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EFFECT OF JUTE LEAVES ON INDOMETHACIN-INDUCED ULCER

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ABSTRACT: *Background: Excessive stomach acid secretion can erode oesophageal tissue, causing swelling, bleeding, and occasionally a peptic ulcer disease. This study aimed to understand the dose-dependent effect of Corchorus olitorius to ameliorate the effects of gastric acid secretion. 44 rats were divided into 2 groups; pre- and post-treatment groups: which were further subdivided into 6 groups each. The pretreatment group had the normal control, negative control, standard group receiving 0.2ml/kg of oral sucralfate and 3 groups receiving Corchorus olitorius extract at doses of 0.9ml/kg, 1.8ml/kg and 3.6ml/kg respectively. All animals in the pretreatments were orally administered the extract for 7 days. On the 8th day, all except the normal group were induced with a gastric ulcer using indomethacin. After 24hours, the rats were sedated using diethyl ether, their stomachs were all harvested, and then, blood samples were collected. It was observed that indomethacin caused severe gastric ulcers, which were reversed by Corchorus olitorius extract. Visible changes were observed in immunoglobulin levels, indicating the anti-inflammatory and mucosal protective properties of this treatment.*

KEYWORDS: *Corchorus olitorius*, gastric ulcer, indomethacin, immunoglobulin, anti-ulcerative property.

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INTRODUCTION

Stomach acid, also known as gastric acid, is a vital digestive fluid produced by the parietal cells in the gastric glands of the stomach lining (Carolyn 2019). Its main constituent is Hydrochloric acid (HCl), which is essential for food digestion (Hsu et al., 2023). Gastric acid secretion is an intricate and dynamic process regulated by neural, hormonal and pancreatic pathways as well as chemical and mechanical stimuli (Schubert et al., 2010). According to Laine et al. (2008), while the physiological generation of gastric acid is beneficial in food digestion and prevents colonization by ingested bacteria, acidity in the stomach lumen can harm the gastric epithelial lining when it crosses the mucosal barrier. Acid-producing cells in the digestive system can sometimes produce too much acid, causing health problems such as reflux disease and stomach ulcers. Some medicines that may cause a decrease in stomach acid secretion include H_2 blockers like Pepcid (famotidine) and Proton Pump Inhibitors (PPIs) (Shamard 2023). Increased stomach acid secretion can be caused by bacterial infections, stress, or the rebound effects of worm infestation (Lee et al., 2017; Shamard, 2023). Prostaglandins are essential for preventing acid damage to the mucosal lining of the stomach and duodenum, and a decrease in the release of this chemical can cause peptic ulcers, gastrointestinal bleeding, and gastroesophageal reflux disease (GERD) (Greenwood-Van et al., 2017).

Peptic ulcer disease (PUD) is the most common condition affecting the submucosal layer in the stomach and duodenum (Chouhan and Lal, 2023; Srivastav et al., 2023). It is usually caused by a defect in the mucosa of the stomach or duodenum that extends beyond the muscularis mucosa (Srivastav et al., 2023). PUDs are also known as sores or lesions on the mucosal lining caused by persistent erosion and injury to the walls of the stomach and duodenum (Quader et al., 2022). It includes ulcers in the oesophagus, stomach, and duodenum. One of the most prevalent causes of PUDs is long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (Sameh et al., 2019; Aladainan et al., 2021; Chaudhry et al., 2023). Indomethacin promotes stomach ulceration by inhibiting protective factors, such as prostaglandin E_2 (PGE_2), bicarbonate, and mucus, while also increasing aggressive variables, including increased gastric hyperacidity and oxidative stress (Bhalke et al., 2010; Sameh et al., 2019). *Helicobacter Pylori* (*H. pylori*) infection is another common cause of this condition. Other causes include Zollinger-Ellison syndrome, stress, chemotherapy, and viral infection; however, these are rare (Malik et al., 2023). The most typical symptoms are stomach discomfort (characterized by a burning sensation after eating), nausea, appetite changes, and heartburn (Mohammad 2022).

The therapy of peptic ulcer disease is primarily focused on lowering gastric acid secretion and strengthening the gastric mucosal barrier. Omeprazole and other Proton Pump Inhibitors (PPIs) are commonly used to treat and manage PUDs caused by stress, NSAIDs, and H. pylori infection. PPIs act by suppressing the stomach's ability to produce acid, hence alleviating symptoms and accelerating recovery. It acts by directly inhibiting the proton pump (hydrogen and potassium pump) in the parietal cells of the stomach, decreasing acid secretion. According to Chaudhry et al. (2023), PPIs have higher efficacy and potency, making them the most effective substance for the therapy of gastric acid disorders (Sameh et al., 2019; Chaudhry et al., 2023; Abdullah et al., 2020).

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Misoprostol, a prostaglandin E_1 analogue, is occasionally used to manage acid secretion abnormalities, especially NSAID-induced peptic ulcers. Its mode of action is to suppress acid secretion and increase the prostaglandin content present in the stomach. The use of H_2 receptor antagonists like cimetidine and ranitidine, as well as antibiotics like clarithromycin and metronidazole, are additional techniques used to eradicate *H. pylori* infections (Sameh et al., 2019; Chaudhry et al., 2023). Although their effects are usually short-term and cannot be used for severe peptic disorders, antacids such as sodium bicarbonate and calcium carbonate can be used for the neutralization of gastric acids (Pugazhenthi et al., 2025; Mohammad, 2022).

Despite the identification and efficiency of various pharmacological agents, some have limited efficacy and severe adverse effects (such as hepatotoxicity, anaphylaxis, diarrhoea and nausea), which can occasionally cause the disorder to recur frequently and render the treatment ineffective (Awaad et al., 2013; Jesus et al., 2013; Qader et al., 2022). This has prompted research into alternative treatments involving the use of plant products for the management and therapy of PUDs. Plant products, which form approximately 25% of the prescription medicines in the world, have proven that the phytochemicals isolated from these plants have enormous therapeutic properties, owing largely to the presence of biologically active compounds (Anwar, 2011; Calleja, 2010). An example of a medicinal plant is the *Corchorus olitorius*.

Jute leaf (*Corchorus olitorius*) is a widespread vegetable belonging to the Tiliaceae family. It is native to the tropical regions of Africa, Asia, but has subsequently expanded to South America, Australia, and some parts of Europe (Zakaira and Shuranjan, 2022). It is also referred to as the long-fruited jute, tossa jute, jute mallow and jew's mallow (Al-Batran et al., 2013). In West Africa, where the leaf is widely used, the Yoruba tribe of Nigeria calls it "ewedu", while the Hausa and Fulani call it "rama". Following its prominence as the most important cash crop of Bangladesh, it is referred to as the country's golden fibre (Islam 2013). It imparts a distinct flavour to food and contains a high dietary protein content; hence it is mostly used as a food source and a thickener in soups, stews and sauces. The leaf contains 17 active nutrients, including potassium, phosphorus, ascorbic acid, beta-carotene, vitamin C, lipids, crude fibre, iron and calcium. Furthermore, it contains significant quantities of α-tocopherol, equal to vitamin E, making it an antioxidant and relating it to protection from cancer, hypertension, diabetes and other medical ailments. (Sule et al., 2017; Ali et al., 2019; Ali et al., 2020). *Corchorus olitorius* contains a large quantity of all amino acids except methionine, which is present in low concentrations.

This study focuses on the anti-ulcerative effects of *Corchorus olitorius* extract on indomethacin-induced gastric ulcers in Wistar rats. It also evaluates the impact of immunoglobulins in lowering inflammation and allergy in gastric ulcers, as well as the antioxidative property of sucralfate against *Corchorus olitorius* extract. Overall, the goal of this research is to contribute to the discovery and application of plant-based anti-ulcerative agents in addressing the challenges posed by PUDs.

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Figure 1: Jute Leaves

METHODOLOGY

Procurement of Experimental Animals

The animals were gotten from the University of Port-Harcourt laboratory animal house, known for adhering to ethical breeding and care standards. They were housed in a controlled environment, maintaining specific conditions such as a temperature range of 22-24°C, humidity levels between 40-60%, and a 12-hour light/dark cycle. The housing consisted of a polypropylene cage with sawdust bedding, which was changed regularly to maintain hygiene. A standard rodent diet and water were provided daily. The animals underwent an acclimatisation period of at least one week before the start of the experiment to allow them to adjust to their new surroundings, thereby minimizing stress and ensuring reliable experimental outcomes.

Sample Selection

The plant (*Corchorus olitorius*) was sourced from the vegetable market at Choba in Obio Akpor Local Government Area, Rivers State, Nigeria. It was then carefully transported to the Plant Science and Biotechnology laboratory for authentication. A qualified botanist conducted the authentication process, which involved examining the morphological characteristics of the leaves, such as leaf shape, size, colour, and texture. Additionally, a sample was compared to an herbarium specimen to ensure correct identification. The authenticated leaves were then processed and stored appropriately for further use.

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Extract Preparation

The compound was extracted using the aqueous technique. For this method, 10g of *Corchorus olitorius* were washed, placed in a clean pot, and boiled with 3 litres of distilled water and concentrated to 1litre. The concentrate was decanted and stored in a clean, dry, labelled container at 4^0C until it was ready for use.

Determination of *Corchorus olitorius* Concentration

In this process, a crucible (an evaporating dish) kept at room temperature was weighed using a G&G electronic weighing scale, and its weight was noted to be 52.97g. One ml of the aqueous *Corchorus olitorius* leaf extract was placed into the crucible and was placed over a warm beaker of water on a hot plate set at 40^{o}C. The content of the dish was allowed to evaporate to dryness and was allowed to cool back to room temperature. Its content and the crucible were weighed again, and their weight noted to be 53.02g. The weight of the extract in the crucible was determined by subtracting the weight of the crucible from the weight of the crucible plus the weight of the extract as shown below.

Table 1: Shows the determination of the extract concentration

Weight Determination	*Corchorus olitorius* leaf extract
Weight of Dish + Dry extract	53.0.2g
Weight of Dish	52.97g
Weight Difference	0.05g
Weight in mg	0.05x1000 = 50mg

Since the volume of the extract used was **1ml**, it implies that the concentration of the extract is **50mg/ml.

Oral Toxicity Testing (LD_{50} Determination)

In this study, Bruce, method of 1985 was employed in determining the LD_{50}, with all the animals used weighing 200g. With this method, a nulliparous and non-pregnant female Wistar rat, fasted overnight (food but not water was withheld) prior to dosing, was orally given a single dose by gavage using a suitable stomach intubation cannula, starting with a dose of 120.5mg/kg of the aqueous *Corchorus olitorius* leaf extract (i.e. 24.1mg/200g or 0.48ml/200g of the extract). The female species was chosen in order to reduce variability and as a means of minimizing the number of animals used. After the aqueous *Corchorus olitorius* leaf extract was administered, food was still withheld for a further 3-4 hours. The animal was observed for death for a period of 48 hours. This dose was chosen since there was no knowledge of the probable toxicity of the extract. At this dose, no death was observed. Since no death was observed, the dose for the next animal was increased by a factor of one-half log times the original dose; (Note: 3.2 is the default factor corresponding to a dose progression of one-half log unit). This was calculated to be 385.6mg/kg or 77.12mg/200g of the extract. The animal was again observed carefully for up to 48 hours before deciding on whether and how much to dose the next animal, and still there was no death. The process of progressive increment was continued with the following doses of 1233.92mg/kg or 246.784mg/200g extract, still without death. Again, another animal was treated with 3948.48mg/kg or 789.7088mg/200g of the aqueous extract**,** and was again observed for 48 hours still there was no death observed. The dose was increased to 5000mg/kg, since it is scientifically accepted

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that a substance is said to be non-toxic at a dose of 5000mg/kg. Thus, 5000mg/kg or 1000mg/200g was administered, and there was still no death observed. This lack of toxicity was confirmed by administering this last dose to three other animals, and still no death was observed in any of them. It therefore implies that the aqueous *Corchorus olitorius* leaf extract is safe at 5000mg/kg, using this method of LD_{50} determination. Where death occurs, the LD_{50} is usually determined using the geometric mean of the last two doses, the highest dose that did not cause death, and the lowest dose that caused death as follows;

The LD_{50} is determined from the formula

$$LD_{50} = \sqrt{D_1 x D_2}$$

Where D_1 is the highest dose that did not cause death, and D_2 is the lowest dose that caused death in the test animals.

Study Design

The study was structured into several experimental groups to assess the therapeutic potential of the plant extract against indomethacin-induced gastric ulcer in Wister rats. A total of 44 animals were involved in the study. Eight of them were used for the LD50 test, leaving 36 animals for the main study. These 36 were then split into two main categories: pre-treatment and post-treatment, with each category further divided into 6 subgroups, consisting of 3 animals per group. For the pre- and post-treatment groups, group 1 received a low dose of extract; group 2 received medium dose of extract; group 3 received high dose of extract; group 4 received standard drug (sucralfate); group 5 received no drug treatment (negative control), while group 6 received distilled water (normal control). All animals in each group except group 6 were all induced with pathologic ulcer.

Method of Carrying out the Study

In the post-treatment group, the animals were fasted and subjected to gastric ulcer induction for 24 hours with indomethacin at a dose of 600mg/70kg before treatment began. The treatments were administered as follows: the low dose group received 250mg/kg; the medium dose group received 500mg/kg; the high dose group received 1000mg/kg; the positive control group received 1000 mg/70kg of sucralfate; the negative control group was induced with ulcers and received no treatment, while animals in normal control group received no induction but were given distilled water. The treatment period lasted for one week, after which the animals were euthanised with diethyl ether. Ulcers were counted; blood samples were collected for immunoglobulins level evaluation, and stomach tissues were taken for histopathological studies. For the pre-treatment group, the same dosage schedule as described above was administered for a period of one week before the animals were exposed to indomethacin according to the scheduled groups for a period of 24 hours, and thereafter, the animals were anaesthetized with diethyl ether and blood samples collected as above as well as stomach tissue. The blood samples were collected into EDTA tubes, and stomach tissues were preserved in universal containers containing 10% formalin for pathohistological evaluation.

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RESULTS

Ulcer Count in Post-Treated Animals

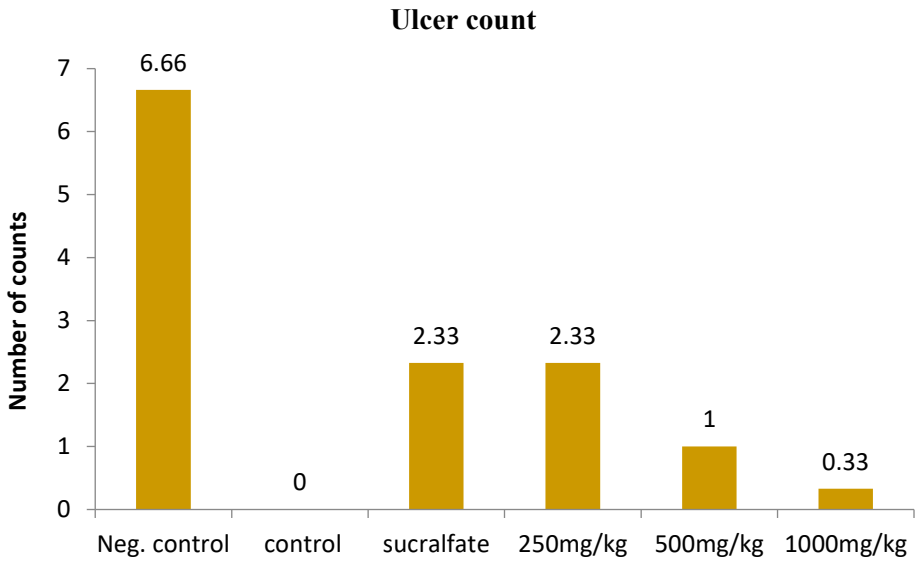

Figure 2: Effect of jute leave extract on ulcer counts in post treated Wistar rats

Ulcer Count in Pre-Treated Animals

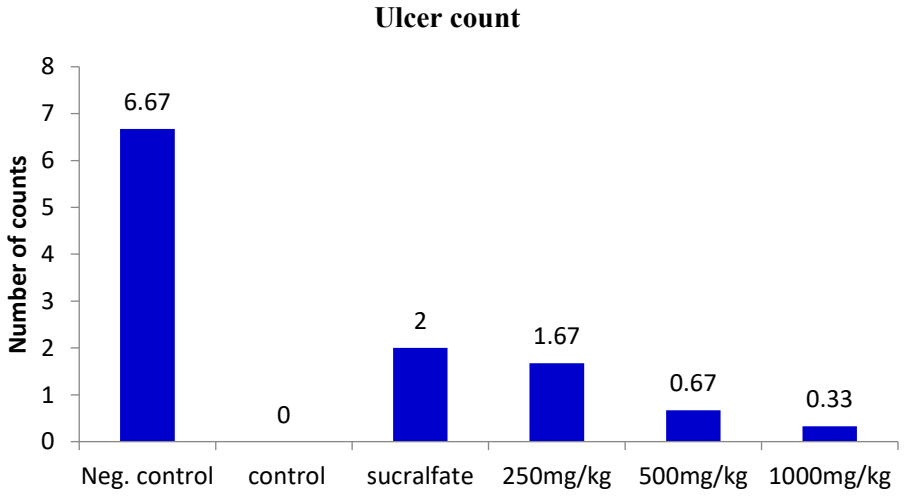

Figure 3: Effect of jute leave extract on ulcer counts in pre-treated Wistar rats

In post-treated animals (figure 2), the negative control group showed the highest average number of ulcer count (6.66±0.88) which was significantly different from all other groups, while the normal control group had no ulcers (0.00±0.00). Sucralfate reduced ulcers counts to an average of 2.33±0.52, demonstrating its efficacy. *Corchorus olitorius* extract exhibited a

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dose-dependent reduction in ulcer counts, with 1000 mg/kg achieving the lowest average number of ulcer count of 0.33±0.13, which is significantly lower than sucralfate. While in pre-treated animals (Figure 3), similar trends were observed. The negative control group maintained significantly highest ulcer count (6.67±0.88), while pre-treatment with 1000 mg/kg of *Corchorus olitorius* extract reduced the average number count to 0.33±0.33, significantly surpassing sucralfate (2.00±0.58). This suggests that both post and pre-treatment with the extract at 1000 mg/kg was more effective than all other group.

Immunoglobulin E (IgE) level in Post-Treated Animals

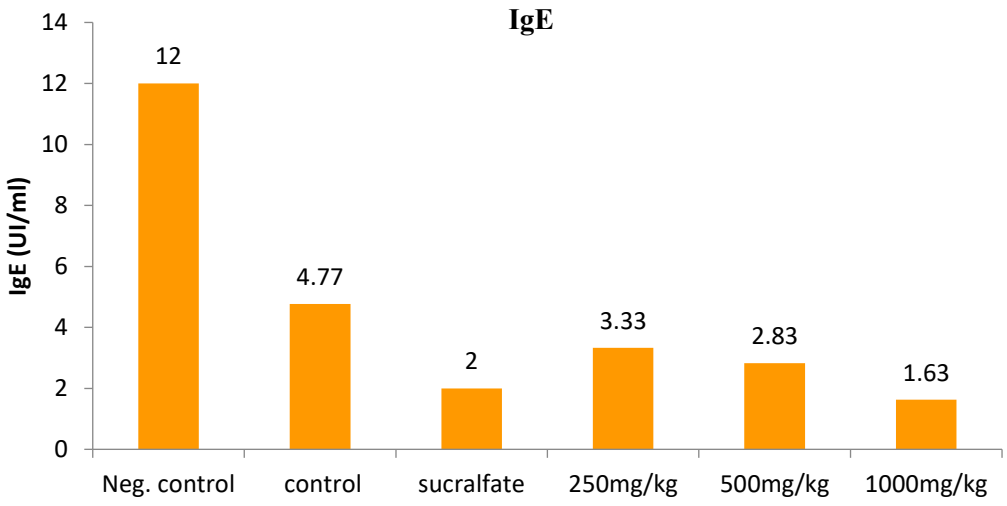

Figure 4: Effect of jute leave extract on immunoglobullin E level in post treated Wistar rats

Immunoglobulin E (IgE) level in Pre-Treated Animals

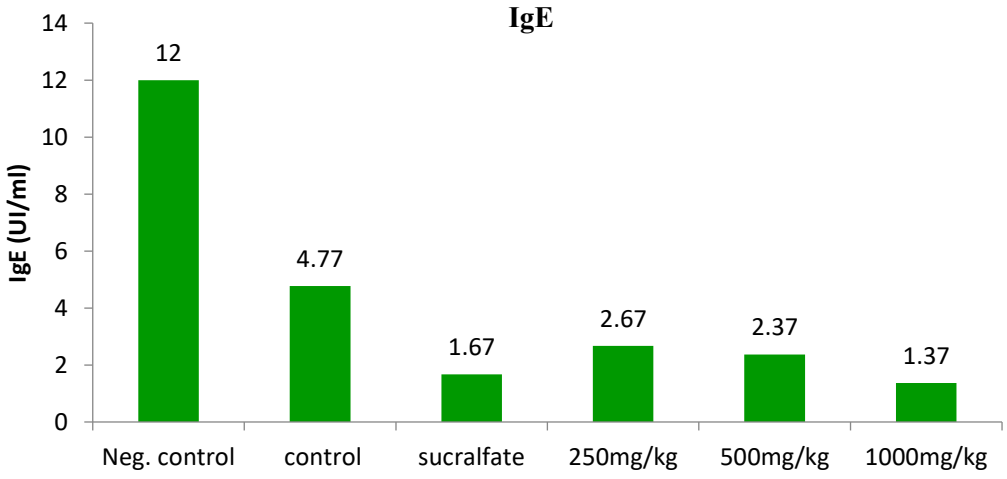

Figure 5: Effect of jute leave extract on immunoglobullin E level in pre-treated Wistar rats

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Immunoglobulin Levels in Post-Treated Animal

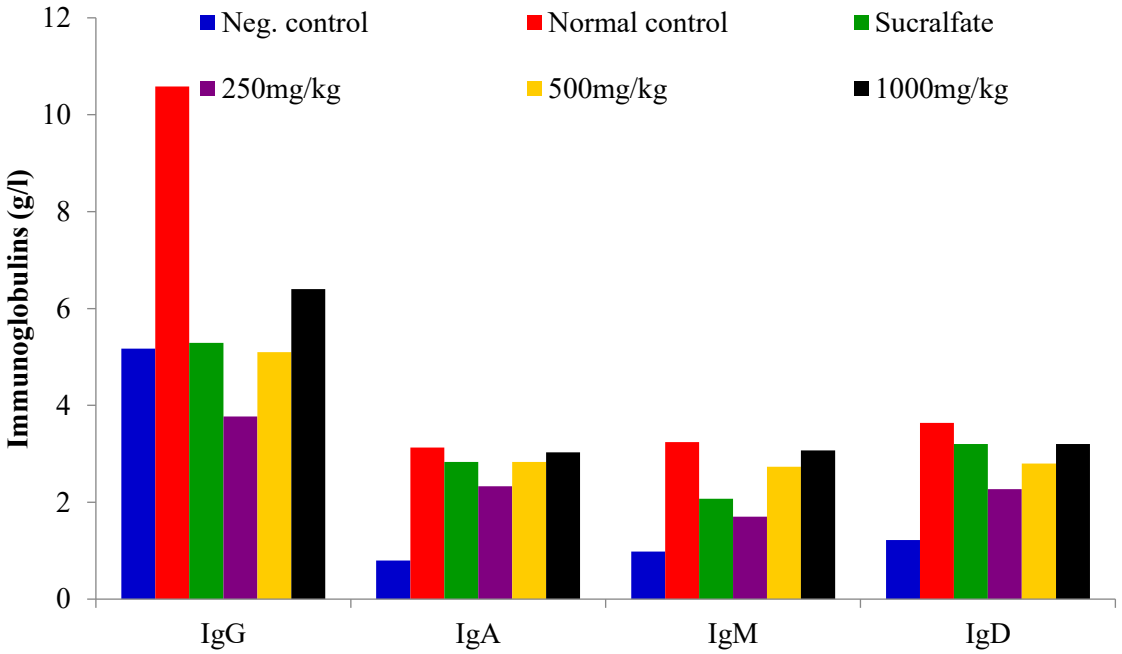

Figure 6: Effect of *Corchorus olitorius* extract on immunoglobulin levels in post treated Wistar rats

Immunoglobulin Levels in Pre-Treated Animals

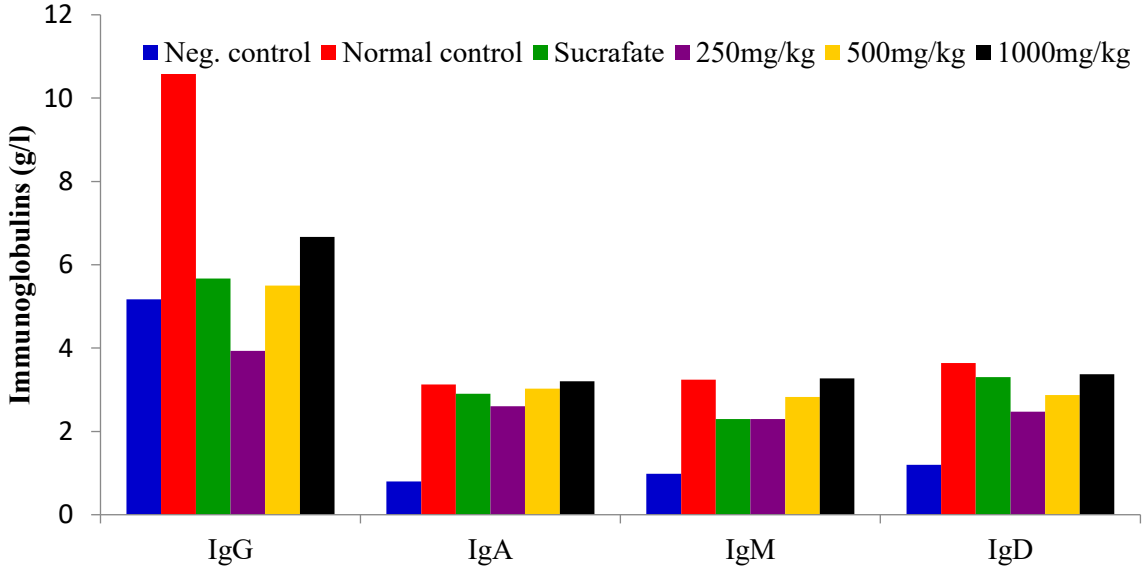

Figure 7: Effect of *Corchorus olitorius* extract on immunoglobulin levels in pre-treated Wistar rats

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The results for immunoglobulin E (IgE) are presented in figures 4 and 5, while those for IgG, IgA, IgM, and IgD are presented in figures 6 and 7 for post-treatment and pre-treatment, respectively. It was observed that the extract exhibited a significantly reduced IgE (1.63±0.19) at the dose of 1000 mg/kg when compared to the negative control (12.00±0.58) and all other groups except sucralfate (2.00±0.58). On immunoglobulin G (IgG), the normal control showed the highest level (10.58±0.05), which was significantly higher than all other groups. This pattern was also observed for IgA, IgM, and IgD. The second highest level of the different immunoglobulins was found in animals treated with the extract at the dose of 1000 mg/kg, indicating that both the extract at 1000 mg/kg and sucralfate normalised the immunoglobulin levels, particularly IgE. *Corchorus olitorius* extract showed significant immunomodulatory effects, especially at a dose of 1000 mg/kg. This pattern of results was also observed in the pre-treatment groups.

Post-Treatment Phase

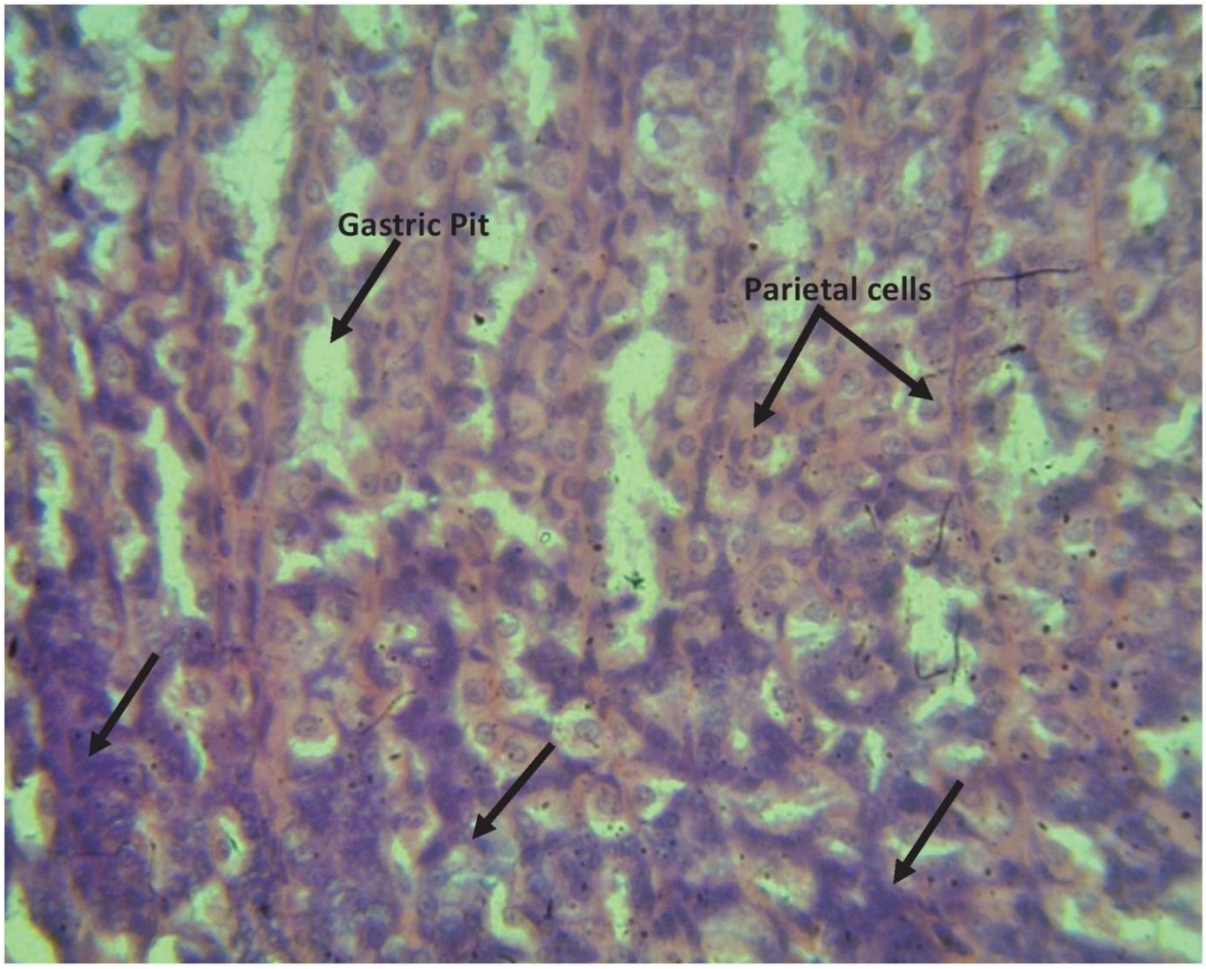

Plate 1: Shows photomicrograph (H&E X400) of the basal layer of the stomach of low dose of post treatment group. It shows decreased thickness of the gastric folds with mucosal gland loss with inflammatory cells activities (arrows), indicating moderate mucosa wall atrophy.

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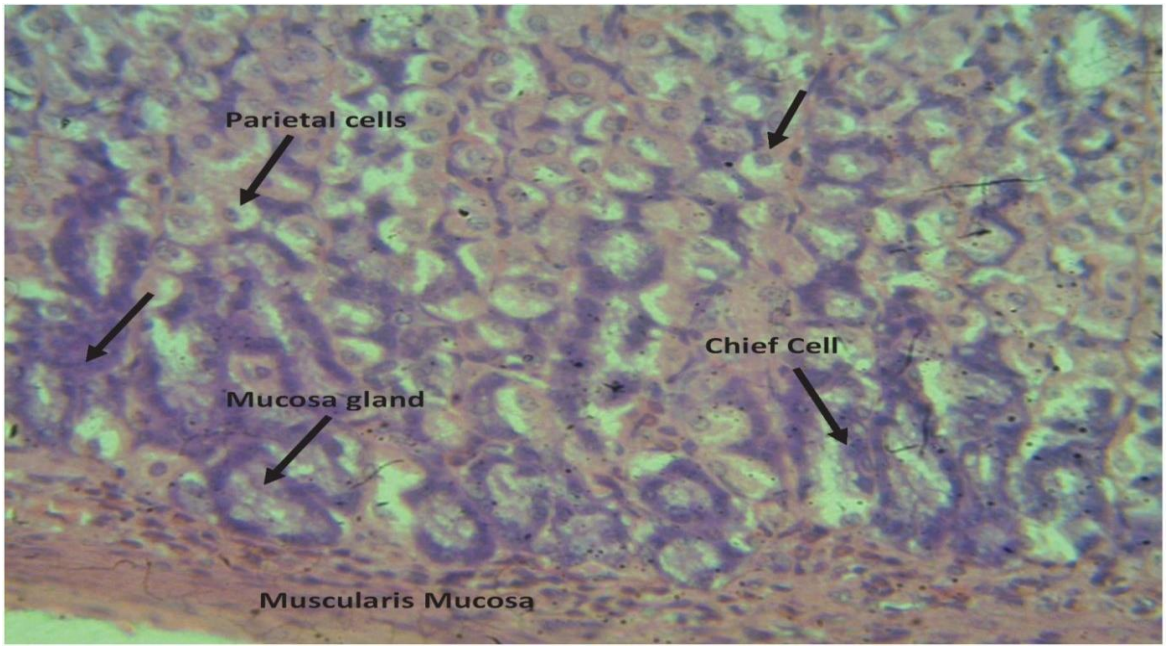

Plate 2: Shows photomicrograph (H&E X400) of the basal layer of the stomach of medium dose of post treatment group. It shows mild vacuolation on the deep layer of the mucosa with reduced parietal cells, indicating moderate atrophic gastritis of the stomach.

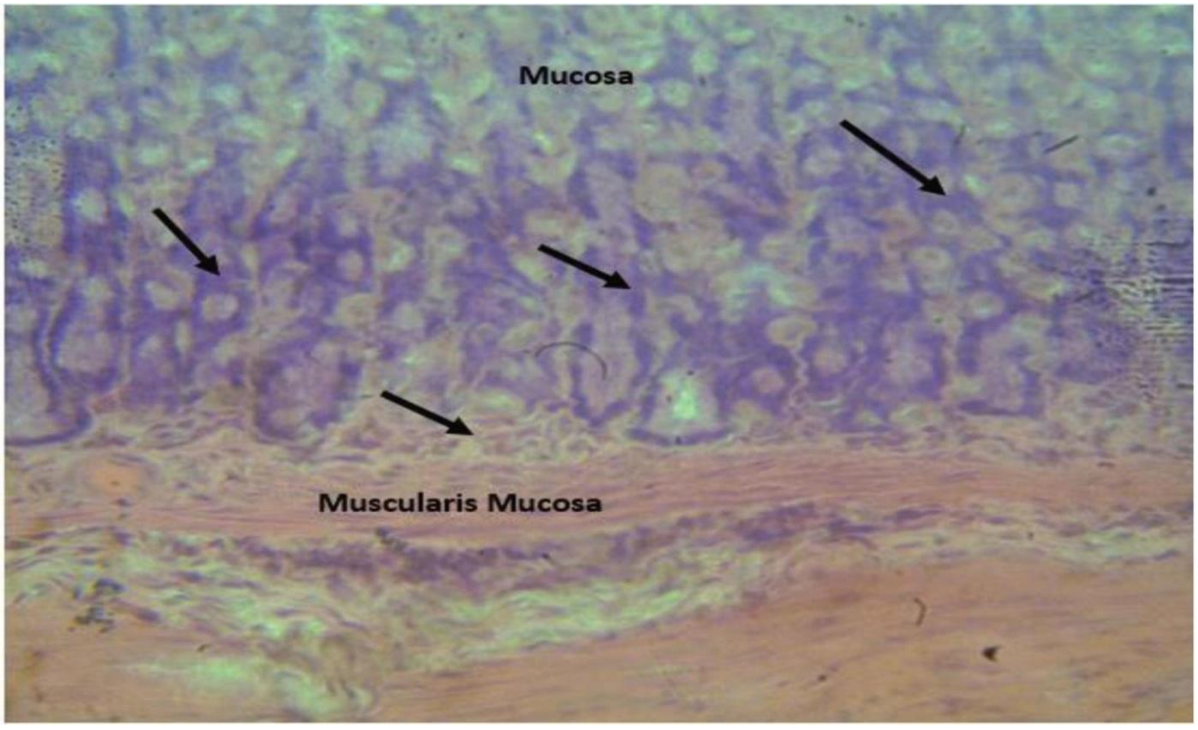

Plate 3: Shows photomicrograph (H&E X400) of the basal layer of the stomach of high dose of post treatment group. It shows thickened mucosal gland with mild haemorrhagic deposit and reduced inflammatory cells infiltration (arrows), indicating moderate distortion of the mucosa wall.

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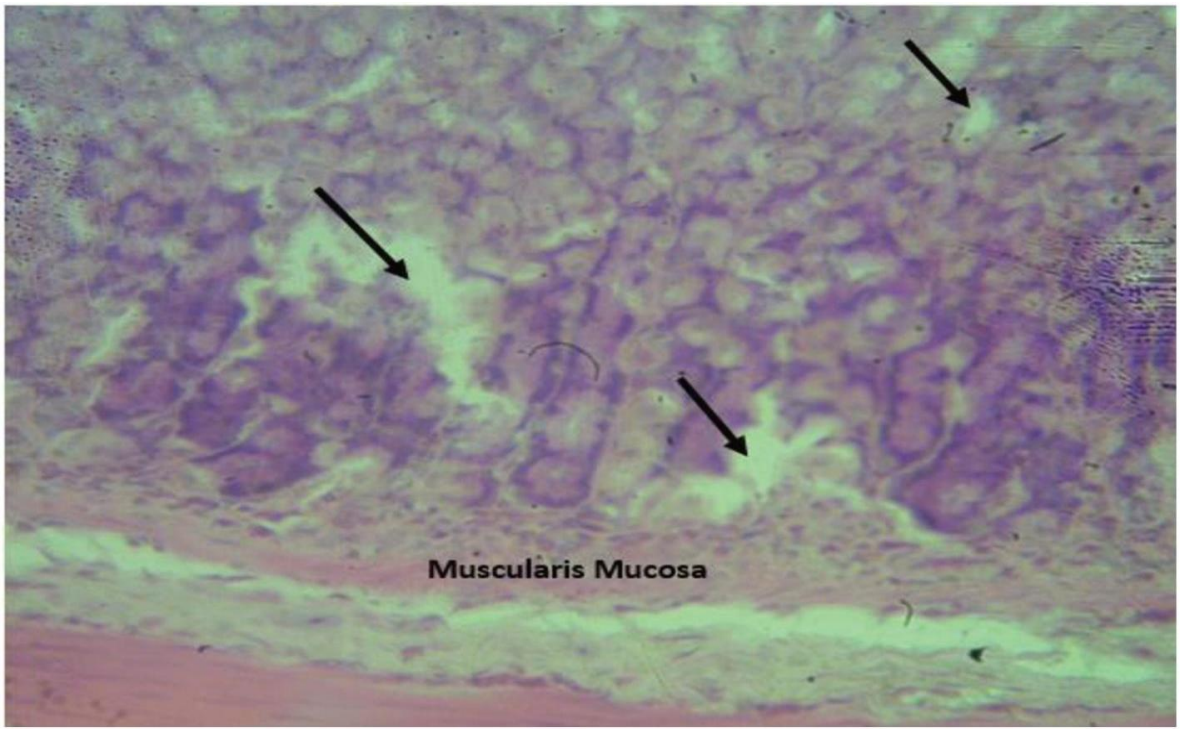

Plate 4: Shows photomicrograph (H&E X400) of the basal layer of the stomach of animals treated with sucralfate. It shows mild disruption of mucosal wall of the stomach with no inflammatory activities (arrows), indicating mild distortion of the stomach tissue

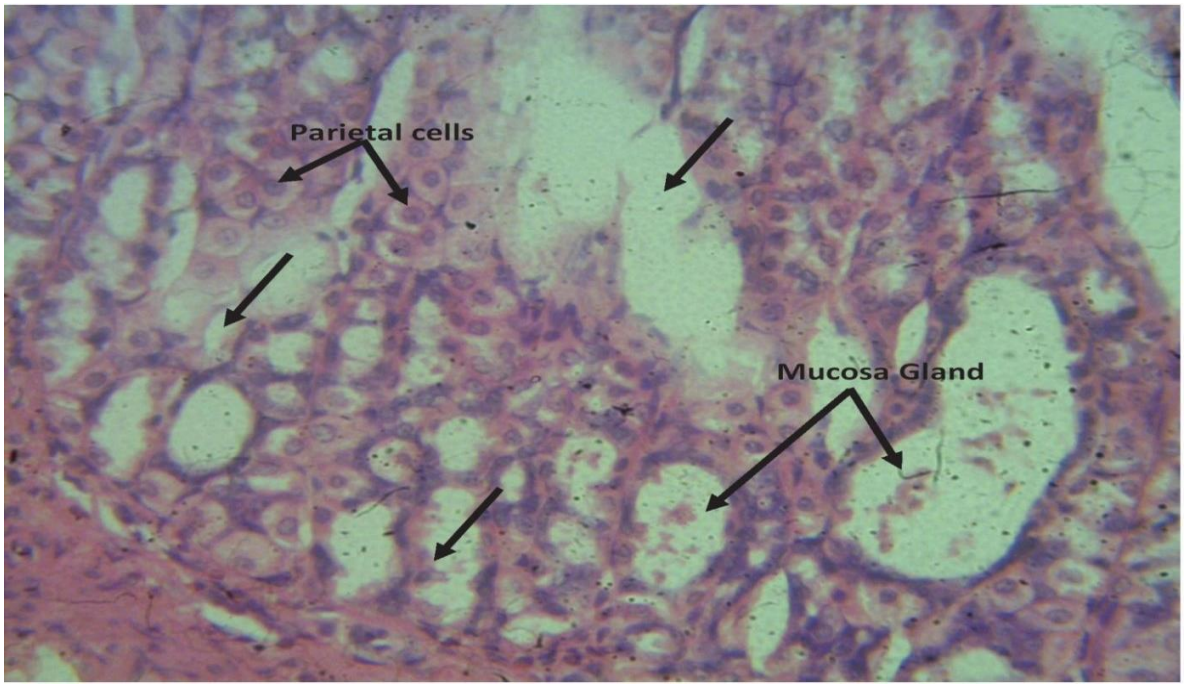

Plate 5: Shows photomicrograph (H&E X400) of the basal layer of the stomach of animals in the negative control group. It shows multifocal disruption of the mucosal glands and cells associated with enlargement of the lamina propria epithelium (arrows), indicating an eroding and degeneration of the mucosa wall

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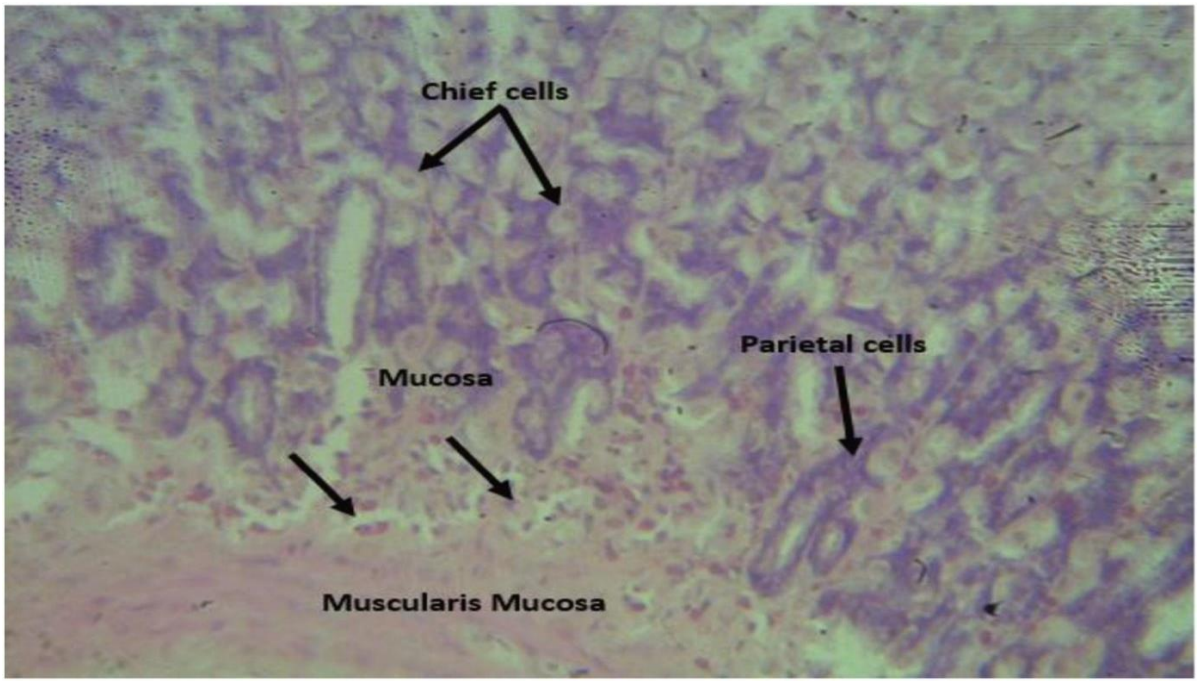

Plate 6: Shows photomicrograph (H&E X400) of the basal layer of the stomach of animals in the normal control group. It shows mucosal gland cells with minimal haemorrhagic deposit (arrows), indicating normal stomach appearance

Pre-Treatment

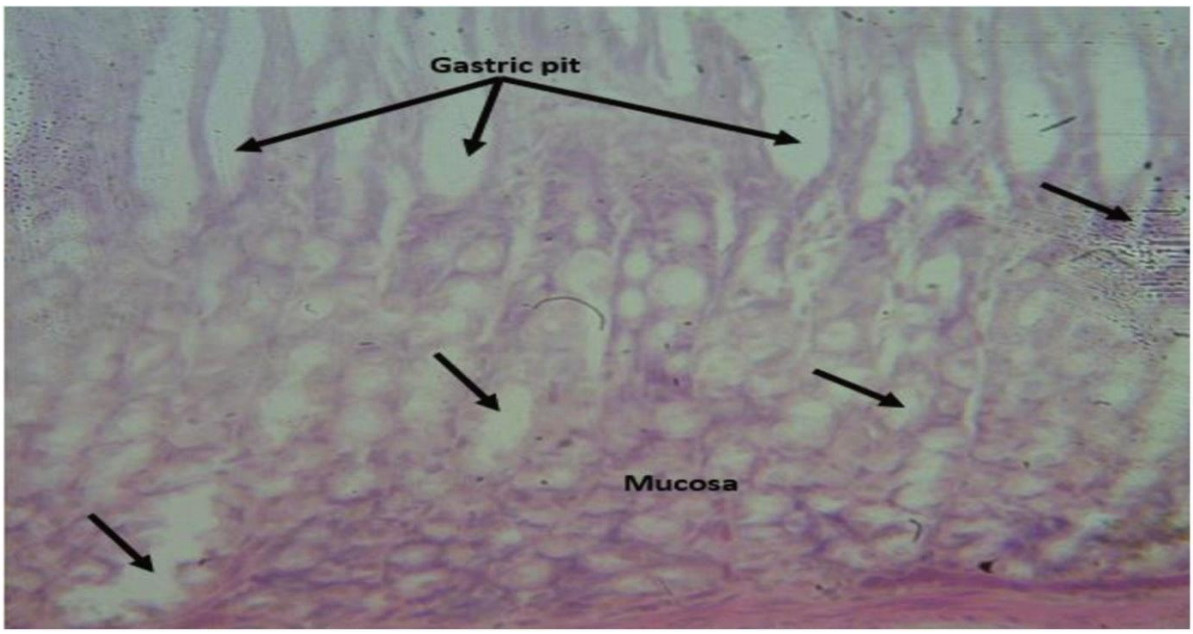

Plate 7: Shows photomicrograph (H&E X400) of the basal region of the stomach of low dose of pre-treatment group, revealing decreased thickness of the gastric folds with mild mucosal gland loss with no inflammation, thus indicating moderate mucosa wall atrophy

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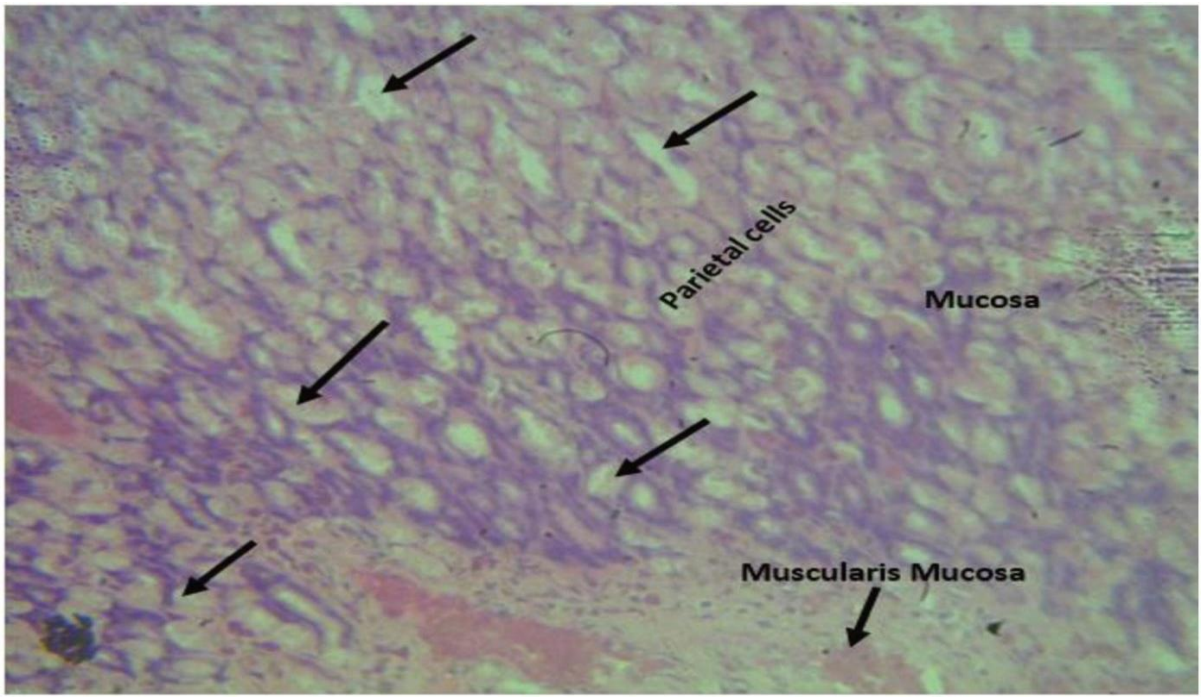

Plate 8: Shows photomicrograph (H&E X400) of the glandular stomach of medium dose of pre-treatment group. It shows mild vacuolation on the deep layer of the mucosa with reduced parietal cells, thus implying mild atrophic gastritis of the stomach.

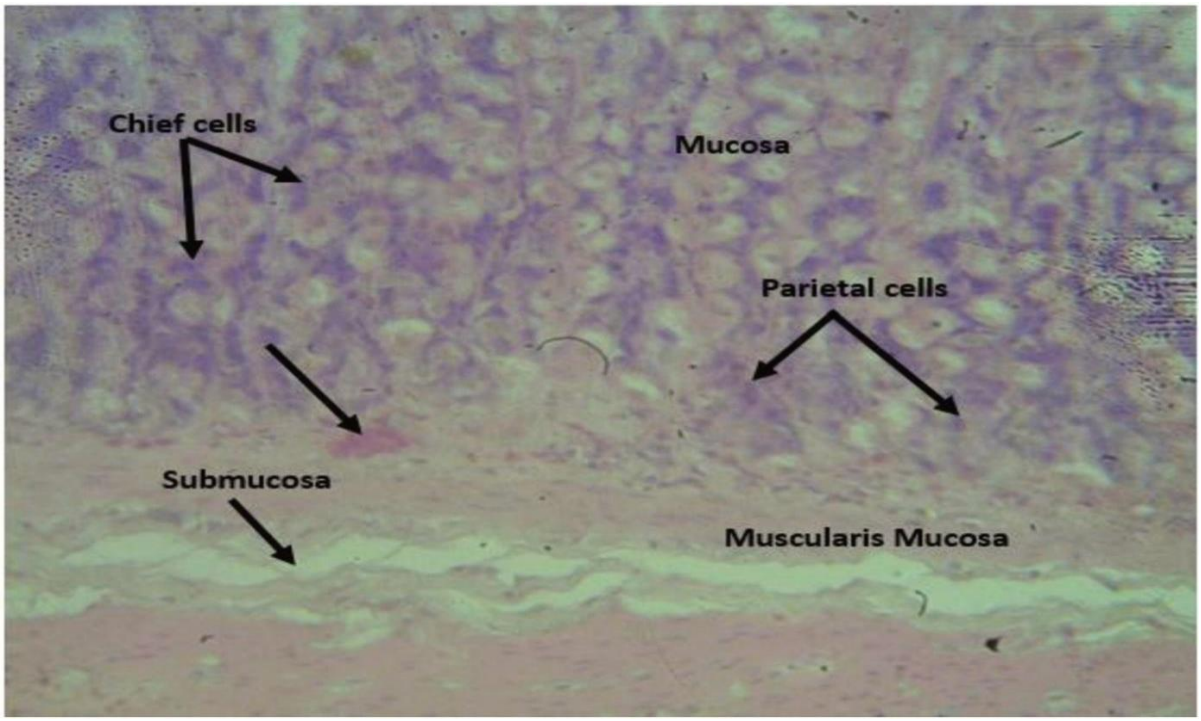

Plate 10: Shows photomicrograph (H&E X400) of the basal layer of the stomach of animals treated with sucralfate. It shows thickened mucosal with increased number of mucosal gland cells and minimal focal haemorrhagic deposit the stomach wall (arrows), thus indicating normal tissues with mild distortion and haemorrhage within mucosal

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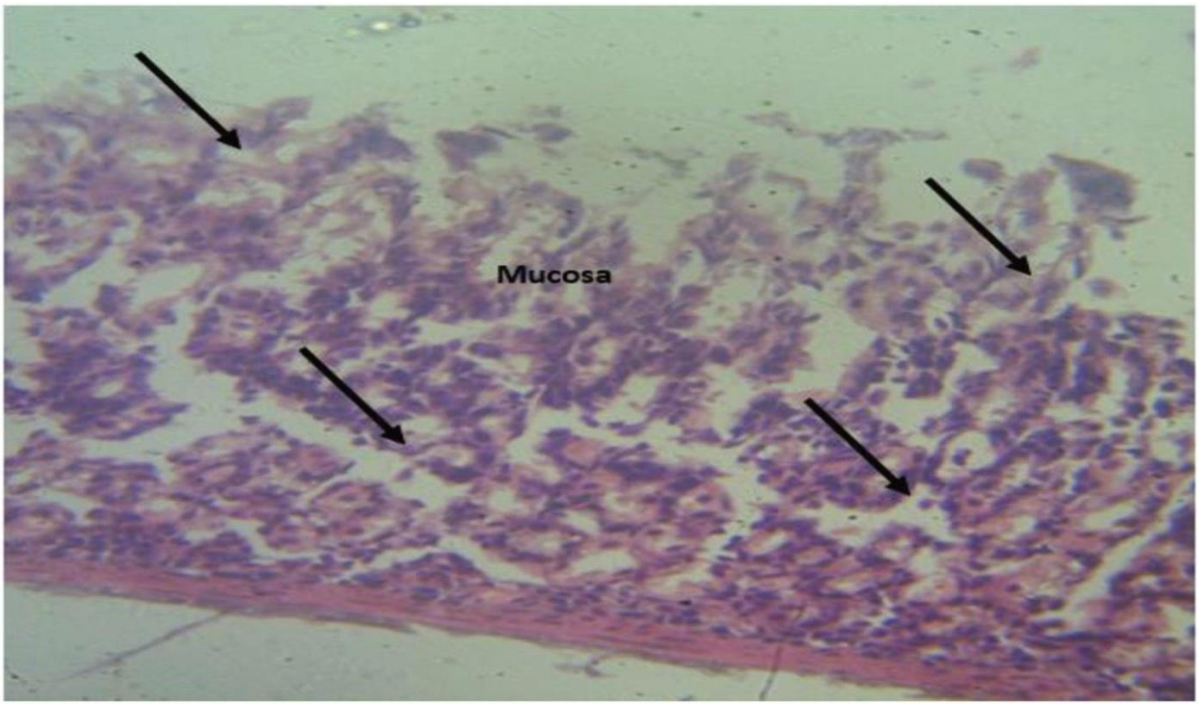

Plate 11: Shows photomicrograph (H&E X400) of the superficial region of stomach of animals in the negative control group, revealing severe multifocal disruption of the mucosal glands and cells (arrows), implying severe distortion of the mucosa wall.

DISCUSSION

This study investigated the effects of *Corchorus olitorius* extract on indomethacin-induced gastric ulcers in Wistar rats. The results showed that the *Corchorus olitorius* extract had a significant dose-dependent decrease in ulcer counts in both the pre-treatment and post-treatment groups, demonstrating its potential for both preventive and therapeutic applications. The ulcer count analysis indicated that the high dosage (1000 mg/kg) of *Corchorus olitorius* was just as effective as the standard drug sucralfate. This suggests that the plant extract has potent cytoprotective and antioxidant properties, most likely because of its bioactive compounds, which include flavonoids and phenolic acids. While the post-treatment results indicate effective healing properties, the pre-treatment result suggests that *Corchorus olitorius* may enhance the gastric mucosa's resilience against elements that act as ulcer-inducing factors. IgG (Immunoglobulin G) levels were generally suppressed in all treatment groups when compared to the normal control; dose-dependent increases were observed at higher doses, signalling a possible immune regulation and reduced inflammation by the extract. All groups, including the sucralfate-treated group, had considerably decreased IgE (Immunoglobulin E) levels, indicating that the extract aids in lowering inflammatory and allergic responses linked to ulcer development. Particularly in the high-dose group, IgA (Immunoglobulin A) levels were higher, indicating an improvement in mucosal immunity, which is essential for protecting the stomach lining against damage. Furthermore, IgM (Immunoglobulin M) and IgD (Immunoglobulin D) levels showed partial restoration but fell short of normal control levels, suggesting that although the extract helps to modulate the

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immune system, not all immunoglobulin responses are fully normalized. The comparative analysis shows that *Corchorus olitorius* has dose-dependent effects, particularly at high doses, with outcomes comparable or superior to sucralfate, making it a promising candidate for alternative ulcer therapy. The findings indicate that *Corchorus olitorius* may function through a variety of mechanisms, including mucosal protection, antioxidative action, and immune response modulation, making it a viable natural therapeutic alternative for NSAID-induced gastric ulcers.

CONCLUSION

Corchorus olitorius extract has potent anti-ulcerative and immunomodulatory properties, effectively lowering ulcer counts and regulating immunological responses in a dose-dependent manner. The study supports the potential use of *Corchorus olitorius* as a natural alternative to standard anti-ulcer drugs like sucralfate, which can provide both preventive and therapeutic benefits against indomethacin-induced gastric ulcers.

FUTURE RESEARCH

Further studies, including clinical trials in human subjects, are recommended to validate the efficacy and safety of *Corchorus olitorius* extract. Research should focus on isolating the bioactive compounds responsible for its therapeutic effects, exploring the specific mechanisms of action, and determining the optimal dosing strategy for maximising benefits while minimising risks.

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