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Efficacy of Powder and Hydroalcoholic Extract of Pharmacopoeial Formulation in Ethanol-Induced Gastric Ulcer in Rats

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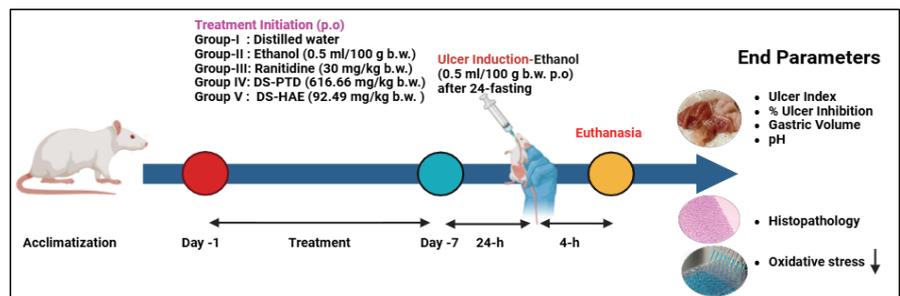
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ABSTRACT



Graphical Abstract

Gastric ulcer is a major public health problem globally and associated with severe complications including haemorrhages, perforations, gastrointestinal obstruction, and malignancy. *Dawa-e-Sahaj* (DS) is a notable Pharmacopoeial formulation used to treat stomach-related ailments in Traditional Unani medicine. However, its efficacy has to be established through scientific validation. The aim of the study was to determine the gastroprotective effects of powder test drug (PTD) and Hydroalcoholic extract (HAE) extract of *Dawa-e-Sahaj* (DS) against ethanol-induced gastric ulcer in rats. Ulceration was induced by a single oral administration of ethanol (0.5 ml/100 g body weight). Wistar rats were pre-treated with ranitidine (standard drug) at a dose of 30 mg/kg/day body weight, PTD and HAE at 616.66 mg/kg and 92.49 mg/kg body weight respectively once daily for 7 days prior to ulcer induction. At the end of the experiment, gastric secretions and antioxidant parameters were evaluated. The obtained data was statistically analysed using GraphPad Prism 8.0.2 software. We observed that the significantly increased ($p < 0.05$) ulcer index, gastric volume, malondialdehyde and myeloperoxidase level were effectively reduced following treatment with PTD and HAE of *Dawa-e-Sahaj*. The PTD and HAE also markedly attenuated the reduced activity of superoxide dismutase3 and prostaglandin E2 as well as pH in the ulcerated rats. These findings are indicative of gastroprotective and antioxidative potentials of the PTD and HAE of *Dawa-e-Sahaj* which is also evident in the degree of % inhibition against ulceration. The available data in this study suggest that the PTD and HAE extracts of *Dawa-e-Sahaj* proved to be capable of ameliorating ethanol-induced gastric ulceration due to its antioxidant potential and presence of bioactive molecules.

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INTRODUCTION

Gastric ulcers are characterized by the erosion or disruption of the inner lining of the stomach, upper small intestine, or lower esophagus, primarily due to excessive acid and peptic secretions [1, 2]. A gastric ulcer is a benign lesion in the stomach that arises from an imbalance between protective and aggressive factors. Protective factors include mucosal blood flow, the mucosal bicarbonate barrier, cell regeneration, motility, enzymatic and non-enzymatic antioxidants, prostaglandins, and

other defensive mechanisms. Conversely, aggressive factors encompass hydrochloric acid, certain foods, pepsin, medications, bile reflux, *Helicobacter pylori* infection, leukotrienes, smoking, hypoxia, caffeine, and alcohol. Among these, excessive alcohol consumption is a leading cause of gastric mucosal injury [3,4]. The primary objective in treating and preventing acid-related disorders is to reduce stomach acid levels or enhance mucosal protection [5]. Each year, approximately 500,000 new cases of duodenal ulcers and four million new cases of gastric ulcers are reported. Additionally, complications associated with peptic ulcer disease (PUD) result in approximately 15,000 deaths annually [2, 6]. In India, PUD is a significant public health concern due to its high morbidity and mortality rates, with Tamil Nadu, Andhra Pradesh, and Jammu & Kashmir

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being identified as high-risk regions [7]. In Unani literature, peptic ulcers (*Quruh Hadmia*) are classified into gastric ulcers (*Quruh al-Mi'da*) and intestinal ulcers (*Quruh-Am'a*) [8]. According to Ibn Sina and Hakim Azam Khan, gastric ulcers develop due to the excessive accumulation of rancid humour (*Khilt-e-Haad*) in the stomach. This accumulation may result from an internal build-up of acidic secretions or acute nasal discharge from the brain (*Nazla-e-Haad Dimaghi*), leading to chronic irritation of the gastric mucosa. Prolonged exposure to these irritants eventually results in infection. Intestinal ulcers (*Qarah-e-Ama'a*) may develop due to excessive bile secretion, which progressively irritates the intestinal lining, or due to alkaline phlegm (*Balgham Shour*), which, because of its alkaline nature, disrupts the integrity of the intestinal walls, leading to damage [9, 10, 11]. In conventional medicine, peptic ulcers are treated with a range of anti-ulcer medications, including antimicrobial agents (e.g., metronidazole, clarithromycin), proton pump inhibitors (e.g., omeprazole, lansoprazole), H2-receptor antagonists (e.g., cimetidine, ranitidine), and bismuth salts, which disrupt bacterial cell walls. However, these drugs are often associated with adverse effects such as gynecomastia, hypersensitivity, impotence, arrhythmia, hematopoietic alterations, and renal disorders, along with significant drug-drug interactions that limit their widespread use [12]. These limitations have led to a growing interest in discovering non-toxic, widely available, and cost-effective anti-ulcer medication [13, 14]. Numerous studies have underscored the anti-ulcer properties of medicinal plants, making them promising candidates for drug development. Investigating the phytotherapeutic potential of medicinal plants commonly used in traditional medicine may provide effective formulations for improved ulcer management.

In Unani medicine, peptic ulcers are treated with therapeutic agents that first cleanse the ulcer site (*Tanqiya-e-Meda*), followed by the removal of morbid matter (*Muzliq*). Once this process is complete, healing agents (*Mudammil-e-Qurooh*), astringents (*Qabiz*), hemostyptics (*Habis-i-Dam*), and absorbents (*Jaazib*) are administered alongside dietary therapy (*Zu'ud Hazam Aghzia*). Additionally, stomach-strengthening agents (*Muqawwi-e-Meda*) are used to enhance gastric resilience [9-11]. *Dawa-e-Sahaj* (DS) is a significant Unani Pharmacopoeial formulation (UPF) listed in the *National Formulary of Unani Medicine* (Part-II, Vol. 1, pp. 126-127) for its efficacy in treating intestinal ulcers (*Sahaj-e-Ama*), gastric ulcers (*Qurooh-e-Meda*), and gastro-duodenal ulcers (*Qurooh-e-Isna Ashri*) [15]. This formulation comprises thirteen medicinal ingredients, including *Triticum aestivum* L. (*Nishasta-e-Gandum Biryani*), *Vachellia nilotica* (*Samagh-e-Arabi*), *Cydonia oblonga* Mill. (*Behidana Biryani*), *Bambusa bambos* (L.) (*Tabasheer Kabood*), *Papaver somniferum* L. (*Khashkhaash Safaid*), *Bole armenia* (*Gil-e-Armani*), *Vateria indica* L. (*Kahruba Shami*), *Corallium rubrum* L. (*Busud*), *Dracaena cinnabari* Balf.f. (*Damm-ul-Akhwain*), *Ocimum basilicum* L. (*Tukhm-e-Rehan*), *Plantago major* L. (*Tukhm-e-Bartang*), *Phyllanthus maderaspatensis* L. (*Tukhm-e-Kanoch*), and *Plantago ovata* Forssk. (*Asapghol Musallam*). These ingredients exhibit sedative (*Musakkin*), astringent (*Qabiz*), antiseptic (*Dafe-Ta'affun*), hemostyptic (*Habisuddam*), cicatrizing (*Mudammil*), resolving (*Muhallil*), and stomach-strengthening (*Muqawwi-i-Meda*) properties, helping to balance gastric pH, form a protective mucosal layer, and reduce the likelihood of ulceration while promoting healing [16-23].

Given the remarkable therapeutic properties of DS, particularly in treating stomach-related disorders, this study aims to validate the traditional use of DS for its anti-gastric ulcer efficacy, comparing its effectiveness against ranitidine (standard drug) in ethanol-induced gastric ulcers in rats.

Materials and Methods

Chemicals and drugs

All chemicals utilized in this study were sourced from Sigma Chemicals, Bengaluru, India. Distilled water was obtained from Department of Ilmul Advia (Unani Pharmacology), faculty of Unani Medicine, AMU, Aligarh, India. The Rat PGE2 Prostaglandin E2 (PGE2) (Cat NO: ELK8711; Sensitivity: 0.97 pg/mL; Detection range: 3.3-200 pg/mL), Rat Myeloperoxidase (MPO) (Cat NO: ELK1661; Sensitivity: 0.122 ng/mL; Detection range: 0.32-20 ng/mL), Rat Malondialdehyde (MDA) (Cat NO: ELK8612; Sensitivity: 5.39 pg/mL; Detection range: 15.63-1000 pg/mL), Rat Superoxide Dismutase 3, Extracellular (SOD3) (Cat NO: ELK2821; Sensitivity: 0.32 ng/mL; Detection range: 0.79-50 ng/mL) ELISA kits were purchased from ELK Biotechnology Co., Ltd, (Wuhan, China).

Collection and authentication of ingredients of Formulation

The crude drug materials used in the formulation were sourced from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh, India in August 2023. The identification of these drugs was carried out based on ethnobotanical literature and further validated in the pharmacognosy unit of Ilmul Advia (Unani Pharmacology). Specimens of all individual drugs included in DS were submitted to the *Ibn-e-Baitar Museum* for future reference in the Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh, India with their corresponding voucher numbers listed in Table 1.

Table 1: Constituents of *Dawa-e-Sahaj*.

S. No.	Scientific Name	Part Used	Quantity (gm)	Voucher No.
	<i>Triticum aestivum</i> L.	Seed	10	SC- 365/23
2.	<i>Vachellia nilotica</i> (L.)	Gum	10	SC- 363/23
3.	<i>Cydonia oblonga</i> Mill.	Seed	10	SC- 361/23
4.	<i>Bambusa bambos</i> (L.) voss	Silicious secretion	10	SC- 362/23
5.	<i>Papaver somniferum</i> L.	Seed	10	SC-358/23
6.	<i>Bole armenia</i>	Clay	10	SC- 367/23
7.	<i>Vateria indica</i> L.	Gum	10	SC- 364/23
8.	<i>Corallium rubrum</i>	Coral	10	SC- 366/23
9.	<i>Dracaena cinnabari</i> Balf.f.	Asparagaceae	10	SC - 368/23
10.	<i>Ocimum basilicum</i> L.	Seed	15	SC- 356/23
11.	<i>Plantago major</i> L.	Seed	15	SC -359/23
12.	<i>Phyllanthus maderaspatensis</i> L.	Seed	15	SC- 360/23
13.	<i>Plantago ovata</i> Forssk.	Seed	10	SC- 357/23

Approval from animal ethics committee

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the Department of Ilmul Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh, India, during its second meeting on August 16, 2022, under registration number 1979/GO/Re/S/17/CPCSEA/35. All experimental procedures and animal care practices adhered to the guidelines established by the Committee for Control and Supervision of Experiments on Animals (CCSEA) and IAEC.

Experimental animals

The experiment was carried out on Wistar albino rats of both sexes, obtained from the Central Animal Facility at AIIMS, New Delhi. The rats were housed in polypropylene cages in groups of five and maintained under standard laboratory conditions in accordance with CCSEA guidelines. These conditions included a controlled temperature of 25±2°C, relative humidity of 50-60%, and a 12-hour light/dark cycle within the animal house facility of the Department of Ilmul Advia, A.M.U, Aligarh. The animals were kept under strict hygienic conditions and provided with a standard pellet diet (Lipton-India Ltd.), along with ad libitum water. All experimental procedures were conducted following international standards, as specified in the OECD Guide for the Care and Use of Laboratory Animals [24].

Preparation of Powder and extract of drug

The ingredients of DS were initially cleaned to eliminate any foreign matter, then shade-dried and stored in sealed containers for future use. Each drug was individually ground using an electric grinder in the Pharmacy Laboratory of the Department of Ilmul Advia (Unani Pharmacology), AMU, Aligarh. The powdered drugs were then passed through a No. 80 sieve to ensure a uniform particle size. Subsequently, the powders of different drugs were mixed in specific proportions according to the dosage prescribed in the National Formulary of Unani Medicine (NFUM) (Table 1). A hydroalcoholic extract (50% alcohol v/v) of DS was prepared using a Soxhlet apparatus with a drug-to-solvent ratio of 1:10 (w/v). The extraction process was conducted at a temperature range of 60°C to 80°C for six hours, following the Standard Operating Procedure (SOP) outlined in the Unani Pharmacopoeia [25,26].

After extraction, the extract was filtered and subsequently dried through evaporation using a water bath. The final yield percentage of the extract, calculated based on the air-dried drug, was determined to be 15% for the hydroalcoholic extract.

Dose determination of formulation

The clinical dose of the powdered form of Dawa-e-Sahaj (DS), as mentioned in the National Formulary of Unani Medicine (NFUM) (Part II, Vol. 1, pp. 126-127), is 6 g per 60 kg of body weight. In the present study, two dosage forms of DS were used: powdered and hydroalcoholic extract. The dose of the powdered form of DS for Wistar rats was determined by applying the human equivalent dose (HED) of formulation using a conversion factor of 6 [27]. Similarly, the dose of the hydroalcoholic extract of DS was calculated based on its yield percentage.

The animal dose for the powdered form and the hydroalcoholic extract was found to be 616.66 mg/kg and 92.499 mg/kg (for a 15% yield), respectively, as detailed in the Table 2.

$$\text{Animal dose (mg/kg)} = \text{Human Equivalent Dose (HED)} \left(\frac{\text{mg}}{\text{kg}} \right) \times 6/37$$

Table 2: Dose Calculation of powdered and hydroalcoholic extract of Dawa-e-Sahaj.

Forms of Dawa-e-Sahaj	Quantity of test drug taken (gm)	Solvent (ml)	Yield of extract (gm)	Yield percent of extract (%)	Conversion factor (rat) 6.16	Animal dose (mg/kg)
Powder	100 mg/kg	-	-	-	37/6	616.66
Hydroalcoholic	100	1000	15	15	37/6	92.49

Animal grouping and treatments

Thirty Wistar rats were randomly assigned to five groups, each consisting of six animals. The test drugs and vehicle were administered orally via gavage for seven consecutive days. Group 1 (normal control) animals received only distilled water. Rats in group 2 (ulcerated group) were given only absolute ethanol orally (0.5 ml/100 g b.w.) after 24 hours of fasting, administered via gastric gavage. Group 3 (standard group) was given ranitidine (30 mg/kg/day, p.o.) for seven days. Group 4 received the powdered form (PTD) 616.66 mg/kg b.w. and Group 5 received hydroalcoholic extract (HAE) 92.49 mg/kg b.w. of DS, via oral administration for seven days.

On Day 7, after a 24-hour fasting period (with *ad libitum* access to water), all animals were subjected to a single dose of absolute ethanol (0.5 ml/100 g body weight), administered one hour after the final treatment. Four hour after ethanol administration, the rats were euthanized by cervical dislocation. Their stomachs were excised, cut open along the greater curvature, gently rinsed with cold normal saline, and spread on a sheet. Gastric lesions in the glandular region of the stomach were examined using a magnifying glass and scored according to previously established methodology.

Determination of anti-ulcer activity

Macroscopic evaluation of stomach

Stomachs were examined by a 10× magnifier lens to assess the formation of ulcers. The number of ulcers was counted and scored as follows:

Table 3: Ulcer scores and descriptive remark.

Remark	Score
Normal coloured stomach	0
Red coloration	0.5
Superficial mucosal injury spot ulcer	1
Hemorrhagic streak	1.5
Deep ulcer	2
Perforation	3

Quantification of ulceration

Degrees of ulceration in the ethanol-treated animals were quantified using the procedure outlined by Szabo and Hollander (1989) [28]. Briefly, cleaned stomachs were pinned on a corkboard and ulcers were scored using dissecting microscope with square-grid eyepiece based on grading on a 0–5 scale (depicting severity of vascular congestions and lesions/hemorrhagic erosions) as presented in Table 4. Areas of mucosal damage were expressed as a percentage of the total surface area of the glandular stomach estimated in square millimeters. Mean ulcer score for each animal was expressed as ulcer index (U.I) and the percentage of inhibition against ulceration was determined using the expressions:

$$\text{U.I} = [\text{Ulcerated area}/\text{total stomach area}] \times 100.$$

$$\% \text{Ulcer inhibition} = [\text{U.I. in control} - \text{U.I. in test}] \times 100/\text{U.I. in control}.$$

Table 4: The evaluation index of ulcer index.

Evaluation index	Score
Almost normal mucosa	0
Vascular congestions	1
One or two lesions	2
Severe lesions	3
Very severe lesions	4
Mucosa full of lesions	5

Determination of gastric pH and volume

The gastric juice volume of each rat was measured after centrifugation at **1,000 rpm for 10 minutes** and subsequently analyzed. A **1 ml aliquot** of the gastric juice was diluted with **1 ml of distilled water**, and the **pH** of the resulting solution was determined using a **pH meter** [1].

Histological examination of stomach

Stomach samples were preserved in 10% neutral buffer formalin immediately after scoring and subjected to histopathological examination. Tissues were subjected to dehydration in increasing concentrations of alcohol, cleared in xylene and then embedded in paraffin wax blocks. Sections of 3 μm thickness were prepared and examined after staining with hematoxylin and eosin (H & E) for detecting any structural changes. Tissues examination was performed using light microscope. A scoring system with a scale of 0–4 was used to assess the histopathological changes by a histopathologist who was blind to all treatments. The scoring system used in the study assessed gastric mucosal edema, gastric hemorrhage, erosion and necrosis.

Preparation of stomach homogenate and assay of antioxidant indices

Immediately after ulcer scoring, whole stomach tissues were ground with liquid nitrogen in a mortar. The ground tissues (0.5 g each) were then homogenized in ice-cold 0.1 M phosphate saline buffer (1:4 w/v, pH 7.4). The homogenates were subsequently centrifuged at 2,500 rpm for 10 minutes at 4°C.

The resulting supernatants were frozen at –20°C to ensure the maximum release of tissue-associated enzymes before being used for the enzyme assay. The levels of prostaglandin E2 (PGE2), myeloperoxidase (MPO), superoxide dismutase 3 (SOD3), and malondialdehyde (MDA) in the stomach homogenate were measured using standard commercial ELISA kits (ELK Biotechnology Co., Ltd, Wuhan, China).

Statistical Analysis

Data are presented as the mean values along with the Standard Error of Mean (± SEM). Intergroup comparison was made by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test using Graph Pad Prism (version 8.0.2) software. A value of p<0.05 was considered as statistically significant.

Results

This study was conducted to evaluate the gastroprotective activity of both the powdered form (PTD) and hydro-alcoholic extracts (HAE) of *Dawa-e-Sahaj* (DS). The model used in this study is independent of gastric acid secretion and closely mimics acute peptic ulcers in humans. The effects of the PTD and HAE of DS on the ulcer index and percentage inhibition of ulcers in experimental animals are presented in Table 5, as well as Figure 1 and 2 respectively.

Oral administration of ethanol (0.5 ml/100 g body weight) led to a significant (p < 0.05) increase in the degree of ulceration (ulcer index) in the rats. However, treatment with PTD and HAE extract of DS resulted in a significant reduction in ulceration. Notably, HAE provided superior protection against ulceration compared to PTD and showed efficacy comparable to the standard drug (Ranitidine).

Table 5 and Figure 3 illustrate the effects of PTD and HAE on gastric secretions in ethanol-induced ulcerated rats. Ethanol administration significantly (p < 0.05) reduced gastric pH while significantly (p < 0.05) increasing gastric volume. Pretreatment with HAE and PTD significantly elevated pH levels while concurrently reducing gastric volume compared to the ulcerated control group. These alterations were significantly mitigated (p < 0.05) in the PTD- and HAE-treated groups.

Table 7 and Figure 5 depict the effects of PTD and HAE of DS on lipid peroxidation (MDA), prostaglandin E2 (PGE2), myeloperoxidase (MPO), and superoxide dismutase 3 (SOD₃) activities in the gastric mucosa of ethanol-induced ulcerated rats. MDA and MPO levels were significantly elevated ($p < 0.05$) in the ulcerated animals. Additionally, a significant ($p < 0.05$) reduction was observed in the activity of PGE2 and SOD₃ in ethanol-induced ulcerated animals. Encouragingly, both PTD and HAE treatments led to significant ($p < 0.05$) improvements in these parameters, with effects comparable to both the normal control group and the standard drug (Ranitidine).

Table 5: Effect of Dawa-e-Sahaj (PTD and HAE) on ulcer index, % ulcer inhibition, gastric Volume and gastric pH in ethanol-induced gastric mucosal lesions in rats.

Groups	Macroscopic Ulcer Score	Ulcer Index (mm ²)	Ulcer Inhibition (%)	Gastric Volume	pH
NC	0	0.00 ± 000	-	2.62±0.19	3.24±0.80
Ethanol	2	28.47 ± 0.5	-	3.15±0.17	2.50±0.28
Ran + Ethanol	0.5	5.72 ± 0.5	79.89	2.55±0.10	4.14±0.18
PTD + Ethanol	0.5	9.70±0.91	65.92	2.60±0.12	3.55±0.22
HAE + Ethanol	0.5	8.46±0.37	70.27	2.64±0.18	3.47±0.26

NC: Normal control, Ran: Ranitidine, PTD: Powder Test Drug, HAE: Hydroalcoholic extract, Data were expressed as mean ± SEM (n = 5).

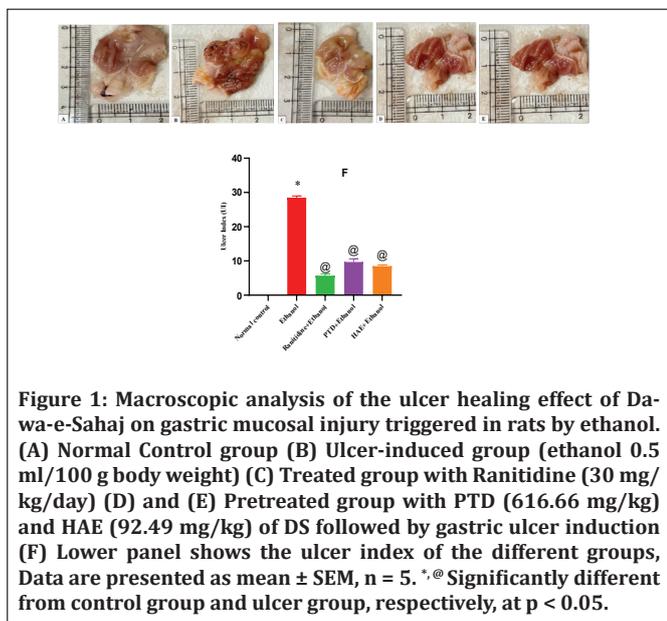


Figure 1: Macroscopic analysis of the ulcer healing effect of Dawa-e-Sahaj on gastric mucosal injury triggered in rats by ethanol. (A) Normal Control group (B) Ulcer-induced group (ethanol 0.5 ml/100 g body weight) (C) Treated group with Ranitidine (30 mg/kg/day) (D) and (E) Pretreated group with PTD (616.66 mg/kg) and HAE (92.49 mg/kg) of DS followed by gastric ulcer induction (F) Lower panel shows the ulcer index of the different groups, Data are presented as mean ± SEM, n = 5. *@ Significantly different from control group and ulcer group, respectively, at $p < 0.05$.

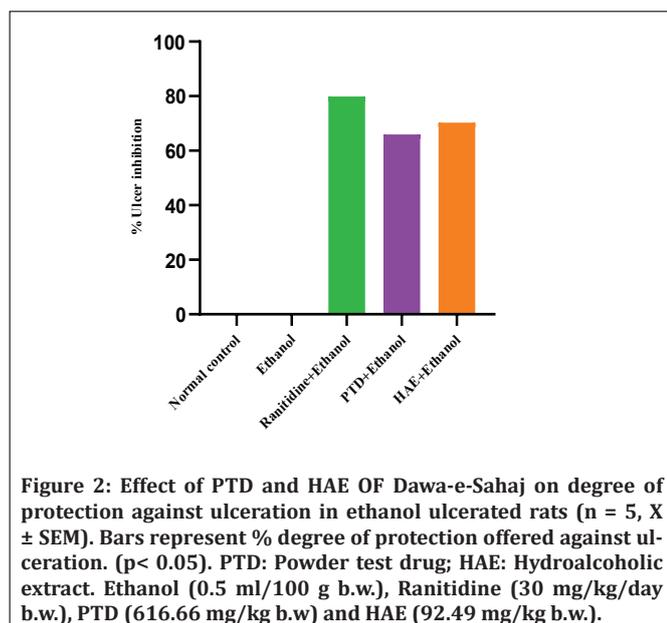


Figure 2: Effect of PTD and HAE OF Dawa-e-Sahaj on degree of protection against ulceration in ethanol ulcerated rats (n = 5, X ± SEM). Bars represent % degree of protection offered against ulceration. ($p < 0.05$). PTD: Powder test drug; HAE: Hydroalcoholic extract. Ethanol (0.5 ml/100 g b.w.), Ranitidine (30 mg/kg/day b.w.), PTD (616.66 mg/kg b.w) and HAE (92.49 mg/kg b.w.).

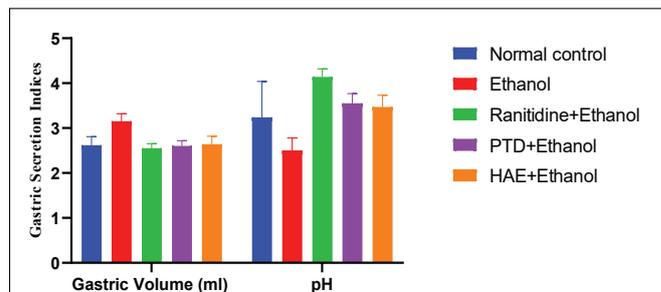


Figure 3: Effects of PTD and HAE of Dawa-e-Sahaj on gastric volume and pH of ethanol ulcerated rats (n = 5). ($p < 0.05$). PTD: Powder test drug; HAE: Hydroalcoholic extract. Ethanol (0.5 ml/100 g b.w.), Ranitidine (30 mg/kg/day b.w.), PTD (616.66 mg/kg b.w) and HAE (92.49 mg/kg b.w.).

Histopathological findings

Histopathological analysis of gastric tissue sections was conducted to evaluate the antiulcer activity of *Dawa-e-Sahaj*. The slides were microscopically examined for pathological and morphological alterations. The gastric mucosa of the normal control group exhibited no pathological abnormalities, with an intact mucosal structure. The mucosal layer contained a sufficient number of secreting epithelial cells, while the submucosal layer appeared normal. The findings from the stomach autopsies of various experimental groups are presented in Figure 4 and Table 6.

In ethanol-treated rats, severe submucosal edema, moderate infiltration of inflammatory cells (lymphocytes and eosinophils), surface epithelium disruption and erosion, as well as mucosal necrosis, were observed. Notable histological alterations were identified in stomach samples from rats treated with PTD and HAE of DS, including mild epithelial disruption, submucosal edema, and leukocyte infiltration. In contrast, the group treated with standard drug (ranitidine) exhibited only mild submucosal edema and minimal infiltration of inflammatory cells in the deeper mucosal layers, with no evidence of necrosis or gastric erosion.

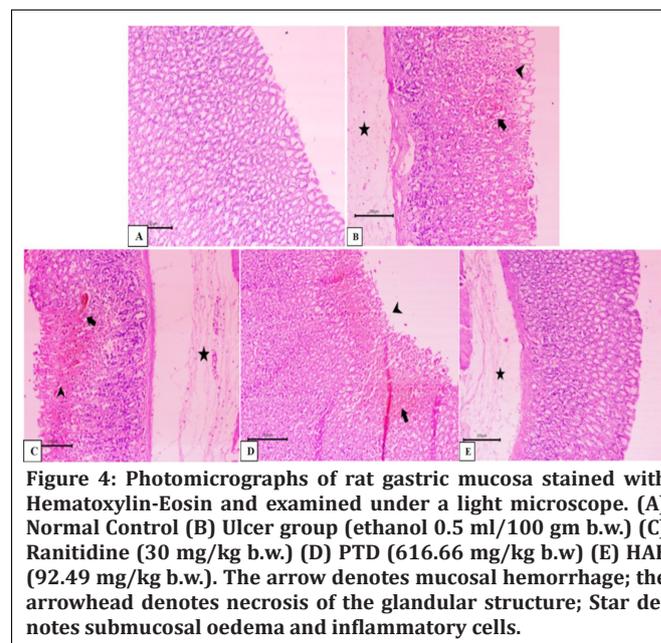


Figure 4: Photomicrographs of rat gastric mucosa stained with Hematoxylin-Eosin and examined under a light microscope. (A) Normal Control (B) Ulcer group (ethanol 0.5 ml/100 gm b.w.) (C) Ranitidine (30 mg/kg b.w.) (D) PTD (616.66 mg/kg b.w) (E) HAE (92.49 mg/kg b.w.). The arrow denotes mucosal hemorrhage; the arrowhead denotes necrosis of the glandular structure; Star denotes submucosal oedema and inflammatory cells.

Table 6: Histopathological Summary of Ethanol induced gastric Ulcer in wistar rat.

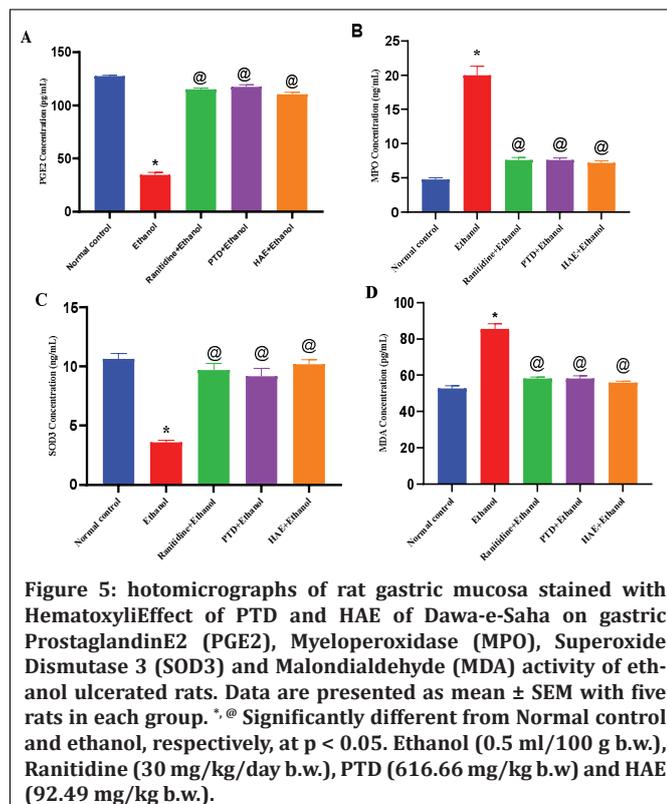
Groups	Erosion	Haemorrhage	Necrosis	Submucosal Oedema
NC	0	0	0	0
Ethanol	2	3	3	2
Ran + Ethanol	2	1	1	3
PTD + Ethanol	1	1	1	1
HAE + Ethanol	1	1	1	1

Ran: Ranitidine, NC: Normal control, PTD: Powder Test Drug, HAE: Hydroalcoholic extract, Data were expressed as mean ± SEM (n = 5).

Table 7: Enzyme activities and oxidative markers in the stomachs of rats administered ranitidine, ethanol and the PTD and HAE of Dawa-e-Sahaj.

Groups	PGE2	MPO	SOD3	MDA
NC	127.4±1.19	4.79±0.25	10.65±0.46	52.64±1.52
Ethanol	34.56±2.29	19.96±1.37	3.60±0.15	85.42±2.85
Ran + Ethanol	115.0±1.13	7.63±0.34	9.72±0.55	58.03±0.89
PTD + Ethanol	117.3±2.07	7.60±0.31	9.18±0.68	58.15±1.57
HAE + Ethanol	110.4±1.90	7.21±0.25	10.20±0.40	55.98±0.84

PGE2=prostaglandin E2, MPO=Myeloperoxidase, SOD3=Superoxide Dismutase 3, MDA=Malondialdehyde, Ran: Ranitidine, NC: Normal control, PTD: Powder Test Drug, HAE: Hydroalcoholic extract, Data were expressed as mean ± SEM (n= 5)



Discussion

According to previous studies, absolute ethanol readily penetrates the stomach's mucosal layer, leading to gastric ulcers within an hour of oral administration. This results in edema, mucosal hyperaemia, necrosis, and mucosal and submucosal bleeding due to increased lipid peroxidation, leukotriene production, oxidative stress, and free radical generation. Additionally, ethanol-induced damage is exacerbated by reduced prostaglandin levels, mucosal blood flow, gastric mucus, bicarbonate secretion, and glutathione levels, leading to cellular and membrane injury [1]. The present study assessed the gastroprotective effects of DS against ethanol-induced peptic ulcers in rats. Oral ethanol administration caused significant macroscopic and microscopic gastric damage, confirming ethanol-induced injury. The results indicate that the DS-treated groups (PTD & HAE) exhibited a significantly lower ulcer index and an improved preventive index compared to the positive control group (ethanol). Additionally, the ranitidine-treated group (standard control) also demonstrated a statistically significant improvement in the ulcer index and ulcer inhibition percentage compared to the ethanol group. Thus, it can be concluded that the Pharmacopoeial formulation DS possesses a notable ulcer-protective effect against ulcers induced through this mechanism.

Oxidative stress is closely linked to the pathophysiology of gastric ulcers [29]. The body's antioxidant defence system, which includes enzymes such as SOD3 and MPO, helps neutralize oxygen-derived free radicals [30]. A reduction in cellular SOD3 and MPO activity impairs the recovery process from ethanol-induced gastric oxidative damage [31]. Prostaglandin E2 (PGE2) plays a crucial role in maintaining gastric mucosal integrity and promoting ulcer healing [32]. It regulates gastric acid secretion, stabilizes

mast cell membranes, and stimulates tissue repair, making it vital for ulcer prevention and healing [33,34]. A decline in PGE2 levels within the gastric mucosa contributes to ulcer formation and aggravation [35].

Consistent with prior research, our findings revealed that ethanol-induced gastroduodenal mucosal injury disrupts the oxidant-antioxidant balance. This was demonstrated by elevated lipid peroxidation (MDA activity) and an inability to neutralize oxygen-derived free radicals, which react with lipids to form lipid peroxides. Lipid peroxidation compromises membrane fluidity, ion transport, and membrane integrity, ultimately impairing cellular function [36]. ELISA assay results confirmed that ethanol administration significantly increased oxidative stress in stomach tissue, as evidenced by heightened lipid peroxidation (MDA) and MPO levels and reduced antioxidant enzyme activity, including PGE2 and SOD, in the positive control (ethanol) group. DS administration enhanced cellular antioxidant defences by increasing PGE₂ and SOD levels while reducing lipid peroxidation (MDA) and MPO levels. These findings suggest that DS's gastroprotective effects may be partially attributed to the stimulation of PGE₂ and SOD₃.

Flavonoids are known to stimulate PGE₂ production in gastric mucosal cells. Exogenous prostaglandins, particularly those of the E series, provide protection against gastrointestinal (GI) mucosal damage caused by various irritants. Research suggests that endogenous prostaglandins are essential for maintaining gastroduodenal integrity [37].

The observed anti-ulcer activity in the ethanol-induced ulcer model may be attributed to the known anti-ulcer potential of DS's ingredients. The aqueous extract of *P. ovata* seeds has been reported to exhibit anti-ulcer potential in indomethacin-induced ulcers in rats at a dose of 100 mg/kg [38]. Similarly, the ethanolic extract of *P. ovata* seeds at 400 mg/kg body weight demonstrated gastric ulcer protection in rats with ethanol-induced ulcers [39]. Aqueous and methanolic extracts of *P. major* have shown significant anti-ulcer potential in indomethacin-induced ulcers in rats, attributed to the plants rich flavonoid and phenolic content with antioxidant properties [40]. Furthermore, *P. major* seed extract at 400 and 700 mg/kg displayed protective effects against acetic acid-induced ulcerative colitis in rats.

The fixed oil of *O. basilicum* seeds has exhibited protective effects against various chemically and stress-induced gastric ulceration models in animals. *O. basilicum* aqueous extract (400 mg/kg) significantly protected against aspirin-induced ulcers in rats (Singh et al., 1999). Additionally, studies have reported that the fixed oil of *O. basilicum* possesses significant anti-ulcer activity, possibly due to its lipoxygenase inhibitory, histamine antagonistic, and antisecretory effects. However, further research is necessary to elucidate its precise mechanism of action [41,42].

Acacia gum has been shown to dose-dependently prevent ethanol-induced ulcer formation in rats when incorporated into a standard diet at concentrations of 2.5%, 5%, or 10% powder [43]. Gum Arabic (GA) contains fibers, total sugars, polysaccharides, phenolic compounds, and flavonoids. Studies suggest that GA contains arabinogalactan, which may contribute to its anti-ulcer activity [44]. According to Cipriani et al. (2006) [45], the anti-ulcer activity of polysaccharides is likely due to their mucosal surface-binding ability, forming a protective coating against acid or scavenging free radicals. GA has demonstrated ulcer-protective effects by suppressing gastric inflammation through the reduction of cytokines such as TNF- α and IL-6, while increasing IL-10 levels in ethanol-induced rats [46]. Flavonoids, tannins, terpenoids, and saponins contribute to the gastroprotective effects of plant extracts [47]. The ulcer-preventive effects of DS may be due to the flavonoids present in *P. ovata*, *P. major*, and *Phyllanthus maderaspatensis* L., as research has shown that flavonoids possess anti-ulcer properties, including antacid activity, pepsin inhibition, and enhanced gastric mucus and bicarbonate secretion. Flavonoids improve mucosal defence by exerting antioxidant, anti-inflammatory, and antibacterial effects against gastric ulcers. Thus, the anti-ulcer activity of DS can be attributed to the presence of these phytochemicals in its formulation.

The constituents of DS contribute to gastric lesion healing and may act synergistically to enhance its therapeutic efficacy. Several studies suggest that flavonoids, particularly garcinol, rutin, and quercetin, exert ulcer-healing effects primarily through their antioxidant activity. Their efficacy is attributed to hydroxyl groups at positions 3, 5, and 7, the double bond at the 2,3 position in conjugation with a 4-oxo function, and the presence of an o-dihydroxy group in the B ring [48].

Saponins and triterpenoids have demonstrated anti-ulcer activity in multiple experimental models by promoting the formation of protective mucus over the gastric mucosa. They also safeguard the mucosa from gastric acid by selectively inhibiting prostaglandins [49]. Tannins exhibit anti-ulcer effects due to their astringent properties and vasoconstrictive

effects [50]. By precipitating microproteins at the ulcer site, tannins form a protective layer that shields the mucosa from gastric secretions and other irritants.

Conclusion

This study demonstrated that both the powdered test drug (PTD) and the hydroalcoholic extract of Dawa-e-Sahaj exhibit significant gastroprotective effects against ethanol-induced ulcers. These gastroprotective effects are attributed to the reduction of oxidative stress markers, anti-secretory properties, and the cytoprotective activities of the phytoconstituents present in the ingredients of Dawa-e-Sahaj. These findings scientifically validate the traditional use of Dawa-e-Sahaj in the treatment of gastric ulcers and suggest that this formulation could serve as a potential source for the development of novel anti-gastric ulcer agents.

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Conflict of Interest

There is no Conflict of interest.

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Data availability

All the data supporting the findings of this study are available from the corresponding author upon request.

CRedit Authorship Contribution Statement

Mohd Haris: Data curation, Writing- original draft preparation, Methodology, Conceptualization, Reviewing, Editing; **Sumbul Rehman:** Supervision, Investigation and Validation; **Abdur Rauf:** Project Administration, Visualization.

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