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ANDROGRAPHIS PANICULATA AMELIORATES INDOMETHACIN-INDUCED GASTRIC ULCERATIONS IN WISTAR RATS: A COMPARATIVE STUDY

Kokelu Anthony Nduka[1], Ani Elemi John[2], Okon Udemeobong Edet[3*], Ime Akaninyene Ubong[4], David Chibuike Ikwuka[5], Tijani Abiola Stephanie[6], Onaadepo Olufunke[7], Twizeyimana Eric[8]

[1,2,3*]Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria.
[4,5,7,8]Department of Physiology, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda.
[6,]Department of Biochemistry, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda

Corresponding Email: [3*]udemeokon@unical.edu.ng

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ABSTRACT:

This research was aimed at investigating the ameliorative effect of *Andrographis paniculata (AP)* following indomethacin-induced gastric ulcer. A total of 20 male albino Wistar rats (150-180g) used for this study were grouped into four (n=5): Normal Control, Negative Control, induced with gastric ulcer using indomethacin at 20mg/kg but untreated; Positive Control, induced with gastric ulcer and treated with omeprazole at 20mg/kg; Test Group, induced with gastric ulcer and treated with aqueous extract of *AP* at a dose of 16.7mg/kg. Body weight changes of the animals were measured every three days while water and food intake were measured daily. After the treatment protocol, the animals were used for estimation of ulcer parameters. The LD_{50} value of aqueous extract of *AP* was 50mg/kg (bw). Water intake was significantly reduced in omeprazole treated group compared to control and ulcer untreated groups but significantly increased in *AP* treated group. Body weight change was significantly reduced in omeprazole treated group compared to all other groups while extract treated group significantly increased body weight change. Gastric acid secretion was significantly reduced in the treated groups when compared to the ulcer untreated group. Gastric mucus was significantly increased in omeprazole and *AP* groups compared to ulcer untreated and control groups. Pepsin was significantly increased in ulcer untreated group compared to control. However, omeprazole and AP significantly reduced pepsin compared to ulcer untreated group. Pepsin was also significantly reduced in AP group compared to omeprazole group. Cross sectional macroscopic examination of the gastric mucosa in ulcer untreated group showed two or more haemorrhagic ulcers while this effect was seen to be ameliorated in omeprazole and *AP* treated groups. From the result of this study, AP has proved to be more potent in protecting against peptic ulcer disease than omeprazole. If this result is applicable to humans, the use of AP in ameliorating the debilitating consequences of peptic ulcer should be researched and encouraged.

Keywords: peptic ulcer disease, omeprazole, *Andrographis paniculata*, gastric acid, LD

1. INTRODUCTION

Peptic ulcer disease (PUD) is a gastrointestinal disease characterized by sores in the lining of the stomach and/or duodenum (Shell, 2021). The most common types of peptic ulcers are “gastric” and “duodenal” ulcers”. This gastrointestinal disease has been shown to result from an imbalance between aggressive factors (hydrochloric acid (HCl), refluxed bile, leukotrienes, (LTs), pepsin, reactive oxygen species etc) and protective factors (mucus-bicarbonate barrier, cell renewal and migration, prostaglandins (PGs), mucosal blood flow, non-enzymatic and enzymatic antioxidants and some growth factors etc) (Amandeep *et al*, 2012). Some factors have been implicated in the pathogenesis of gastric ulcer. Some of the most important factors are; a bacteria, Helicobacter Pylori (H. Pylori) infection, chronic use of Non-steroidal anti-inflammatory drugs (NSAIDs), chemicals (HCl/ethanol), and gastric cancer (Ali *et al.*, 2019; Dunlap and Patterson, 2019; Pandey *et al.*, 2019; Bereda, 2022). A plethora of reports have shown that non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the synthesis of protective prostanoids in the gastric and duodenal mucosa, leaving the mucosa susceptible to ulceration by gastric acid (Bindu *et al.*, 2020; Maziero Alves *et al.*,

2021; Traoré et al., 2021). Gastric ulcer is the most prevalent gastrointestinal disorder ever known, accounting for an estimated 15,000 mortality yearly (Shristi *et al.*, 2012).
NSAIDs are commonly prescribed both in the hospital and by patent medicine dealers as an effective relief of pain and inflammation caused by a variety of clinical disorders, including different types of headache, arthritis, non-arthritic musculoskeletal conditions and dysmenorrhea. These drugs cause erosion of the gastrointestinal tract as a side effect, especially the gastric epithelium due to the imbalance between the aggressive and protective factors. However, various synthetic antiulcer drugs like cimetidine, misoprostol, omeprazole etc. are employed to manage and cure NSAID-induced gastric ulcer (Mehrabani *et al.*, 2009).
Currently, extensive scientific evidence shows that the two major etiological factors involved in PUD are infection with H. pylori and ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) (Melcarne *et al*, 2016; Satoh *et al*., 2016; Lanas and Chan, 2017). NSAIDs have been shown to inhibit the synthesis of protective prostanoids in the gastric and duodenal mucosa, leaving the mucosa susceptible for subsequent ulceration by gastric acid (García-Rayado *et al.*, 2018). NSAIDs initiate mucosal injury topically by their acidic properties as well as via oxidative stress and generation of reactive oxygen species (ROS) (Liu *et al.*, 2016).
There are also incidences of relapses and danger of drug interactions during ulcer therapy, prompting a search for non-toxic, easily accessible, and affordable antiulcer medications. Generally, research in herbal medications has been on the increase as various herbs have been found to influence outcome of a number of disorders across different body systems. Examples include the effect Moringa Oleifera has on altered blood parameters (Ofem *et al,* 2015), Citrus sinensis on thyroid diseases (Uduak *et al,* 2014), Aloe barbadensis on altered acid secretion (Ani *et al,* 2005) and a host of others over the years. *Andrographis paniculata* (*AP*) is a cheap, easily accessible/readily available bitter tasting annual plant used by traditional practitioners to meet primary healthcare needs of the people especially those who cannot afford the orthodox drugs. This plant also attenuates oxidative stress (Kokelu *et al*., 2023). There have been folklore beliefs on the efficacy of *AP* as an alternative complementary medicine for the remedy of gastric problems. Many individuals have given undocumented testimonies on the efficacy of this plant in the treatment of ulcer. In view of this, the study is therefore designed to assess the role of *AP* on the development/treatment of indomethacin-induced gastric ulcers in albino Wistar rats.

2. MATERIALS AND METHODS

Ethical approval
All authors hereby declare that "principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. Research proposal was submitted and ethical approval granted by the Animal Research Ethics Committee of the college of Medical Sciences, University of Calabar, Calabar-Nigeria before commencement of the research.
Experimental Animals and treatment
A total of 20 male albino Wistar rats (150-180g) used for this study were grouped into four (n=5): **Group1** (**Normal Control)** - (fed with normal chow + water)
Group2 (Negative Control) – Induced with ulcer using indomethacin at dose of 20mg/kg bw
Group3 (Positive Control) (Ulcer+Om) - Induced with ulcer using indomethacin at dose of 20mg/kg bw and treated with omeprazole.
Group4 (Test Group) - Induced with ulcer using indomethacin at dose of 20mg/kg bw and treated with *AP* at a dose of 16.7mg/kg bw from the result of acute toxicity (LD_{50}) study of the plant as determined by the method of Lorke (1983).

Group 3 and 4 were pre-treated with omeprazole (20mg/kg) and *AP* (at a safe dose of 16.7mg/kg, which was 1/3 of LD_{50}) respectively for 21 days prior to ulcer induction. Then on days 22 to 24, ulcer was induced twelve hourly in groups 2, 3 and 4 at a dose of 20mg/kg of indomethacin (Satoh *et al.,* 1981). During this period of ulcer induction, treatment continued in groups 3 and 4 while group 2 remained untreated.

Induction of gastric ulceration

Induction of gastric ulcer in rats using indomethacin was done after subjecting the animals to 24 hours fasting. Within 2 hours post-fasting, they were re-fed with pellet diet for one hour. After one hour of re-feeding, Indomethacin was administered orally at a dose of 20mg/kg body weight twelve hourly as reported by Satoh *et al.* (1981). Gastric ulcer was induced for three days with concurrent treatments.

Measurement of ulcer parameters

Gastric acid secretion

Gastric acid secretion was measured by a continuous perfusion method of Ghosh and Schild (1958) as modified by Osim *et al.* (1991). After an overnight fast, anaesthesia was induced using 25% urethane at a dose of 6ml/kg bw intraperitoneally. A cannula was inserted into the trachea to maintain airflow while an oesophageal cannula for infusion of saline was passed through the mouth to the stomach. Another cannula was introduced into the stomach through an incision in the duodenum and was ligated at 0.5 cm from the pylorus. The stomach was first flushed using 10ml saline at room temperature through the esophageal cannula and then flushed again with normal saline (pH; 7.0) at 37◦C at the rate of 1 ml/min using infusion pump (Harvard apparatus, MA, USA). The stomach perfusate was collected every 10 min, and acid output was measured by the titration of the perfusate with 0.01 N NaOH to a pH 7.0 using phenolphthalein as indicator. Basal collections were done in all four groups. All groups were later challenged with subcutaneous injections of histamine (100mg/kg body weight) as reported by Ani *et al.* (2005).

Gastric mucus secretion

Adherent mucus weight was determined by the method of Tan *et al. (2006)*. Gastric mucus covering the walls of the stomach was carefully scraped using a glass slide into a small sample tube containing 1mL of water whose weight was predetermined. The weight of the container and mucus was taken using a digital electronic balance and the difference taken as the weight of the mucus.

Measurement of gastric pepsin level

The determination of proteolytis of gastric secretion (which is the basis for measurement of pepsin) was performed using casein as a substrate according to the method reported by Osim *et al.* (1991). 1ml of various concentrations of bovine casein ranging from 0.1-1.0mg/100ml in 0.1N HCl was transferred to a tube and intubated for 30mins. With 3.9mls of 25g/100mls 0.1N HCl of the substrate in a water bath at 37^0C. 10mls of 10% trichloroacetic acid (TCA) was added and the tubes allowed to stand for 10mins before they were filtered using Whatman's filter paper no.1 Blanks were made for each concentration by adding 10mls TCA before addition of the enzyme. Duplicate determinations were performed for each enzyme concentration. The optical density of the filtrate was measured at 200nm wavelength. For determination of the proteolytic activity of gastric secretion, the same procedure was followed at a concentration of 2% of 0.1N HCl (by adding 1ml of 2% of 0.1N HCl into each). A standard curve was constructed from which pepsin content of gastric secretion was determined.

Determination of pH of gastric juice

The pH of gastric juice was done by the use of pH strips.

Macroscopic assessment of ulcers

Before examination of ulcers, the rats were anaesthetized using chloroform and the stomachs were removed and opened along the greater curvature. The stomach of each animal was spread on a sheet of cork so as to have a clear macroscopic view of the gastric mucosa for assessment of ulcers.

Statistical analysis

All data collected were subjected to statistical analyses using analysis of variance (ANOVA) of the SPSS statistical software (V.17) and Microsoft excel 2010. Turkey post hoc test was used to determine the difference among group means. Results were expressed as Mean ± standard error of mean (SEM). A value of $P< 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

Results

LD_{50} of *Andrographis paniculata*

Experiment	Dose (mg/kg bw)	Number of Mortality after 24hours
Phase 1	25	0/3
	50	1/3
	100	3/3
Control	0	0/3

TABLE 1: Acute lethal effect of extract of *Andrographis paniculata* administered intraperitoneally (I.P) to Wistar rats

From the result above, the LD_{50} was calculated using the formular below:

$LD_{50}= \sqrt{(LD_0 \times LD_{100})}$

Where,

LD_0= The highest dose that caused zero mortality (that is, no mortality)

LD_{100}= The least dose that caused 100% mortality

Therefore, $LD_{50}= \sqrt{(25 \times 100)}= 50$mg/kg (bw)

Mean daily food and water intake

Mean daily food intake (In grams) was 23.04±0.77; 23.33±1.43; 24.43±1.71 and 26.20±0.74 for Control, Ulcer Untreated, Ulcer+Om and Ulcer+*AP* respectively. From this result, there was no significant difference in mean food intake across the groups (FIG. 1).

Mean daily water intake (in mls) was 35.19±0.64; 37.50±1.37; 26.21±0.56 and 46.45±1.22 for Control, Ulcer Untreated, Ulcer+Om and Ulcer+*AP* respectively. This result presented a significant decrease ($P<0.001$) and increase ($P<0.001$) in daily water intake of Ulcer+Om and Ulcer+AP when compared to control respectively. More so, there was a significant decrease in water intake in Ulcer+Om group when compared to ulcer untreated group. Meanwhile, Ulcer+AP group induced a significant increase ($P<0.001$) in water intake when compared to ulcer untreated group as well as Ulcer+Om (FIG. 2).

Mean body weight change

Mean body weight change (in grams) was 57.00±4.82; 48.70±4.57; 4.20±2.76 and 59.40±3.87 for Control, Ulcer Untreated, Ulcer+Om and Ulcer+*AP* respectively. Body weight change was significantly reduced ($P<0.001$) in Ulcer+Om group when compared to control and ulcer untreated groups. However, Ulcer+*AP* caused a significant increase in body weight change when compared to Ulcer+Om group (FIG. 3).

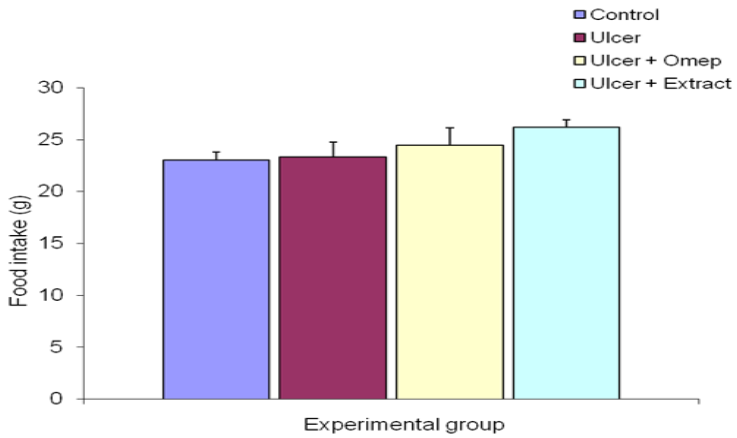

FIG. 1: Comparison of mean food intake of control, ulcer and ulcer treated groups.

Values are expressed as mean ± SEM, n = 5.
No significant differences among groups

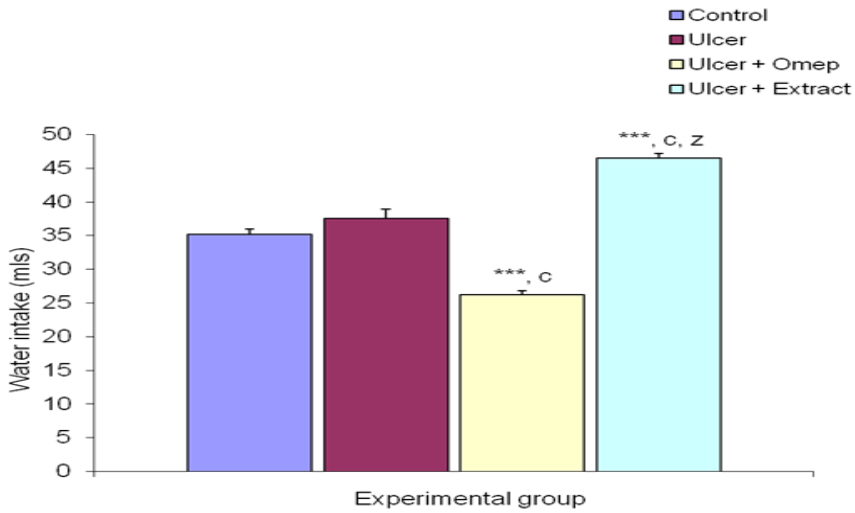

FIG. 2: Comparison of mean water intake of control, ulcer and ulcer treated groups.

Values are expressed as mean ± SEM, n = 5.
*** = significantly different from control at p<0.001;
c = significantly different from ulcer at p<0.001;
z = significantly different from ulcer + omep at p<0.001

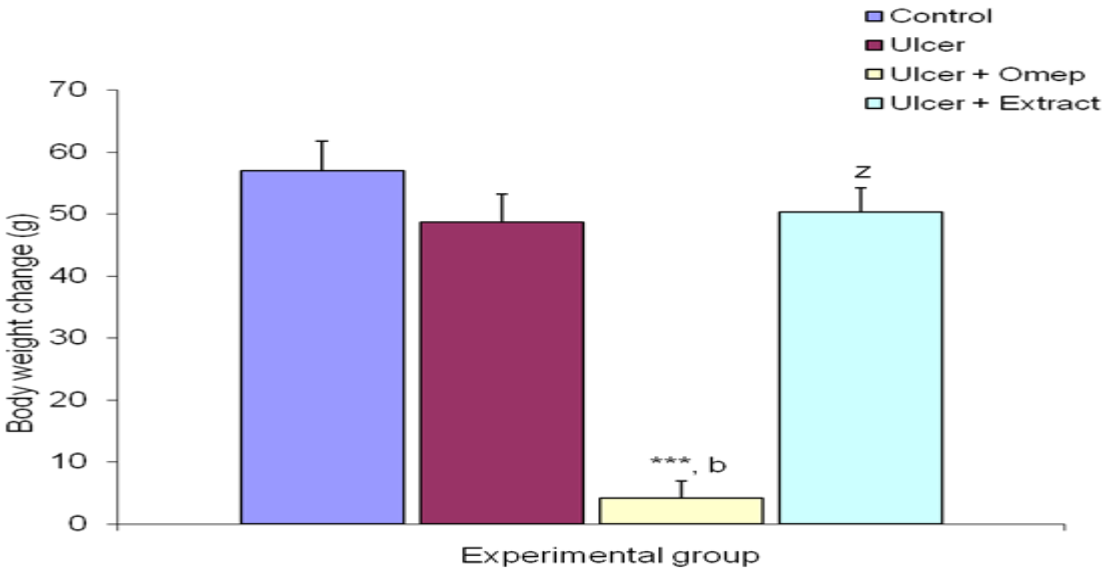

FIG. 3: Comparison of body weight change of control, ulcer and ulcer treated groups.

Values are expressed as mean ± SEM, n = 5.
*** = significantly different from control at p<0.001;
b = significantly different from ulcer at p<0.01;
z = significantly different from ulcer + omep at p<0.001

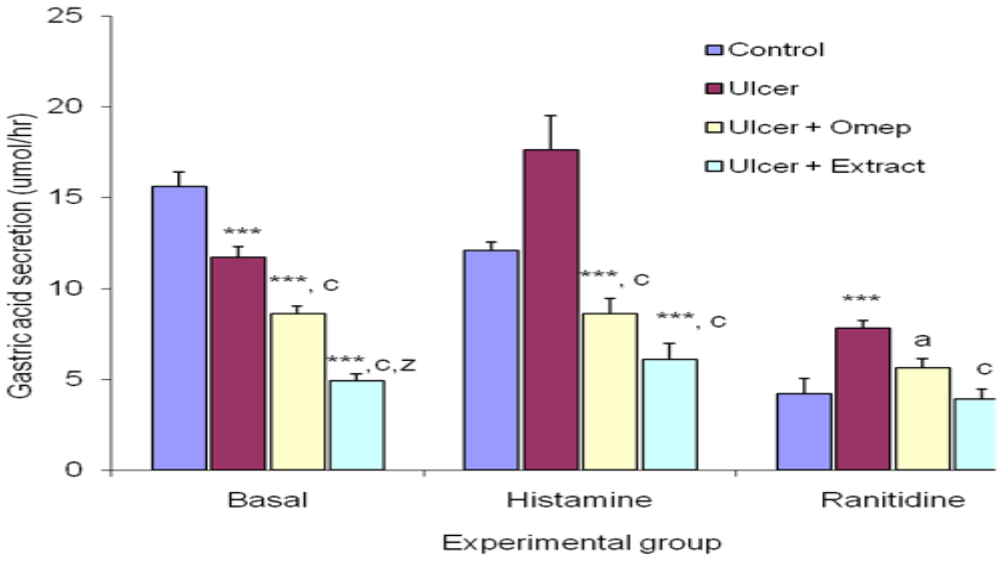

FIG. 4: Effect of ulcer treatment with omeprazole and extract on basal and challenged gastric acid secretion in rats.

Values are expressed as mean + SEM, n = 5.
*** = significantly different from control at p<0.001;
a = p<0.05, c = p<0.001 vs ulcer;
z = significantly different from ulcer + omep at p<0.001

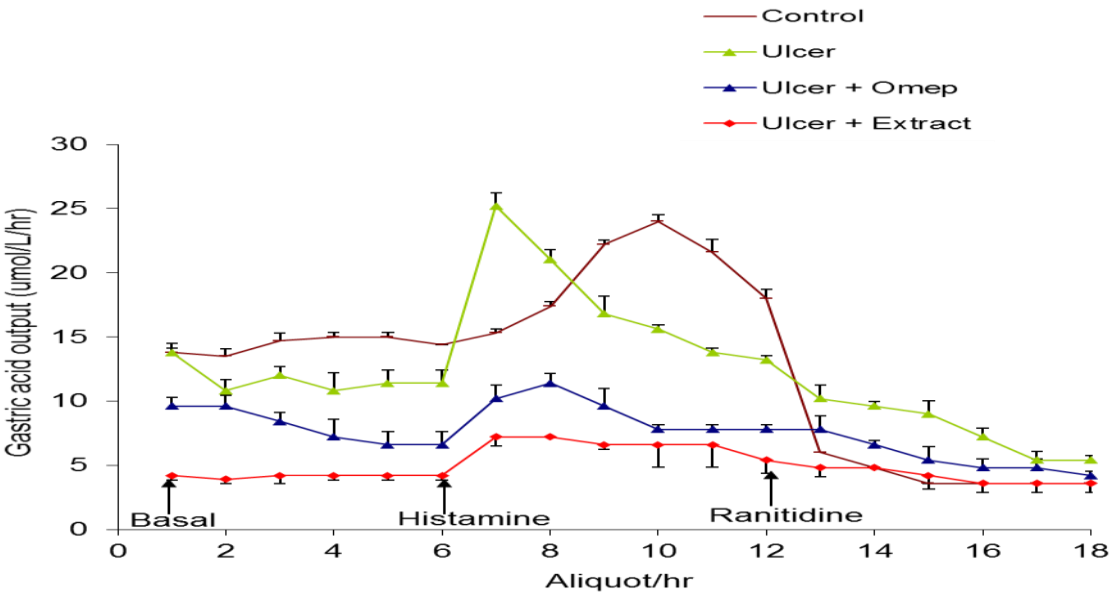

FIG. 5: Comparison of gastric acid output in control, ulcer and ulcer treated rats.

Values are expressed as mean ± SEM, n = 5.

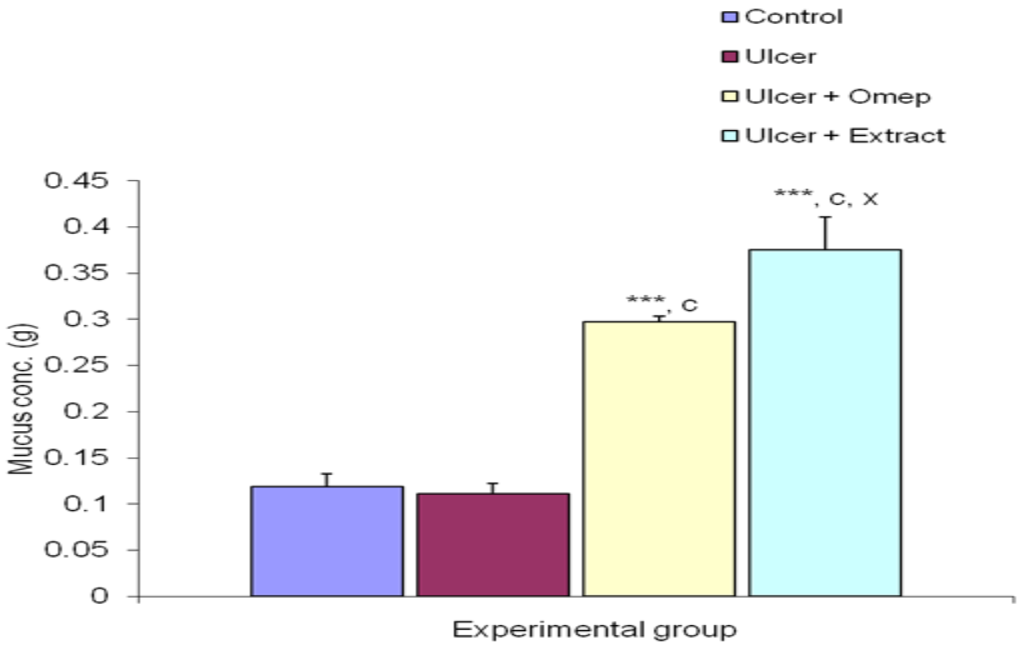

FIG. 6: Comparison of mucus concentration in control, ulcer and ulcer treated groups.

Values are expressed as mean ± SEM, n = 5.
*** = significantly different from control at p<0.001;
c = significantly different from ulcer at p<0.001;
x = significantly different from ulcer + omep at p<0.05

Ulcer Parameters

Mean gastric acid output in all experimental groups for basal, histamine and ranitidine

Mean gastric acid secretion (µmol/hr) in basal for control, ulcer untreated, ulcer+Om and Ulcer+*AP* are 15.60±0.26, 11.70±0.46, 8.60±0.58 and 4.90±0.05 respectively. This result showed a progressive significant decrease (P<0.001) in mean gastric acid output of ulcer

untreated, ulcer+Om and Ulcer+*AP* respectively when compared to control. However, ulcer+Om group significantly reduced gastric acid output when compared to ulcer untreated group. More so, extract treated group showed a marked reduction (P<0.001) in mean gastric acid output when compared to ulcer, ulcer untreated as well as ulcer+Om groups.

Upon administration of histamine, mean gastric acid output was 12.10±1.84, 17.60±1.89, 8.60±0.63 and 6.10±0.27 for control, ulcer untreated, ulcer+Om and Ulcer+*AP* respectively. From this result, there was a significant increase in mean gastric output of ulcer untreated group when compared to the two treated groups, although this was also higher than control but the difference was non-significant. Both Omeprazole and extract treated groups significantly reduced (P<0.001) gastric acid output when compared to control and ulcer untreated groups.

Upon administration of ranitidine, mean gastric output were 4.20±0.41, 7.80±00.87, 5.60±0.55 and 3.90±0.24 for control, ulcer untreated, ulcer+Om and Ulcer+*AP* respectively. Mean gastric acid output in ulcer untreated group was significantly higher (P<0.001) when compared to control. However, Omeprazole and extract treated group had significantly reduced (P<0.05 and P<0.001) gastric acid output when compared to ulcer untreated group respectively (FIG. 4/5).

Gastric Mucus output

Mean gastric mucus (grams) was 0.12±0.01; 0.11±0.01; 0.30±0.01 and 0.38±0.04 for control, ulcer untreated, ulcer+Om and Ulcer+*AP* respectively. This result presented a significant increase (P<0.001) in mucus output of omeprazole and extract treated groups when compared to control as well as ulcer untreated groups. More so, extract treated group had significantly increased (P<0.001) mucus output than ompeprazole treated group. (FIG. 6)

PH of gastric juice

Mean pH of gastric juice were 5.58±0.11; 3.40±0.47; 4.20±0.84 and 5.32±0.39 for control, ulcer untreated, ulcer+Om and Ulcer+*AP* respectively. From this result, mean pH of gastric juice was significantly decreased (P<0.01) in ulcer untreated group compared to control. Interestingly, the extract treated group caused a significant elevation (P<0.05) in mean pH of gastric juice when compared to ulcer untreated group (FIG. 7).

Gastric pepsin concentration

Mean pepsin level (mg/ml) was 0.47±0.01; 0.61±0.01; 0.57±0.02 and 0.49.±0.01 for control, ulcer untreated, ulcer+Om and Ulcer+*AP* respectively. This result showed that mean pepsin concentration was significantly higher (P<0.001) in ulcer untreated group as well as omeprazole treated group when compared to control. However, omeprazole and extract treated group had significantly reduced (P<0.05 and P<0.001) pepsin concentration when compared to ulcer untreated group. More so, the extract treated group had significantly reduced (P<0.001) pepsin concentration when compared with omeprazole treated group (FIG. 8).

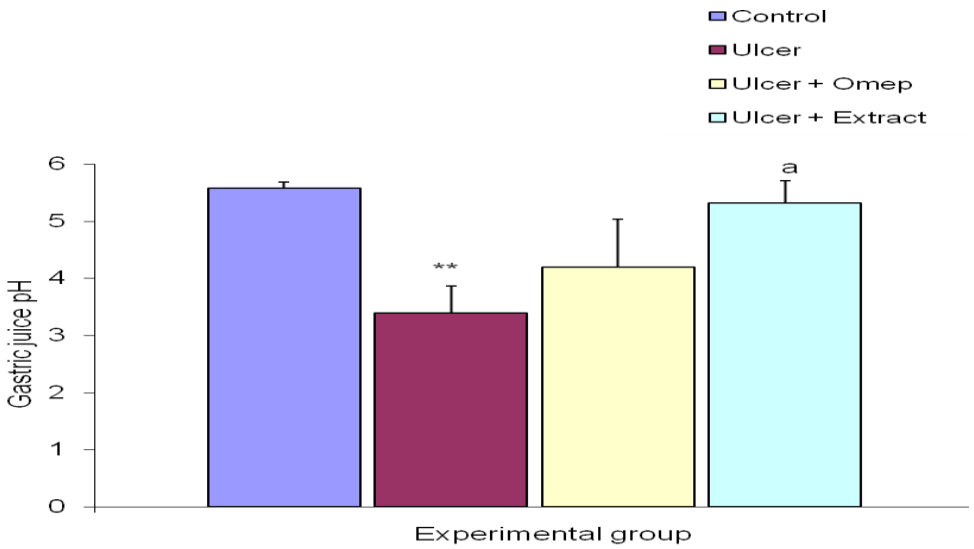

FIG. 7 : Comparison of gastric juice pH in control, ulcer and ulcer treated groups.

Values are expressed as mean ± SEM, n = 5.
** = significantly different from control at p<0.01;
a = significantly different from ulcer at p<0.05

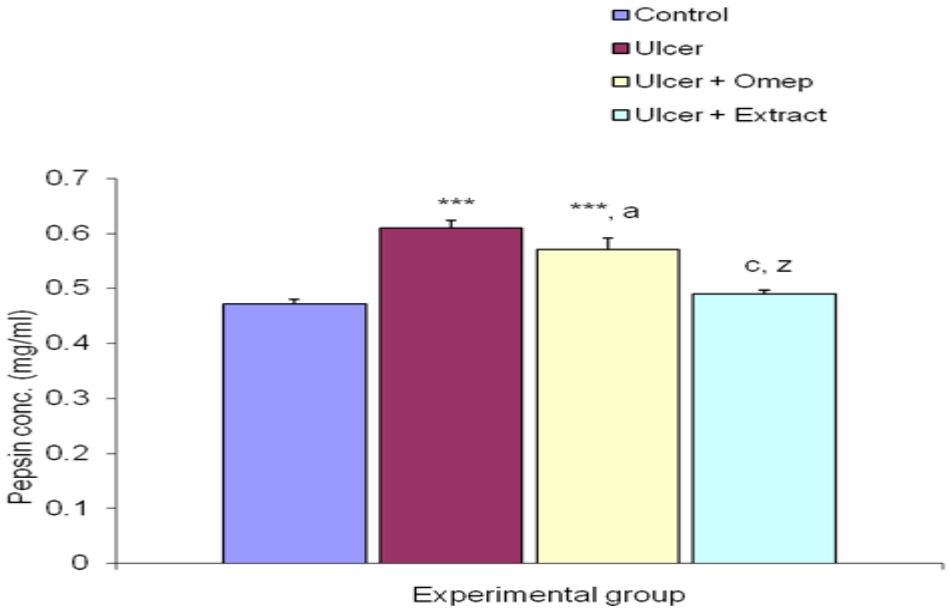

FIG. 8: Comparison of pepsin concentration in control, ulcer and ulcer treated groups.

Values are expressed as mean ± SEM, n = 5.
*** = significantly different from control at p<0.001;
a = significantly different from ulcer at p<0.05;
c = significantly different from ulcer at p<0.001;
z = significantly different from ulcer + omep at p<0.001

Macroscopic assessment of ulcer

Ulcer assessment is shown on plates 1-4 below. Cross sectional macroscopic examination of the gastric mucosa in ulcer untreated group showed two or more haemorrhagic ulcers while this effect was seen to be ameliorated in omeprazole and *AP* treated groups.

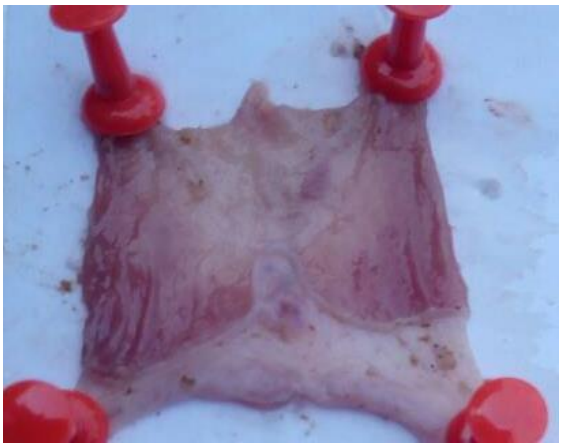

Plate 1: Control

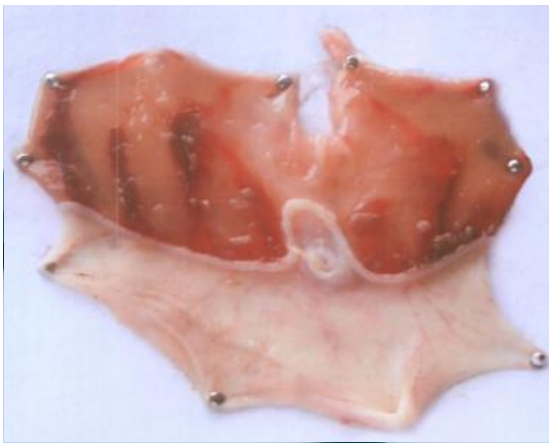

Plate 2: Ulcer untreated

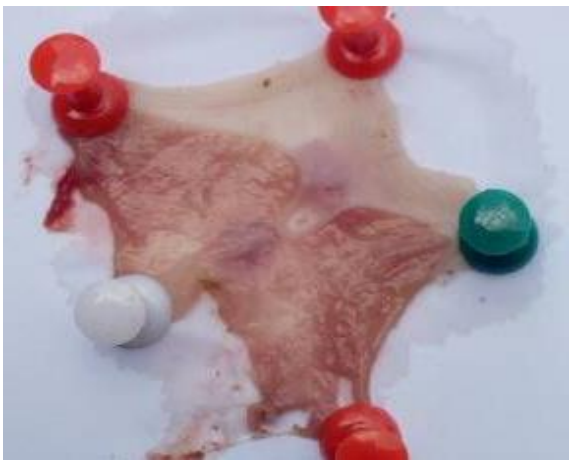

Plate 3: Ulcer+Om

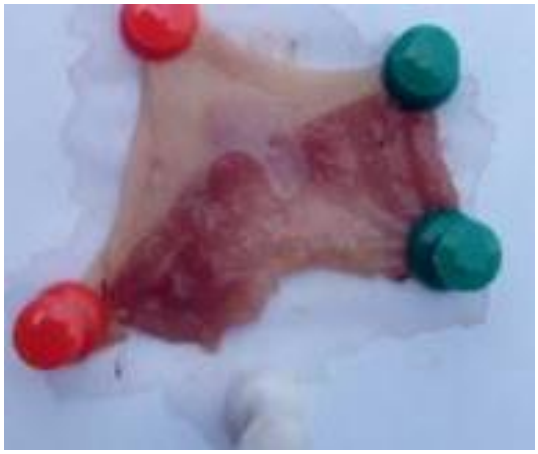

Plate 4: Ulcer+AP

4. DISCUSSION

From the acute toxicity study, the LD_{50} was arrived at 50mg/kg body weight. According to Ahmed (2015), any compound (in rat) with LD_{50} of 5000mg/kg or more should be considered as practically harmless. Due to the low LD_{50} value, there is need to caution herbal medicine practitioners who prescribe this extract indiscriminately without having the knowledge of the lethal dose and the effective dose.

Food intake across all the groups showed no significant difference while water intake was significantly reduced in omeprazole treated group compared to the control, as well as ulcer untreated group. The mechanism by which omeprazole reduces water intake following ulcer treatment is not clear. However, Omeprazole has been reported to increase water absorption in the gastrointestinal tract (Jeppesen *et al.,* 1998; Suksridechacin *et al.,* 2020). This may greatly increase extracellular fluid volume and thus sets a negative feedback on the thirst centre which could be a possible explanation to the reduced water intake in omeprazole treated group seen in this study. Meanwhile, water intake was greatly increased in the extract treated group compared to the other three groups but the mechanism of this action was not investigated in this study. Body weight change was markedly reduced in omeprazole treated group than all three groups while extract treated group caused a marked improvement in body weight change. This result is consistent with several reports that proton pump inhibitors

(PPIs) are risk factors of bone loss (Elaine *et al.*, 2008; Khalili *et al.*, 2012; Ursomanno *et al.*, 2020) which in turn reduces body weight. The mechanism in which AP increases body weight is not yet clear but this result is in contrast with the study by Niranjan *et al.* (2010) who reported that AP had no effect on body weight.

Gastric acid secretion was significantly increased in all test groups compared to control. However, the two treated groups had significantly reduced gastric acid output when compared to ulcer untreated group. Meanwhile *Andrographis paniculata* treated group showed more reduction in gastric acid output than omeprazole treated group. This result is in line with the numerous folklore beliefs in the potency of *AP* against gastric ulcer as well as the reported gastroprotective effects of *AP* (Saranya *et al.,* 2011; Umashanker *et al.,* 2011; Wang *et al.,* 2021). The pH of gastric juice was significantly decreased in ulcer untreated group but the extract treated group caused a significant increase in the pH of gastric juice. This may also contribute to its gastroprotective effect against ulcers.

Gastric mucus was significantly raised in omeprazole and extract treated group than ulcer untreated group and even control. Meanwhile, this increase was more in extract treated group than omeprazole treated group. From this study, omeprazole besides being a proton pump inhibitor is seen to exhibit another antiulcer effect by causing an increase in gastric mucus. Meanwhile, *AP* was seen to induce a more potent effect thus, showing its efficacy against gastric ulcer. The mechanism by which *AP* increases production of gastric mucus in this study is unknown

Pepsin concentration was significantly higher in ulcer untreated group as well as omeprazole treated group when compared to control. However, omeprazole and extract treated group had significantly reduced pepsin concentration when compared to ulcer untreated group. The extract treated group also had a significantly reduced pepsin concentration when compared to omeprazole treated group. This increase of pepsin in ulcer untreated group may have resulted from decreased pH of gastric juice and increased gastric acid output which may have enhanced the increased conversion of pepsinogen to pepsin. Meanwhile, the decrease in pepsin concentration in omeprazole treated group is confirmation of its effect as a proton pump inhibitor but the mechanism of decreased pepsin concentration caused by the extract was not determined in this research.

Cross sectional macroscopic examination of the gastric mucosa in ulcer untreated group showed 2 or more hemorrhagic ulcers while this effect was seen to be ameliorated in omeprazole and extract treated groups

5. CONCLUSION

From the result of this study, *Andrographis paniculata* which is readily available and affordable has proved to be very potent in prevention/management of peptic ulcer disease. Its effect has been seen to be more potent when compared with the conventional proton pump inhibitor (omeprazole). If this result is applicable to humans, the use and further research on this plant extract in ameliorating the debilitating consequences of peptic ulcer should be encouraged.

Conflict of interests

Authors declare that there are no existing competing interests.

Authors' contributions

This work was carried out in collaboration with all authors. Author AEJ designed the study and wrote the protocol. Authors OUE and KAN managed the literature search, carried out the feeding regimens while TAS did the biomedical analysis of samples. Authors IAU and OO wrote the first draft of the manuscript. Authors DCI and TE carried out the statistical

analysis. Author AEJ edited the manuscript. All authors read and approved the final manuscript before submission.

6. REFERENCES

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