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PHYTOPHARMACOLOGICAL SCREENING JUICE OF *TRITICUM AESTIVUM* AND *CURCUMA LONGA* LEAVES AGAINST INDOMETHACIN INDUCED PEPTIC ULCER

Imran Hussain, Hargovind Garg*, Rajesh Singh Pawar

Department of Pharmacology, Truba Institute of Pharmacy, Karod- Gandhi Nagar Bypass road, Bhopal-462038 (MP)

*Corresponding Author's E mail: **hargovindgarg@gmail.com**

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ABSTRACT

This study investigated the anti-ulcerogenic activity of juice of *Triticum Aestivum* and *Curcuma Longa* leaves against indomethacin-induced peptic ulcer in rats. Thirty-six rats were separated into 6 groups and each groups containing six rats. For 7 days before ulcer induction with indomethacin, group first is normal group received the normal saline 1 ml for 7 days. Group second served as inducer group indomethacin (30 mg/kg) was administered orally. Group third was served as *Triticum aestivum* (1 mL/kg b/w) group administered orally. Fourth group served as *Curcuma longa* (1 mL/kg b w). Fifth group was treated with 1:1mL/kg b/w of *Triticum aestivum*+ *Curcuma longa*. Sixth group was served as Standard (Omeprazole 20 mg/kg b/w). The animals were sacrificed; their stomachs were removed and examined for ulcer index, volume of gastric juice, pH of gastric juice and free acidity. Fifth group combination of *Triticum aestivum* + *Curcuma longa* (1:1 mL/kg b/w) showed the significant result as compared to *Triticum aestivum* and *Curcuma longa* individual dose. Thus, from the present study concluded that the combined juice of *Triticum aestivum* and *Curcuma longa* stimulate the anti-ulcer activity.

Keywords: Anti-ulcerogenic, Indomethacin, Stimulate and Induction.

INTRODUCTION

The principal function of the stomach is to mix the food with acid, mucus and pepsin and then release the resulting chyme, at a controlled rate into the duodenum for the process of absorption. Gastric motility is controlled by both neural and hormonal signals. Nervous control originates from the enteric nervous system as well as the parasympathetic (predominantly vagus nerve) and sympathetic systems. A number of hormones have been shown to influence gastric motility for example, both gastrin and cholecystokinin act to relax the proximal stomach and enhance contractions in the distal stomach. Other functions of the stomach include the secretion of intrinsic factor necessary for the absorption of vitamin B12 [1].

The stomach lies largely in the left hypochondrial region under cover of the lower part of the rib cage. The lower and distal parts of the stomach, however, lie in the epigastric and upper umbilical regions of the abdomen. The stomach is a distensible organ. In the adult, it has an average capacity of 1.5 litres. The stomach is approximately J shaped, although in certain individuals it may lie transversely when it is known as a steer-horn stomach. The size, shape and position of the stomach can vary considerably, depending on the posture of the individual and on the state of fullness of the stomach. The empty stomach appears flattened. It presents anterior and posterior surfaces, which are demarcated from each other by the greater and lesser curvatures (Figure 1). The lesser curvature forms the upper right border of the stomach while the greater curvature forms the lower left border. The stomach has two openings or orifices. The proximal one is termed the cardiac orifice through which the stomach communicates with the oesophagus. The distal orifice is the pyloric orifice through which the stomach communicates with the duodenum. The regions of the stomach adjacent to the cardiac and pyloric openings are known as the cardia and pylorus, respectively. The main parts of the stomach are the fundus, body and pyloric part [2-3]**.** Phytochemical screening of *Triticum aestivum* using various extracts like aqueous, methanol, ethylacetate and chloroform. Phytochemical qualitative analysis of *Triticum aestivum*. The screening analysis of *Triticum aestivum* using various solvents revealed the presence of carbohydrate, protein, alkaloids, tannins, phenols, in the methanolic and aqueous extracts. While the presence of saponins was noted in chloroform extract. The qualitative phytochemical analysis results explored the presence of a wide range of phytochemical constituents which signifies the *Triticum aestivum* as a valuable therapeutic natural source which will serve as an effective herbal option to compact dreadful infectious diseases [4]**.** Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are polyphenols and are responsible for the yellow color of turmeric. Curcumin can exist in at least two tautomeric forms, keto and enol. The enol form is more energetically stable in the solid phase and in solution. Curcumin can be used for boron quantification in the so-called curcumin method. It reacts with boric acid forming a red colored compound, known as rosocyanine. Curcumin is brightly yellow colored and may be used as a food coloring. As a food additive, its E number is E100 [5-8].

MATERIALS AND METHOD

Selection and collection of Plant

Plant and plant parts was selected on the basis of ethno-botanical survey. Pharmacological investigations report and recent investigations were considered in respect of selected Plant. Fresh leaves *Curcuma longa* and *Triticum aestivum* leaves of free from disease were collected from local area.

Antiulcer activity

Acute toxicity study

The acute oral toxicity study was carried out according to OECD 423 guidelines. Four ranges of dose were used for toxicity studies, i.e., 5mg/Kg, 50 mg/Kg, 300mg/Kg, 2000mg/Kg. animals were observed individually for next 48 hours after dosing for the presence of mortality during this period and 24 hours after sample administration. So, present experimental studies the $1/10^{th}$ and $1/5^{th}$ dose of *Rosa indica*was selected *i.e.* no mortality observed.

Preparation of animals

A total number of 36 healthy albino wistar rat aged weeks and weighing 200±40 grams were housed into cages for 15 days. They were housed in a controlled room temperature environment (25± 2^0C) and light with alternate 12-hour light/dark cycles. The animals had free access to rats pellets (Keval sales corporation) and water *ad libitum.* After 15 days of acclimatization, rats were randomly divided into six experimental groups of six rats each groups. This research work approved by Institutional animal ethical committee of PBRI Bhopal. The approval number is PBRI/IAEC/22-10-21/001.

Indomethacin Induced peptic ulcer in Rats

All rats were fasted for 24 h before ulcer induction to ensure theirstomachs are empty. During the fasting period, rats were allowed to havefree access to water only.Rats were divided into six groups each containing 6 animals.Group first is normal group received the saline daily for 7 days. Group second served as inducer group indomethacin (30mg/kg) was administered orally for 7 days. Group third was served as *Triticum aestivum* (1 mL/kg bw) administered orally. Fourth group served as *Curcuma longa* (1 mL/kg bw). Fifth group was treated with*Triticum aestivum* + *Curcuma longa* (1:1 mL/kg bw) daily for 7 days. Sixth group was served as Standard (Omeprazole 20 mg/kg bw).Then the parameters mentioned below were measured [9]**.**

Table 1: Indomethacininduced peptic ulcer

Groups	Treatment groups
Group I	Normal Control (Normal saline)
Group II	Inducer Indomethacin (30 mg/kg bw)
Group III	*Triticum aestivum* (1 mL/kg bw)
Group IV	*Curcuma longa* (1 mL/kg bw)
Group V	*Triticum aestivum* + *Curcuma longa* (1:1mL/kg bw)
Group VI	Standard (Omeprazole 20 mg/kg bw)

Measurement of various parameters

Ulcer index

The following arbitrary scoring system was used to grade the incidence and severity of lesion. The stomachs were then cut along the greater curvature, rinsed with normal saline to remove gastric contents, and examined by using a 10x magnifier lens to assess the formation of ulcers [10]. Numbers of ulcers were counted and then scored by using the Kulkarni method (0 = no ulcer, 0.5 = red coloration, 1 = spot ulcers, 2 = Haemorrhagic streaks, and 3 = Ulcers > 3 but < 5 and 5 = Ulcers > 5)

The ulcer Index and percentage of ulcer inhibition were determined as follows:

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

Where, UN = Average number of ulcers per animal, US = Average of severity score, UP = Percentage of animals with ulcers

Collection of gastric juice

After postoperative period, animal was sacrificed by cervical dislocation and the stomach was dissected out as a whole by passing a ligature at the esophageal end. Gastric content was evacuated into graduated tube by cutting along the greater curvature of the stomach, and was centrifuged at 3000 rpm for 10mins[11]**.**

Volume of gastric juice

The volume of the centrifuged sample was expressed as ml/ 100g body weight.

pH of gastric juice

pH of gastric juice was measured with the help of pH meter.

Determination of free acidity

The phenolphthalein indicator was used. Aliquot of gastric juice was titrated with 0.01N NaOH until pink colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity [12].

Preparation of plant sample

Wheat grass leaf was harvested from local market, Bhopal and was identified by a botanist. The leaves were removed from the stem and washed thoroughly with running water to remove contaminants. The fresh leaves were milled into powder and stored in a plastic container. Tumeric leaf extract in the form of powder, was purchased from local market Bhopal. It was weighed accordingly and administered in aqueous solution.

RESULTS & DISCUSSION

Indomethacin induced peptic ulcer in rats

Effect of the oral treatment with juice of *Triticum aestivum* and *Curcuma longa* on indomethacin induced peptic ulcer in rats.

Table 2: Indomethacin induced peptic ulcer in rats

Treatment Groups	Ulcer Index	
	Mean	SD
Group I- I Normal Control (Saline)	0	0
Group II- Inducer Indomethacin (30 mg/kg bw)	10.8666	0.0516
Group III – *Triticum aestivum* (1 mL/kg bw)	3.3666	0.1211
Group IV – *Curcuma longa* (1 mL/kg bw)	3.45	0.281
Group V– *Triticum aestivum* + *Curcuma longa*(1:1 mL/kg bw)	1.7166	0.2857
Group IV – Standard (Omeprazole 20 mg/kg bw)	1.6833	0.2041

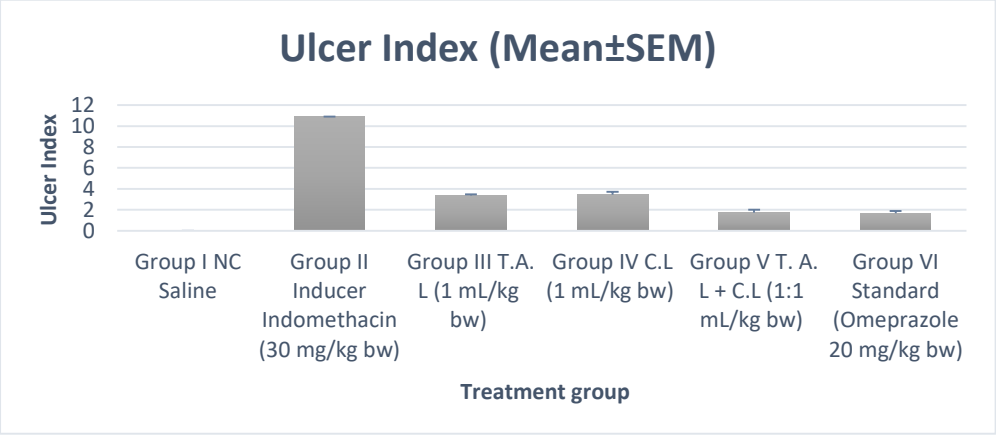


Fig. 1: Bar chart represents ulcer index in indomethacin induced peptic ulcer in rats

Determination of total volume of gastric juice:

Table 3: Observation of total volume of gastric juice

Treatment Groups	Total volume of gastric juice (ml)
Group I- I Normal Control (Saline)	1.6466±0.6288
Group II- Inducer Indomethacin (30 mg/kg bw)	5.8883±0.9783
Group III – *Triticum aestivum* (1 mL/kg bw)	3.8966±1.0525
Group IV – *Curcuma longa* (1 mL/kg bw)	3.9766±1.2119
Group V – *Triticum aestivum* + *Curcuma longa*(1:1 mL/kg bw)	1.9733±0.5268
Group IV – Standard (Omeprazole 20 mg/kg bw)	1.2333±0.5051

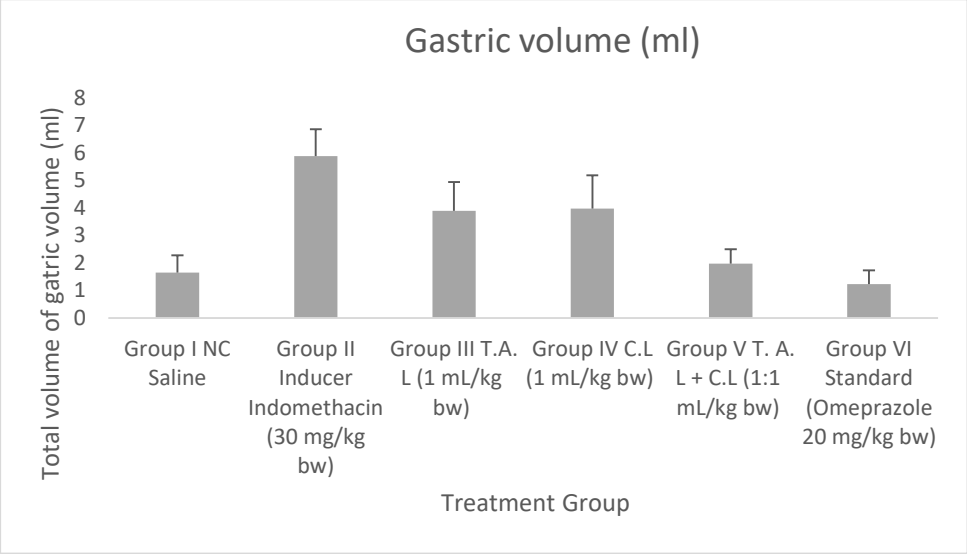


Fig. 2: Bar chart represents total volume of gastric juice in indomethacin induced peptic ulcer in rats

Determination of pH of gastric juice:

Table 4: Observation of pH of gastric juice

Treatment Groups	pH of gastric juice (ml)
Group I- I Normal Control (Saline)	4.035±0.457
Group II- Inducer Indomethacin(30mg/kgbw)	2.4666±0.8564
Group III – *Triticum aestivum* (1 mL/kg bw)	3.2266±1.0497
Group IV – *Curcuma longa* (1 mL/kg bw)	3.3316±0.5977
Group V – *Triticum aestivum*+ *Curcuma longa* (1:1 mL/kg bw)	4.005±0.6091
Group IV – Standard (Omeprazole 20 mg/kg bw)	4.0333±0.8594

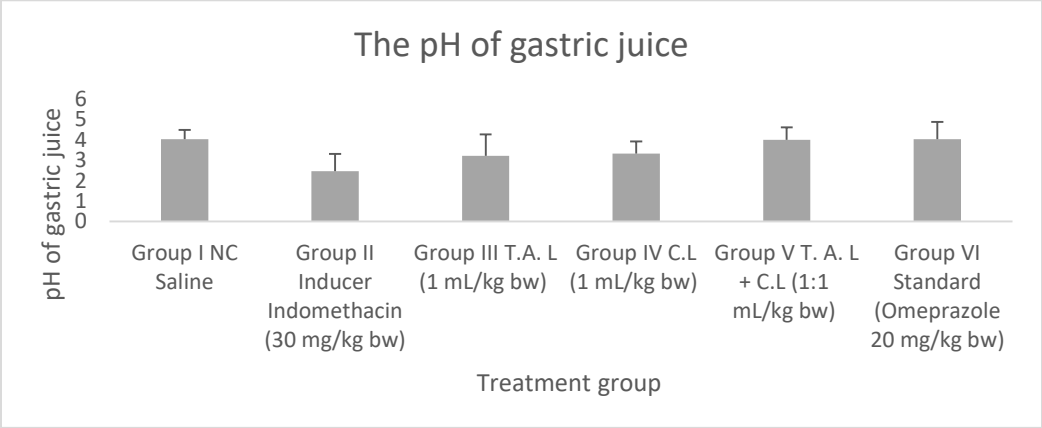


Fig. 3: Bar chart represents pH in indomethacin induced peptic ulcer in rats

Free acidity determination

Table 5: Observation of free acidity in indomethacin induced ulcer peptic in rats

Treatment Groups	Free acidity determination (mE/L)
Group I- I Normal Control (saline)	17.3333±3.2659
Group II- Inducer Indomethacin (30 mg/kg bw)	20.1666±2.8577
Group III – *Triticum aestivum* (1 mL/kg bw)	13.8333±2.3166
Group IV – *Curcuma longa* (1 mL/kg bw)	11.6666±2.6583
Group V – *Triticum aestivum* + *Curcuma longa* (1:1 mL/kg bw)	10.1666±2.1369
Group VI – Standard (Omeprazole 20 mg/kg bw)	10.3333±2.16024

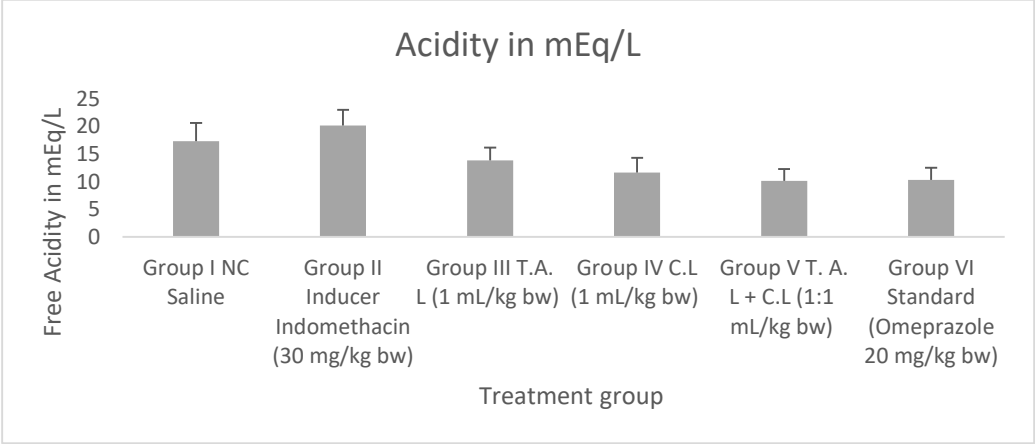


Fig. 4: Bar chart represents free acidity in indomethacin induced peptic ulcer in rats

Histopathology Group I Normal Control (Saline)

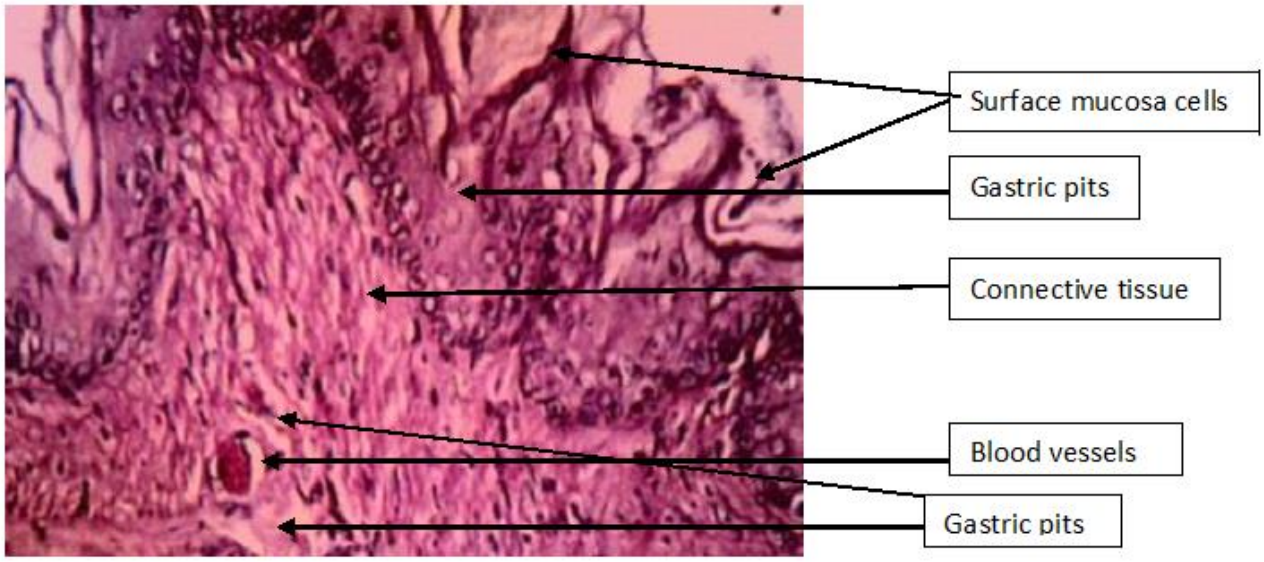


Fig. 5 (a): Photomicrographs of stomach of rats showing surface mucosa cells, gastric pits, connective tissue, blood vessels, gastric pits

Group II Inducer Indomethacin (30 mg/kg bw)

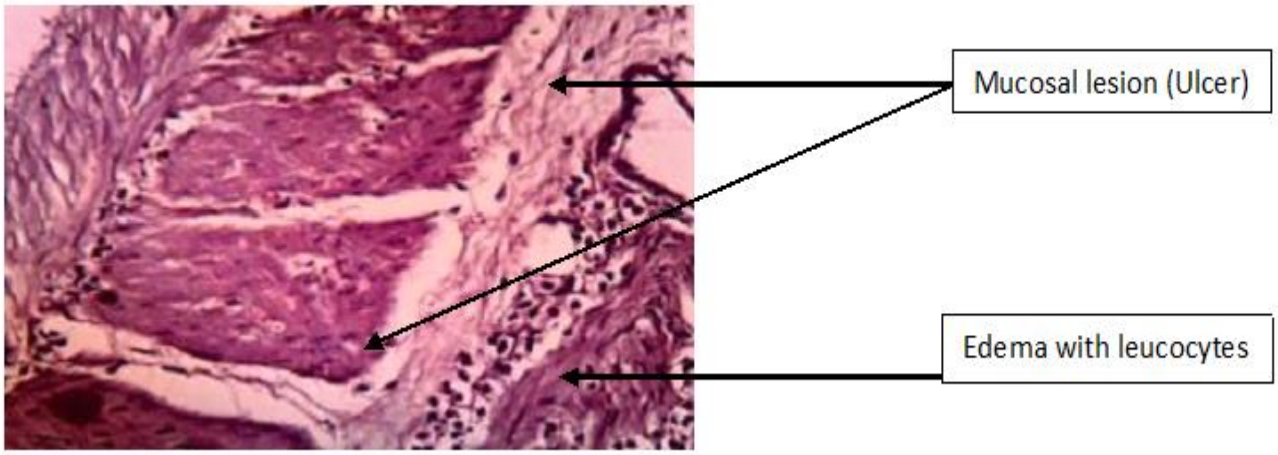


Fig. 5 (b): Photomicrographs of stomach of rats showing mucosal lesion (ulcer) and edema with leucocytes

Group III *Triticum aestivum* (1 mL/kg bw)

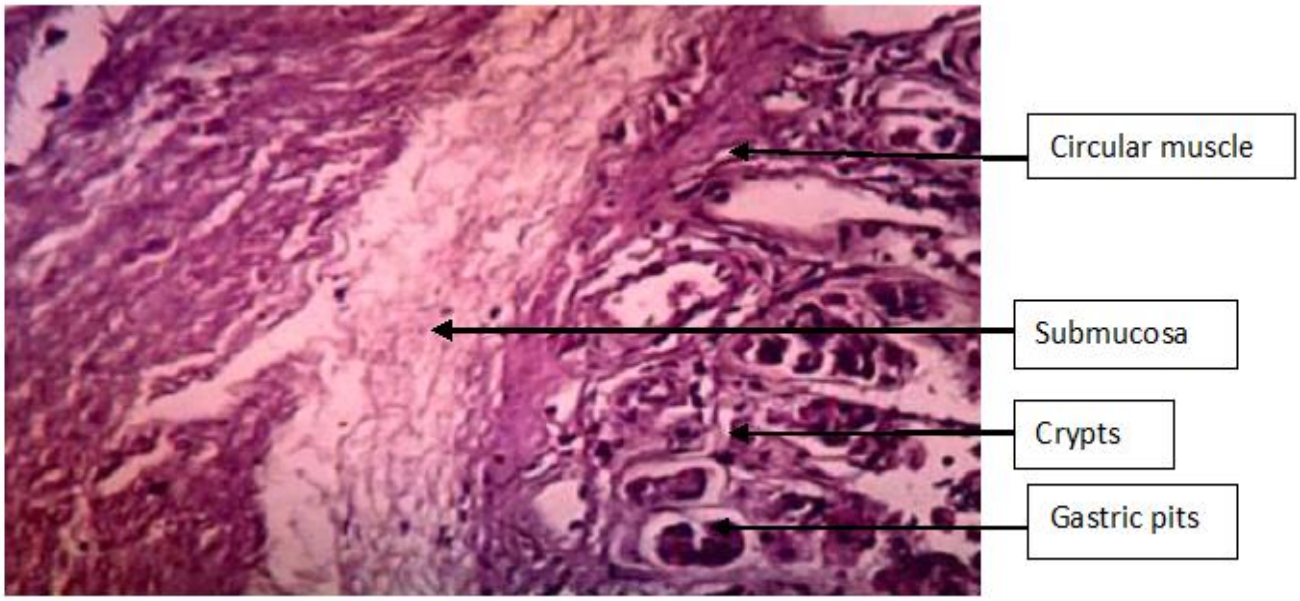


Fig. 5 (c): Photomicrographs of stomach of rats showing circular muscle, submucosa, crypts and gastric pits

Group IV *Curcuma longa* (1 mL/kg bw)

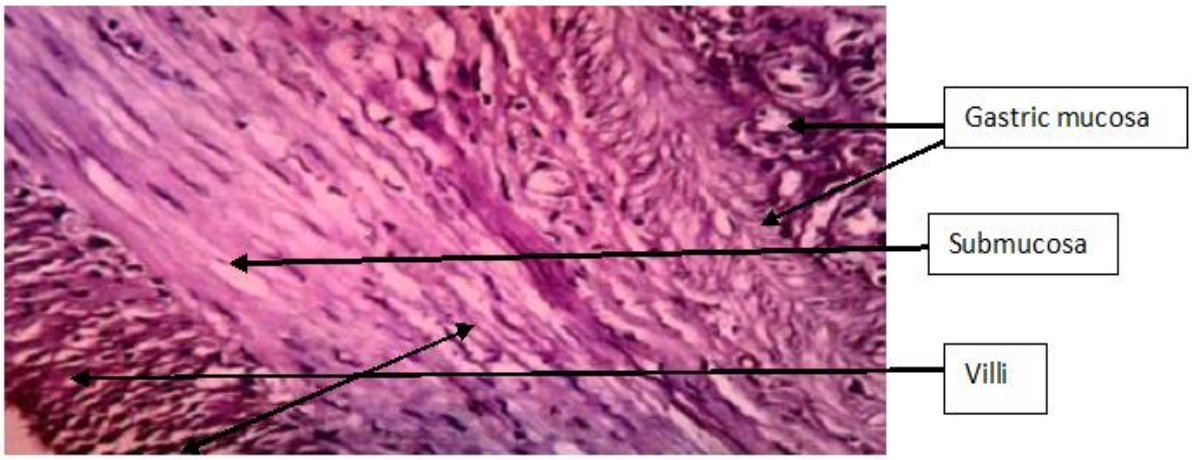


Fig. 5 (d): Photomicrographs of stomach of rats showing gastric mucosa, submucosa and villi

Group V Triticum aestivum + Curcuma longa (1:1 mL/kg bw)

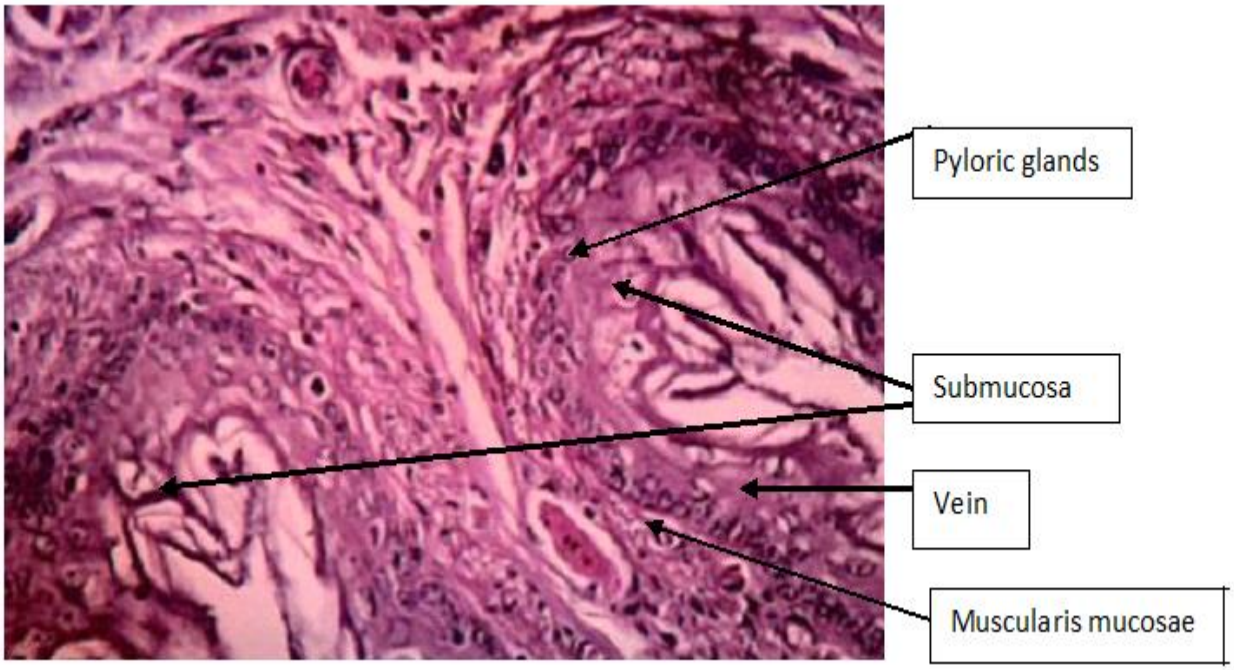


Fig. 5 (e): Photomicrographs of stomach of rats showing pyloric glands, submucosa, vein and muscularis mucosae

Group VI Standard (Omeprazole 20 mg/kg bw)

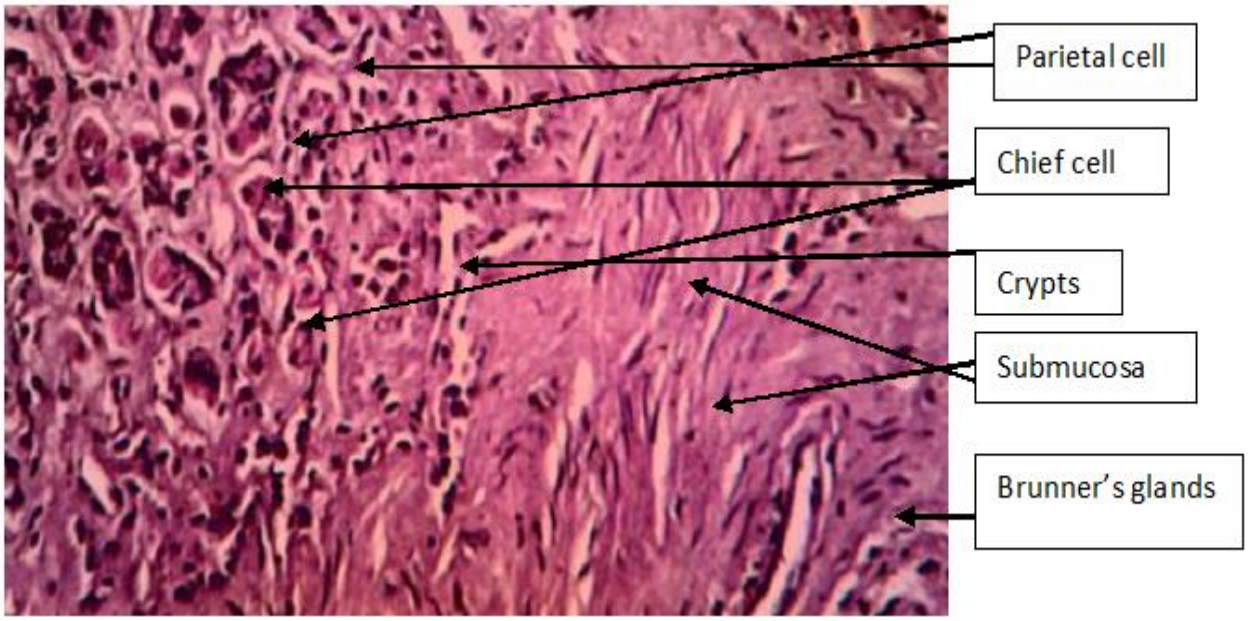


Fig. 5 (f): Photomicrographs of stomach of rats showing parietal cell, chief cell, crypts, submucosa and brunner's glands

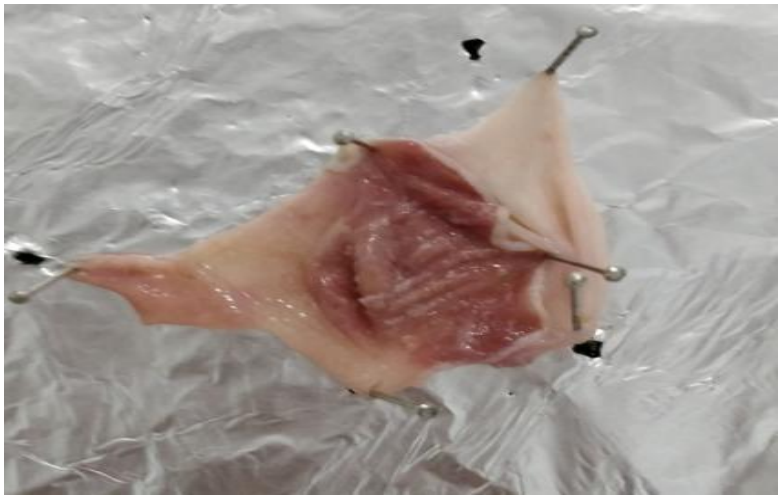


Fig. 6: Group I Normal Control (Saline)

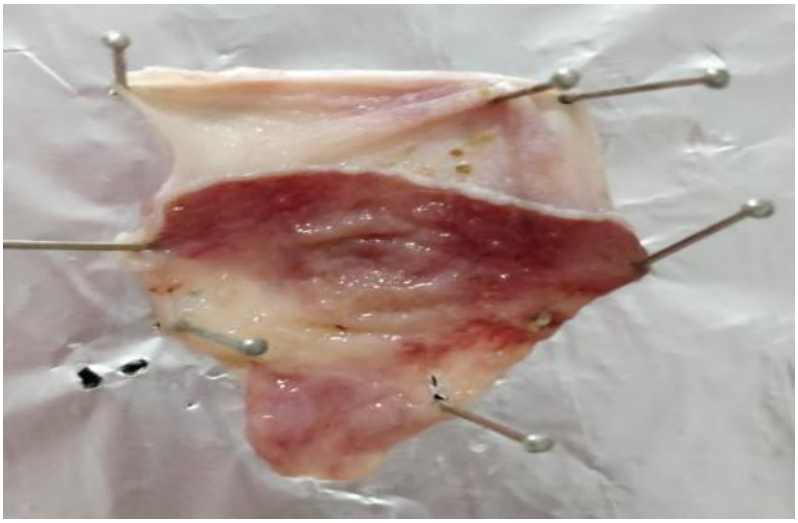


Fig. 7: Group II Inducer Indomethacin (30 mg/kg bw)

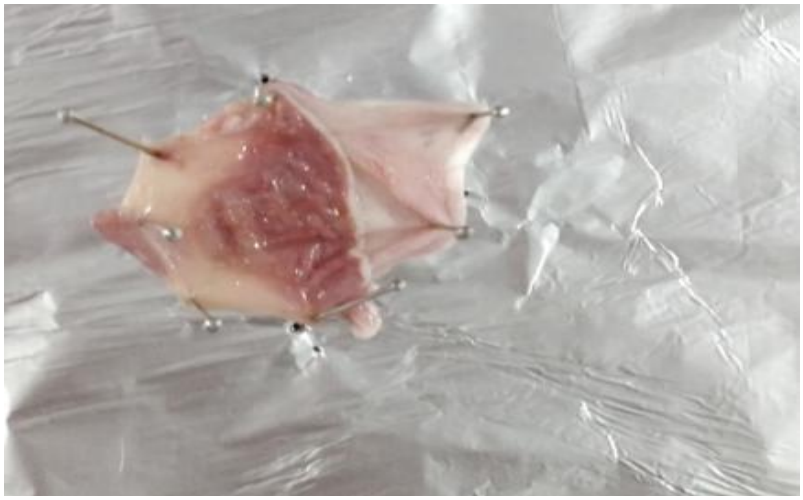


Fig. 8: Group III *Triticum aestivum* (1 mL/kg bw)

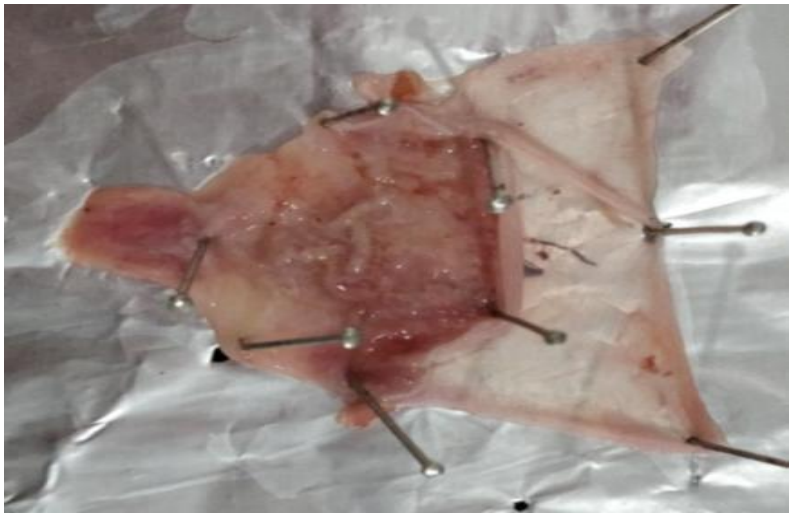


Fig. 9: Group IV *Curcuma longa* (1 mL/kg bw)

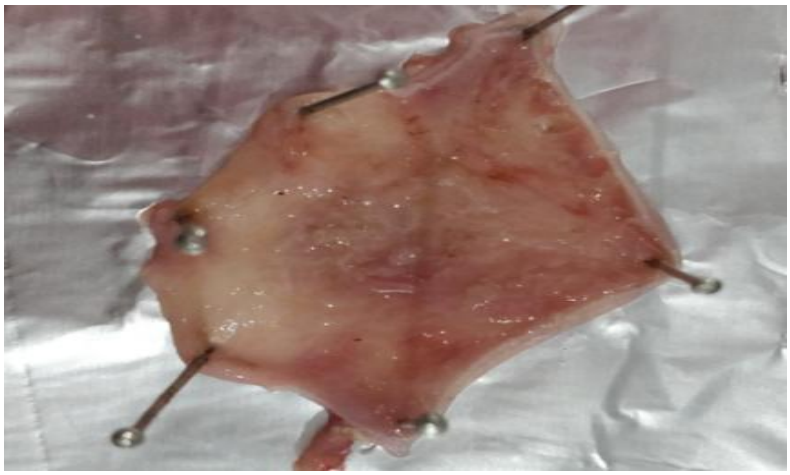
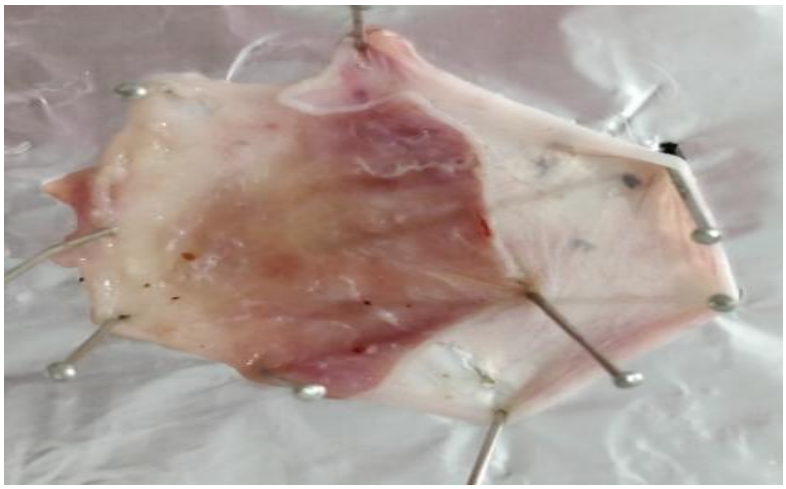


Fig. 10: Group V *Triticum aestivum* + *Curcuma longa* (1:1 mL/kg bw)



Peptic ulceration, considered to be one of the modern age epidemics, has been affecting approximately 10% of world population [12]**.** Earlier study suggested that peptic ulcer is due to an imbalance between acid

and pepsin along with the weakness of the mucosal barrier [13]. Due to these, it is commonly associated with damage of the stomach's mucosal layer, which is simply generated via excess generation of exogenous and endogenous active oxygen and free radicals. Some of the main causes of gastric ulcers include chronic use of alcoholic beverages and anti-inflammatory drugs, as well as stress and Helicobacter pylori infection [13].

The Indomethacin-induced ulcer model was employed for screening anti-ulcerogenic activity because the model shows cytoprotection and gastric acid secretion activities [14]. IND, a nonsteroidal anti-inflammatory drug (NSAID), induced gastroduodenal ulceration via its ability to suppress prostaglandin synthesis [15]. The suppression of prostaglandin, which plays vital role in stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair, results in increase susceptibility to mucosal injury and gastroduodenal ulceration [15].

Curcumin has been defined as the most active component in Curcuma longa and has been shown to have considerable gastroprotective, anti-ulcerogenic and therapeutic effect in gastric ulcer disease. It has been reported to heal peptic ulcer. Observed that the use of wheat grass (*Triticum aestivum*) juice is very effective and safe as a single or adjuvant treatment of active distal Ulcerative colitis (UC). Green juice and fractions from green juice of leaves of *Triticum aestivum*containing water soluble proteins and water soluble organic compounds showed anti- stomach ulcer activity in stressed rats. Since the juice of *Curcuma longa*and *Triticum aestivum* demonstrated significant reduction of the ulcer lesions under experimental models. In this study, several parameters such as ulcer index, volume of gastric juice, pH and free acidity were evaluated in rats.

Six groups of adult albino wistar rats were taken for the study. Rats were divided into six groups each containing 6 animals. Group first is normal group received the saline for 7 days. Group second is indomethacin inducer group (30 mg/kg bw).Group third is*Triticum aestivum* (1 mL/kg bw). Fourth group is*Curcuma longa* (1 mL/kg bw). Group fifith *Triticum aestivum* + Curcuma longa (1:1 mL/kg bw) and Group sixth standard (Omeprazole 20 mg/kg bw). The juice of *Triticum aestivum* and *Curcuma longa*was evaluated by using indomethacin induced peptic ulcer model.Ulcer produced in this model was seen as red sores. The stomachs of rats in the control (saline) group showed higher inductions of gastric ulcers due to increased levels of gastric juice in the rat's stomachs. There was a significant decrease in the measured gastric ulcer index in the stomach of *Triticum aestivum* + *Curcuma longa* (1:1mL/kg bw)treated animals when compared with the Curcuma longa (1 mL/kg bw) treated, *Triticum aestivum* (1 mL/kg bw) and Standard (Omeprazole 20 mg/kg bw) animals. The combination of Curcuma longa and *Triticum aestivum* exhibited antiulcerogenic effect against indomethacin-induced gastric ulcer as the value of ulcer index was observed as 1.7166 in the rats pre-treated for 7 days respectively when compared

with the standard group which showed the value of ulcer index 1.6833 treated with Omeprazole 20 mg/kg bw. The volume of gastric juice was observed as 1.9733ml of *Triticum aestivum* + Curcuma longa (1:1 mL/kg bw) in decreased level as compared to *Triticum aestivum* (1 mL/kg bw) and *Curcuma longa* (1 mL/kg bw) treated group. The pH of gastric juice was observed as 4.005 of *Triticum aestivum* + *Curcuma longa* (1:1 mL/kg bw) treated group and it showed the reduction in acidic pH as compared to *Triticum aestivum* (1 mL/kg bw)showed 3.2266pH and *Curcuma longa* (1 mL/kg bw) treated group showed 3.3316pH.The free acidity was observed as mE/L of *Triticum aestivum* + *Curcuma longa* (1:1 mL/kg bw) treated group 10.1666and it showed the reduction in acidity as compared to *Triticum aestivum* (1 mL/kg bw) showed 13.8333mE/L and *Curcuma longa* (1 mL/kg bw) treated group showed 11.6666mE/L.

CONCLUSION

The plant sample kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders.

The fresh juice of *Triticum aestivum* and *Curcuma longa*were extracted. These bio-active components like carbohydrates, glycosides, flavonoids, alkaloids, triterpenoids and phenolic components are present in the juice of *Triticum aestivum* and *Curcuma longa*reportedly. They exhibit potential effect on anti-ulcer activity. Experimental results have revealed that the combination of *Triticum aestivum* and *Curcuma longa*juice have various degrees of anti-ulcer activity depending upon the dose level and the bioactive components present in it.

Thus, from the present study concluded that the combined juice of *Triticum aestivum* and *Curcuma longa* stimulate the anti-ulcer activity. With respect to this study, the findings showed that the treatment of *Triticum aestivum* and *Curcuma longa*maintain the normal range of acidity and also maintain the pH level of stomach. Present study supports the use of combined juice of *Triticum aestivum* and *Curcuma longa* by local healers as traditional medicine in treatment of ulcer. This effect can be attributed to presence of various bioactive components present on extract and also be due to protective potential of juice confirms the mechanism of anti-ulcer activity against indomethacin induced potential of *Triticum aestivum* and *Curcuma longa* plant. Thus, the combination of *Triticum aestivum* and *Curcuma longa* juice should be preferred over omeprazole.

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