Section A-Research paper

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Evaluation of the antiulcer activity of hydroalcoholic extract of *Curcuma longa* L. and *Glycyrrhiza glabra* L. with vitamin M & B_{12} on albino rats

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Abstract

Gastric ulceration is one of the common GIT troubles. Numerous orthodox medicines are practiced worldwide for its treatment, but prolonged use is associated with several adverse effects. Today doctors have shown a growing interest in herbal therapies due to fewer side effects. The present study investigates the antiulcer activity of hydroethanolic extracts of *Curcuma longa* L. (CE) and *Glycyrrhiza glabra* L. (GE) with Vitamin M & B_{12} on albino ratsagainst indomethacin-induced gastric ulcer model. Animals were divided into 11 different groups (n=6) i.e. normal control (I), negative control (II), vehicle control (III), positive control (IV), and treated groups (V-XI). Doses were prepared as suspension in 1% w/v CMC in dH_2O. Gastric ulceration was induced via indomethacin suspension (20 mg/kg, p.o.) in II to XI besides I group. Ranitidine (20 mg/kg, p.o.) was used as a reference anti-ulcer drug as per prior reports and literature reviews. Suspensions of single-dose/day were administered to animal groups IV to XI for 15 days via oral gavage. Stomach was instantly removed after animal sacrifice. Gross lesion and histological examinations were carried out. Mean ulcer score, ulcer index, and percentage of ulcer inhibition were tabulated for the II-XI group. Gastric contents were also assessed to find total acid output, pepsin activity, and mucin contents. The obtained results revealed that oral doses of CE and GE markedly lowered ulcer index as compared to control groups. The extracts also noticeably decreased the total acid output and pepsin activity in treated groups. Pre-treatment's of animals with 2 mg/kg of vitamin M and 50 µg/kg of vitamin B_{12} prevented ulcer formation. These findings demonstrated that hydroethanolic CE and GE extracts as well as Vitamin M & B_{12} also exhibiting marked gastric ulcer protection.

Keywords: Antiulcer, Indomethacin induced ulcer model, Ranitidine, *Curcuma longa* L., *Glycyrrhiza glabra* L.

INTRODUCTION

Gastric ulcer is one of the prevalent disorders of stomach. It manifests itself as an erosion/sore in the lining of gastric mucosa via exogenous and endogenous factors i.e. gastric acid and /pepsin, mental stress, noxious agents, NSAIDs, bacterial infection, smoking and excessive alcoholic consumption **[1, 2, 3].** The above factor/s may elevate lipid per oxidation and formation of ROS in the gastric mucosal tissues. Elevated levels of these free radicals harm the gastric tissues that can cause abdominal inflammation, bleeding and sometimes even to death **[4, 5].** Several synthetic drugs as PPIs, antacids, H-2 receptor antagonists, anticholinergics are medicinally used for the ulcer treatment **[6, 7].** But prolonged usage of these drugs is associated with undesirable/adverse effects. Today, there is an increasing need of safe and effective medicine for the treatment and prevention of gastric ulcer. In recent decades, the safety and efficacy profile of the herbs with respect to their herbal formulations has been widely assessed by many researchers across the globe. Approximately 80% of Asian population is relied on herbal medicine for some aspects of primary health care **[8]**. Majority of herbal practitioners believe that herbal drugs maintain the state of natural balance of body so that body can heal itself **[9]**. Several previous exploration reports and safety records have also indicated that Curcuma and Glycyrrhiza herbs are quite safe in experimental research explored utilizing numerous animal models **[10, 11]**. Moreover, vitamin M helps in RBC production at cellular level. Further it is essential for cell replication, DNA synthesis and tissue repair. Vitamin M also plays an important role in the chemoprevention of gastric carcinogenesis by enhancing gastric epithelial apoptosis in patients with premalignant lesions. However, it is not fully understood whether consuming recommended or higher amounts of Vitamin M from foods or in supplements can lower the risk of gastric ulcer or gastric carcinogenesis **[12, 13]**. Also, the scarcity of Vitamin B_{12} creates irritation and inflammation to stomach due to malfunctioning of stomach cells that may stop the production of IFs **[14, 15]**. The present study was carried out to investigate the gastroprotective potential of hydroethanolic extract of *Curcuma longa L, Glycyrrhiza glabra L*, vitamin M and vitamin B_{12} against gastric mucosal lesions induced by indomethacin on albino rats. Ranitidine drug has been utilized here as reference drug for comparing anti-ulcer parameters.

MATERIALS AND METHODS

Drugs

Ranitidine and indomethacin were purchased from Sigma Aldrich, India. These drugs were dissolved in 1% w/v CMC and administered orally to the rats according to the recommendations made by Abdulla *et al.* (2010) with slight modification **[16].**

1% w/v (CMC)

Pure CMC (1.00 g) powder was mixed with distilled water (100 mL, Thermo Fischer) and it was further stirred to form clear solution **[17].**

Chemicals and Instruments

Soxhlet apparatus (Singhla scientific industries), Eppendorf tubes (Alkon), Oral gavage needle (16 G x 3 mm ball point, Orchid Scientific; India), plastic syringe (Alkon), petroleum ether AR (Rankem), carboxy methyl cellulose (Sigma-Aldrich), hematoxylin (H) and eosin (E) dyes (Sigma-Aldrich). Other analytical grades chemicals for the above study were provided by store of the DOPS, Kumaun University, Nainital, Uttarakhand.

Plant material collection and authentication

Dried roots of Curcuma longa and stolons of Glycyrrhiza glabra were procured from local market of Bijnor, Uttar Pradesh, India. These drugs were identified and authenticated by Senior scientists- **Dr. Sunita Garg**, CSIR-NISCAIR and **Mr. R.S. Jayasomu**, RHMD,CSIR-NISCAIR, India.

Preparation of hydroethanolic herbal extract

Procured crude drug materials were properly cleaned and washed separately with deionised water. After proper washing, each raw material was sliced and air dried in the sun but under the shade for 07 days and again dried at 50^0C in hot air oven for 6 hours. Each dried raw material was cut down in number of small pieces and powdered separately by hand grinding mill (Kalsi). The resulting powdered material was screened through 40 mesh size sieves to obtain powdered sample of uniform size. Weighed quantity of powder drug material (i.e. 100 gm for each drug) was defatted with petroleum ether; after then it was extracted separately with 500 ml of hydroethanolic solvent at room temperature by using Soxhlet apparatus for 48 hours to 72 hrs. Each resultant extract was filtered separately and concentrated in a rotary evaporator (Wkie Lab) under reduced pressure to obtain a thick and dried semi solid brown mass. The dried ethanolic mass was put into the mortar and grinded again with pestle to convert it into powdered form. The dried powdered extracts were properly packed and stored in deep freezer maintained at −20°C **[18, 19].**

Experimental animals

Healthy Wistar albino male rats weighing b/w 200-250 g were used for the present study. All the animals were acclimatized in animal house of DOPS, Kumaun University, Nainital, Uttarakhand, India. These animals were separated randomly and housed into polyacrylic cages (n=6) with flat bottom to limit their mobility and prevent coprophagy. Animals were housed at ambient temperature (23 ± 2^0C), relative humidity (55±15%) and 12/12-hour light and dark conditions for one week prior to start of experiment. Animals were fed standard diet and water *ad libitum.* The experimental protocol was approved by IAEC (Approval Number- KUDOPS/103) as per CPCSEA, New Delhi, Government of India. Animals were fasted for 24 hours but provided water *ad libitum* up to 2 hours prior to experiment [**20, 21].**

Acute toxicity studies

Acute toxicity study was performed at the limit test dose of 2000 mg/kg hydroethanolic extract in 1% w/v CMC suspension as per AOT-425, OECD guidelines. Three healthy adult male albino rats (200-250 g) were randomly grouped and housed into polyacrylic cage. After being fasted for 2 hours, suspension of 2000 mg/kg herbal extract in 1% w/v CMC polymer was administered perorally in albino rat. After then, these animals were observed

continuously for 2 hours for behavioural/autonomic profiles and for any other sign of toxicity or mortality up to a period of 7 days **[22].**

Animal grouping, dosing and examination of anti-ulcer activity

The experimental protocols were carried out as per the method of Srikanta *et al*., 2007 & Morimoto *et al*., 1991 with slight modification. Healthy albino Wistar male rats were divided into 11 animal groups with 6 rats in each group.

Group-I – Normal control [dH_2O, 1ml/100 gm, p.o.]

Group-II- Ulcerated control (Indomethacin-induced) [Negative control, 20 mg/kg in 1% w/v CMC, p.o.],

Group-III- Vehicle control [1% w/v CMC in dH_2O, 10 ml/kg, p.o.],

Group-IV - Positive control [ranitidine 20 mg/kg in 1% w/v CMC, p.o.],

Group-V – Vitamin M treated [2 mg/kg of vitamin M in 1% w/v CMC, p.o.],

Group-VI- Vitamin B_{12} treated [50 µg/kg of vitamin B_{12} in 1% w/v CMC, p.o.],

Group-VII- Treated with co-administration of vitamin M plus vitamin B_{12} [2 mg/kg of vitamin M and 50 µg/kg of vitamin B_{12} in 1% w/v CMC, p.o.],

Group-VIII- Treated with CE-200 [200 mg/kg CE in 1% w/v CMC, p.o.],

Group-IX- Treated with CE-400 [400 mg/kg CE in 1% w/v CMC, p.o.],

Group-X- Treated with GE-200 [200 mg/kg GE in 1% w/v CMC, p.o.],

Group-XI- Treated with GE-400 [400 mg/kg GE in 1% w/v CMC, p.o.],

All groups were received single dose per day for seven days via oral gavage. The drug dose of different groups was formulated as suspension via dissolving in 1% w/v CMC. The selection of dose was based on previous literature and reports which demonstrated ameliorative and marked actions in animal models **[23, 24].**

On 7th day, 6 h after the last dose of the medication, the animals were euthanized byketamine-xylazine anaesthesia and their abdomens were immediately dissected out. These stomachs were removed from abdomen and its gastric contents were drained into graduated centrifuge tube. The stomachs were incised and opened along the greater curvature. Stomachs were gently rinsed with distilled water to eliminate remnants of gastric contents and blood clots. Thereafter, it was stretched and flattened on a piece of card board. The inner surface of stomach was examined by a magnifier lens (10x) to see mucosal integrity and occurrence of ulcer. Then number of ulcers, UI and % inhibitions were counted **[25].** Subsequently, gastric mucosal tissues were fixed in 10% buffered formalin for histological studies **[26].**

Table (1) is used for counting of ulcer score on the gastric mucosa **[27].**

Table (1)

Ulcer Score	**Observation parameter**
0	Normal coloration
0.5	Red coloration
1.0	Spot ulceration
1.5	Hemorrhagic streak
2.0	Ulcers/Hemorrhagic streaks $\geq$ 3mm but $\leq$ 5 mm
3.0	Ulcers/Hemorrhagic streaks $>$ 5 mm

Calculation Percentage of inhibition is calculated by the following formula **[28],**

% of inhibition = [(UIC-UIT)/UIC] x 100

Where,

UIC = Ulcer Index of control group

UIT= Ulcer index of treatment group

UI= Ulcer Index that is calculated by the formula **[29],**

UI= A+B+C x 10^{-1}

Where,

A= average number of ulcers per animal (mean ulcer score/animal)

B= Mean severity of ulcer score

C= Percentage of animals with ulcers

Statistical analysis

The various statistical data were expressed as mean ± SD. Graph Pad prism 7.0 is used for statistical analysis of these results. The statistical analysis was carried out using one-way ANOVA followed by Dunnett's test. P values <0.05 were considered as significance.

Histological studies of gastric mucosa during control and different treated groups Histological inspections were performed according to the method formerly explained by Ogihara and Okabe (1993). Autopsy sections were collected from stomach of different groups and preserved in 10 % formalin **[30]**. At autopsy, small pieces of gastric mucosal tissue were embedded in paraffin and sectioned at 5µm in an automated microtome (LEICA RM 225). Disruption of gastric mucosa, regeneration of ulcerated mucosa (ulcer re-epithelialization), tissue arrangements and inflammatory exudates were observed under the light microscope (Nikon E 200). Hematoxylin and eosin dyes were used for staining the gastric tissues [**31]**.

Biochemical analysis of gastric contents

Gastric contents from each experimental animal were centrifuged at 1000 rpm for ten minutes to eliminate any solid debris and then pH and volume of the supernatant was measured. The supernatant fluid was then analyzed for total acid output, pepsin activity, and mucin content. The above findings were expressed as mean± SEM [**32-34]**.

RESULTS

Percentage yield of herbal extract

The yield of the dried hydroethanolic CE and GE were found to be 11.87 % w/w and 10.45% w/w respectively.

Acute oral toxicity study Acute oral toxicity study as per the AOT-425 of OECD guideline, clearly indicated that herbal extract of GE and CE caused no mortality at the peroral dose of 2000 mg/kg within the first 24 hours and for the next 7 days. At each 2 hours, physical and behavioural observations of the experimental albino rats also revealed no visible signs and symptoms of acute oral toxicity.

Effects of test substances on the values of UI and % of ulcer inhibition parameters

Table (2) Effects of CE, GE, Vit. M and Vit. B_{12} on ulcer index & percentage of ulcer inhibition in indomethacin ulcerated albino rats (n=6, mean± SD

Animal group	Dose	No. of animals	Average no. of ulcers per animal (A)±SD	Mean Severity of ulcer score (B) ±SD	% of animals with ulcers (C)	Ulcer index (UI)	% of Inhibition
I	-	6	-	-	-	-	-
II	20 mg/kg, p.o.	6	9.750±0.274	7.417±0.861	100 (6/6)	27.167±1.362	0
III	10 ml/kg, p.o.	6	9.167±0.258	7.416±0.492	100 (6/6)	26.583±0.988	2.15
IV	20 mg/kg, p.o.	6	4.750±5.203	3.583±3.930	50 (3/6)	13.333±4.438	50.92
V	2 mg/kg, p.o.	6	9.083±4.454	6.917±3.397	83.33 (5/6)	24.333±3.942	10.43
VI	50 µg/kg, p.o.	6	8.917±6.909	6.417±4.974	66.67 (4/6)	22.001±5.886	19.02
VII	2 mg/kg, p.o.(Vit.M); 50 µg/kg, p.o. (Vit. B_{12});	6	8.250±6.393	6.250±4.845	66.67 (4/6)	21.167±5.508	22.09
VIII	200 mg/kg C.E. in 1% w/v vehicle, p.o.	6	5.250±4.071	4.333±3.357	66.67 (4/6)	16.250±3.589	40.18
IX	400 mg/kg C.E. in 1% w/v vehicle, p.o.	6	4.916±3.813	4.083±3.169	66.67 (4/6)	15.667±3.371	42.33
X	200 mg/kg G.E. in 1% w/v vehicle, p.o.	6	5.917±4.587	4.833±3.751	66.67 (4/6)	17.417±4.035	35.89
XI	400 mg/kg G.E. in 1% w/v vehicle, p.o.	6	5.583±4.329	4.667±3.615	66.67 (4/6)	16.917±3.832	37.73

These results clearly showed that peroral administration of indomethacin (20 mg/kg in 1% w/v CMC suspension) has caused gastric erosion in experimental animals. Pre-treatment with hydroethanolic CE and GE, vitamin M and B_{12} has lowered the value of gastric ulceration in albino rats [Table (2)]. CE-200 and CE -400 pre-treated group have reduced the significant gastric ulceration as compared to negative control group($p<0.05$). GE at the dose of 200 mg/kg (Group X) and 400 mg/kg (Group XI) has also reported significant ulcer protection but less as comparison to group VIII and Group IX ($p<0.05$). Each extract at a dose of 400 mg/kg has shown meager elevation in the values of the percentage of ulcer inhibition as compared to the dose of 200 mg/kg (fig.1). Group III (vehicle control,10 ml/kg CMC p.o.) has showed negligible change in the values of UI and % of ulcer inhibition. Group IV (positive control; ranitidine 20 mg/kg in a 1% w/v CMC vehicle) has shown a reduction in UI values when compared to Group II (negative control). Approximately 51% ulcer inhibition has been reported in positive control group. Group V (Vit M; 2 mg/kg) and VI (Vit. B_{12}; 50 µg/kg) have shown 10.43% and 19.02% of ulcer protection respectively. The combined dose of Vit M and Vit B_{12} (Group VII) revealed 22.09% ulcer inhibition in comparison to Group II. The CE-200 and CE-400 treated groups have shown 40.18% and 42.33% of ulcer inhibition. Similarly, the GE-200 and GE-400 treated groups have shown 35.89% and 37.73% of ulcer inhibition, respectively, when compared to group II [Table (2)].

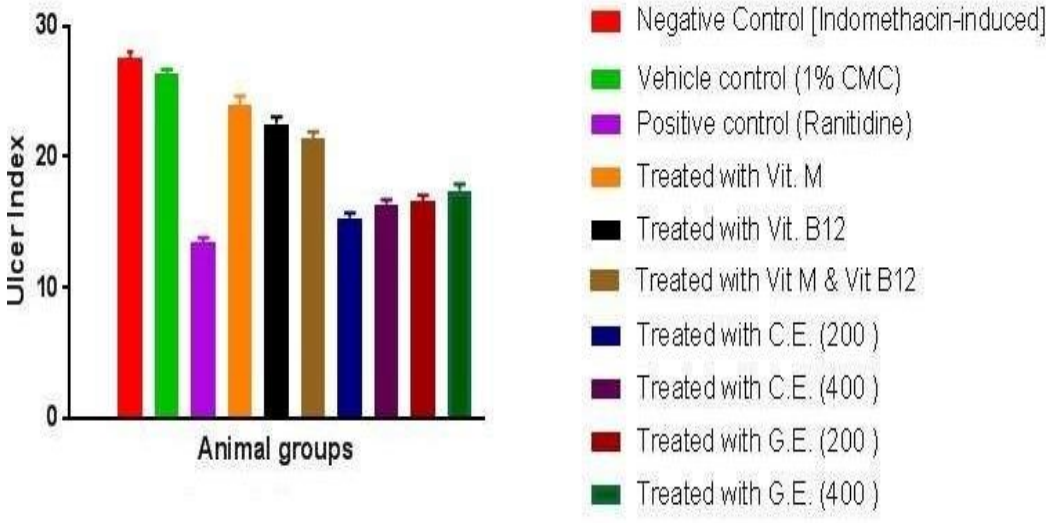
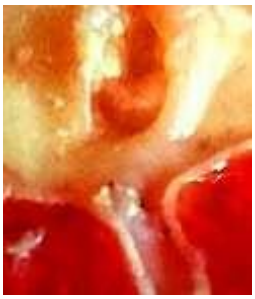
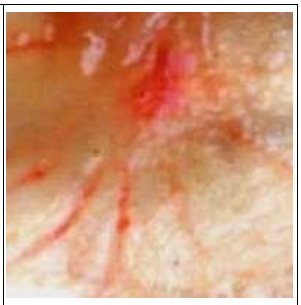
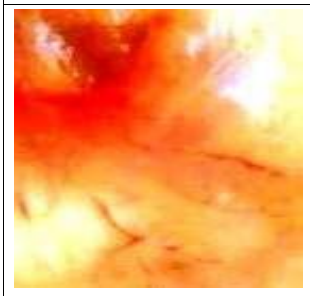
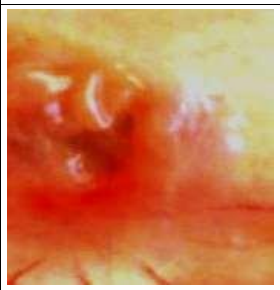
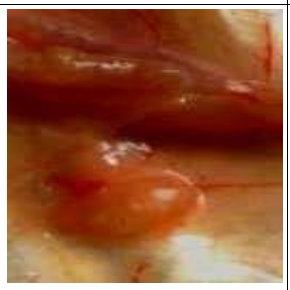
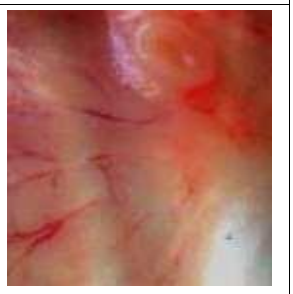


Fig. 1: Effects of CE, GE, Vit. M and Vit. B_{12} on ulcer index & percentage of ulcer inhibition in indomethacin ulcerated albino rats (n=6, mean± SD) with P values <0.05 [one-way ANOVA followed by Dunnett's test, Graph Pad Prism 7.0]

			
a) Group I (Normal gastric mucosa)	b) Group II (Ulcerated mucosa)	c) Group III (Vehicle control)	d) Group IV (Positive control)
			
e) Group V (Vit. M treated)	e) Group VI (Vit. B_{12} treated)	f) Group VII (Treated with Vit. M & B_{12})	g) Group VIII (Treated with CE -200)

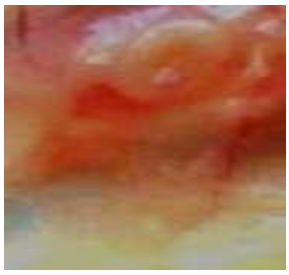
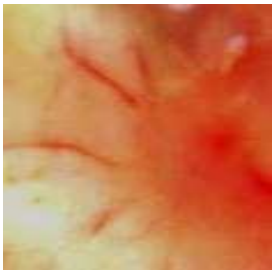
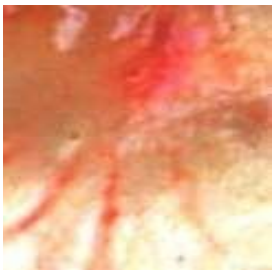
		
h) Group IX (Treated with CE -400)	i) Group X (Treated with GE- 200)	j) Group XI (Treated with GE - 400)

Fig. 2: Observation images of the inner surface of the stomach in different animal groups viewed from a 10X magnifier lens.

Histological evaluation of gastric lesions

Group I: Majority of rats in normal control group exhibited no disruption to gastric mucosal epithelium with neither oedema nor leucocytes infiltration of gastric tissues. Well defined oxyntic cells were also prominently observed on gastric mucosal epithelium. White double headed arrow in image (a) showed the presence of normal surface mucous epithelium (SME).

Group II: Majority of rats in ulcerated control group (indomethacin-induced) exhibited extensive gastric mucosal erosion (ulcers) with oedema and leucocytes infiltration. White double headed arrow showed sub-mucosal oedema and sky-blue arrows showing sub-mucosal leukocyte infiltration in rat; while white single headed arrow indicated mucosal lesion [image (b)].

Group III: Majority of rats in Group III (**Vehicle control)** exhibited nominal reduction in total ulcerated area. Black arrow showed the presence of infiltration in gastric mucosal layer and green arrow indicated dilation of gastric lumina. While red double headed arrow showed the slight increment in number of peptic cells [image (c)].

Group IV: Histology of ranitidine-treated group (**Positive control**) showed a marked improvement in healing of surface mucosal erosions. White double headed arrow in image of this group exhibited a marked reduction in sub-mucosal oedema, while green arrow indicated the contraction of gastric lumina [image (d)].

Group V: Majority of rats of **Vitamin M treated group** didn't have enough protection towards the gastric mucosal layer as compared to indomethacin group. Each rat of this group showed the presence of mucosal erosions (ulcers) and leucocytes infiltration of gastric tissues [image (e)].

Group VI: Albino rats of **Vitamin B_{12} treated group** had comparatively better protection of the gastric mucosal layer that was clearly observed by the microscope and showed a marked reduction in ulcerated area, reduced or absent of sub-mucosal oedema and leucocytic infiltration [image (f)].

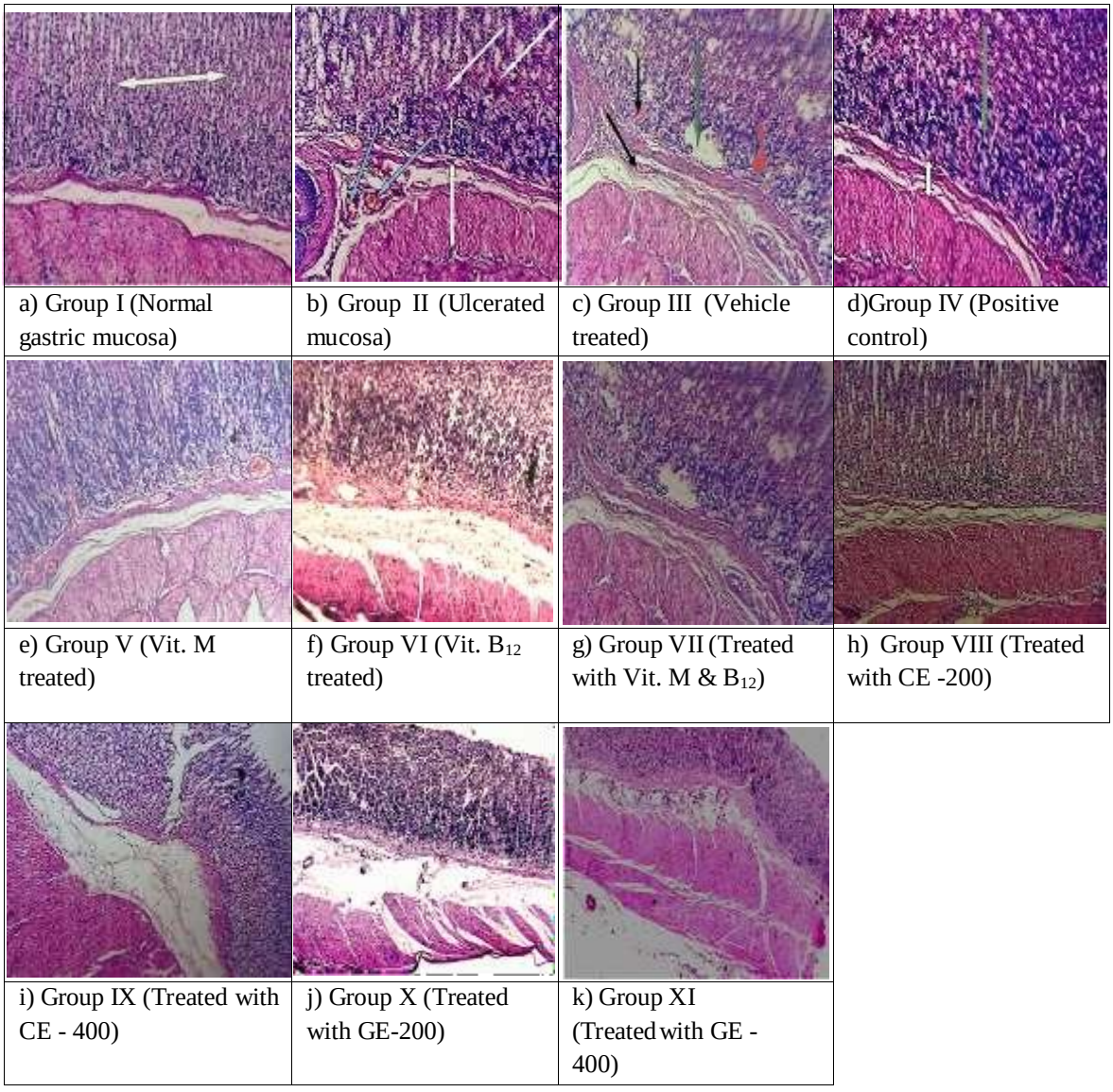
Group VII: This group was designed for inspecting the summative effect of Vit. M & Vit. B_{12} on ulcerated mucosal layers of albino rats. The combined effect revealed a marked synergistic and cytoprotective action towards injured mucosal area. Majority of albino rats of this group experienced a meaningful reduction in gastric mucosal erosion and in the values of

ulcer index as compared to ulcerated control group [image (g)].

Group VIII: One-hour prior treatment with CE-200 before administering indomethacin drug marked a statistically significant reduction in mean gastric erosion score. Surface mucosal disruptions and abnormalities are less observed. Submucosal oedema is reduced or absent when compared to the ulcerated control. [image (h)].

Group IX: One-hour prior treatment with CE-400 [i.e., a two-fold elevated dose of CE] before administering the indomethacin drug revealed mild reduction for the mean gastric erosion score as compared to group VIII. Minor disruptions were seen in histological images of the gastric mucosal layer. Submucosal oedema and leukocytes infiltration are rarely observed in this group of albino rats [image (i)].

Fig. 3: Histological sections of the gastric mucosa in albino rat from Group I to XI (H& Estain, 10 X magnifications).



a) Group I (Normal gastric mucosa)	b) Group II (Ulcerated mucosa)	c) Group III (Vehicle treated)	d)Group IV (Positive control)
e) Group V (Vit. M treated)	f) Group VI (Vit. B_{12} treated)	g) Group VII (Treated with Vit. M & B_{12})	h) Group VIII (Treated with CE -200)
i) Group IX (Treated with CE - 400)	j) Group X (Treated with GE-200)	k) Group XI (Treated with GE - 400)	

Group X: One-hour prior treatment with GE-200 before administering indomethacin drug marked a statistically meaningful lowering of mean gastric ulcer score. Surface mucosal disruptions, inflammatory and broken gastric mucosal barriers are rarely seen. Submucosal oedema is reduced or absent when compared to the ulcerated control [image (j)].

Group XI: One-hour prior treatment with GE-400 [i.e., a two-fold elevated dose of GE] before administering the indomethacin drug revealed mild reduction for the mean gastric erosion score as compared to group X. Minor disruptions were seen on gastric mucosal layer. Submucosal oedema and leukocytes infiltration are rarely observed in this group of albino rats [image (k)].

Results of biochemical analysis of gastric contents

The effect of indomethacin, vehicle, ranitidine, Vit. M, Vit. B_{12}, GE and CE at specific dose levels on different component of gastric fluid are listed in table (3). Rats pre-treated with Vit. M plus Vit. B_{12} lowers the gastric juice volume (ml), total acid output (mEq/3hr), and pepsin activity (µg/ml) up to 3.942±0.022, 134.77±1.158 and 252.668±0.827 respectively as compared to ulcerated control.

Table (3) Total acid output, pepsin activity and mucin content of gastric juice in Group (I-XI)

Animal group	Gastric juice volume (ml) ±SEM	Gastric pH ±SEM	Total acid output (mEq/3hr)±SEM	Pepsin activity (µg/ml tyrosine)±SEM	Mucin content(µg/ml hexose)±SEM
I	3.443±0.019	3.893±0.043	57.898±2.621	112.053±1.089	396.247±0.358
II	4.222±0.020	2.395±0.038	172.105±2.151	302.110±1.160	226.445±0.510
III	4.193±0.024	2.462±0.013	170.085±2.564	298.348±0.654	356.140±0.716
IV	2.885±0.016	3.205±0.070	82.677±2.412	167.137±0.920	338.421±0.468
V	4.108±0.019	2.485±0.052	163.672±1.931	280.228±0.964	362.852±0.455
VI	4.163±0.008	2.500±0.032	161.55±1.672	274.452 ±1.362	368.057±0.440
VII	3.942±0.022	2.525±0.017	134.77±1.158	252.668±0.827	370.935±0.289
VIII	3.692±0.024	3.725±0.019	88.102±1.664	172.558±0.413	390.432±0.871
IX	3.827±0.028	3.508±0.012	87.085±1.279	170.992±0.908	391.118±0.687
X	3.975±0.012	3.780±0.009	92.822±0.916	175.050±0.489	386.997±0.790
XI	4.097±0.027	3.620±0.020	90.490±0.812	173.603±0.262	388.038±0.604

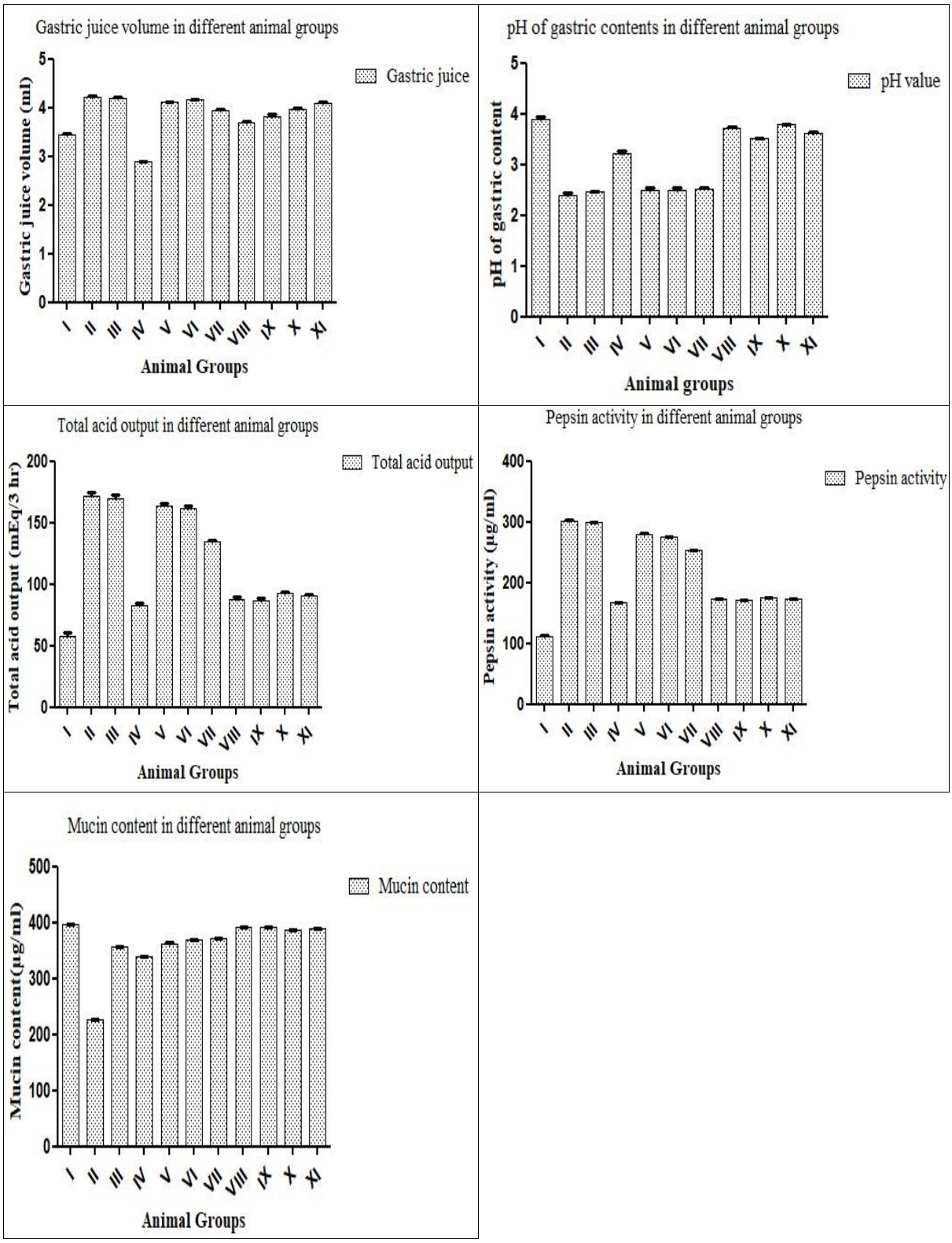


Fig. 4 : Analysis of gastric juice volume, pH, total acid out put, pepsin activity and mucin contents in gastric fluids of different Control (i.e. Group **I**- Normal control, Group **II** -Ulcerated control (Indomethacin-induced), Group **III**-Vehicle control, Group **IV**-Positive control (Ranitidine), and **Treated** (i.e. Group **V**- Vit. M treated, Group **VI**- Vitamin B_{12} treated, Group **VII**- Vit. M & B_{12}

treated,Group **VIII**- CE-200 treated, Group **IX**- CE-400 treated, Group **X**- GE-200 treated, Group **XI**- GE-400 treated) animal groups [n=6 (albino rats) mean± SEM with P values <0.05, Graph Pad Prism 7.0].

Peroral administration of CE-200 (i.e. 200 mg/kg) & CE-400 (i.e. 400 mg/kg) with 1% CMC vehicle to albino rat(s) showed 3.692±0.024 & 3.827±0.028 ml (gastric juice volume), 88.102±1.664 & 87.085±1.279 mEq/3hr (total acid output) and 172.558±0.413 & 170.992±0.908 µg/ml (pepsin activity) as compared to ulcerated control. Peroral administered dose of GE-200 (i.e. 200 mg/kg) & GE-400 (i.e. 400 mg/kg) with 1% CMC vehicle to albino rat(s) exhibited 3.975±0.012 & 4.097±0.027 ml (gastric juice volume), 92.822±0.916 & 90.490±0.812 mEq/3hr (total acid output) and 175.050±0.489& 173.603±0.262 µg/ml (pepsin activity) as compared to ulcerated control. Hence, it has been clearly observed that Vitamin M and B_{12} and different doses of CE and GE significantly improved the values of gastric pH and mucin content when compared to the ulcerated control.

DISCUSSION

Most of scientific research preceding already proved that indomethacin (a potent NSAID) is capable of producing injury to the surface of GI mucosa in various experimental models. In the current study, peroral administration of indomethacin (20 mg/kg dose in 1% CMC) caused an elevation in ulcer acuity, number of ulcers, and ulcer index, as well as a decrease in mucin content (approximately 43%) when compared to the normal control group or Group I (distilled H_2O, 1ml/100 gm, p.o.). Photomicrographs of unstained and stained sections [with Hematoxylin (H) & Eosin (E) tissue stain] showed comparatively acute gastric mucosal damage, oedema with leucocytic infiltration in most of the indomethacin induced animals as comparison to Group I. There was a statistically significant difference reported on mean severity of ulcer score b/w negative control [Indomethacin- induced] and other controlled groups ($p < 0.05$). Gastric mucosal toxicity of NSAIDs is mainly believed to their interdiction opposed to cyclooxygenase (COX) enzymes that hindering in the release of prostaglandins (PGEs). The release of PGEs is higher in gastric mucosal secretion and exhibiting crucial role in pathophysiology of GUD. PGE_2 of gastric mucosa and gastric juice provides cytoprotection to gastric mucosal layer via suppression of gastric acid, elevating the release of mucin content and boosting up mucosal barrier resistance that check the reverse-diffusion of gastric juice from gastric lumina to submucosal tissues. Indomethacin (a commonly prescribed NSAIDs) significantly reduces PGE_2 concentration and lipid peroxidation with neutrophil activation that associated with an increase in gastric motility with microvascular permeability. Hyper gastric motility arises as per rhythmic hypercontraction of the gastric mucosa that's mediated via a vagal-cholinergic mechanism. Severe physical stress, overdose or prolonged use of NSAIDs, and Helicobacter pylori infection may cause gastric mucosal disruption. Oxidative damage through reactive oxygen species (i.e., O_2•- and OH•) are currently thought to be one of the potential reasons for the above mucosal disruption. Lipid peroxidation is an important component of OH•-induced oxidative damage to the gastric mucosal membrane, which is often elevated in indomethacin-induced gastric ulceration. As a result, an increase in protein oxidation and a decrease in glutathione levels are reported. Increased OH• generation in mucosal cells induces cell death, or apoptosis, which triggers gastric mucosal ulceration.

Vitamin M at a 2 mg/kg dose also showed gastroprotective action against the lipid peroxidative activity of indomethacin. Folate has significant role in de novo synthesis of nitrogenous bases i.e. purines & thymidine. Folic acid boosts up the DNA stability and apoptosis process that helps in healing of gastric ulcer. Peroral administration of vitamin M in group V showed the elevated level of mucus concentration and superoxide dismutase enzymes (SODs) that ultimately protects the gastric mucosal cells from free radical attacks. The presence of oxidative stress and lipid peroxidation in mucosal tissue samples helps to evaluate MDA (malondialdehyde) concentration. Higher the value of MDA level reflects an increase in the production of free radicals. Uplifting of MDA level acts as marker of oxidative stress and lipid peroxidation in gastric ulcer model in albino rat. It ultimately notifying that Vitamin M suppresses the lipid peroxidation activity of indomethacin in treated groups. Most of the aforementioned literature survey clearly indicated the usage of Vitamin M (2mg/kg dose) for 21 days. Maximum literature search revealed its gastroprotective action as per the antioxidative and antisecretory properties of Vitamin M. But in the current study the above dose was administered perorally for 7 days and didn't shows noticeable change b/w the Vitamin M treated (Group V) and indomethacin-group (Group II). Peroral administration of 20 mg/kg dose of ranitidine for 7 days showed a significant gastroprotective action against the indomethacin induced ulcer (Group II). Ranitidine treated group (Group V) lowers the value of ulcerative Index (UI) which results in an increase in the value of % of inhibition. Statistically significant difference was reported while decreasing ulcer acuity and elevating mucin content b/w ranitidine group and indomethacin group ($p < 0.05$). In this study, it is clearly seen that the group pre-treated with ranitidine (group IV) drug has lower value of ulcer index as compared to indomethacin-induced (group II). The combination of Vitamin M and B_{12} was able to attenuate gastric ulceration induced by indomethacin drug. Lower value of UI was reported in these combination group (i.e. vitamin M & B_{12}) as compared to indomethacin control group. The combination of the above two vitamin resulted with an elevated level of mucin content and exhibited a statistically difference as compared to group II. The combined dose of vitamin M & B_{12} enhances approximately 64 % mucin content as compared to indomethacin control (Group II). Also, a significant change was reported in their mean severity of ulcer score and ulcer index when this group compared to group II ($p < 0.05$).

CONCLUSION

In recent decades, there have been great advancements towards the treatment of GUD patients. Nowadays, multiple therapeutic agents have become available worldwide that provide protection against GUD. In the present study, indomethacin-induced gastric ulcer model in albino rats were employed to mark the gastroprotective activity of the hydroethanolic plant extracts, vitamin M and vitamin B_{12}. The study steadfastly revealed that the hydroethanolic extract of *C. longa* and *G. glabra* have shown significant antiulcer activity which upholds the traditional claim of the above-mentioned plant species. Healing mechanism of gastric mucosal tissues was prominently displayed by the above extracts due to scavenging of free radicals and regulation of gastric mucosal membrane permeability that nullifies the distress of indomethacin drug on the gastric acid release. The research findings clearly exhibited that ethanolic extract of *C. longa* and *G. glabra* provides cytoprotective action by lowering of acid pepsin and increasing the amount of mucus secretion. The combination dose

of vitamin M (2 mg/kg, p.o.) with vitamin B_{12} (50 µg/kg, p.o.) was found to uplift the gastric healing action synergistically. On summing up the above histological and pharmacological findings, it could be concluded that combination of vitamin M plus B_{12} and *C. longa* & *G. glabra* extracts possesses ameliorative gastroprotective activity that may be a milestone towards the better treatment of GUD patients.

Abbreviations GE, *Glycyrrhiza glabra extract;* CE, *Curcuma longa extract;* Vit M, Vitamin M*;* Vit B_{12}, Vitamin B_{12}; GIT, Gastrointestinal tract; GUD, Gastric Ulcer Disease; SEM, Standard Error of Mean; SD, Standard deviation; n, Number of animals; *p.o.*, peroral; *COX*, Cyclooxygenase; MDA, Malondialdehyde; PPI, Proton pump inhibitors; UI, Ulcerative or ulcer Index; NSAIDs, Non-Steroidal Anti-Inflammatory Drugs; mg/kg, milligram/kilogram; µg/kg , microgram/kilogram; H & E, Hematoxylin & Eosin; ANOVA, Analysis of Variance; PGEs, prostaglandins; CMC, Carboxy Methyl Cellulose; GI, gastrointestinal; DNA, Deoxyribonucleic acid; mEq, milliequivalents; dH_2O , Distilled water; hrs, Hours; AOT, Acute Oral Toxicity; OECD, Organization for Economical Co-operation and Development; IAEC, Institutional Animal Ethics Committee; DOPS, Department Of Pharmaceutical Sciences; RHMD, Raw material Herbarium and Museum; NISCAIR, National Institute of Science Communication And Information Resources; CSIR, Council of Scientific and IndustrialResearch; ROS, Reactive Oxygen Species; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; SODs, Super Oxide Dismutase; IFs, Intrinsic factors.

Research Ethics Ethical clearance was attained from IAEC (Approval Number-KUDOPS/103) as perCPCSEA, New Delhi, Government of India.

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