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ANTI-INFLAMMATORY EFFECTS OF UVARIA OVATA ROOT EXTRACT ON WISTAR RATS VIA PRO-INFLAMMATORY CYTOKINES AND STOMACH HISTOLOGICAL ASSAY.

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Abstract

The anti-inflammatory effects of Uvaria ovata on pro-inflammatory cytokines and stomach histology of aspirin ulcer-induced rats were studied. A total of 25 Wistar rats weighing 100g -140g were used for this study. The rats were divided into 5 groups of 5 rats each. Group 1 received no administration; group 2 was used as the negative control and was treated with 800mg/kg of Aspirin per day. Group 3 was the positive control and was administered 4mg/kg of Omeprazole, followed by 800mg/kg Aspirin. Group 4 rats were given low dose (400mg/kg) of the extract of Uvaria ovata, followed by 800mg/kg of Aspirin, while group 5 was given high dose (800mg/kg) Uvaria ovata followed by 800mg/kg of Aspirin. The result revealed that the extract showed significant anti-inflammatory effect on the stomach and pro-inflammatory cytokines.

Keywords: Parasites, Bioaccumulation, Heavy metals, Total Hazard Quotient and Hazard Index.

1.0 Introduction

By virtue of their role in inflammation, cytokines can be divided into pro-inflammatory such as interleukin-1 beta (IL-1β), tumor necrosis factor- alpha (TNF-α), interleukin-6, etc., or anti-inflammatory such as interleukin-10. The pro-inflammatory cytokines are mainly secreted by monocytes and endothelial cells, especially following injury. Cytokine expression is associated with cellular intercommunication via autocrine, paracrine and endocrine mechanisms, culminating in the recruitment of different types of cells into the injured area, including neutrophils and monocytes, thereby favoring additional infection and thus creating a vicious circle (Zhang and An, 2009). Cytokines interact with specific membrane-associated receptors through a complex network linked to the feedback loops and cascades involving reactive oxygen species (ROS), toll-like receptor (TLR) activation, and the nuclear factor system (Korneev et al., 2017). Due to their wide array of inflammatory effects, cytokines have been implicated in various disease processes, including peptic ulcers (Haghazali et al., 2011). As a result of the crucial part cytokines play in influencing inflammatory response during peptic ulcer disease, targeting these inflammatory mediators and their signaling pathways may represent a promising therapeutic strategy for ameliorating the development of peptic ulcer symptoms by promoting the anti-inflammatory cytokines as well as inhibiting the pro-inflammatory ones. More than 50 interleukins are encoded by the human genome (Brocker et al., 2010). The interleukin-1 family of cytokines is made up of 11 members, but the most important one by far is the pro-inflammatory cytokine interleukin-1 beta (IL-1β) (Agyare et al., 2018). IL-1β is produced mainly by macrophages, dendritic cells and mononuclear cells of the tissues, skin and blood respectively. Activated IL-1β activates vascular endothelial growth factor, which promotes the growth of blood vessels, and simultaneously induces chronic inflammation of blood endothelial cells. IL-1β binds to its receptor to activate MyD88 and IL-1 receptor associated kinase-4, which leads to phosphorylation of IL-1 receptor associated kinase-2 and IL-1 receptor associated kinase-1, and then activates NF-ĶB. The activated NF-ĶB enters the nucleus and promotes transcription of some inflammatory genes, which give rise to an increase in the level of inflammatory factors in the body (Qian et al., 2019). Furthermore, the interaction of Helicobacter pylori with the gastric epithelium induces the production of IL-1β. The IL-1β is chemotactic for neutrophils, mononuclear cells and macrophages in the gastric mucosa. Simultaneously, H. pylori also stimulate gastric mucosal infiltration by T cells, B cells and dendritic cells, and induce the release of tumor necrosis factor-alpha (Sanchez-Zauco et al., 2017).

Tumor necrosis factors (TNF) are a superfamily that contains 19 members. The members of this superfamily play a vital role in inflammation and immune response (Aggarwal et al., 2012). The most potent TNF involved in inflammation is TNF-alpha (Gough and Myles, 2020). Tumor necrosis factor-alpha (TNF-α) was originally identified as a factor that leads to the rapid necrosis of transplanted tumors. However, now it is referred to as a major pro-inflammatory cytokine sourced from vascular endothelial cells, keratinocytes, and fibroblasts, that is involved in the very first phase of most inflammatory events in the body. Immediately after the creation of a wound, TNF-α level surges, and it initiates inflammation by promoting the recruitment of inflammatory leucocytes which ultimately prolongs the inflammation and reduces the wound healing potential. TNF-α also plays a key role in regulating the synthesis of extracellular matrix proteins and matrix metalloproteinase (Agyare et al., 2018). Suganuma et al. (2008), showed that a special protein, tumor necrosis factor-alpha protein (Tip-alpha), secreted from H. pylori (causative agent of over 80% of peptic ulcer disease cases) is a potent inducer of TNF-α, mediated through NF-kappaB activation, and also induces tumor-promoting activity in Bhas 42 cells.

Many diseases occur at the tissue level. Histology provides important insight into the pathogenesis of various disease conditions. It is the study of the structure and function of a tissue by correlating the differentiation of the tissue structure to their specific function (Ross and Pawlina, 2016). The stomach is a digestive organ that receives content from the esophagus through the gastroesophageal sphincter and empties into the duodenum through the pyloric sphincter. The junction between the stomach and the esophagus is marked by a sharp transition from the stratified squamous epithelium to simple columnar epithelium. The cell types found in the stomach include mucous cells, parietal cells, chief cells, endocrine cells, etc., with each cell carrying out specific functions (Dorland's Medical Dictionary, 2012).

Uvaria ovata is a shrub in the annonaceae family that grows about 1-2m tall (LinLin et al., 2009). The branches are marked by rusty hairs, the leaves are ovate-shaped, and the flowers are yellow-colored, borne singly in small clusters. Uvaria ovata plants are predominantly found in the coastal regions of West Africa. They are referred to as "ire" in Igbo Language (Burkil, 2004). Currently, very little printed information is available about the therapeutic potential of this plant. In some local parts of Southern Nigeria, the roots of Uvaria ovata plants are applied in the treatment of inflammatory conditions in the gut. This study is motivated by this acclaimed benefit of Uvaria ovata on the gastrointestinal system.

2.0 AIM

To investigate the anti-inflammatory effect of Uvaria ovata root extract by its effect on proinflammatory cytokines and stomach histology.

3.0 MATERIALS AND METHODS

3.1 Sample identification and collection

Fresh roots of Uvaria ovata were collected from fallow grassland in Umuahia, Umuahia Local Government Area of Abia State, Nigeria, and they were authenticated at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria, where they were assigned the requisite voucher numbers.

3.2 Preparation of plant extract

The root extract was prepared according to the method used by Jensen, (2007). The collected fresh roots of Uvaria ovata were dried under shade for 14 days, after which they were pulverized to powder using a manual blender. Fifty grams of the powdered sample was introduced into the extraction chamber of the Soxhlet extractor and extraction was done using ethanol as solvent. Temperature was maintained at 60^0C throughout the extraction period of 48 hours. At the end of the period, the collected extract in ethanol was dried in a laboratory oven at 40^oC to obtain an extract of 9.4g, which was preserved in a freezer until needed, after ascertaining its percentage yield (18.8%).

1g of dried extract was dissolved in 10ml of water to give a stock solution of 0.1g/ml (100mg/ml).

3.3 Experimental design:

Twenty-five rats were assigned into 5 groups of 5 rats each. The rats were starved for 24 hours before the experiment. Group 1 served as control and was not treated. Group 2 was the negative control (no treatment before ulcer induction). Group 3 was the positive control, and the rats were administered 4mg/kg of Omeprazole (before ulcer induction). Groups 4 and 5 received 400 and 800 mg/kg, respectively, of Uvaria ovata root extract before ulcer induction. Thirty minutes later, the rats in groups 2, 3, 4 and 5 were administered aspirin (800mg/kg) to induce ulcer. After another two hours the animals were sacrificed and tested for proinflammatory markers (cytokines: interleukin 1-beta and tumor necrosis factor-alpha), and histology of the stomach was also conducted.

This is illustrated on Table 1 below:

Table 1: Experimental design

Animal Grouping		**Treatment**	**Duration**	**No. of rats**
Group 1	Control	No administration	1 day	5
Group 2	Negative control	800mg/kg Aspirin	1 day	5
Group 3	Positive control	4mg/kg Omeprazole, followed by 800mg/kg Aspirin	1 day	5
Group 4	Low dose	400mg/kg Uvaria ovata, followed by 800mg/kg Aspirin	1 day	5
Group 5	High dose	800mg/kg Uvaria ovata, followed by 800mg/kg Aspirin	1 day	5

3.4 Pro-inflammatory cytokine assay

Blood was collected and centrifuged at 3000 rpm for 5 minutes. The serum was extracted and stored at a temperature of -30^{o}C. The pro-inflammatory cytokines tumour necrosis factor-alpha (TNF-α) and interleukin 1-beta (IL-1β) were assayed using enzyme-linked immunosorbent assay (ELISA).

3.5 Determination of the effect of Uvaria ovata on stomach histology

Sections of the stomach were collected for histopathological examination. The samples were fixed in preservative for a minimum of 48 hours. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in a waterbath and incubated at 60˚C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80% and 70%). The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX. The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were randomly taken using a Motic™ 5.0 megapixels microscope camera at x100 magnifications.

The procedures involving the animal models conformed to the guiding principles in the care and the use of animals by the American Physiological society (American Physiological society, 2002).

3.6 Statistical analysis

Data were expressed as mean±SEM and their group will be evaluated by one-way analysis of variance (ANOVA).

4.0 RESULTS

4.1 Effect of Uvaria ovata on proinflammatory cytokines

Table 2 below shows the effect of Uvaria ovata root extract on the pro-inflammatory cytokines: interleukin-1 beta and tumor necrosis factor-alpha.

The mean values of interleukin 1 beta in group 1 (control), group 2 (negative control), group 3 (Omeprazole 4mg/kg after aspirin ulcer-induction), group 4 (U.o 400mg/kg after aspirin ulcer-induction) and group 5 (U.o 800mg/kg after aspirin ulcer-induction) were 0.72±0.05, 1.78±0.17, 1.36±0.11, 1.17±0.13 and 0.94±0.04 respectively. Group 2 mean IL-1β concentration was significantly higher than the values for each of the group 1 (control), 3, 4 and 5. Group 3 (omeprazole) mean IL-1β concentration was significantly higher than each of the group 1, group 4 and group 5. Group 4 mean value was significantly higher than the values for groups 1 and 5. Group 5 mean value was significantly higher than the control ($P<0.05$).

The mean values of tumor necrosis factor-alpha concentrations in groups 1, 2, 3, 4 and 5 were 66.0±2.95, 85.8±2.35, 82.6±2.20, 77.7±1.48 and 71.1±0.78. . Group 2 mean IL-1β concentration was significantly higher than the values for each of the group 1 (control), 3, 4 and 5. Group 3 (omeprazole) mean IL-1β was significantly higher than each of the group 1, group 4 and group 5. Group 4 mean value was significantly higher than the values for groups 1 and 5. Group 5 mean value was significantly higher than the control ($P<0.05$).

Table 2: effect of Uvaria ovata on pro-inflammatory cytokines (IL-1β and TNF-α)

Treatment groups	IL-1B	TNF- α
Normal control	$0.72{\pm}0.05^{a}$	$66.0{\pm}2.95^{a}$
Ulcer control	$1.78{\pm}0.17^{e}$	$85.8{\pm}2.35^{e}$
Omeprazole 20 mg/kg	$1.36{\pm}0.11^{d}$	$82.6{\pm}2.20^{d}$
U. O root extract 500 mg/kg	$1.17{\pm}0.13^{c}$	$77.7{\pm}1.48^{c}$
U. O root extract 1000 mg/kg	$0.94{\pm}0.04^{b}$	$71.1{\pm}0.78^{b}$

Values are presented as mean ± standard deviation (n = 5) and values with different superscripts are significantly ($P<0.05$) different from any paired mean

4.2 Effect of Uvaria ovata treatment on stomach histology

Plate 1a showed sections of the stomach histology of control rats. The pictograph presented in this slide showed the normal histo-architecture of the cardiac gastric mucosa. Normal gastric pits (arrow); gastric glands (GG); muscularis mucosa (MM); submucosa and muscularis layers were observed. Lumen (L); Esophageal mucosa (yellow arrow). H&Ex100.

Plate 1b showed histological sections of the stomach of group 2 rats (negative control rats induced ulcer without treatment). The pictograph presented on this slide showed multifocal areas of mucosal necrosis with hemorrhage (arrow). Lumen (L); Muscularis mucosa (MM), Submucosa (SM). H&Ex100

Plate 1c showed sections of the stomach of group 3 rats (administered 4mg/kg of omeprazole). The pictograph presented on this slide showed multifocal areas of mucosal necrosis with marked inflammatory cellular infiltration and sloughing of necrotic debris into the luminal space (arrow). Lumen (L); Muscularis mucosa (MM), Submucosa (SM); Muscularis layer (ML). H&Ex100

Plate 1d showed sections of the stomach histology of group 4 rats (administered 400mg/kg of Uvaria ovata extract). The pictograph presented on this slide showed mild multifocal areas of mucosal necrosis involving mostly the upper layers of the gastric mucosa (arrow). Lumen (L); Muscularis mucosa (MM), Submucosa (SM), Muscularis layer (ML). H&Ex100

Plate 1e showed sections of the stomach of group 5 rats (administered 800mg/kg of Uvaria ovata). The pictograph presented on this slide showed mild multifocal areas of mucosal necrosis involving mostly the upper layers of the gastric mucosa (arrow). Lumen (L); Muscularis mucosa (MM), Submucosa (SM). H&Ex100

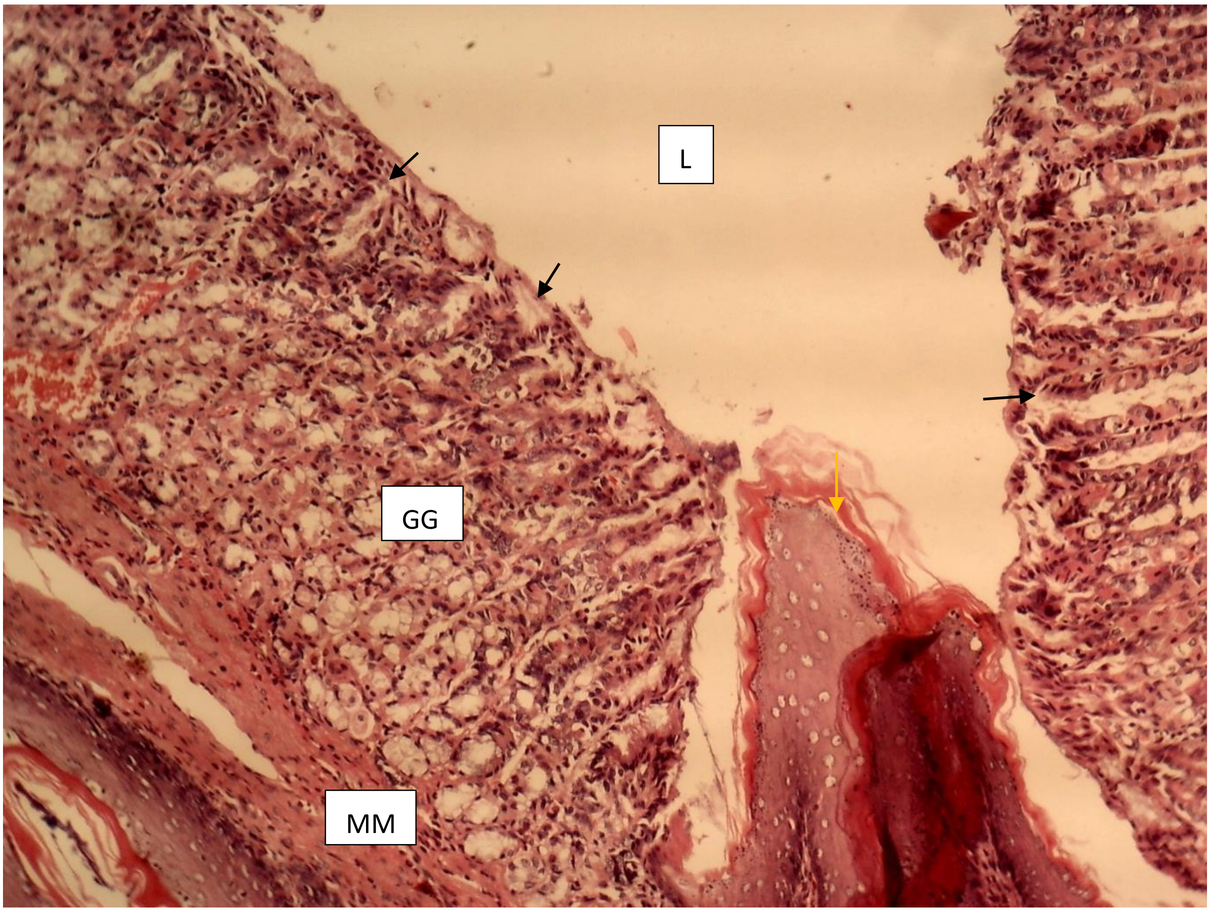

Plate 1a: stomach histology of control rats showing normal histo-architecture of gastric mucosa

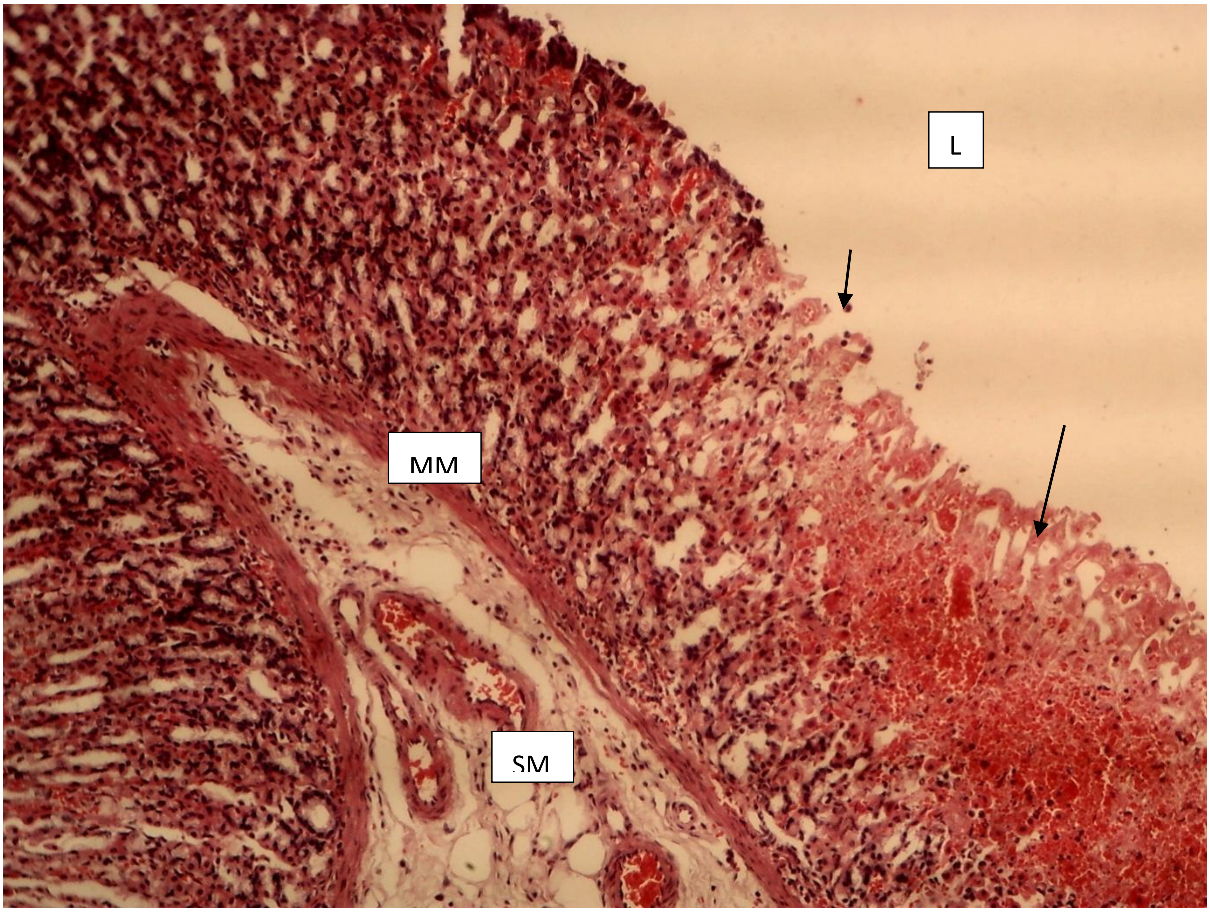

Plate 1b: stomach histology of group 2 (negative control) rats showing extensive multifocal areas of mucosal necrosis with hemorrhage

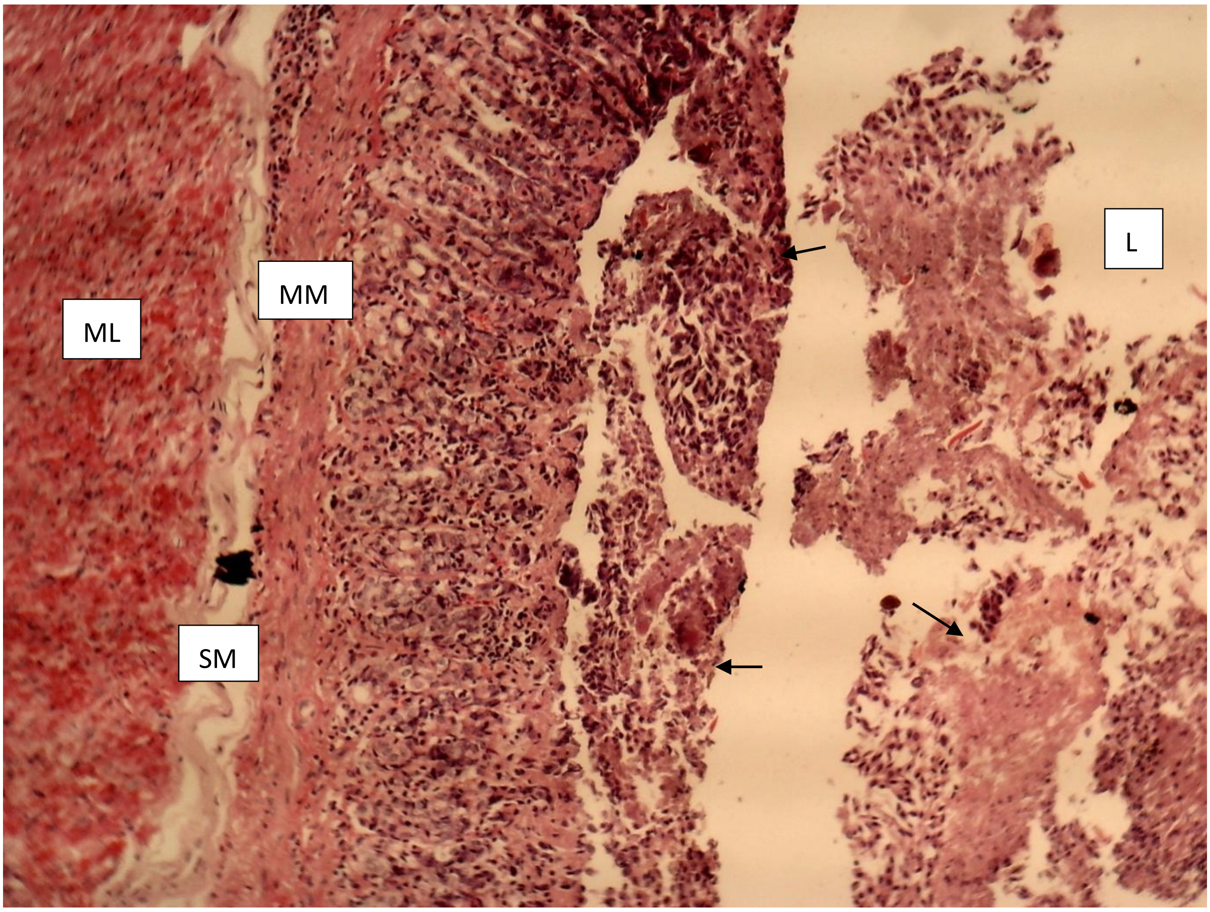

Plate 1c: stomach histology of group 3 (omeprazole) rats showing multifocal areas of mucosal necrosis with marked inflammatory cellular infiltration and sloughing of necrotic debris into the luminal space

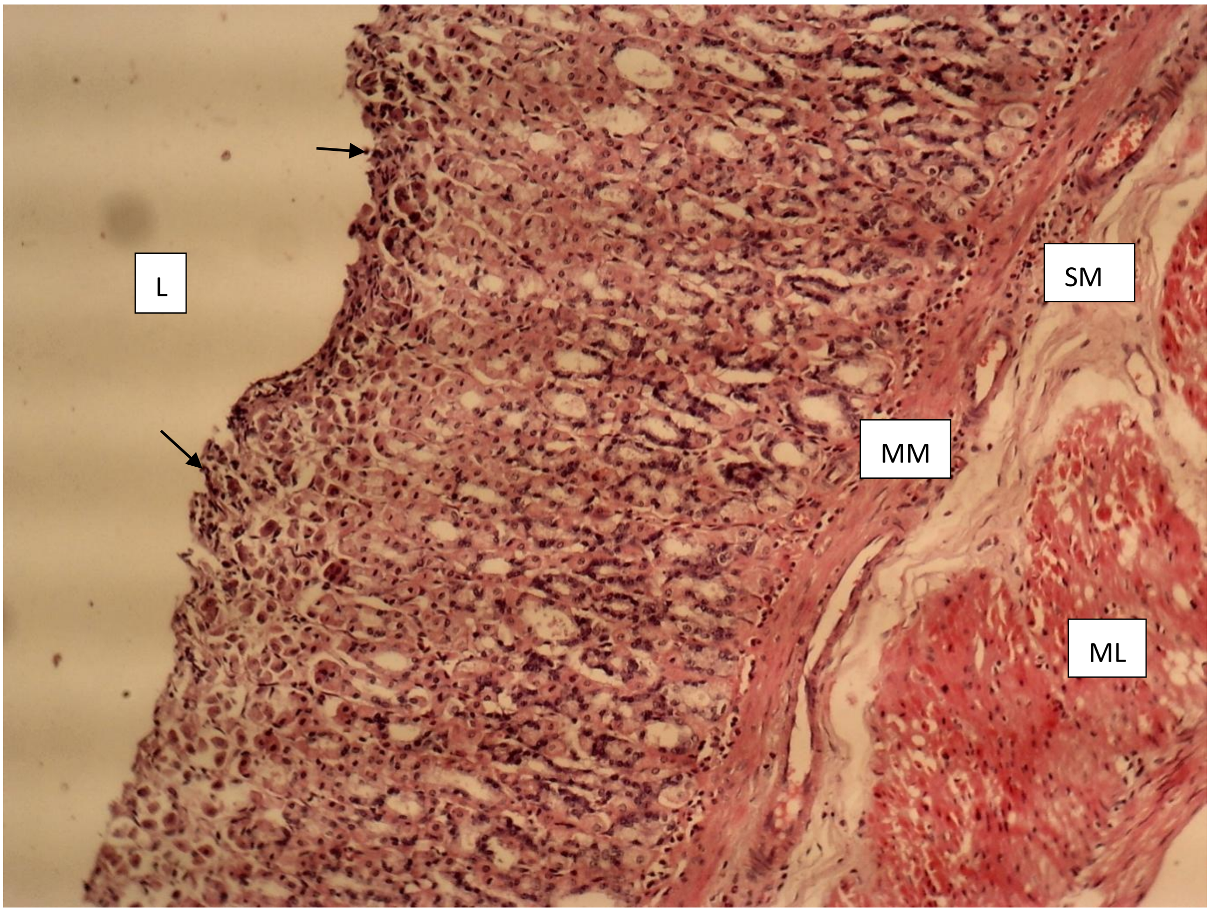

Plate 1d: stomach histology of group 4 rats (400mg/kg of UO) showing mild multifocal areas of mucosal necrosis involving mostly the upper layers of the gastric mucosa

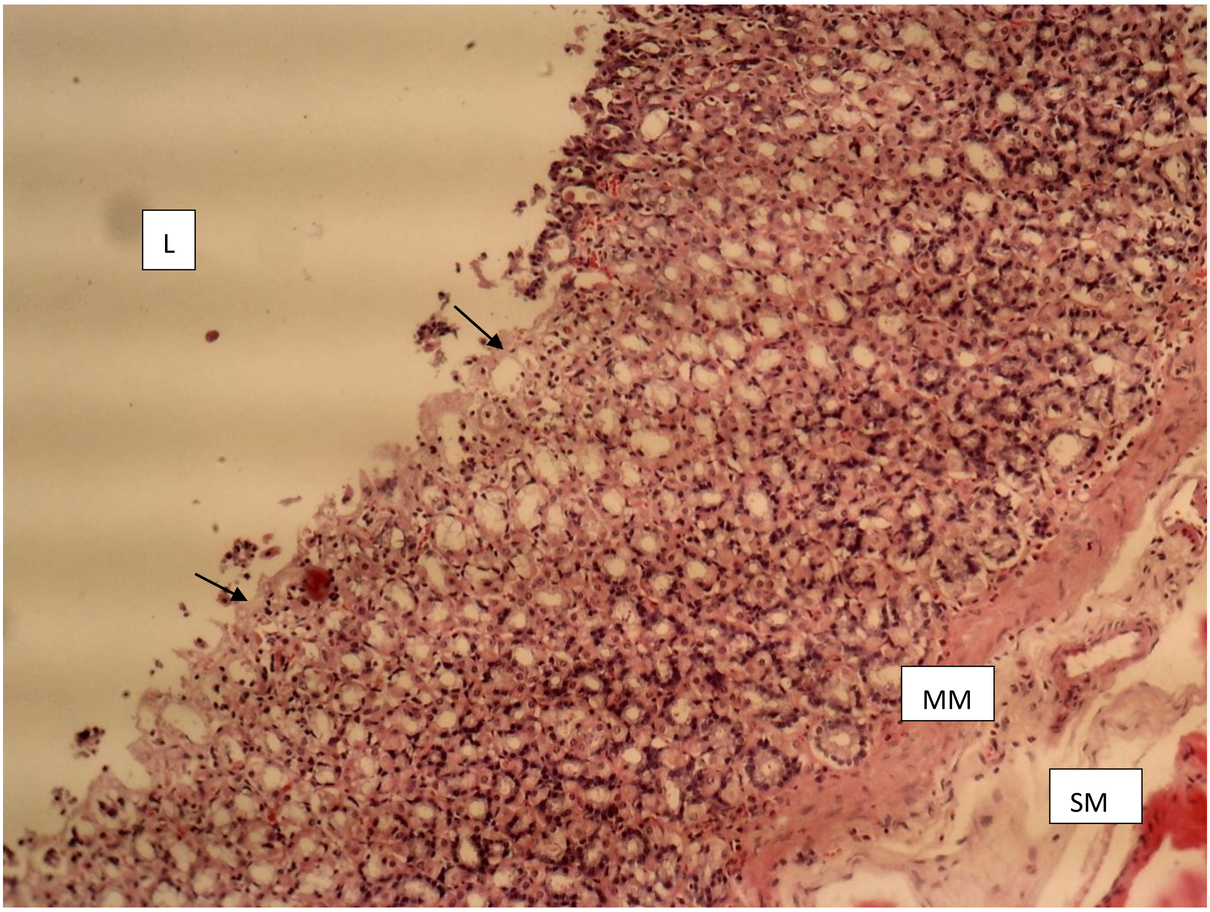

Plate 1e: stomach histology of group 5 (800mg/kg of UO) rats showing very mild multifocal areas of mucosal necrosis involving mostly the upper layers of the gastric mucosa.

5.0 DISCUSSION

The results from stomach histology showed badly inflamed gastric cells in the negative control group. The extract was able to reduce some of the aspirin-induced gastric mucosal ulcerations in the treated animals. A study by Idam and Ebisintei (2022), indicated that Uvaria ovata roots showed significant gastroprotection against aspirin-induced gastric ulcers. These effects may have been brought about by the wound-healing potentials of the flavonoid, alkaloid and tannin-rich fractions, which are richly contained in Uvaria ovata roots, as these phytocomponents have been observed to significantly cause wound contraction and angiogenesis owing to their high collagen and fibroblast presence. A study by Chen et al. (2019) showed that tannins successfully accelerated reepithelialization of lesions by increasing hair follicles, growth factors such as transforming growth factor-beta, basic fibroblast growth factor and vascular endothelial growth factor around wounds. Peng et al. (2019), showed that a wide range of alkaloids, including indoles, isoquinolines, quinolizidines, xanthines, etc. modulated gut microbiota, restored epithelial barrier function and regulated gastrointestinal oxidative and inflammatory status via several mechanisms including reducing facultative anaerobes, lipid peroxidation products, ROS production, and NF-ĶB and mitogen-activated protein kinase (MAPK) signaling pathways, and increasing the levels of obligate anaerobes, claudins, zonula occludins, etc. (Peng et al., 2019).

It has already been established in the course of this work that tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) are two of the most powerful proinflammatory biomarkers. Their levels in the serum rise precipitously following any condition that causes inflammation, and their increased secretion signals further inflammation in a positive feedback loop (Agyare et al., 2018; Qian et al., 2019). Inflammation is the major characteristic of a wound due to the release of leukotrienes, cytokines, prostaglandins, reactive oxygen species and eicosanoids. The results from this study showed a significant reduction in TNF-α and IL-1β levels in the treated groups compared with the control. This may imply that Uvaria ovata root extract significantly inhibited the synthesis and activities of new proinflammatory mediators, showing even higher anti-inflammatory effects than omeprazole. Alkaloids, flavonoids and tannins have been found to possess anti-inflammatory activity in both proliferative and exudative phases of inflammation. Tannins reduced significant neutrophil recruitment and proinflammatory cytokines in a zymosan-induced peritonitis mouse model (Yeo et al., 2020), and reduced myeloperoxidase enzyme activity in another study (Soyocak et al., 2019). Souto et al. (2011) demonstrated that alkaloids inhibit eosinophil recruitment, production of nitric oxide mediators, cyclooxygenase activity, and carrageenan-induced paw edema, all of which are known causes of inflammation. Flavonoids potentiate their anti-inflammatory effects by inhibiting the formation of different proinflammatory mediators and activating the anti-inflammatory ones (Souto et al., 2011).

6.0 CONCLUSION

The results obtained from the study showed that Uvaria ovata root extract exhibited tremendous anti-inflammatory effect by significantly reducing the levels of the proinflammatory biomarkers (interleukin-1β and tumor necrosis factor-α), and also showed significant alleviation of gastric inflammation on the stomach histology results.

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