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Original Research Article

Protective Effect of Aqueous Extract of *Hypoestes rosea* Against Indomethacin-Induced Gastric Ulcer in Sprague Dawley Rats

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ABSTRACT

The leaves of *Hypoestes rosea* contain phytochemical compounds such as saponins, hypoestoxide, flavonoids and terpenoids amongst others. These compounds have been reported to be effective anti-diabetic, anti-ulcerogenic, anti-inflammatory and anti-oxidative agents. The leaves of *H. rosea* are also used in folk medicine for management of stomach related ailments. This study explores the effectiveness of aqueous leaf extract of the plant as anti-ulcerogenic potentials against indomethacin-induced gastric ulcer in Sprague Dawley rats. The LD_{50} of the crude extract was determined to be 3.25 g/kg bw. The rats were pretreated with 100, 200 and 300 mg/kg bw of the extract for 21 days prior to ulcer induction with indomethacin (40 mg/kg bw). The extract of *H. rosea* caused a significant ($p<0.05$) decrease in biochemical parameters of the gastric secretions analyzed as follows: Pepsin level of the gastric juice decreased from 126.30 ± 1.25 to 66.70 ± 1.20 µg/mL, gastric secretion volume decreased from 4.1 to 2.1 mL and the ulcer lesion index decreased from 13.40 to 1.67. Gross examination and histological investigation revealed that *H. rosea* extract prevented gastric mucosa erosion and hemorrhagic streaks, typical features of ulceration. It also prevented loss of mucin from the gastric mucosa. In conclusion, the results suggest that aqueous extract of *H. rosea* protects against indomethacin-induced gastric ulceration in Sprague Dawley rats with the most effective dose being 300 mg/kg bw.

Keywords: *Hypoestes rosea,* Aqueous extract, Indomethacin, Gastric ulcer, Anti-ulcer.

Introduction

Gastric ulcers are among a group of diseases collectively called Peptic Ulcer Disease (PUD) together with esophageal and duodenal ulcers.[1] PUDs are characterized by lesions, sores or breaks along the inner lining of the gastrointestinal tract due to increased pepsin or acid secretion. Physiologically, peptic ulcers result from an imbalance between mucosal aggressive and protective factors.[2] Mucosal protective factors include increased blood flow, increased bicarbonate secretion and increased mucin production. Mucosal aggressive factors include increased gastric acid secretions and pepsin activity.[3] Gastric ulcers have four major causative agents namely: infection with *Helicobacter pylori*, use of Non-steroidal anti-inflammatory drugs (NSAIDs), acid hyper-secretory disease like Zollinger-Ellison syndrome and idiopathic ulcers. Minor causes of gastric ulcers include: chronic alcohol consumption, viral infections like Herpes Simplex Virus (HSV), cytomegalovirus (CMV), stress (burns, acute illnesses) and malignant tumors of the lungs or gut.[4,5]
Management of gastric ulcer in modern medical practice involves the use of anti-ulcer medications with their main objectives being to prevent gastric acid secretion, relieve pain, heal the ulcerative lesion and delay or prevent its recurrence.[6] These anti-ulcer drugs exert their actions on the gastric mucosa through different mechanisms. Drugs like cimetidine, ranitidine and famotidine are Histamine-2-Receptor Antagonists (H_2RA).

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They serve to inhibit acid secretion by blocking H_2 receptors on the parietal cells of the stomach.
Antacids such as aluminum hydroxide function by neutralizing the acidity of the stomach and reducing heart burn symptoms, Drugs classed under the category of Protein Pump Inhibitors (PPI) such as esomeprazole and omeprazole exhibit their action by inhibition of the proton pump. Other drugs like sucralfate cover the ulcer site and protect against attack form the acidic components. Also, misoprostol is a synthetic prostaglandin that simulates the action of natural prostaglandins in the stomach and intestine thus protecting the architectural and structural integrity of the stomach mucosa.[7] These drugs though effective have been reported to confer some side effects on patients ranging from headaches caused by H_2 blockers to kidney failure caused by chronic PPI use and induced abortion in pregnant women caused by misoprostol.[8] These adverse effects coupled with other societal considerations such as high cost of living, high cost of synthetic anti-ulcer medicines, inadequate medical coverage and inaccessibility of rural dwellers to appropriate medical care has prompted a growing interest in the formulation of non-toxic, anti-ulcer medications sourced from easily obtainable natural plants and herbs.[9] Some of these plants are already used in traditional healing and folklore medicine.[10] One of such plants is *Hypoestes rosea.*
Hypostes rosea (Acanthacea family) is an evergreen shrub. It is abundantly available in the southern part of Nigeria and other countries in West Africa.[11] It is commonly known as 'Iyip Abasi' in Efik language spoken by indigenous tribes in Akwa Ibom and Cross River States, Nigeria. 'Iyip Abasi' is interpreted in English to mean 'Blood of Jesus'. The leaf is so named due to the dark red exudate obtained from its hot water decoction. *H. rosea* contains some bioactive compounds such as roseanolone, isoroseanolone, roseadione, hypoestoxides and lupeol.[12-16] These compounds are classified under the general family of flavonoids, phenols and terpenes that have been researched and reported by several studies to play major roles as

antioxidants, anti-inflammatory, anti-fungal and anti-ulcer agents.[11,12,14,17] Although, several studies have been done on *Hypostes rosea*, there has been no known research done to investigate its potential properties in gastric ulcer management, hence the purpose for this study.

Materials and Methods

Chemicals and drugs
All chemicals and drugs used were of analytical standard. Omeprazole was obtained from Alapharm Nigeria Ltd (Aba, Nigeria), indomethacin was procured from Embassy Pharmaceuticals and Chemicals Ltd Reagents (Lagos, Nigeria). Hematoxylin, eosin, Schiff reagent and per-iodic acid were purchased from Sigma Chemical Co. Ltd (St. Louis, Missouri, USA). Absolute ethanol and Hcl were obtained from Merck Pharmaceutical and Life Sciences Ltd (Distribution subsidiary, Lagos, Nigeria) and distilled water was obtained from the Department of Physiology, University of Calabar, Calabar.

Plant collection and authentication
Fresh leaves of *Hypoestes rosea* were collected from farms within Calabar metropolis in January, 2022. They were identified and authenticated at the Herbarium, Department of Botany and Ecological Studies, University of Calabar and assigned a voucher number HERB/BOT/UCC/035.

Preparation of plant extract
The leaves of *H. rosea* were removed from the plant stalk, washed properly in water to remove any sand and debris. The leaves were then dried in a shade environment. After a period of two weeks, the drying process was completed. The dried leaves were pulverized into powder using a commercially available milling machine. The dried leaf powder (724 g) was macerated in three liters of distilled water and allowed to stand for 48 hours. The extract was decanted and double filtered using chess cloth and Whatman number 1 filter paper to obtain a clear extract. The filtrate was concentrated under reduced pressure at 45°C using a rotary evaporator and dried using a Biobase drying oven.[11] The resulting crude extract was weighed and stored. It was preserved for the duration of the experiment by storage in an airtight container and placed in a refrigerator. The percentage yield was determined according to the following formula.

$$\text{Percentage yield} = \frac{\text{weight of crude extract}}{\text{weight of powder}} \times 100$$

LD_{50} determination
The LD_{50} was determined according to a previously described method.[18] The animals were divided into 3 stages. In stage one, they were divided into four groups of two rats each and administered 50 mg/kg bw, 200mg/kg bw, 400 mg/kg bw and 800 mg/kg bw of the extract, they were observed after one hour and then at two-hour interval for 24 hours. In stage 2, two animals each were placed in 3 groups and administered with 1000 mg/kg, 1500 mg/kg and 2000 mg/kg. The animals were observed for 24 hours. In Stage three, 2 rats each were placed in 2 groups administered 3000mg/kg and 3500mg/kg of the extract and observed for 24 hours.

Experimental animals
A total number of 48 Sprague-Dawley rats (100 - 130g) were used. The rats were purchased from the Animal House, Department of Physiology, University of Calabar, Calabar. Ethical approval for animal use was obtained from the Ethical Committee, Faculty of Basic Medical Sciences, University of Calabar with ethical approval number FAREC/PA/UC/049. The animals were allowed an acclimatization period of 2 weeks while being fed with standard rat chow (Vital feed grower pellets. Produced by UAC, Grand Cereals Ltd. Nigeria) and they had water as often as they desired. All the animals were handled according to international guidelines for animal usage.[19,20]

Experimental protocol
Animal grouping,
Forty-eight (48) rats were allocated into 6 groups of 8 rats each. The groups were as follows:

Group 1: (Normal control: rat chow + water alone).
Group 2: (Ulcer control: Gastric ulcer induced with indomethacin (40mg/kg bw). No extract or drug treatment).
Group 3: Omeprazole group (omeprazole 20mg/kg bw).
Group 4: Extract 100mg/kg bw.
Group 5: Extract 200mg/kg bw.
Group 6: Extract 300mg/kg bw.

Anthropometric measurement
The weights of the experimental animals were measured using an electronic scale (MSP digital electronic scale: range 0.05g to 12kg). The initial weight range of the animals was 100 – 130g. At the end of the extract administration period, the weight range was 120 – 140g.

Ulcer induction, isolation of stomach and collection of gastric juice
The animals were pretreated for 21 days with the appropriate dose of extract. Gastric ulceration was induced by a single dose of indomethacin (40mg/kg bw) administered orally.[10] Prior to indomethacin administration, the animals were fasted for 24 hours. The animals had free access to water during this fasting period.
Eight hours after ulcer induction, the animals were anaesthetized with 1mL intraperitoneal injection of ketamine and humanely sacrificed by cervical dislocation. The abdomen was cut open and the stomach excised. Upon excision, the stomach was opened along the greater curvature and the gastric content collected into designated pre-labelled plain sample containers and used for biochemical analysis.

Gross evaluation of rat stomach and ulcer quantification
Post animal sacrifice, the excised stomachs were opened, remnant of stomach content washed off with normal saline. The cleaned stomachs were spread open and pinned to a corkboard. The degree of ulceration was evaluated with a dissecting microscope to determine the ulcer lesion index.[21] The ulcerative lesions were graded as follows: Grade 0 – For gastric mucosa with no ulceration; Grade 0.5 – For gastric mucosa with small/minor ulceration; Grade 1 – For gastric mucosa with moderate ulcer lesions; Grade 2 – For gastric mucosa with severe ulcer lesions.

Evaluation of gastric secretion parameters
pH values of the gastric fluid obtained from the various experimental groups were determined using a defined method.[22] An aliquot of gastric fluid was diluted with equal volume of distilled water and measured using a digital pH meter. Gastric secretions for pepsin analysis were collected after a three-hour duration into a plain sample container after ligation of the pyloric junction to prevent flow of gastric content into the small intestine. Pepsin activity was analyzed using a defined method.[23] Volume of gastric secretions from the various groups were also determined.

Histological evaluation
Longitudinal sections of stomach tissues were obtained from the anterior part of the animals stomachs. The tissues were fixed in 10% neutral buffered formalin for 24 hours, processed in an Automated tissue processor, embedded in molten paraffin wax, cut into 4µm sections using a rotary microtome and floated out onto clean labeled glass slides. The tissue sections were stained with routine Hematoxylin and Eosin (H and E)[24] to show general tissue architecture and Periodic Acid Schiff (PAS) to demonstrate mucin on the gastric mucosa. After staining, the tissues were examined microscopically at x 100 magnification.

Statistical analysis
Data obtained were expressed as Mean ± Standard deviation (SD). One-way ANOVA and Student t-test were done using SPSS software package for windows (version. 23) for differences between means. Values of $p \leq 0.05$ were considered statistically significant.

Results and Discussion

Acute toxicity study
The result of the LD_{50} determination is shown in Table 1. The animals displayed no sign of toxicity and mortality up to a dose of 3000 mg/kg bw. Mortality was recorded at 3500 mg/kg bw. Hence, the LD_{50} of aqueous extract of *H. rosea* was determined to be 3250 mg/kg bw.

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With an LD_{50} of 3.25 g/kg bw. The most effective dose with regards to prevention of ulceration in this study was 300mg/kg bw, a previous study reported that administration of *H. rosea* at that dose may cause detrimental effects to kidney health.[16]

Weight changes after extract administration

The weight of the experimental animals measured before and after 21-day extract administration are shown in Table 2. Weight gain was observed in all the groups. It ranged from the lowest gain of 6% in group 3 (omeprazole group) to the highest gain of 12.8% in group 1 (normal control). Increase in weight of rats after administration with *H. rosea* extract was similarly reported in another study.[16]

Macroscopic examination and ulcer lesion index

Plate 1 shows the gross appearance of the stomachs of animals in groups 1 to 6. Administration of indomethacin (40 mg/kg bw) induced varying degrees of ulceration in the different groups. The ulcerative lesions formed appeared as grooves of erosion and visible hemorrhagic streaks along the stomach mucosa. There were very few visible spots of ulceration on the stomach mucosa in Group 6 treated with the extract (300 mg/kg bw). Figure 1 shows the ulcer index of the various groups. Indomethacin (40mg/kg) caused a significant ($p \leq 0.05$) increase in the ulcer index of animals in group 2 (ulcer control) (13.3 ± 0.47). *H. rosea* at 300 mg/kg bw produced a significant decrease in the ulcer index value when compared with the positive control.

Indomethacin causes inflammation and accumulation of reactive oxygen species that may result in damage to the gastric mucosa by oxidation. It also inhibits the synthesis of prostaglandins and elevates the ulcer index[25-27] *H. rosea* showed a remarkable degree of protection of the stomach mucosa against ulceration as it reduced the ulcer index and prevented visible ulcerative lesions on the stomach mucosa. This effect may be due to the presence of flavonoids and terpenoids present in the leaf. Several studies have highlighted the activity of these biochemical compounds as anti-oxidative and antiulcer agents.[28,29]

Biochemical analysis of gastric secretions

As shown in Table 3, the mean values of pH for groups 1 to 6 were 3.4 ± 0.08, 2.5 ± 0.08, 4.8 ± 0.05, 3.5 ± 0.08 and 3.6 ± 0.05 respectively. There was a significant difference in the pH values between the various groups. ($p \leq 0.05$). Post hoc analysis with Turkey HSD determined a significant difference in mean pH values between all the group pairs except between groups 1 and 4, groups 1 and 5 and groups 1 and 6. Indomethacin caused a marked increase in acidity of the gastric fluid. The mean pepsin values for groups 1 to 6 as shown in Table 3 were 43.3 ± 1.28µg/mL, 126.3 ± 1.25 µg/mL, 59.0 ± 0.8 µg/mL, 70.7 ± 1.2 µg/mL, 73.7 ± 2.1 µg/mL and 66.7 ± 1.2 µg/mL respectively. Indomethacin caused a significant increase ($p \leq 0.05$) in activity of pepsin enzyme across the groups. In the groups administered with *H. rosea*, an incremental elevation of pepsin activity was observed. The mean volumes of gastric secretions are shown in Figure 2. The least volume of gastric secretion (1.6mL) was recorded in group 1 (normal control) while the highest volume of gastric secretion (4.1 mL) was observed in group 2 (ulcer control).

Biochemical analysis of gastric secretions is usually required after exposure of the gastric mucosal surface to potential ulcerogenic agents. This gives information about the integrity of the mucosal environment.[30] Indomethacin administration caused an increase in pepsin activity and volume of gastric secretion. It also caused reduction in gastric pH.[31] Elevated pepsin activity results in an overall increase in acidity of the gastric environment.

Table 1: Acute toxicity study of *H. rosea*

Phase	Group	Dose (mg/kg bw)	Mortality	%Mortality
I	1	50	0/2	-
	2	200	0/2	-
	3	400	0/2	-
	4	800	0/2	-
II	1	1000	0/2	-
	2	1500	0/2	-
	3	2000	0/2	-
III	1	3000	0/2	-
	2	3500	½	50%

LD_{50}: 3250 mg/kg bw

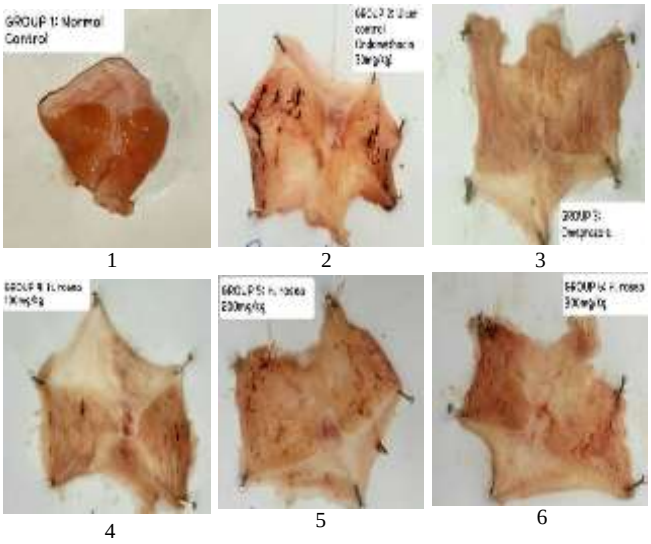

Plate 1: Gross representation of the stomachs of 1) Negative control, 2) Positive control, 3) Omeprazole treated group, 4) *H. rosea* 100mg/kg bw, 5) *H. rosea* 200mg/kg bw, 6) *H. rosea* 300mg/kg bw. Red arrow indicates ulcerative hemorrhagic streaks.

Table 2: Mean weights of experimental animals

Group	Initial mean weight (g) (n = 8) ± SD	Final mean weight (g) after 21 days extract administration (n = 8) ± SD	P-value	% weight gain
1 (Normal control)	106.1 ± 3.7	121.7 ± 2.2	<0.001*	12.8%
2 (Ulcer control)	113.7 ± 7.1	126.4 ± 6.0	0.004*	10%
3 (Omeprazole)	127.1 ± 2.4	135.3 ± 3.2	<0.001*	6.0%
4 (*H. rosea* 100mg/kg)	121.1 ± 2.0	130.9 ± 2.0	<0.001*	7.5%
5 (*H. rosea* 200mg/kg)	113.4 ± 3.3	121.6 ± 2.9	<0.001*	6.7%
6 (*H. rosea* 300mg/kg)	127.0 ± 2.1	138.4 ± 3.5	<0.001*	8.2%

All results are expressed as Mean ± SD, (*) represents statistical significant values

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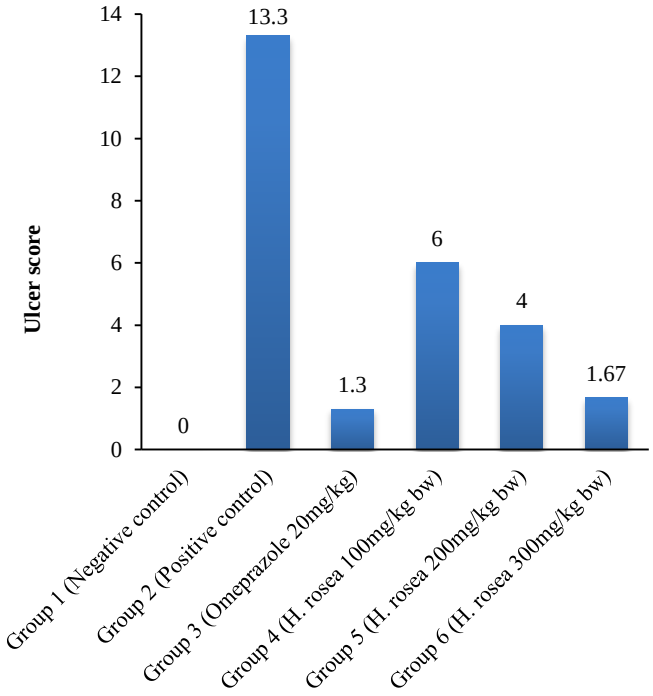

Figure 1: Ulcer index of the various experimental groups

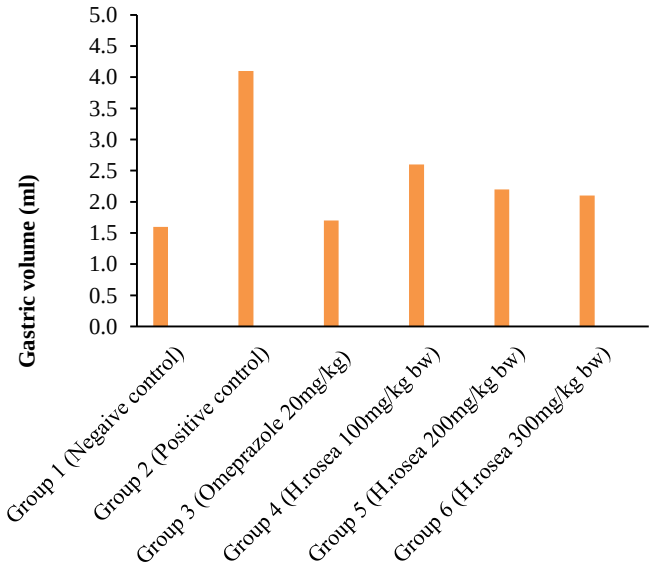

Figure 2: Volume of gastric secretions

Previous studies have established a strong correlation between hyper acidity and ulcer formation.[10,32] *H. rosea* mitigated this effect of induced hyperacidity possibly due to the presence of flavonoids. A study reported that flavonoids may prevent ulceration by stimulation of prostaglandin secretion and production of bicarbonates.[33]

Histological findings

Plate 2 depicts the photomicrographs of H and E stained representative sections of stomach tissue from groups 1 to 6. Tissue sections in Group 1 (normal control) showed normal histological architecture. The traditional three layers of the stomach (mucosa, submucosa and muscularis propria) and by extension other parts of the GIT were well highlighted. Indomethacin administration caused a substantial ulceration of the gastric mucosa as seen in group 2 (Ulcer control). There was presence of prominent ulcerative lesions on the mucosal surface which was indicated by areas of massive mucosal erosion. Also seen were massive proliferation and influx of inflammatory leukocytes and loss of glandular structures. Groups 4, 5 and 6 treated with 100mg/kg 200mg/kg, 300mg/kg respectively of *H. rosea* extract showed varying degrees of minimal ulceration. Group 6, treated with 300mg/kg bw of the extract showed a similar histological picture as the omeprazole group (Group 3).

As observed in the Hematoxylin and Eosin photomicrographs, *H. rosea* prevented aggregation of inflammatory cells in the gastric mucosa environment, this may be due to the presence of hypoestoxide, a major biochemical compound contained in the leaves of the plant. A study reported hypoestoxide to be a potent anti-inflammatory agent.[12] Gastric mucus (mucin) is present on the entire surface of the gastric mucosa. The mucus is a viscous, transparent gel-like substance. It is mainly composed of 95% water and 5% high molecular weight glycoprotein. In addition to gastric surface protection against exogenous and endogenous toxins that are capable of causing gastric mucosa damage, mucins also act as antioxidants against build-up of reactive oxygen species that may cause oxidative damage to the stomach mucosa and they contribute to healing of ulcerative lesions.[34,35] Mucin depletion is one of the major aggressive factors that contribute to gastric ulcer formation. Indomethacin causes decrease in mucin secretion and depletion of the secreted mucus thus adversely affecting the protective function of mucin on gastric mucosa.[10] In this study, mucin was qualitatively demonstrated by specialized histological staining with PAS reagent. *H. rosea* administration prevented mucin depletion as the experimental groups administered with the extract showed intense PAS staining reaction which highlights the abundance of mucin in the gastric mucosa. This may be due to the activity of flavonoids as they have been reported to enhance the production of mucin.[33,36]

Plate 3 shows the photomicrographs of PAS stained representative sections of stomach tissue from groups 1 to 6. In the PAS staining reaction, the staining intensity is indicative of the concentration of mucin present. There was a pale staining PAS reaction in group 2 (ulcer control). This pale staining reaction indicates the loss of mucin in the gastric mucosa. Groups 1 (negative control) and 6 (300mg/kg bw) had the most intense PAS staining reaction, this is indicative of abundant mucin presence in the gastric mucosal cells.

Table 3: pH values and pepsin activity of the various experimental groups

Treatments	pH value	Pepsin activity(µg/mL)
Group 1 (Normal control)	3.4 ± 0.08	43.3 ± 1.28
Group 2 (Ulcer control)	2.5 ± 0.08	126.3 ± 1.25
Group 3 (Omeprazole treatment)	4.8 ± 0.05	59.0 ± 0.80
Group 4 (*H. rosea* 100mg/kg)	3.5 ± 0.05	70.7 ± 1.20
Group 5 (*H. rosea* 200mg/kg)	3.5 ± 0.08	73.7 ± 2.10
Group 6 (*H. rosea* 300mg/kg)	*3.6 ± 0.05*	66.7 ± 1.20
	[F(5,12) = 250.4, p = < 0.001]*	**[F(5,12) = 856.6,p = < 0.001]***

All values are mean ± SD. * represents significant level at $p \leq 0.05$

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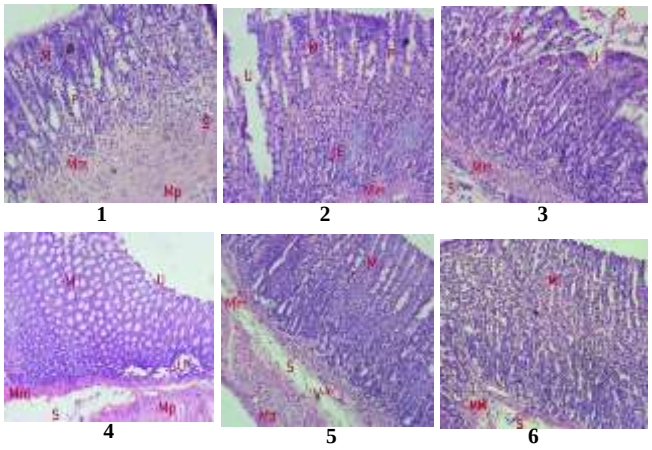

Plate 2: Hemtoxylin and eosin photomicrographs of groups 1 to 6 showing representative sections of the stomach (H&E x100 magnification). Group 1(Normal control) showing normal stomach histology, Group 2(Ulcer control) showing deep penetrating ulceration, Group 3 (Omeprazole treated group), Group 4(*H. rosea* 100mg/kg bw) showing mild ulceration and edema, Group 5(*H. rosea* 200mg/kg bw) and Group 6(*H. rosea* 300mg/kg bw) showing normal stomach architecture

KEY: M:Mucosa layer, Mm:Muscularis mucosa, S:Sub-mucosa, P:gastric pits, Mp: Muscularis propria, V: blood vessel, U:Ulcer, P: gastric pits, E:epithelial cells.

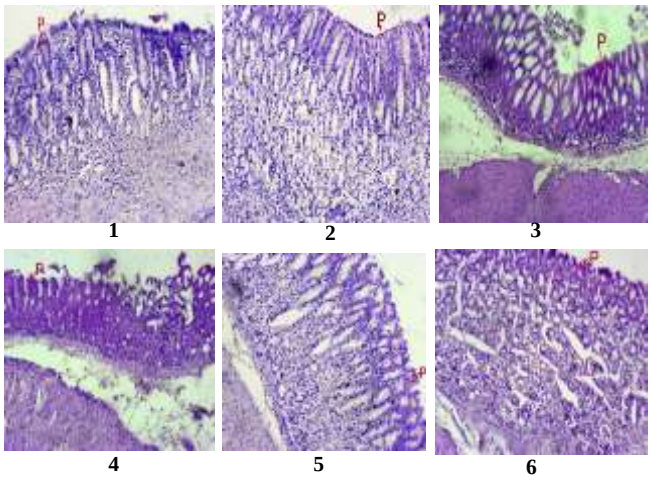

Plate 3: Periodic acid Schiff (PAS) photomicrographs of groups 1 to 6 (PAS x100 magnification). Group 1(Normal control) showing positive PAS staining, Group 2(Ulcer control) pale PAS staining, Group 3 (Omeprazole treated group), Group 4(*H. rosea* 100mg/kg bw), Group 5(*H. rosea* 200mg/kg bw) and Group 6(*H. rosea* 300mg/kg bw) showing normal positive PAS staining.

Conclusion

The findings of this study indicate that aqueous extract of the leaves of *H. rosea* possess anti-ulcerogenic activity on indomethacin induced gastric ulcer in Sprague-Dawley rats. This may be due to the presence of phytochemical compounds such as flavonoids, terpenes and hypoestoxide present in the leaves.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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