ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Pharmacodynamic Interaction of *Coccinia Indica* with Omeprazole in Experimentally Induced Ulcers in Rats

Hansa Gupta[1], Jyothi Y[2]*, Vamsee Veena A[3], and Deepika Bora[4]

Department of Pharmacology, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India.

ABSTRACT

The study evaluates the possible gastroprotective properties of combination therapy using Omeprazole and *Coccinia Indica* using three different gastric ulcer models. Gastric ulcers in SD rats were induced by Indomethacin (25mg/kg), Pylorus ligation model and Stress-induced Ulcer. Various parameters like Acidity, ulcer index, pepsin and mucin content, Antioxidant parameters like SOD and Catalase were evaluated. Omeprazole (2mg/kg, oral) was used as the standard drug. *Coccinia Indica* was administered at two dose levels, 200mg/kg and 400mg/kg. Statistical analysis was done by ANOVA followed by Dunnets Multiple comparison test. *P*<0.05 was considered statistically significant. Oral administration of combination of Omeprazole and *Coccinia Indica* at 200 and 400mg/kg produced significant (*p*<0.01 & p<0.001) decrease in acidity, ulcer index and severity of ulceration in the pylorus ligation model as well as protection against stress and indomethacin-induced ulcerations compared to control. It also shows significant (P<0.001) decrease in pepsin content and significant (p<0.001) increase in mucin content compared to control in pylorus ligation model. In Indomethacin induced model, combination therapy at high dose shows significant increase (p<0.001) in antioxidant parameters like SOD and catalase compared to control. The anti-ulcer effects of combination of Omeprazole and *Coccinia Indica* at both the dose levels were significantly higher than that of Omeprazole alone. Combination therapy was found to be an effective antiulcerogenic agent, minimizing any possible side effects. The results of this study suggest that combination therapy causes an inhibitory effect on release of gastric hydrochloric acid and protects gastric mucosal damage.

Keywords: Ulcer; Indomethacin; Pylorus ligation; Stress; *Coccinia indica*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Peptic ulcer disease (PUD) refers to a disruption of the mucosal integrity of the stomach, duodenum, or both, caused by local inflammation, which leads to a well-defined mucosal defect. PUD results from an imbalance between factors promoting mucosal damage (gastric acid, pepsin, *H. pylori* infection, NSAIDS) and those influencing gastro duodenal defense (mucus, bicarbonate, prostaglandin, and mucosal blood flow) [1]. People who take NSAIDs, smoke cigarettes, excessive intake of alcohol, or use cocaine, high intake of spicy foods or coffee, food poisoning, presence of *Helicobactor pylori* or secondary due to pathological conditions such as Zollinger- Ellison syndrome are at increased risk of developing PUD [2].

In the U.S., PUD affects between 3.5 and 7.5 million people, with approximately one-half million new cases diagnosed every year. Despite improvements in therapy, the widespread use of NSAIDs and low-dose aspirin, the economic burden of PUD remains a significant issue.

Due to the ineffectiveness as well as the potential side effects of modern drugs, patients are often led to explore complementary/ alternative medicines such as herb, and medicinal botanicals in particular [3]. However, simultaneous administration of herbs and drugs may mimic, magnify or oppose the pharmacological effects of each other [4]. Reports indicate that about 15–20% of individuals on prescription medications also use herbal supplements [5]. The problem is further compounded by the fact that many physicians are themselves not always familiar with the potential for herb drug interactions [6]. It is imperative to promote credible research on the safety and efficacy of combined herb-drug treatment for variety of ailments. It is believed that although herbs hold promise as therapeutically effective medicaments, appropriate studies should be carried out to confirm their efficacy in the presence of modern medicines [4].

Coccinia indica commonly called as Ivy gourd is a unique tropical plant which grows well in India. Classified as a medicinal herb in Ayurveda medicine, Ivy gourd grows quickly as trailing vines. It has ivy like leaves, white flowers and a small white gourd which turns red and sweet when ripe. The whole plant has been traditionally used for various medicinal purposes, and the leaves in particular have been used in folk medicine for the treatment of a number of ailments including diabetes, wounds, ulcers, inflammations, skin problems, fever, asthma and cough. In traditional medicine, fruits have been used to treat leprosy, fever, asthma, bronchitis and jaundice. The fruit possesses mast cell stabilizing, anti-anaphylactic and antihistaminic potential [7]. The leaf and its constituents have been reported to possess hypoglycemic, hypolipidemic and antioxidant properties and are also used to treat infective hepatitis [8].

Despite the popular use of this species as a medicinal plant there is no study on pharmacodynamic interaction with conventional drug in gastrointestinal system. *Coccinia indica* was effectively employed for curing ulcer-induced inflammation in traditional Indian system of medicine [9]. Moreover, it is also our interest to determine the possible interaction of ivy gourd with conventional antiulcer drugs such as proton pump inhibitor as most patients opt for concurrent administration of traditional remedies with these drugs. Hence the current study is designed to determine the effect of *Coccinia indica* leaf extract on experimentally induced gastric ulcers in presence and absence of conventional antiulcer drug Omeprazole using ethanolic extract of this plant.

MATERIALS AND METHODS

Plant Extract and Chemicals:

The *Coccinia indica* leaf extract was obtained as a gift sample from Green Chem, Bangalore, India. Omperazole and Indomethacin was obtained as gift sample from Akkums pharma, India. Nitroblue tetrazolium, trichloro acetic acid, Alcian blue dye, Folins reagent, HCl and other chemicals used were of analytical grade.

Preliminary phytochemical screening::

The phytochemical examination of the METC was performed by the standard methods [26].

Experimental Animals:

All the experiments were carried out with 8-9 weeks old Male SD rats of 220-250g obtained from Central Animal house, Krupanidhi College of Pharmacy, Bangalore, India. Animals were housed, under controlled conditions of temperature (23±2ºC), humidity (50±5%) and 12 h light-dark cycle. Animals were fed with rat diet pellet and water *ad libitum*. All the animals were acclimatized for seven days before to start the experimental studies. Experimental protocols were followed as per Institutional Animal Ethical committee guidelines and Animal Ethical committee clearance (CPCSEA No. 2013/PCOL/005) was obtained for the procurement of animals.

Preparation of doses:

Animals were administered a dose *Coccinia indica* at 200 and 400 mg/kg body weight based on literature survey. Omeprazole was administered at 2mg/kg body weight dissolved in distilled water and Indomethacin was dissolved in normal saline and administered at 25mg/kg body weight based on literature survey. Daily freshly prepared doses of *Coccinia indica* (*CI*) (200 and 400mg/kg b.w *p.o*) were given to the different groups of experimental animals.

Indomethacin induced ulcers:

The animals were treated with *Coccinia indica* at 200 and 400 mg/kg body weight orally for 5 days. On the 4^{th} day animals were kept for fasting for 24 hr. On the 5^{th} day animals were administered Indomethacin 25 mg/kg to induce ulcer. Rats were sacrificed after the 6 hrs of the last dose of Indomethcin. Animals were sacrificed by cervical dislocation method. The stomachs were removed and gastric juice was collected, they were cut open along the greater curvature of stomach, ulcer score and ulcer index were determined. The glandular portion of the stomach was taken and was used for estimation of mucin content [10], total proteins [11], super oxide dismutase activity and catalase activity [12].

Pylorus ligation induced ulcers:

The antiulcer activity was evaluated in pylorus ligated rats. The animals were treated with *Coccinia indica* at 200 and 400 mg/kg body weight orally for 5 days. Animals were fasted for 36 hr before pylorus ligation with water *ad libitum* by placing them individually in metabolic cages to avoid coprophagy and cannibalism. Under Ketamine HCl (90 mg/kg, i.p) & xylazine HCl (10 mg/kg, i.p) anesthesia, the abdomen was opened by midline incision below sternum. The pyloric portion of stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal wall was closed with sutures. The animals were deprived of food and water during the postoperative period and were sacrificed 6 hr after pylorus ligation by cervical dislocation method. The stomach was isolated and the contents of the stomach were collected and centrifuged. The gastric juice was used for estimation of free acidity, total acidity, pepsin content, total proteins & mucin content was determined. The stomach was cut open along the greater curvature and the ulcer index and ulcer score was determined [13, 14, 15].

Stress induced gastric ulcers:

The anti ulcer activity of plant extract was investigated by water immersion stress induced model. Rats were fasted for 24 hr. On the test day, *Coccinia indica* at 200 and 00 mg/kg body weight was administered orally to the rats and was subjected to swimming for 3 hr in a standard glass cylinder (height 45 cm, diameter 25 cm with water up to 35 cm. After 3hr, rat was sacrificed by cervical dislocation method, stomach was excised, and cut opened along the greater curvature. The ulcer index and ulcer score was determined [16, 17].

Methods for Biochemical estimations like free acidity, total acidity, mucin content and pepsin secretion in gastric juice:

Collection of Gastric juice:

Gastric juice was collected from pylorus-ligated rats as mentioned earlier. The gastric juice collected was centrifuged for 1000 rpm for 10 minutes and the volume of gastric juice was measured. This gastric juice was used for biochemical estimations as follows.

Determination of free acidity and total acidity:

1ml of gastric juice was pipette out and was diluted to 10 ml with distilled water, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01 N sodium hydroxide till the solution turns to orange color (end point). The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. The volume of alkali added was noted which corresponds to total acidity [18]. Acidity was calculated by using the formula

$$\text{Acidity} = \frac{\text{volume of NaOH x Normality of NaOH x 100}}{0.1} \text{ mEq/litre.}$$

Estimation of pepsin:

Peptic activity was determined by a modification of the method of Anson (1938). For estimation of pepsin, placed 4 test tubes (1) and (2) containing 5ml of substrate, (3) and (4) containing 10ml of trichloro acetic acid. The gastric juice was mixed with an equal volume of 0.01M hydrochloric acid, warmed to 37^0 C, 1ml of this mixture was added to each of test tubes of (1) and (4). Incubated for 15 minutes at the end mixed tube (1) with (4) and (2) with (3). Allow standing for about four minutes. (1) + (4) give test and (2) + (3) give blank. The mixture was filtered. To 2ml of the filtrate, 10 ml of NaOH was added. Then 1 ml of phenol reagent was added and mixed by gentle rotation. After 30 minutes, the absorbance was measured at 680 nm. The difference between test and blank gives the measures of peptic activity [15, 20].

Estimation of Mucin :

After the collection of the gastric juice, the glandular portions of the stomach was excised and opened down the lesser curvature. The weight of the tissue is noted. The everted stomachs were soaked for 2 hours in 10 ml of 0.1% Alcian blue 8GX dissolved in 0.16 M sucrose solution buffered with 0.05M sodium acetate. Uncomplexed dye was removed by two successive washes with 10 ml of 0.25 M of sucrose for 15 minutes and 45 minutes. Dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5 M magnesium chloride for two hours. The resultant solution was vigorously shaken with an equal volume of diethyl ether and the emulsions were then centrifuged at 3000 rpm for10 min. The absorbance of the aqueous layer was measured against a buffer blank at 580 nm. The quantity of blue dye recovered per gram of wet glandular tissue was then calculated from a standard curve [21].

Estimation of super oxide dismutase:

Rats were sacrificed using ether anesthesia and the stomach was dissected out. The glandular portion of the stomach was perfused with cold normal saline. 250mg of stomach tissue was sliced and was homogenated in 5 ml of 0.25 % sucrose in phosphate buffer pH 7.4 and the homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was used for estimation of super oxide dismutase. 100 µl of 5 % tissue homogenate in 0.2 M sucrose in phosphate buffer (0.25 M, pH 7.4) was taken in test tube to this a mixture containing 1 ml of sodium carbonate, 0.4 ml of NBT and 0.2 ml of EDTA was added and zero minute reading was taken at 560 nm. The reaction was initiated by addition of 0.4 ml of 1mM hydroxylamine hydrochloride to the test tube. The reaction mixture was incubated at 25^0 C for 5 minutes; the reduction of NBT was measured at 560 nm. A parallel control without tissue homogenate was also treated in similar manner as test. One enzymatic unit of SOD is the amount in the form of proteins present in 100 µl of 5 % tissue homogenate required to inhibit the reduction of 24 mM NBT by 50 % and is expressed as units/mg of protein [15]. The concentration of the enzyme is calculated using the formula

$$\text{Units/mg} = \frac{\text{Absorbance of Test X Absorbance of Standard}}{\text{Absorbance of Standard Conc}}$$

Estimation of catalase :

Rats were sacrificed using ether anesthesia and the stomach was dissected out. The glandular portion of the stomach was perfused with cold normal saline. 250 mg of tissue was sliced and was homogenated in 5 ml of cold 0.15 M KCl and the tissue homogenate was centrifuged at 800 rpm for 10 minutes, the supernatant was collected and used for the estimation of catalase. 100 µl of 5 % tissue homogenate in 0.15 M KCl buffer was added to 1.9 ml of phosphate buffer (0.25 M, pH 7) and absorbance was measured at 240 nm. To the above reaction mixture 1 ml of Hydrogen Peroxide solution was added and the absorbance measured after allowing standing for 1 minute at 240 nm using phosphate buffer as blank solution. One international unit of catalase utilized is that amount which catalyzes the decomposition of 1mM hydrogen peroxide per minute at 37^0 C and expressed in terms of units/mg of protein [22]. The catalase concentration is calculated using the formula:

$$\text{Units/mg} = \frac{\text{A240/min x 1000}}{\text{43.6 x mg of enzyme/ml reaction mixture}}.$$

Scoring of ulcer was made as follows:

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

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:-

$$\% \text{ Protection} = \frac{\text{Control mean ulcer index-Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Statistical Analysis:

The data were analyzed statistically using analysis of variance (ANOVA) followed by Tukey's post test. Values are expressed as Mean ± Standard errors of mean (S.E.M). $P<0.05$ is considered as significant and $P>0.05$ were considered as non-significant. Statistical comparisons were performed by Dunetts multiple comparision test using Graph Pad Prism version 5.0, U.S.A.

Histopathological evaluation:

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity of *Coccinia indica*. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-µm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for Pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes [25].

RESULTS

Qualitative chemical analysis of extracts of *Coccinia indica:*

Qualitative chemical analysis of extracts of *Coccinia indica* shows presence of Alkaloids, Steroids, Flavonoids, Saponins and Tannins. Certificate of Analysis obtained from the Green Chem shows *Coccinia indica* leaf extract is well established by HPTLC method.

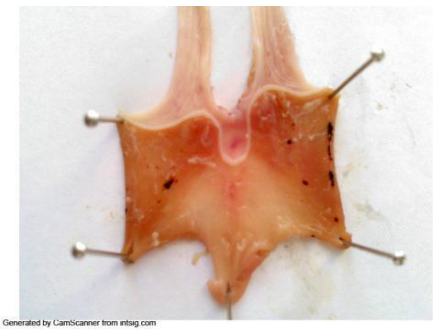
ISSN: 0975-8585

Effect of combination treatment of *Coccinia indica* and Omeprazole on Indomethacin induced ulcers:

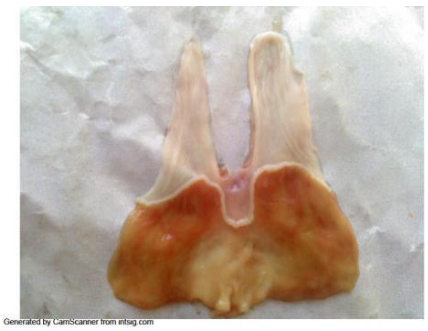
Gastric lesions were analyzed by measuring the number of gastric ulcers on the gastric mucosal surface in all experimental groups. As shown in Table 1 and Fig 1, oral administration of Omeprazole significantly causes the decrease in the Ulcer score and Ulcer index in the mucosa of stomach, compared with the indomethacin group (P<0.001). Combination treatment of Omeprazole and *Coccinia indica* with 200 and 400 mg/kg, significantly reduces (P<0.001) the Ulcer score and Ulcer index when compared to indomethacin alone. Combination treatment of Omeprazole and *Coccinia indica* with 200 and 400 mg/kg significantly increases the Mucin content (P<0.001) compared to Indomethacin alone. Combination treatment of Omeprazole and *Coccinia indica* with 200 and 400 mg/kg significantly increases the antioxidant parameters like SOD (P<0.001) and Catalase (P<0.001) as shown in Table 1. These results were supported by histopathological studies as shown in Fig 4.

Fig 1: Photographs showing effect of *Coccinia indica* extracts & Omeprazole on healing of Indomethacin induced gastric ulcers:

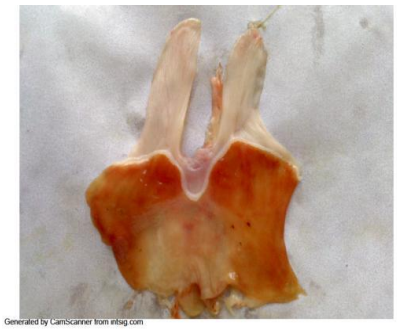
Control



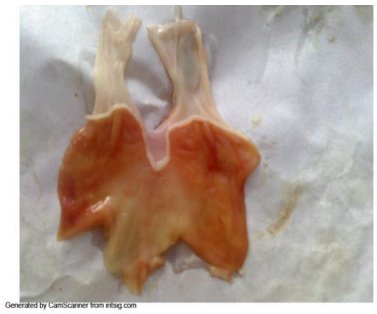
LCIE (200 mg/kg, p.o.)



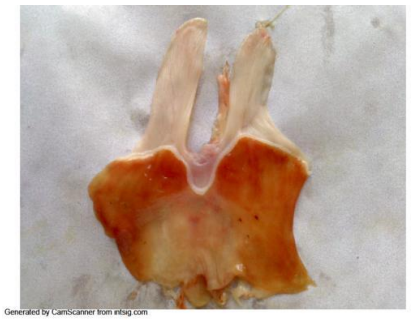
HCIE(400Mg/Kg)



OMPZL(2mg/kg.p.o)



LCIE(200Mg/Kg)+OMPZL(2mg/kg.p.o)



LCIE(200Mg/Kg)+OMPZL(2mg/kg.p.o)

OMPLZ (Omeprazole 2 mg/kg, p.o.); LCIE (Low *Coccinia indica* extract 200 mg/kg, p.o.), HCIE (High *Coccinia indica* extract 400 mg/kg, p.o.)

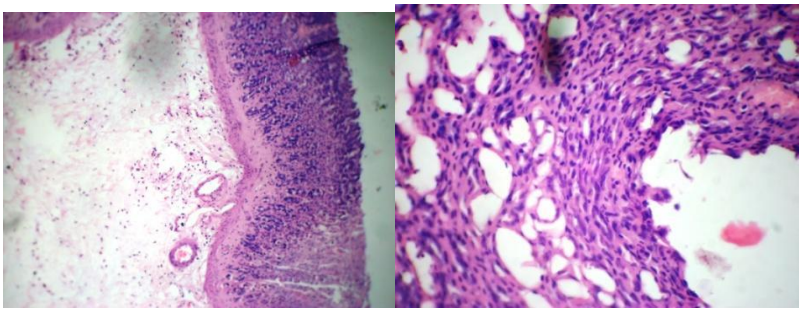
ISSN: 0975-8585

Table 1: Effect of *Coccinia indica* extracts & Omeprazole on healing of indomethacin induced gastric ulcers :

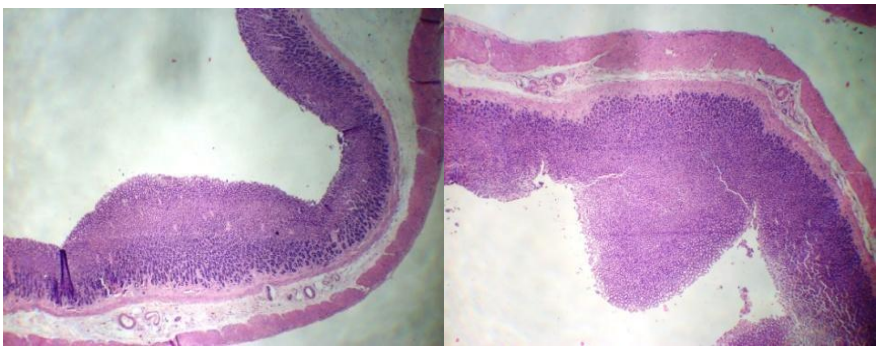
Treatment	Mucin content (µg/gm)	Ulcer index	Ulcer score	Total proteins (mg/dl)	SOD (Units/mg of proteins)	Catalase (Units/mg of proteins)
Control	0.505±0.05	0.791±0.06	13.167±1.17	0.837±0.06	18.073±1.18	19.673±1.91
LCIE	0.766±0.05*	0.721±0.04	10.667±0.88	0.763±0.03	21.667±0.66	29.749±1.68
HCIE	0.912±0.05**	0.594±0.04**	9.000±0.52**	0.782±0.04	25.651±0.69*	35.512±1.16**
OMPZL	1.528±0.05***	0.169±0.04***	6.333±0.67***	0.957±0.10	29.036±0.94***	46.556±4.48***
LCIE+OMPZL	1.583±0.12***	0.134±0.01***	5.167±0.48***	0.678±0.07	31.406±1.79***	51.291±1.80***
HCIE+OMPZL	2.133±0.07***	0.055±0.01***	3.667±0.67***	0.658±0.04	34.896±2.67***	58.391±3.96***

All values are mean ± SEM (n=6), *p< 0.05, ** p<0.01, ***p <0.001 when compared with control; OMPLZ (Omeprazole 2 mg/kg, p.o.); LCIE (Low *Coccinia indica* extract 200 mg/kg, p.o.), HCIE (High *Coccinia indica* extract 400 mg/kg, p.o.)

g 4: Histopathology showing effect of *indica* extracts & Omeprazole on healing of Indomethacin induced gastric ulcers. Light micrographs of rat stomach at 10 X (Hematoxylin and Eosin stain):



(A) CONTROL **(B) LCIE(200mg/Kg,p.o)**



(C) HCIE (400 mg/kg, p.o.) **(D) OMPZL (2 mg/kg, p.o.)**

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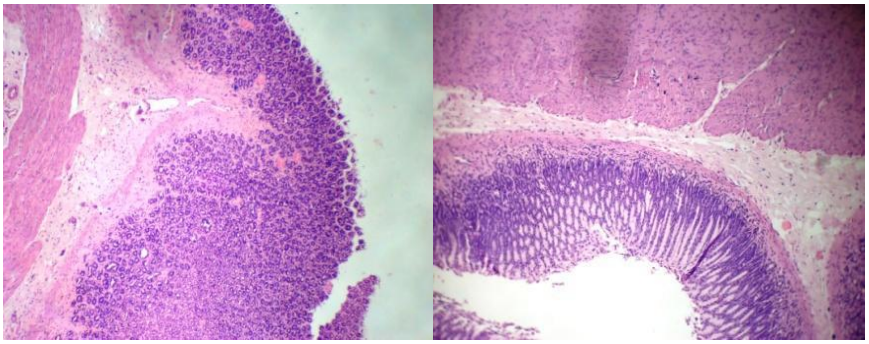


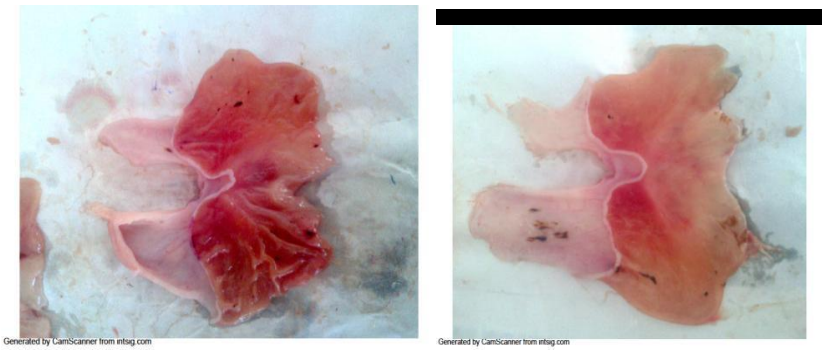
(E) LCIE(200mg/Kg,p.o)+ OMPZL (2 mg/kg, p.o.) (F) HCIE(400mg/kg,p.o)+ OMPZL (2 mg/kg, p.o.)

CONTROL: Epitheliosis, associated with high sub mucosa edema, lymphocytic infiltration in lamina propria.
LCIE : High to moderate epitheliosis, sub- mucosa edema, moderate lymphocytic infiltration in lamina propria
HCIE: Moderate proliferation of epithelial cells
OMPZL: Mild epitheliosis with mild mononuclear cell infiltration
LCIE+OMPZL: Low intensity of proliferation of epithelial cells with less sub mucosa edema.
HCIE+OMPZL: Minimal epitheliosis, with less leukocyte infiltration and sub mucosa edema.

Effect of combination treatment of *Coccinia indica* and Omeprazole on Pylorus ligation induced ulcers:

Gastric lesions were analyzed by measuring the number of gastric ulcers on the gastric mucosal surface in all experimental groups. As shown in Table 2 and Fig 2, oral administration of Omeprazole significantly causes the decrease in the Ulcer score and Ulcer index in the mucosa of stomach, compared with the Pylorus ligation group ($P<0.001$). Combination treatment of Omeprazole and *Coccinia indica* with 200 and 400 mg/kg, significantly reduces ($P<0.001$) the Ulcer score and Ulcer index when compared to Pylorus ligation alone. Combination treatment of Omeprazole and *Coccinia indica* with 200 and 400 mg/kg significantly decreases the Free acidity and Total acidity ($P<0.001$) compared to Pylorus ligation alone. Combination treatment of Omeprazole and *Coccinia indica* with 200 and 400 mg/kg significantly increases ($P<0.001$) the Mucin content and significantly reduced ($P<0.001$) the pepsin content. These results were supported by histopathological studies of various treatement groups as shown in Fig 5.

Fig 2: Photographs showing effect Effect of *Coccinia indica* extracts & Omeprazole on water immersion induced stress gastric ulcers:



CONTROL **LCIE(200mg/Kg,p.o)**

ISSN: 0975-8585

HCIE (400 mg/kg, p.o.)

OMPZL (2 mg/kg, p.o.)

LCIE(200mg/Kg,p.o)+ OMPZL (2 mg/kg, p.o.)

HCIE(400mg/Kg,p.o)+ OMPZL (2 mg/kg, p.o.)

OMPLZ (Omeprazole 2 mg/kg, p.o.); LCIE (Low *Coccinia indica* extract 200 mg/kg, p.o.), HCIE (High *Coccinia indica* extract 400 mg/kg, p.o.)

Table 2: Effect of *Coccinia indica* extracts & Omeprazole on pylorus ligation induced gastric ulcers

Treatment	**Ulcer index**	**Ulcer score**	**Free acidity** (mEq/L)	**Total acidity** (mEq/L)	**Mucin content (µg/gm)**	**Pepsin content** (µmol/6hr)	**Total protein** (mg/dl)
Control	0.437±0.02	17.333±0.84	46.167±1.70	57.500±1.48	12.383±0.57	0.205±0.01	0.665±0.04
LCIE	0.392±0.02	16.500±1.15	37.500±1.71***	50.667±1.20**	25.750±0.91***	0.190±0.01	0.694±0.04
HCIE	0.343±0.01**	13.333±0.88**	30.333±1.43***	35.167±1.40***	39.027±1.08***	0.161±0.01***	0.648±0.02
OMPZL	0.300±0.02***	10.000±0.58***	16.500±1.57***	19.667±1.54***	51.578±0.88***	0.112±0.01***	0.645±0.02
LCIE+OMPZL	0.275±0.01***	7.667±0.67***	11.667±1.28***	15.333±0.99***	58.617±0.98***	0.0840±0.01***	0.685±0.07
HCIE+OMPZL	0.252±0.01***	5.500±0.56***	5.000±0.58***	9.833±0.79***	71.645±2.82***	0.034±0.01***	0.686±0.05

All values are mean ± SEM (n=6), *p< 0.05, ** p<0.01, *** p <0.001 when compared with control; OMPLZ (Omeprazole 2 mg/kg, p.o.); LCIE (Low *Coccinia indica* extract 200 mg/kg, p.o.), HCIE (High *Coccinia indica* extract 400 mg/kg, p.o.)

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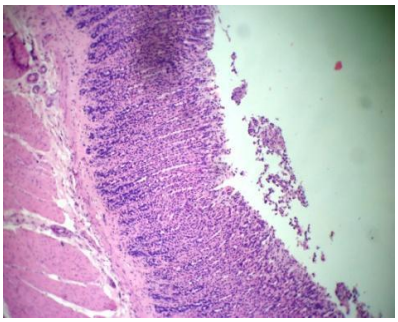
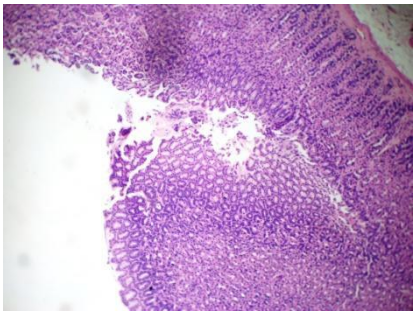
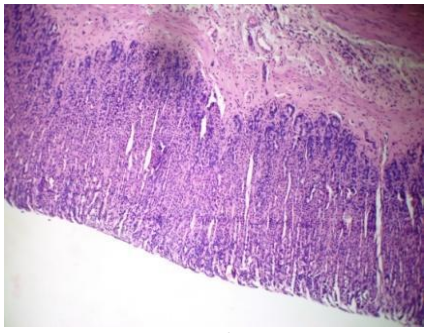
Fig 5: Histopathology showing effect of *Coccinia indica* extracts & Omeprazole on healing on water immersion induced stress gastric ulcers. Light micrographs of rat stomach at 10 X (Hematoxylin and Eosin stain):



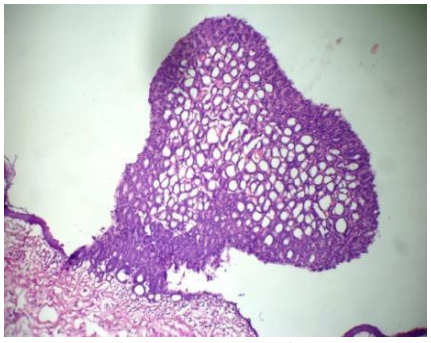
(A) CONTROL



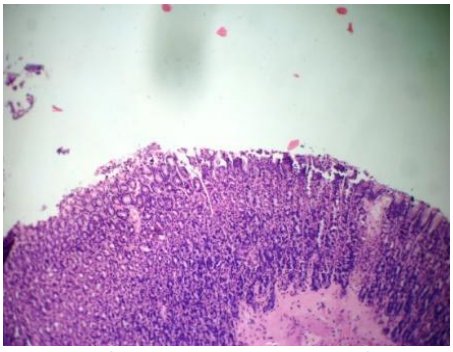
(B) LCIE(200mg/Kg,p.o

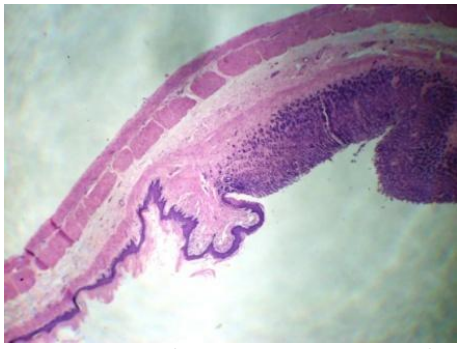


(C) HCIE (400 mg/kg, p.o.)



(D) OMPZL (2 mg/kg, p.o.)





(E) LCIE(200mg/Kg,p.o)+ OMPZL (2 mg/kg, p.o.)(F) HCIE(400mg/kg,p.o)+ OMPZL (2 mg/kg, p.o.)

CONTROL: Mucosal ulceration consisting of degenerated epithelial cell.
LCIE : Disruption of the upper cell layer and lamina propria, erosion of mucosa, infiltration by mononuclear cell.
HCIE: Intact epithelium, shredded lymphocytes and proliferating vascular spaces
OMPZL: Intact epithelium, lamina propria and mucosa
LCIE+OMPZL: Mild proliferation of epithelium, absence of lymphocytic infiltration.
HCIE+OMPZL: Intact mucosa, mild injury of epigastric cells.

Effect of combination treatment of *Coccinia indica* and Omeprazole on Stress induced ulcers:

Gastric lesions were analyzed by measuring the number of gastric ulcers on the gastric mucosal surface in all experimental groups. Oral administration of Omeprazole significantly causes the decrease in the Ulcer score and Ulcer index in the mucosa of stomach, compared with the stress induced group ($P<0.001$). Combination treatment of Omeprazole and *Coccinia indica* with 200 and 400 mg/kg, significantly reduces

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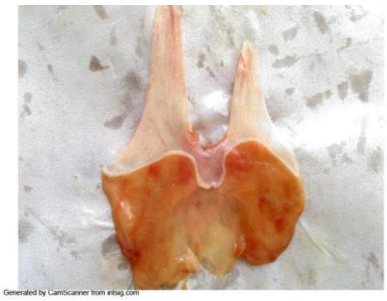
(P<0.001) the Ulcer score and Ulcer index when compared to Stress induced alone as shown in Table 3 and Fig 3. These results were supported by histopathological studies as shown in Fig 6.

Fig 3 : Photographs showing effect of *Coccinia indica* extracts & Omeprazole on healing on Pylorus ligation induced gastric ulcers:

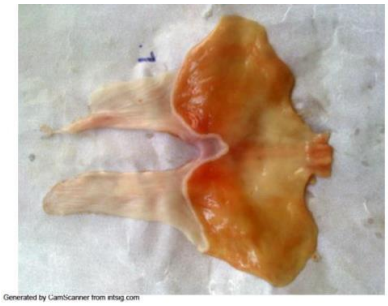
CONTROL

LCIE(200mg/Kg,p.o)

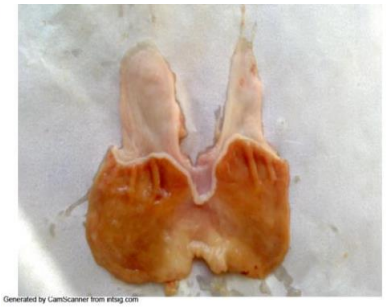


HCIE (400 mg/kg, p.o.)

OMPZL (2 mg/kg, p.o.)



LCIE(200mg/Kg,p.o)+ OMPZL (2 mg/kg, p.o.)



HCIE(400mg/Kg,p.o)+ OMPZL (2 mg/kg, p.o.)

OMPLZ (Omeprazole 2 mg/kg, p.o.); LCIE (Low *Coccinia indica* extract 200 mg/kg, p.o.), HCIE (High *Coccinia indica* extract 400 mg/kg, p.o.).

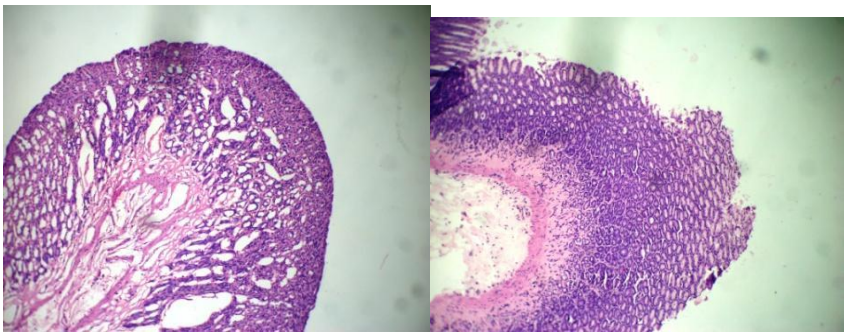
Table 3: Effect of *Coccinia indica* extracts & Omeprazole on ulcer index and ulcer score in stress induced gastric ulcers:

Treatment	Ulcer index	Ulcer score
Control	0.884±0.03	15.167±1.08
LCIE	0.794±0.02	14.500±0.76
HCIE	0.575±0.03***	11.333±0.49**
OMPZL	0.446±0.03***	5.667±0.67***
LCIE+OMPZL	0.353±0.03***	4.833±0.48***
HCIE+OMPZL	0.111±0.01***	2.333±0.56***

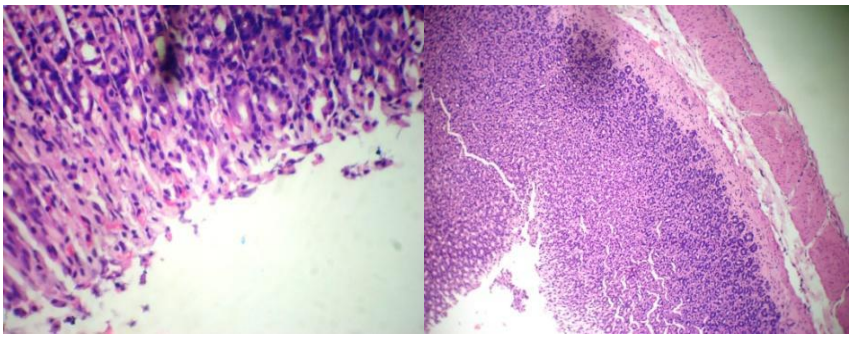
All values are mean ± SEM (n=6), *p< 0.05, ** p <0.01, *** p <0.001 when compared with control; OMPLZ (Omeprazole 2 mg/kg, p.o.); LCIE (Low *Coccinia indica* extract 200 mg/kg, p.o.), HCIE (High *Coccinia indica* extract 400 mg/kg, p.o.)

ISSN: 0975-8585

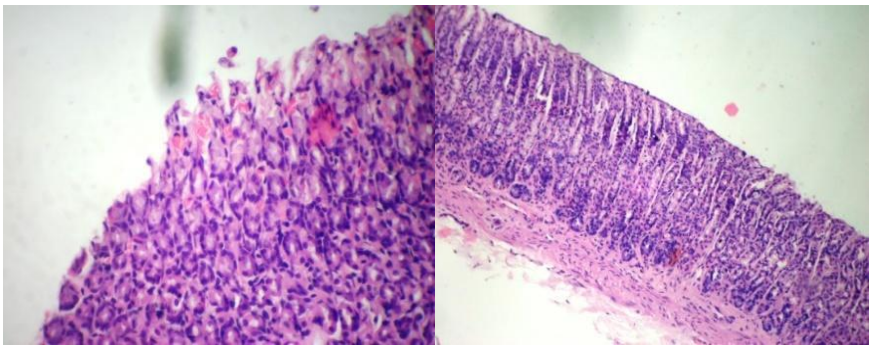
Fig 6: Histopathology showing effect of *Coccinia indica* extracts & Omeprazole on healing on Pylorus ligation induced gastric ulcers:. Light micrographs of rat stomach at 10 X (Hematoxylin and Eosin stain):



(A) CONTROL **(B) LCIE(200mg/Kg,p.o)**



C) HCIE (400 mg/kg, p.o.) **(D) OMPZL (2 mg/kg, p.o.)**



E) LCIE(200mg/Kg,p.o)+ OMPZL (2 mg/kg, p.o.)(F) HCIE(400mg/kg,p.o)+ OMPZL (2 mg/kg, p.o.)

CONTROL: Severe congestion of blood vessels, tissue infiltration, edema, several ulcers.
LCIE : High proliferation of upper layer of epigastric cell.
HCIE: Moderate focal dense infiltration in lamina propria, mild epitheliosis.
OMPZL: Mild blood vessel congestion and mild tissue infiltration.
LCIE+OMPZL: Minimal epitheliosis with less mononuclear cell infiltration in lamina.
HCIE+OMPZL: Partial sub mucosal congestion and infiltration of cell, no necrosis.

DISCUSSION

The present study was done to evaluate the pharmacodynamic interaction of *Coccinia indica* with omeprazole in experimentally induced ulcer in rats. *Coccinia indica* and the combination with Omeprazole showed effect on the various gastric ulcer induced by different methods. The gastric ulcer was induced by three different models viz; Indomethacin induced ulcer, pylorus ligation induced ulcers and water immersion stress induced ulcers model.

Indomethacin an NSAID produces erosions and ulcers in the gastrointestinal tract of experimental animals. A layer of mucus that apparently forms a barrier covers the gastric mucosa. The administration of indomethacin results in the production of gastric mucosal damage mainly in the glandular portion of the stomach. Indomethacin is a known prominent inhibitor of prostaglandin synthesis that in turn damages the mucosal barrier; the damage in the mucosal barrier causes the permeation of sodium ions from the mucosa in to the lumen [23].

The cytoprotective agents prevent the ulcers induced by Indomethacin. The plant extract and the combination with omeprazole were effective in reducing ulcer index and ulcer score. This shows the cytoprotective activity of *Coccinia indica* was also effective in increasing gastric mucus content and activities of endogenous antioxidant enzymes such as SOD and catalase. Hence, it can be suggested that cytoprotective effect in Indomethacin induced gastric ulcers may be due to both increase in cytoprotective mucus secretion and antioxidant property of ivy gourd.

The gastric antisecretory effect was evaluated in pylorus ligated rats. The ligation of pyloric end of the stomach causes accumulation of gastric acid and pepsin in the stomach leading to development of ulcers. *Cocinia indica* leaves extract and omeprazole reduced the secretion of gastric aggressive factors; free acidity, total acidity and pepsin and increased secretion of gastric cytoprotective factor mucin.

Stress induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production. There is also an increase in gastrointestinal motility, which causes folds in the gastrointestinal tract that comes in contact with acid leading to induction of gastric ulcers and stress also brings central nervous system into play. Agents that decrease the G.I motility and that have central actions are helpful in reducing the ulcers due to stress full conditions. The extract of *Cocinia indica* leaves and omeprazole was effective in reducing the ulcers induced due to the stress. This suggests that some of the constituents present in the extract of *Cocinia indica* leaves may have central actions, which are helpful in reducing the gastric ulcers or the reduction may be due to local effect on gastric motility or gastric secretion [24].

CONCLUSION

The *Coccinia indica* extract and its interaction with omeprazole shows effective in decreasing the development of gastric ulcer secretion in stress induced gastric ulcers, Indomethacin induced gastric ulcers, and pylorus ligation induced ulcer in rats. The extract was also found to be effective in increasing the healing of gastric ulcer, the high dose and interaction of ivy gourd with omeprazole shows more effective in healing. The anti-ulcer effect of *Coccinia indica* may be due to both reduction in gastric acid secretion and gastric cytoprotective action.

ACKNOWLEDGEMENT

The authors are sincerely thankful to Green Chem, Bangalore, India, for providing the gift sample of *Coccinia indica* extract.

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