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Original Article

EVALUATION OF ANTI ULCER ACTIVITY OF HYDROALCOHOLIC EXTRACTS OF *GYMNEMA SYLVESTRE* ON ALBINO WISTAR RATS

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ABSTRACT

Objective: The present research is designed to evaluate the anti-ulcer activity of HAGS (Hydroalcoholic extract of *Gymnema sylvestre*) in NSAIDs and pylorus ligation-induced rat models.

Methods: The antiulcer activity of the hydroalcoholic extracts of *Gymnema sylvestre* (100 and 200 mg/kg, p. o.) was evaluated in ethanol, indomethacin, pylorus ligation and acetic acid-induced ulcer models in rats. Parameters such as mean ulcer indices and percentage ulcer inhibition were assessed in ethanol, indomethacin and acetic acid-induced ulcer models, while the gastric volume, pH, and titratable acidity were evaluated in the pylorus ligation ulcer model.

Results: Hydroalcoholic extract of *Gymnema sylvestre* (100 mg/kg) and (200 mg/kg) could significantly ($P<0.001$) reduce the ulcer index, ulcerated area and total acidity compared to standard drug and thereby significantly ($P<0.001$) increase percentage inhibition of ulcers and protected area which was evident by the significant rise in pH of gastric content. A significant increase was observed in pH, NP-SH, GSH, enzymic antioxidants and protein with a significant decrease in volume of gastric juice, free and total acidity, acid output and LPO levels activities in 100 mg/kg and 200 mg/kg of HAGS treated rats compared to disease control rats. The effect of extracts was dose-dependent and results were comparable to that of the standard drug Cimetidine.

Conclusion: It is concluded that the Hydroalcoholic extract of *Gymnema sylvestre* shows a significant effect on NSAIDs and Pylorus ligation-induced rat models. It shows a significant reduction in the lesion index.

Keywords: *Gymnema sylvestre*, Anti-ulcer, Hydroalcoholic extract, Traditional medicine, Pylorus ligation ulcer, NSAIDs induced ulcer

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INTRODUCTION

A peptic ulcer is an acid-induced lesion of the digestive tract that is usually located in the stomach or proximal duodenum and is characterized by denuded mucosa with the defect extending into the submucosa or muscularis propria. The estimated prevalence of peptic ulcer disease in the general population is 5–10%, but recent epidemiological studies have shown a decrease in the incidence, rates of hospital admissions, and mortality associated with peptic ulcer.

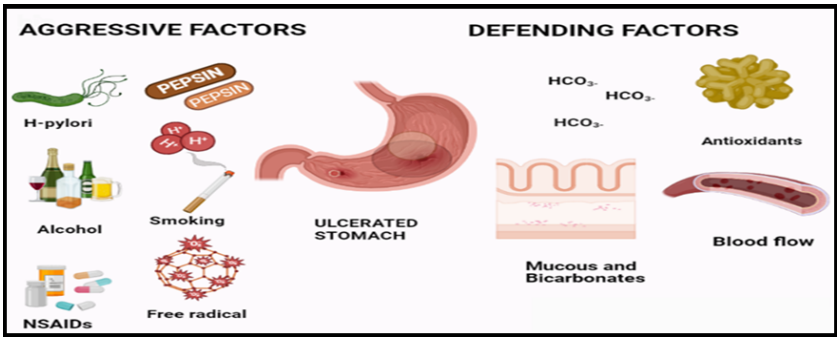


Fig. 1: Schematic representation of peptic ulcer etiology

This is most likely secondary to the introduction of new therapies and improved hygiene, which resulted in a decline in Helicobacter pylori (*H. pylori*) infections.

Traditionally, mucosal disruption in patients with acid peptic disease is considered to be a result of a hypersecretory acidic environment together with dietary factors or stress. Risk factors for developing peptic ulcer include *H. pylori* infection, alcohol and tobacco consumption, non-steroidal anti-inflammatory drugs (NSAIDs) use, and Zollinger–Ellison syndrome. The main risk factors for both gastric and duodenal ulcers are *H. pylori* infection and NSAID use [1, 2].

For more than a century, peptic ulcer disease was most often managed surgically, with resulting high morbidity and mortality rates. Although the incidence of peptic ulcer disease in Western countries has declined over the past 100 y, around 1 in 10 Americans are still affected [3]. The annual financial burden of peptic ulcer disease in the US, including direct

and indirect costs, is estimated at US$3.4 billion.5 Since peptic ulcer disease is still common, and peaks in the elderly, it is expected that its impact on human health and health economics will remain an important issue in the future [4].

An estimated 15,000 deaths occur each year as a consequence of PUD in India. In the USA, approximately 4 million individuals have peptic ulcers, while each year about 350,000 new cases are diagnosed; about 100,000 patients are hospitalized and at least about 3000 people die as a result of the disease [5].

More and more herbal and synthetic drugs are offering newer and better options for peptic ulcers. The drug type varies from a proton pump inhibitor to an H2 antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, enterochromaffin-like cell (ECL), hyperplasia, and hemopoietic changes [6, 7].

Due to the reported side effects of available antiulcer drugs, the focus has been shifted towards natural products as the new sources of antiulcer agents [8]. The development of new anti-ulcer drugs from medicinal plants is an attractive proposition because diverse chemical compounds have been isolated from medicinal plants with anti-ulcer activity and have been shown to produce promising results in the treatment of gastric ulcers [9].

Gymnema Sylvestre R. Br. (Asclepiadaceae) is a herb distributed throughout the world [10]. The leaves of the plant are widely used for the treatment of diabetes and as a diuretic in Indian proprietary medicines. The plant is documented to have beneficial digestive, anti-inflammatory, diuretic, hypoglycemic, and antihelmintic effects. It is believed to be used in dyspepsia, constipation, jaundice, haemorrhoids, cardiopathy, asthma, bronchitis, and leukoderma [11].

Fig. 2: *Gymnema sylvestre* plant, Source: https://images.app.goo.gl/wVg5qiSB8bVrFNBj9

Various parts of the plant are used in the treatment of skin problems, bronchitis, antifungal, eye disease, cancer, diabetes, digestive, diuretic, emetic, expectorant, laxative, stimulant, stomachic, uterine tonic, and urinogenital infection, etc. It has anti-diabetic, anti-sweetener, and anti-inflammatory activity [12]. Due to this, the present research was carried out to examine the anti-ulcer effects of *Gymnema sylvestre* hydroalcoholic extracts against NSAIDS and pylorus ligation-induced ulcers in wistar rats.

MATERIALS AND METHODS

Animals

Healthy adult Albino Wistar rats of 3-4 w old, weighing 200±10 g were obtained from the central animal house of Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode, Tamilnadu. Throughout the acclimatization and experimental period, the animals were housed in autoclavable polypropylene cages (six rats per cage) in standard laboratory conditions (humidity 50-60%, lighting conditions (12–h light/12-h dark cycle) and temperature 21±2 °C). The study protocol was approved by the Institutional Animal Ethical Committee (IAEC), Swamy Vivekanandha College of Pharmacy, and conducted under guidelines set by the CCSEA (Committee for Control and Supervision of Experiment on Animals). The animals were acclimatized for 2 w in the laboratory conditions before the experiment. Rats were provided with a standard pellet diet and water ad libitum freely throughout the study. The study protocol was approved by the Institutional Animal Ethics Committee Reg. No. SCVP/IAEC/PG/18/19/2022.

Plant material

Hydroalcoholic extract of *Gymnema sylvestre* was purchased from ELLESS AROMATICS, Chennai, Tamilnadu, India with an analytical report containing the following specification.

Acute oral toxicity studies

The acute toxicity of the Hydroalcoholic extract of *Gymnema sylvestre* is determined by using albino mice of either sex (20-25 g), maintained under standard conditions. The animals fasted for 3 h before the experiment. Animals are observed for their mortality and toxic symptoms up to 48 hr study period following the starting dose of 2000 mg/kg administration as per OECD guidelines No 423 [13]. From the LD50 dose, 1/10 and 1/5th doses were selected and considered low and high doses respectively.

Anti-Ulcer screening

Non-steroidal anti-inflammatory drug (NSAID)-induced Ulcer screening

The experiment was performed according to the method of (Nwafor *et al.*, 2000) [14] with a few modifications. After 12 h of fasting, the Albino Wistar rats were randomly divided into five groups of six animals each. The first group was given 1 ml of vehicle (1% Tween 80 aqueous solution), the second group was treated with Cimetidine (100 mg/kg B. Wt.), the third group received Indomethacin (100 mg/kg B. Wt.) and the remaining two groups received 100 and 200 mg/kg of GS respectively for five days. All the treatments were administered orally. One hour after treatment, all the rats were administrated Indomethacin (100 mg/kg) to induce Gastric Ulcer. Four hours after treatment with Indomethacin, the animals were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The stomachs were gently rinsed with normal saline to remove the gastric contents and blood clots for subsequent scoring. After scoring, the stomach was stored in normal saline (4 °C) and used for further biochemical analysis.

The ulcers were graded using the following scoring system:

0.5-Blushing

1-Spot ulcers

1.5-Haemorrhage streaks

2-Ulcers>3 mm but<5 mm

2.5-Ulcers>5 mm.

Pylorus ligation (PL)-induced ulcers screening

The Albino Wister rats were kept fasting for 18 h and care was taken to avoid coprophagy. Drugs were administered for 5 d. Animals were anaesthetized using Thiopentone sodium (40 mg/kg B. Wt., *i. p.*), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the postoperative period. After 4 h, stomachs were dissected out; gastric juice was collected in a graduated centrifuge tube and cut open along the greater curvature. The ulcer index was calculated [15]. After scoring, the stomach was stored in normal saline (4 °C) and used further for biochemical analysis.

Determination of secretary parameters

After the pylorus ligation stomach was dissected out, and cut open and the gastric juice was drained into a graduated centrifuge tube, centrifuged at 3000 rpm for 10 min. The supernatant was collected and used for the estimation of the volume of gastric juice, pH, free acidity, total acidity, and protein. The volume was noted and expressed as ml/100 g/4 h and pH was measured using a pH meter. Estimation of free and total acidity was carried out by the method of Card and Marks using gastric juice [16]. Free acidity and total acidity were determined by titrating with 0.01N Sodium hydroxide using Topfer's reagent and Phenolphthalein as indicators respectively. The acidity was expressed as mEq/l/100 g.

Determination of pH

The PH of the gastric juice was determined using an EI-Digital pH meter.

Determination of total and free acidity

Pipette out 1 ml of gastric fluid and diluted to 10 ml with distilled water and it was titrated against 0.01 N Sodium hydroxide using Topfer's reagent as an indicator (Dimethyl-amino-azo-benzene with Phenolphthalein) until the solution turns to orange colour. Note the volume of consumed 0.01 N NaOH which corresponds to the free acidity. Titrate further till the solution regains pink colour. Note the volume of NaOH which corresponds to the total acidity.

Biochemical analysis

Preparation of stomach homogenate

500 mg of the pyloric part of the stomach was homogenized in 5.0 ml of 0.1 M Tris base buffer pH 7.4. The homogenized organs were then centrifuged at 3000 rpm for 10 min. The supernatant was used for the estimation of various enzymatic and non-enzymatic antioxidants including Reduced Glutathione, Glutathione Peroxidase, Protein, Peroxidase, Non-Protein Sulfhydralase, Catalase, and Lipid Peroxidation [17].

Estimation of catalase

Pipette out 4.0 ml of 0.01 M Phosphate buffer, 0.5 ml of homogenate and 0.2 ml of 0.2 M hydrogen peroxide, and 1.2 ml of distilled water was taken. The reaction was stopped by adding 1.0 ml of dichromate acetic acid reagent at 0 and 60 sec. All the tubes were kept in a boiling water bath for 10 min and the absorbance was measured at 620 nm. For standards, 20–100 micromoles of (0.1-0.5 ml) hydrogen peroxide were taken. The volume is made up of 0.5 ml of distilled water. Then 4.0 ml of phosphate buffer and 1.0 ml of dichromate acetic acid reagent were added and the colour intensity was measured at 630 nm in UV/VIS spectrometer [18]. For blank 0.5 ml of distilled water and 4.0 ml of phosphate buffer and 1.0 ml of dichromate acetic acid were taken. The results are expressed as micromoles of hydrogen peroxide used/min/mg of protein.

Lipid peroxidation assay

Pipette out 0.2 ml of the homogenate to 1.5 ml of trichloro acetic acid and 1.5 of thiobarbituric acid reactive substances was added. The tubes were placed in a boiling water bath for 30 min. The pink colour was developed and the colour intensity was measured at 532 nm using a UV/VIS spectrometer [19].

Estimation of reduced glutathione

0.2 ml of sample was mixed with 1.8 ml of precipitating reagent. After 15 min, the tubes were centrifuged at 3000 rpm for 15 min. 1.0 ml of supernatant was added with 4.0 ml of 0.3 M phosphate buffer and 0.5 ml of DTNB. The colour intensity was measured at 405 nm in UV/VIS spectrometer [20].

Histological analysis

Haematoxylin and eosin staining

The stomach was dissected out from the rats and fixed in 10% neutral buffered formalin for 48 h. The organs were then washed in running tap water. The organs were then trimmed and processed in Yorco automatic tissue processor. The processed tissue sections were then embedded in paraffin wax using Leica embedding station. Three-micron-thick sections were prepared using a Leica microtome. The sections were stained using routine haematoxylin and eosin technique. The stained sections were observed for any changes under the light microscope.

Statistical analysis

The results are expressed as mean±SD for six replicated determinations. The results were analyzed statistically by one-way analysis of variance (ANOVA) followed by a Tukey multiple comparison test. The significance levels were analyzed at $p<0.001$, $p<0.01$, and $p<0.05$. All statistical tests were carried out using Prism 8.0 (graph pad) statistical software.

RESULTS

Extractive value of hydroalcoholic extract of *gymnema sylvestre*

Table 1, shows the extractive values of the hydroalcoholic extract of *Gymnema Sylvestre*

Table 1: Extractive values of HAGS

S. No.	Solvent used	Percentage
1	Hexane	3.04%
2	Petroleum ether(60-80 °C)	5.24%
3	Chloroform	2.344%
4	Ethyl acetate	3.92%
5	Methanol	20.8%
6	Water	16.5%

Acute toxicity studies

Acute toxicity study results showed that the ethanolic extract of *Gymnema sylvestre* did not cause any toxic symptoms or mortality up to a dose level of 2000 mg/kg and was considered safe. Hence 100 mg and 200 mg of the dose were selected for further pharmacological screening as sub-maximal and maximal doses.

Secretary parameters

Effect of HAGS on acid secretary parameters using pyloric ligation induced ulcer model

Table 2, shows the levels of acid secretary parameters of the control and experimental group in pyloric-ligated rats. The volume of gastric juice, free and total acidity of gastric juice was increased significantly ($p<0.001$) with a significant decrease ($p<0.01$) in pH compared to Cimetidine-treated animals.

Effect of HAGS changes in lesion index using pylorus ligation and indomethacin induced ulcer model

Table 3, shows the dose-dependent changes in the lesion index of the experimental group of rats. Rats administered with Indomethacin show a marked significant increase ($p<0.001$) in lesion index as compared to Cimetidine-treated animals. Treatment with HAGS 100 mg/kg and HAGS 200 mg/kg showed a significant increase ($p<0.001$) in lesion index when compared to Cimetidine-treated rats. Rats that are Pylorus ligated showed marked increased significance

(p<0.001) in lesion index as compared to Cimetidine-treated animals. Treatment with HAGS 100 mg/kg and HAGS 200 mg/kg showed a slight increase (p<0.001) in lesion index compared to Cimetidine-controlled rats.

Table 2: Effect of HAGS on acid secretary parameters

Groups	Volume (ml)	Free acidity(meq/l)	Total acidity (meq/l)	pH
Normal control	1.28±0.01	12.59±0.03	11.50±0.14	4.30±0.05
Disease control	5.37±0.02***	24.89±0.67***	34.25±0.37***	1.90±0.01***
Cimetidine 100 mg/kg, *p. o*	2.90±0.01	13.83±0.12	15.63±0.31	3.90±0.05
HAGS 100 mg/kg, *p. o*	4.30±0.02***	21.07±0.51***	47.29±0.35***	2.83±0.03***
HAGS 200 mg/kg, *p. o*	3.77±0.05***	16.78±0.13**	34.32±0.34***	3.22±0.04***

The results are expressed as mean±SEM for six animals in each group and statistical significance was calculated by ANOVA followed by Tukey's multiple comparison tests. Note-***p<0.001 and **p<0.01 was considered significant when Groups are compared as follows: Cimetidine vs. Control, Disease Control and Treatment.

Table 3: Effect of HAGS on lesion index

Groups	Pyloric ligation-induced ulcer		Indomethacin induced ulcer	
	Lesion index (mm^2)	Inhibition (%)	Lesion index (mm^2)	Inhibition (%)
Normal	0	-	0	-
Disease control	7.45±0.08***	-	9.38±0.07***	-
Cimetidine 100 mg/kg *p. o*	2.27±0.08	71.15	2.34±0.02	74.82
HAGS 100 mg/kg *p. o*	4.46±0.08***	36.21	5.41±0.06***	40.33
HAGS 200 mg/kg *p. o*	3.36±0.08***	55.91	4.30±0.05***	54.40

The results are expressed as mean±SEM for six animals in each group and statistical significance was calculated by ANOVA followed by Tukey's multiple comparison tests. Note-***p<0.001 was considered significant Groups are compared as follows: Cimetidine vs. Disease and Treatment.

Table 4: Effect of HAGS on NP-SH levels

Groups	NP-SH (µg GSH/g)	
	Pyloric ligation-induced ulcer	Indomethacin induced ulcer
Normal	3.52±0.04	3.83±0.06
Disease control	0.77±0.01***	0.78±0.09***
Cimetidine 100 mg/kg	2.40±0.05	2.35±0.02
HAGS 100 mg/kg	2.27±0.08	1.94±0.03**
HAGS 200 mg/kg	2.90±0.04**	2.72±0.03**

The results are expressed as mean±SEM for six animals in each group and statistical significance was calculated by ANOVA followed by Tukey's multiple comparison tests. Note-***p<0.001 and **p<0.01 was considered significant when compared Groups are compared as follows: Cimetidine vs. Disease Control and Treatment.

Effect of HAGS on the levels of NP-SH in gastric mucosa using pyloric ligation and indomethacin induced ulcer model

Table 4 depicts the levels of NP-SH in control and experimental ulcer models using rats. Disease control rats showed a significant decrease (p<0.001) in NP-SH level compared to Cimetidine-treated animals. Treatment with HAGS 200 mg/kg showed a slight increase (p<0.01) in NP-SH levels compared to Cimetidine-controlled rats.

Effect of HAGS on lipid peroxidation and antioxidant status in gastric mucosa using pylorus ligation and indomethacin-induced ulcer model

Table 5, shows the effect of HAGS on lipid peroxidation and antioxidant status in gastric mucosa using Indomethacin induced ulcer model.

Effect of HAGS on lipid peroxidation level in gastric mucosa

TBARS level was found to be increased significantly in diseased animals (p<0.001) compared with normal control. The low dose and high dose of HAGS were found to exhibit (p<0.001) decreased significantly against Disease control rats. The percentage protection exhibited by a high dose of extract was greater than that of a low dose. Treating animals with Cimetidine the lipid Peroxidation was found to be decreased significantly (p<0.01) against disease control animals.

Effect of HAGS on GSH level in gastric mucosa

GSH level was found to be significantly decreased in diseased animals (p<0. 001) compared to normal control animals. The low dose of HAGS was found to exhibit (p<0.001) increased significance and whereas the high dose of HAGS rats showed a significant increase (p<0.001) in GSH levels when compared to Disease control. The percentage protection exhibited by a high dose of extract was greater than that of a low dose. Thus the dose-dependent increased level of GSH effect was observed in HAGS-treated animals.

Effect of HAGS on catalase level in gastric mucosa

Catalase level was found to be significantly decreased in diseased animals (p<0.1) compared to normal control animals. The low dose of HAGS was found to exhibit (p<0.5) increased significance than disease control animals. Whereas the high dose of HAGS rats showed a significantly decreased (p<0.05) in catalase activity when compared to Disease control. Cimetidine-treated animals increased significantly (p<0.1) against disease control animals.

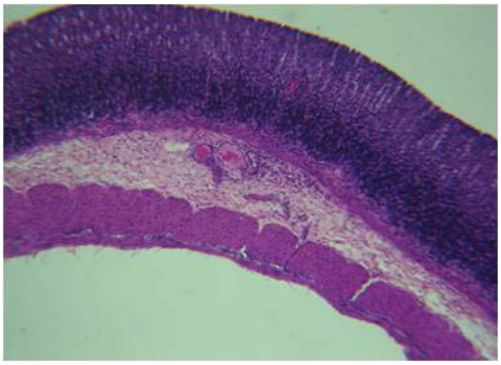
Histological analysis

Fig. 3 shows the histological examination of the stomach of the control and experimental group of rats. The gastric mucosa of control rats (A) revealed a normal mucosal architecture with normal glands, whereas Disease control rats (C) showed mucosal ulceration, Submucosal Oedema, Inflammation and poly morphonuclear infiltration in the ulcer site as well as in the Oedematous submucosa. HAGS 100 mg/kg, p. o. treated rats (E) showed mucosal regeneration with moderate erosion in the glandular stomach and the HAGS 200 mg/kg, p. o. (F) showed only congestion of mucosa with no inflammatory cells. In reducing congestion and haemorrhage, the extract efficacy was comparable to that of Cimetidine whereas this standard drug was found to be better than the extract in normalizing oedema and necrosis.

Table 5: Effect of HAGS on lipid peroxidation and antioxidant status in gastric mucosa

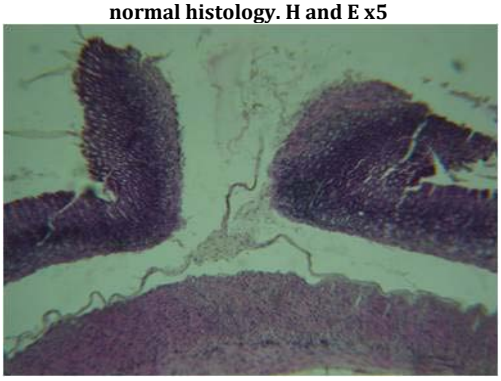
Groups	Pyloric ligation-induced ulcer			Indomethacin induced ulcer		
	LPO (nm of MDA/mg of protein)	GSH (nm of GSH/mg of protein)	Catalase (nm of H2O2 used/min/mg of protein)	LPO (nm of MDA/mg of protein)	GSH (nm of GSH/mg of protein)	Catalase (nm of H2O2 used/min/mg of protein)
Normal	2.743±0.034	164.1±0.512	0.0142±0.0006	2.74±0.03	167.00±1.15	0.0023±0.0001
Disease control	12.06±0.017***	2822±0.4155***	0.0047±0.0001***	4.47±0.03**	85.88±1.05***	0.014±0.0005***
Cimetidine 100 mg/kg	2.977±0.881	5374±0.8756	0.0166±0.0001	2.40±0.06	161.9±0.66	0.027±0.0008
HAGS 100 mg/kg	4.850±0.360***	3877±0.879***	0.0097±8.81***	2.92±0.03***	125.8±0.9597***	0.0173±0.0001***
HAGS 200 mg/kg	3.968±0.008***	5087±3.156***	0.0134±0.0001***	2.16±0.03**	155.0±0.5774**	0.024±0.0008*

The results are expressed as mean±SD for six animals in each group and statistical significance was calculated by ANOVA followed by Tukey's multiple comparison tests. Note-*-P<0.01, **-P<0.1, ***-P<0.05, ****-P<0.5, were considered significant when compared groups are compared as follows: Control vs. Disease Control, Disease control vs. Treatment.

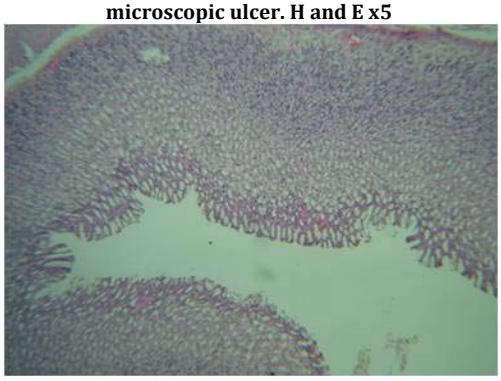


A. Section of the glandular stomach of the control group showing normal histology. H and E x5

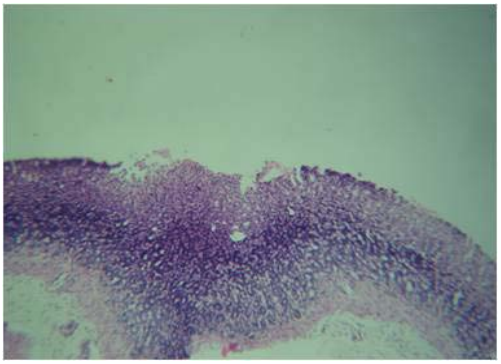
B. Ulcerated gastric mucosa of the diseased control group with the microscopic ulcer. H and E x5



C. Higher magnification of the ulcerated mucosa of the glandular stomach of diseased control. H and E x10



D. Gastric mucosa of standard drug treated showing no ulceration in the mucosa of glandular stomach. H and E x5



E. Low dose group revealing moderate erosion in the glandular stomach. H and E x5

F. Section of the glandular stomach of high dose group showing only congestion of mucosa with no ulceration. H and E x5

Fig. 3: Histological analysis of hags

DISCUSSION

Ulcers are caused due to imbalance between aggressive and defensive factors of the gastric mucosa. Pepsin and gastric acid make up the offensive factors whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation and prostaglandins [21]. Different therapeutic agents including plant extracts are used to inhibit gastric acid secretion or

to stimulate the mucosal defence mechanism by increasing mucus production protecting the surface epithelial cells, or interfering with the PG synthesis [22]. Thus, the present investigation was carried out to evaluate the anti-ulcer activity of the HAGS against different animal models of ulcers.

The etiopathogenesis of peptic ulcer has changed from Schwartz's dictum 'No acid (gastric juice)-No ulcer' to 'No mucosal damage-No ulcers' [23]. It is now believed that peptic ulcers result from an imbalance between defensive (cytoprotective) and offensive factors (gastric acid), association with *H. Pylori* infection and increased use of NSAIDs like Aspirin and Indomethacin drugs causes damage by inhibiting biosynthesis of cytoprotective Prostaglandins [24].

This research work was carried out to evaluate the anti-ulcer activity of the Hydroalcoholic extract of *Gymnemaa sylvestre*. For this purpose, the anti-ulcer activity was evaluated using Indomethacin-induced Ulcer, the most commonly used experimental model for the evaluation of anti-ulcer activity in animal models. Also, the effect of HAGS on gastric secretion was evaluated using the pylorus-ligated ulcer model.

Analysis of HAGS, for the phytochemical parameter such as total ash, insoluble ash, water insoluble ash, sulphated ash, water soluble extractive, loss on drying, pH etc., gives an idea to use the same as a pharmaco-therapeutic agent, if it possesses promising biological activity. It indicates the strength of the samples to be used as a drug. The physicochemical parameters analyzed show within the limit of pharmacopoeia.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), like Aspirin and Indomethacin, are known to induce ulcers during Anti-Inflammatory therapy, by inhibiting Prostaglandin Synthetase through the Cyclooxygenase pathway [25]. In the stomach, Prostaglandins play a vital protective role, stimulating the secretion of Bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair [26]. Thus, suppress Prostaglandin synthesis. Indomethacin coincides with the earlier stages of damage to the cell membranes of mucosal, parietal and endothelial cells. It has been reported that gastric acid secretion is involved in the formation of Indomethacin-induced mucosal lesions and results in increased susceptibility to mucosal injury and gastroduodenal ulceration. It was observed that HAGS extract displayed a significant reduction of the mucosal damage in the Indomethacin-induced ulcer model.

According to the experimental models used in this study, NSAIDs like Indomethacin induce ulcer formation by depleting cytoprotective PGs. PGE2 and PGI2 of gastric and duodenal mucosa are responsible for mucus production and maintaining the cellular integrity of the gastric mucosa [28].

Gastric acid has been shown to play an important permissible role in NSAID-associated mucosal injury. Acid-reducing property is discussed with several anti-ulcer drugs. In the present study, an increase in pH and a decrease in acidity were shown in ulcerated animals treated with HAGS, which is highly desirable for gastroprotection and antiulcer effect. The gastroprotective effect of HAGS may be due to the direct action on the acid-producing cells.

Increased level of LPO is due to an increase in the generation of Reactive Oxygen Species during stress leading to oxidative damage. SOD converts the reactive superoxide radical to H2O2, which if not scavenged by CAT can by itself cause lipid peroxidation by generation of hydroxyl radicals. Hence decrease in CAT levels has led to an increase in the accumulation of these Reactive Oxygen Species and thus, has caused increased lipid peroxidation and tissue damage. GSH is a non-enzymic mode of defence against free radicals. HAGS significantly reversed these oxidative changes induced by stress due to its antioxidant property. HAGS also inhibited the oxidation of reduced glutathione in a dose-dependent manner.

Pyloric ligation significantly induced lipid peroxidation as seen from an increase in LPO levels. This is due to an increase in the generation of Reactive Oxygen Species during stress leading to oxidative damage. Normally the increase in damage due to O2 is contained by dismutation with SOD [29]. SOD converts the reactive O2 to H2O2, which if not scavenged by the CAT can by itself cause lipid peroxidation by increase in the generation of hydroxyl radicals. Hence decrease in CAT levels led to an increase in the accumulation of these reactive products and thus, has caused increased lipid peroxidation and tissue damage. The effect is further aggravated by decreased activity of gastric peroxidases during stress [30]. In HAGS treated animals showed a significant increase in the CAT. So due to the increase in catalase, the hydroxyl free radical has reduced which protects the gastric mucosa.

Potentiation of the mucosal injury by N-ethylmaleimide and SH blocker may be explained by the enhanced microvascular permeability in the gastric mucosa and inhibition of the gastric motility commonly associated with the prevention of mucosal injury [31]. The concentration of NP-SH in gastric mucosa lowers due to the accumulation of toxic free radicals in ulcerated regions associated with NSAID was noted in the present study. Earlier studies on gastric cytoprotection have also revealed the involvement of NP-SH stimulating agents in the cytoprotective effect of several drugs and it has been reported that the increasing levels of NP-SH groups account for ulcer inhibition [32]. The present study demonstrates that HAGS elicited an increase in NP-SH level which could have possibly resulted in the cytoprotective property of the extract.

Histopathological studies further confirmed that pre-treatment with the extract inhibited NSAID-induced ulcers, congestion, oedema, haemorrhage and necrosis in gastric mucosa. In reducing congestion and haemorrhage, the extract's efficacy was comparable to that of Cimetidine, whereas this standard drug was found to be better than the extract in normalizing oedema and necrosis because of both antioxidant [33] and antisecretory activity.

In addition, oxidative damage is considered to be a common factor in the pathogenesis of ulcers by different experimental and clinical models. This antioxidant activity has been described for several triterpenes, such as: α-and β-amyrins, Oleanolic acid, Ursolic acid, Lupeol and Glycirretinic acid, among other related compounds [34]**.** Furthermore, antioxidant activity was observed in HAGS-containing Terpenes. Therefore, the anti-ulcer activity of HAGS extract may be partially due to its relative antioxidant activity.

CONCLUSION

Peptic ulcer disease is one of the most common gastrointestinal disorders. It mainly involves an imbalance between the offensive and defensive factors. Many synthetic drugs such as H2 blockers, gastroprotective and proton pump inhibitors are available in the market but they are showing many side effects. Medicinal plants and their products are considered to less side effects and more efficacy when compared to synthetic drugs. Many medicinal plants and natural analogues showed prominent anti-ulcer and gastro-protective activities. Based on this literature review the plant *Gymnema sylvestre* was selected for screening antiulcer activity. The present results indicate that the Gastroprotective effects of the Hydroalcoholic extract of *Gymnemaa sylvestre* in the experimental ulcers induced by NSAID could be related to its significant reduction in lesion index observed in ulcer-induced animals treated with HAGS compared to disease-control rats. A significant increase was observed in pH, NP-SH, GSH, and CAT with a significant decrease in the volume of gastric juice, free and total acidity, acid output and LPO levels activities in HAGS-treated rats compared to disease-control rats. Histological studies confirmed the gastroprotective activity of the Hydroalcoholic extract of *Gymnemaa sylvestre.*

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AUTHORS CONTRIBUTIONS

The conception and formulation of the research methodology were done by the first author who was also responsible for the execution

of the experiments and drafting of the manuscript under the supervision of the second author. The other three co-authors were jointly involved in the design of the work, supervision, conducting of a literature search and writing of the manuscript.

CONFLICT OF INTERESTS

The author declares that they have no conflict of interest regarding the publication of this paper.

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