impact on health. It is more common in the industrialized society.[1] Both sexes are at equal risk of developing the ulcer with male to female ratio of 3:1 for duodenal ulcer and1.5:2.1forgastric ulcer. Women are most often affected by a peptic ulcer at or after menopause,[2] which is 2–3 times frequent in lower social classes.[3] Old age group patients are more prone to develop a gastric ulcer as chances of development of peptic ulcer disease (PUD) increases with advancing age.[4] Young individuals are more prone to develop duodenal ulcers.[5] In India, its incidence is 4–10 per thousand populations. Around four million new cases of peptic ulcers occur every year around the globe.[4]

Precise etiology of this disease is yet to be known; however multifactorial pathogenesis including stress, NSAIDs, consumption of alcohol, smoking, and extensive burn have been described as predisposing factors.[5] *H. pylori* is claimed to be common in more than 90% of cases of duodenal ulcer and 70% in gastric ulcer.[6] In Conventional medicine, H_2blockers, proton pump inhibitors, antacids, ulcer protective, ulcer healing, and anti-*H. pylori* drugs are used as anti-ulcer agents but very often these drugs produce adverse effects such as headache, dizziness, constipation, tiredness, and muscular pain.[7]

According to Unani medicine, gastric ulcer results either due to penetration of rancid humour into the gastric mucosa or due to its irritant action.[8] Moreover, the role of microorganism in its pathogenesis may also be considered, as IbneSina and RabbanTabri, two well-known scholars of Unani Medicine, have considered sepsis as one of the causes of the peptic ulcer.[8,9] In Unani medicine, PUD is treated with drugs having demulcent, astringent, hemostatic, and cooling properties. Single drugs having properties such as *Aegle marmelos*

Address for correspondence:
Abdul Wadud, National Institute of Unani Medicine, Bengaluru, Karnataka, India.
E-mail: drwadud87@gmail.com

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Linn,[10] *Mimosa pudica* Linn.,[11] *Pongamiapinnata* Linn.,[12] *Moringa oleifera* Lam.,[13] and *Rhuscoriaria* Linn.[14] have been scientifically evaluated for their antiulcer activity. *SafoofeVaj* consisting of *A.calamus* Linn., *Z.officinale* Rosc., *B. serrata* Roxb. ex Colebr., *N. sativa* Linn., and *C. sativum* Linn is used in various gastric ailments since long.[15,16] Its ingredients VajTurki (*Acoruscalamus*Linn.),[17] *Zanjabeel*(*Zingiber officinale*Rosc.),[18] *Kundur* (*Boswallia serrata* Roxb. ex Colebr.),[19] *Shooneez* (*Nigella sativa* Linn.),[20] and *KishneezKhushk* (*Coriandrum sativum* Linn.)[21] have been reported to possess significant anti-ulcer activity in animal studies, but as a compound formulation, it has not been evaluated for its anti-ulcer activity.

MATERIALS AND METHODS

Chemicals

Chemicals and reagents used for the study were of analytical grade procured from Micro Labs Ltd. Ranitidine was purchased from J. B. Chemicals and Pharmaceutical Ltd. Carboxyl methylcellulose (CMC) manufactured by HIMEDA was procured from the local market of Bengaluru.

Plant Collection

A. calamus Linn., *Z.officinale* Rosc., *B. serrata* Roxb. ex Colebr., *N. sativa* Linn., and *C. sativum* Linn. were procured from the local market of Bengaluru. They were authenticated at Centre for Repository of Medical Resources at Trans-disciplinary University, Bengaluru vide Reference No. FRLHT Acc. 3486, 3487, 3488, 3489, and 2490, respectively. A voucher specimen has also been deposited in the Drug Museum of National Institute of Unani Medicine (NIUM), Bengaluru with No. 36/IA/Res/2015.

Preparation and Dose of the Test Drug

The ingredients were dried in shade, powdered in an electrical grinder. The dose of test drug was calculated from the human therapeutic dose that is 5 gas mentioned in classical Unani literature.[15] The doses for a rat was calculated by the conversion factor of 7 and was found as 600 mg/kg.[22] Since the test drug was studied at three different dose levels, two more doses were taken based on the calculation by the formula of Miller and Tainter[23] and were found as 400 mg/kg and 1000 mg/kg, respectively. Ranitidine was used as the standard drug in the dose of 50 mg/kg. All drugs were administered orally as suspension of 0.3% in CMC.

Animals

A total of 60 Wistar rats of either sex were used in this study. Animals were divided into 10 groups of 6 animals each. Groups were named as negative control, positive control, two standard control one as pre-treated and other one as post-treated with ranitidine, three test groups as pre-treated treated with 400 mg/kg, 600 mg/kg, and 1000 mg/kg and three curative groups as post-treated with 400 mg/kg, 600 mg/kg, and 1000 mg/kg. Ulcer was induced by administration of Indomethacin.

Before the study, the protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide Registration No. IAEC/11/01/IA. The study was conducted on healthy Wistar rats of either sex; weighing 150–250 g, which were procured from Sri Venkateshwara Enterprises, (Reg. No. 233), Bengaluru. The animal care procedures and experimental protocol were in accord with the guidelines of CPCSEA. The animals were acclimatized to the laboratory condition for 7 days before the experiment. They were kept in polypropylene cages under standard environmental conditions at temperature 23 ± 2°C, humidity at 45–55% with a 12 h light and 12 h dark cycle fed with standard commercial food pellets (Hindustan Unilever Ltd) and water *ad libitum*.

Indomethacin Induced Gastric Ulcer

The study was carried out in Indomethacin induced gastric ulcer model[24] with minor modification in the treatment schedule. The animals were divided into 10 groups of 6 animals each. Animals of Group I (Negative control) were administered only with 0.3% CMC suspension throughout the study; Group II (Positive control)was treated with Indomethacin 20 mg/kg once orally for 5 days after 24 h of fasting; and Group III (Pre-treated standard group) was given standard drug Ranitidine 50 mg/kg once orally for 5 days. Groups IV, V, and VI (Pre-treated test group A, B, and C) were treated with test drug in doses 400 mg/kg, 600 mg/kg, and 1000 mg/kg, respectively. The treatment was continued for 5 days. On 6th day after 24 h of fasting (Coprophagy was prevented during fasting by putting the animals in cages grating on the floor), the ulcer was induced by Indomethacin 20 mg/kg for next 5 days once given orally. Food was withdrawn for 2 h after Indomethacin administration. On 5th day after 12 h of fasting, the animals were administered the last dose of Indomethacin; after 5 h, the animals were sacrificed. In the remaining four groups, namely, post treated standard group and test groups of three different doses, first, the ulcer was induced by inducing Indomethacin in 20 mg/kg once daily for 5 days. Thereafter the animals were treated with standard and test drugs for the next 5 days in the same dose and same manner as first six groups. On 6th day, the animals were sacrificed under Thiopentone anesthesia, 50 mg/kg IP after 12 h of fasting; the abdomen was opened by midline incision; stomach was taken out and opened along with the greater curvature; washed with fresh water and spread on cardboard with the mucus surface upward. The stomach of one rat from each group was preserved in 10% formalin for histopathology.

Scoring of the Ulcer

Scoring was done by the method of Brzozowski *et al*., (1998).[25] The surface was examined for ulcer scoring under a magnifying lens (10 fold magnifications).

Determination of the degree of ulceration

Degree of ulceration was calculated asunder:

Normal stomach.....	(0)
Red coloration....	(0.5)
Spot ulcer....	(1)
Hemorrhagic streak....	(1.5)
Ulcers.....	(2)
Perforation.....	(3)

The average degree of single ulceration (AUD) for each group was determined by adding together the degree of single ulceration (DSU) and dividing it by the number of animals. Based on the percentage of rats with ulceration (%RU), the ulcer index was calculated by the following formula.

$$\text{Ulcer index} = \frac{(\text{AUD})(\%\text{RU})}{100}$$

(AUD) - The average degree of single ulceration
(%RU) - Percentage of rats with ulceration

The percentage of ulcer protection was determined by the following formula:

$$\%\ \text{Protection} = \frac{\text{Controlmeanulcerindex} - \text{Testmeanulcerindex}}{\text{Controlmeanulcerindex}} \times 100$$

Statistical Analysis

Data were expressed as Mean ± SEM, analyzed using ANOVA one-way with Tukey Kramer comparison of all pairs of columns. The difference of mean was considered significant at $P < 0.05$. The ulcer score and index of various groups were compared with the positive control group.

RESULTS

Effects of Test Drug on Indomethacin Induced Gastric Ulcer

Animals of Negative control (Group I) showed no gross pathological sign. In positive control (Group II), pre-treated standard group (Group III), and pre-treated Group A (Group IV), ulcer score was found to be non-significant respect to the positive control. In pre-treated test Group B (Group V), pre-treated test group C (Group VI) ulcer score was found to be significant ($P < 0.05$) concerning positive control. In post-treated standard group (Group VII), post-treated Group A (Group VIII), post-treated test Group B (Group IX), post-treated test Group C (Group X), and ulcer score was found to be non-significant concerning positive control [Table 1].

Histopathology

Histopathological examination of the gastric mucosa of the negative control [Figure 1a] showed no pathological signs. In positive control [Figure 1b], the mucosal layer showed a mild decrease in secreting epithelial cells along with mild inflammatory infiltrations. The submucosal layer showed mild edema with congested blood vessels and hemorrhagic areas were seen. In pre-treated standard [Figure 1c] showed intact gastric mucosa. The mucosal layer showed increased secreting epithelial cells, regenerative cells and scant inflammatory infiltrations. In pre-treated test A [Figure 1d] showed most of the mucosal layer decrease in secreting lining epithelial cells. In pre-treated test B [Figure 1e] showed most of the mucosal layer regenerating secreting epithelial cells with moderate inflammatory infiltrations. The submucosal layer shows moderate edema with inflammatory infiltration. In pre-treated test C [Figure 1f] showed most of the mucosal layer showed an increase in secreting epithelial cells. In post-treated standard [Figure 1g] the mucosal layer showed a mild decrease in secreting epithelial cells with scant inflammatory infiltration. In post-treated test A [Figure 1h] showed intact gastric mucosa. The mucosal layer showed a moderate decrease in secreting epithelial cells with inflammatory infiltration. In post-treated test B [Figure 1i] the mucosal layer showed a mild decrease in secreting epithelial cells with inflammatory infiltration. The submucosal layer appeared within normal limits. In post-treated test C [Figure 1j] the mucosal layer showed adequate secreting epithelial cells. The submucosal layer showed mild edema [Table 2].

DISCUSSION

As per Unani concept, peptic ulcer is produced due to penetration of rancid humor into the gastric mucosa or its irritant action, emotional stress, strain, over exhaustion, and sepsis.[8,9] Accordingly, treatment is given by using drugs having reverse properties to the above-mentioned causes. In Unani Medicine, the concept of *Ilaj-biz-zid*, that is, treatment of diseases by drugs having a *mizaj*(temperament) opposite to the temperament of the diseases. In most of the studies, peptic ulcer being a hot disease, has been treated with cold drugs such as *Mimosa pudica,*[11] *Rhuscoriaria* Linn.,[14] *Terminalia chebula*Retz.,[26] *Solanum nigrum* Linn.,[27] *Centellasciatica*(L.) Urban,[28] and *QurseTabasheer*.[29]

But in modern viewed point, the effect of test drug may be due to its ingredients. These drugs have siccative and

Table 1: Effect of *SafoofeVaj* on indomethacin induced gastric ulcer

Groups	Treatment	ADU	%RU	Ulcer index	%Reduction
Group I Negative control	Suspension of 0.3% CMC	0.42±0.33	33	0.14	89
Group II Positive control	IM 20 mg/kg (in suspension of 0.3% CMC)	3.9±0.57**a	100	3.92	00
Group III Pre-treated Stand.	Ranitidine 50 mg/kg (in suspension of 0.3% CMC)	2.42±0.54	100	2.42	38
Group IV Pre-treated test A	Test drug 400 mg/kg+IM 20 mg/kg (in suspension of 0.3% CMC)	1.58±0.42	83	0.35	60
Group V Pre-treated test B	Test drug 600 mg/kg+IM 20 mg/kg (in suspension of 0.3% CMC)	1.25±0.53*b	67	0.84	68
Group VI Pre-treated test C	Test drug 1000 mg/kg+IM 20 mg/kg (in suspension of 0.3% CMC)	1.33±0.62*b	50	0.67	66
Group VII Post-treated Stand.	IM 20 mg/kg+Ranitidine 50 mg/kg (in suspension of 0.3% CMC)	2.25±0.64	100	2.25	43
Group VIII Post-treated test A	IM 20 mg/kg+Test drug 400 mg/kg (in suspension of 0.3% CMC)	1.92±0.65	83	1.59	51
Group IX Post-treated test B	IM 20 mg/kg+Test drug 600 mg/kg (in suspension of 0.3% CMC)	2±0.55	83	1.66	37
Group X Post-treated test C	IM 20 mg/kg+Test drug 1000 mg/kg (in suspension of 0.3% CMC)	2±0.53	83	1.66	37

IM: Indomethacin, Stand: Standard, CMC: Carboxyl methyl cellulose, %RU: Percentage of rats with ulceration, ADU: Average degree of ulceration. (n=6 in each group). Analyzed by ANOVA one way with Turkey: comparison all pairs of columns. *P<0.05, **P<0.01, [a]With respect to negative control; [b]With respect to positive control

Table 2: Histopathological summary of indomethacin induced gastric ulcer

Groups	Congestion	Hemorrhage	Edema	Inflammation	Mucosal cell secretion
Group-I	-	-	-	-	++
Group-II	+	+	++	+++	-
Group-III	-	-	-	++	+
Group-IV	-	-	-	+	-
Group-V	-	-	++	+	++
Group-VI	-	-	-	-	++
Group-VII	-	-	-	+	-
Group-VIII	-	-	-	+	-
Group-IX	-	-	-	+	-
Group-X	-	-	+		++

Normal; (-), Moderate; (+), severe; (++), intensely severe; (+++)

detergent actions; *Z. officinale* and *B.serrate* being antiseptic may have acted upon sepsis. *C. sativum* being febrifuge may have reduced intensity of irritation because of its cooling action. *B.serrate* has glutinous action and the probable effect may be due to the formation of a protective layer.

Although, in our study, the ingredients of test drug are mostly hot in temperament even though they are reported effective in a hot disease. It reveals that simply temperament of the drug, as opined by some scholars, is not the only mechanism of action. The temperament of a drug is not the only entity for the determination of the action of the drug. In our study, the compound formulation having mostly hot drugs was found effective in a hot disease, which justifies chemical constituents along with physical presence of a matter are responsible for drug action.[30].

Phytoconstituents such as alkaloids, phenolic compounds, polysaccharides, saponins, tenpins, tannins, and steroids are responsible for pharmacological actions.[19] Scientific studies carried out on some of the ingredients of *SafoofeVaj* have reported them as antiulcer. Active compounds in the test drug were phenolic compounds. Phenolic glycosides have the anti-secretory effect, while quinones are cytoprotective and increase PG synthesis. Terpenoids with their antiulcer effect are mostly cytoprotective leading to increased mucus production

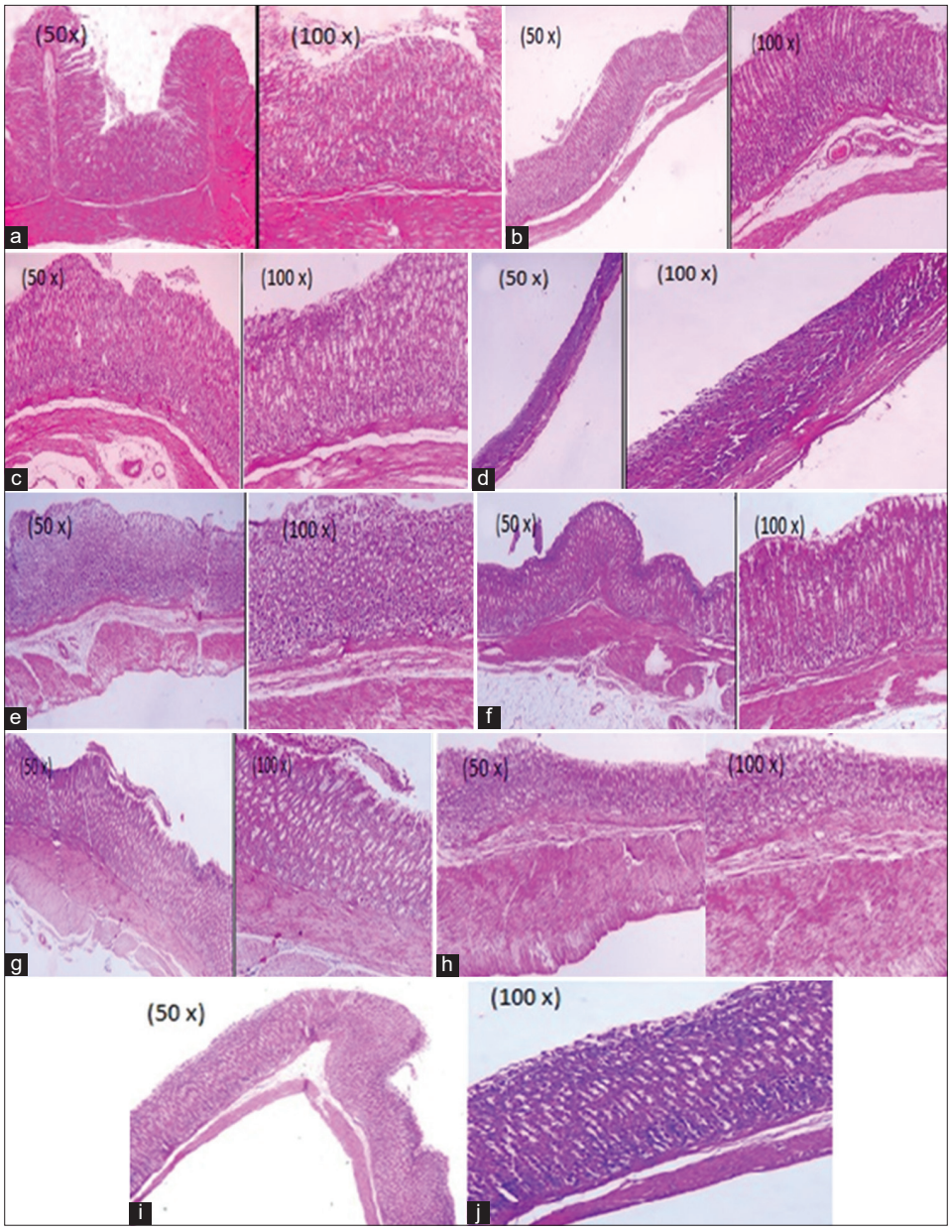

Figure 1: Microscopic images of gastric mucosa showing (a) negative control with normal mucosa, (b) positive control showing inflammation, edema, and congestion. (c) Pre-treated Standard Group, (d) pre-treated test Group A, (e) pre-treated test Group B, (i) pre-treated Test Group C, and (j) post-treated Standard Group showing Regeneration cells. (f) Post-treated Test Group A showing almost Normal mucosa. (g) Post-treated Test Group B and (h) Post-treated Test Group C showing inflammatory cells

in the stomach through different mechanisms; among these is enhanced mucosal PG content. Flavonoids exert gastro-protective action in mammals by increasing endogenous prostaglandin levels, decreasing histamine secretion, inhibiting *H. Pylori* and scavenging oxygen-derived free radicals. Polysaccharides exhibit cytoprotective action by stimulating mucosal regeneration, proliferation and increasing PG synthesis and thus normal gastric mucus levels are achieved.[31]

Since the etiology of peptic ulcer in itself is not confined to a single cause; conventional medicine also treats the disease symptomatically for relief of pain, ulcer healing, prevention of complication, and prevention of relapse. Hence, the approaches targeted at its management comprise multidrug modalities for reduction of gastric acid secretion, neutralization of gastric acid, ulcer protection and antimicrobial therapy for eradicating *H. pylori* infection. The same is also applied in Unani medicine.

CONCLUSION

The present study revealed that *SafoofVaj* is effective in peptic ulcer disease

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