



***Andrographis paniculata* as Promising Novel Protective Therapy of Oxidative Stress in Indomethacin-Induced Gastric Ulcer in Rats**

**Antony Nduka Kokelu^a, John Elemi Ani^{a,b},
Akaninyene Ubong Ime^{b*}, Stephanie Tijani Abiola^c,
Abdullateef Isiaka Alagbonsi^b, Bidemi Kazeem Okesina^b
and Alphonse Niyodusenga^b**

^a Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria.

^b Department of Physiology, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda, Rwanda.

^c Department of Biochemistry, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda, Rwanda.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2023/v34i51138

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/99110>

Original Research Article

Received: 01/03/2023
Accepted: 02/05/2023
Published: 05/06/2023

ABSTRACT

This research investigated the effect of *Andrographis paniculata* (AP) on oxidative stress following indomethacin-induced gastric ulcer in rats. A total of 20 male albino *Wistar* rats (150-180g) used for this study were grouped into four (n=5): 1, Negative Control; 2, Positive Control and 3, test group treated with normal chow, 20mg/kg indomethacin, 20 mg/kg indomethacin plus omeprazole at 20mg/kg and 20mg/kg indomethacin plus AP at 16.7 mg/kg respectively. After treatment period,

*Corresponding author: E-mail: akaninyeneime1@gmail.com, i.akaninyene@ur.ac.rw;

estimation of oxidative stress parameters was carried out on the animals. The LD₅₀ of aqueous extract of AP was 50mg/kg bw. Body weight change was significantly reduced in omeprazole treated group compared to all other groups while extract treated group had significantly increased body weight change. There was a significant increase in malondialdehyde (MDA) level of ulcer untreated group compared to other groups. The two treated groups had significantly reduced MDA compared to ulcer untreated group. There was a significant decrease in the levels of GPx and SOD of ulcer untreated group compared to control. Meanwhile, these were significantly increased in extract and omeprazole treated groups compared to ulcer untreated group. Catalase was significantly increased in all three groups when compared to control but its level was significantly increased in extract treated group compared to ulcer untreated and omeprazole treated groups. From this study, AP has proved to protect against oxidative stress implicated in the pathogenesis of ulcer. If this result is applicable to humans, further research and use of AP in ameliorating debilitating consequences of peptic ulcer should be encouraged.

Keywords: Peptic ulcer disease; oxidative stress; antioxidants; indomethacin; *Andrographis paniculata*; LD₅₀; rats.

1. INTRODUCTION

Peptic ulcer disease (PUD) is often defined as a mucosal break greater than 3-5 mm in the stomach or duodenum with a visible depth. It is therefore an endoscopic diagnosis in contrast to dyspepsia, which is a clinical diagnosis based on symptoms alone. 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). Some factors have been implicated in the pathogenesis of gastric ulcer, among which are oxidative stress [1,2,3] and chronic use of Non-steroidal anti-inflammatory drugs (NSAIDs) [4,5,6].

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 [7,8,9]. Gastric ulcers associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs) remain a major clinical problem and considered to cause a substantial socioeconomic burden and negatively impacts the quality of life [10].

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) [11,12,13]. 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 [14]. NSAIDs initiate mucosal injury topically by their acidic properties as well as via oxidative stress and generation of reactive oxygen species (ROS) [15]. Indomethacin, which is one of the commonest NSAIDs used in Nigeria has been implicated in the pathogenesis of gastric ulcer in rats much more than other NSAIDs [16,17,18], although without clearly defined mechanism of action. However, there have been suggestions that indomethacin induces gastric damage by inhibiting the release of protective factors like prostaglandin E₂ (PGE₂), bicarbonate, and mucus; increasing aggressive factors like acid; and increasing oxidant parameters while decreasing antioxidant parameters [19].

In spite of the rapidly changing concept of gastric ulcer management from the conventional vagotomy, prostaglandin analogues, H₂ receptor antagonists, and antacids to proton pump inhibitors, gastrointestinal toxicity remains an impediment to their application in clinical practice. Generally, research in herbal or medicinal plants 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 beneficial effects of *Moringa Oleifera*, *Allium cepa*, *Aloe barbadensis* and *Citrus sinensis*, with various documented therapeutic properties [20,21,22,23,24]. *A. 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. 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 oxidative stress in albino *Wistar* rats suffering from indomethacin-induced gastric ulcer.

2. METHODOLOGY

2.1 Experimental Animals and Treatment

A total of 20 male albino *Wistar* rats (150-180g) used for this study were grouped into four (n=5):

Group 1 (Normal Control) - (fed with normal chow + water)

Group 2 (positive Control) – Induced with ulcer using indomethacin at dose of 20 mg/kg bw

Group 3 (Ulcer+Om) - Induced with ulcer using indomethacin at dose of 20 mg/kg bw and treated with omeprazole.

Group 4 (treated 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 [25].

Groups 3 and 4 were pre-treated with omeprazole (20 mg/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 [26]. During this period of ulcer induction, treatment continued in groups 3 and 4 while group 2 remained untreated.

2.2 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. [26]. Gastric ulcer was induced for three days with concurrent treatments.

2.3 Measurement of MDA, GPx, SOD and Catalase

Malondialdehyde level was measured by the method as reported by Omodanisi, Aboua and Oguntibeju [27]. Glutathione peroxidase level was measured by the method as reported by Abarikwu et al. [28]. Superoxide dismutase level

was measured by the method as reported by Gorzi and Asadi [29]. Catalase level was measured by the method as reported by Safaeian et al. [30].

2.4 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 \pm standard error of mean (SEM). A value of $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1 LD_{50} of *Andrographis paniculata*

From the result above, the LD_{50} was calculated using the formula 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, $LD_{50} = \sqrt{(25 \times 100)} = 50\text{mg/kg (bw)}$

3.2 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. Moreover, there was a significant decrease in water intake in Ulcer+Om group when compared to ulcer untreated group. Meanwhile, Ulcer+*AP* group showed a significant increase ($P < 0.001$) in water intake when compared to ulcer untreated group as well as Ulcer+Om (Fig. 2).

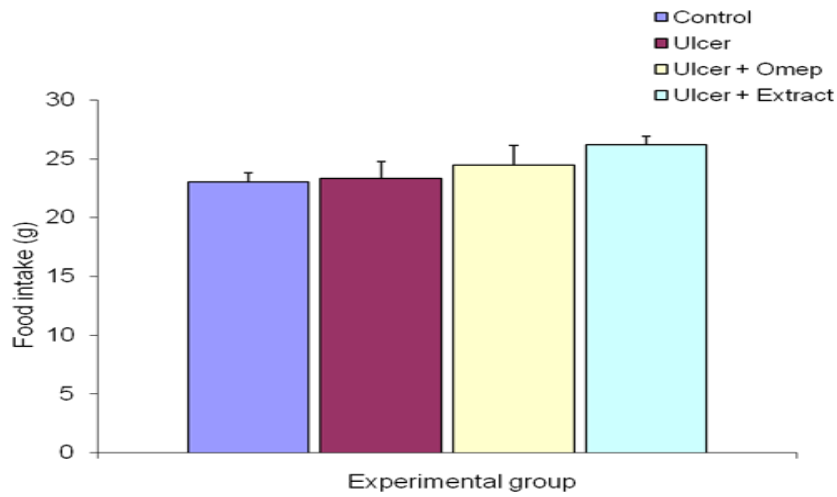


FIG. 1: Comparison of mean food intake of control, ulcer and ulcer treated groups.

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

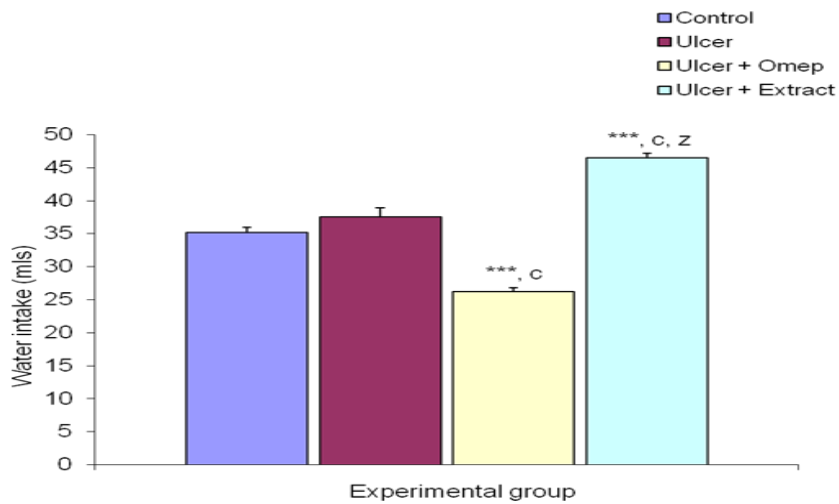


FIG. 2: Comparison of mean water intake of control, ulcer and ulcer treated groups.

Values are expressed as mean \pm 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 + omeprazole at $p < 0.001$

Table 1. Acute lethal effect of extract of *andropogon paniculata* administered intraperitoneally (I.P) to wistar rats

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

3.3 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).

3.4 Malondialdehyde and Antioxidant Levels

3.4.1 Serum malondialdehyde level in the different groups

Malondialdehyde level (nmol/min/ml) were 0.011 ± 0.000 , 0.014 ± 0.000 , 0.13 ± 0.001 and 0.012 ± 0.001 for control, ulcer untreated, ulcer+Om and Ulcer+AP respectively. From this result, there was a significant increase ($P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively) in MDA levels of extract treated group, omeprazole treated group and ulcer untreated group when compared to control. Meanwhile, there was a significant decrease ($P < 0.05$ and $P < 0.001$ respectively) in MDA levels of omeprazole and extract treated groups when compared to ulcer untreated group. The extract treated group showed a significant decrease in MDA level when compared to omeprazole treated group (Fig. 4).

3.4.2 Serum glutathione peroxidase level in the different groups

Glutathione peroxidase ($\mu\text{mol/l}$) was 1567.00 ± 2.59 , 1419.20 ± 28.07 , 1424.40 ± 18.18 and 1563.20 ± 4.81 for control, ulcer untreated, ulcer+Om and Ulcer+AP respectively. From this, there was a significant reduction ($P < 0.001$) in Gpx concentration of ulcer untreated and omeprazole treated groups when compared to control. However, extract treated group showed a significant increase ($P < 0.001$) in GPx level when compared to ulcer untreated and even omeprazole treated groups (Fig. 5).

3.4.3 Serum superoxide dismutase level in the different groups

SOD concentration (ng/ml) was 17.00 ± 0.07 ; 12.96 ± 0.20 ; 14.94 ± 0.09 and 16.38 ± 0.16 for control, ulcer untreated, ulcer+Om and Ulcer+AP respectively. This result presented a significant decrease ($P < 0.001$, $P < 0.001$ and $P < 0.01$ respectively) in SOD concentration of ulcer untreated, omeprazole and extract treated groups when compared to control. The result also showed a significant increase ($P < 0.001$) in SOD levels of omeprazole and extract treated groups when compared to ulcer untreated group. Moreover, the extract treated group had significantly increased ($P < 0.001$) SOD concentration when compared to omeprazole treated group (Fig. 6).

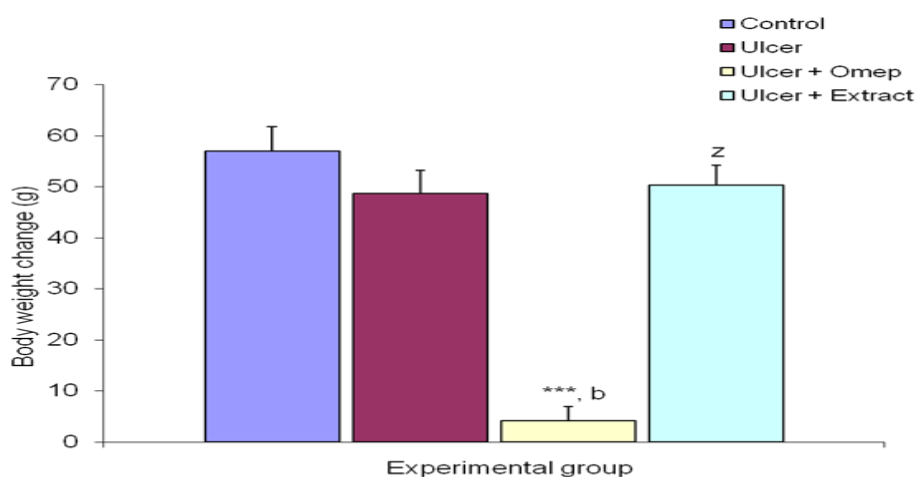


FIG. 3: Comparison of body weight change of control, ulcer and ulcer treated groups.

Values are expressed as mean \pm 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 $p < 0.001$

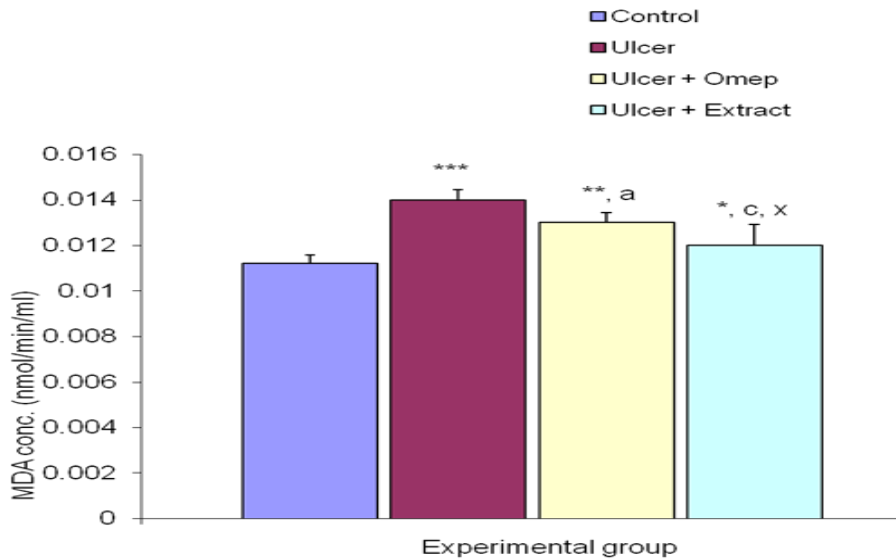


Fig. 4. Comparison of malondialdehyde concentration in control, ulcer and ulcer treated groups.

Values are expressed as mean \pm SEM, n = 5.
 * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ vs control;
 a = $p < 0.05$, c = $p < 0.01$ vs ulcer
 x = significantly different from ulcer + omeprazole at $p < 0.05$

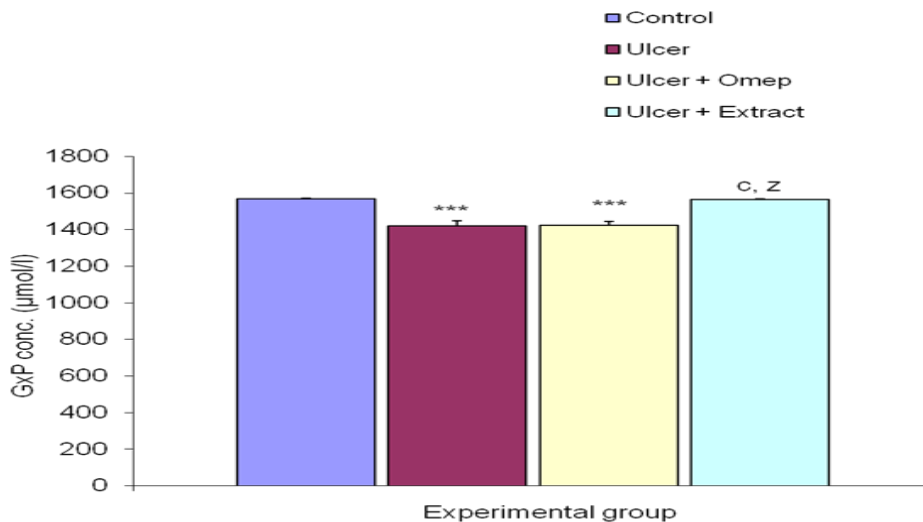


Fig. 5. Comparison of glutathion peroxidase concentration in control, ulcer and ulcer treated groups.

Values are expressed as mean \pm 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 + omeprazole at $p < 0.001$

3.4.4 Serum catalase level in the different groups

Catalase concentration (nmol/min/ml) was 1.14 ± 0.05 , 2.20 ± 0.07 , 2.14 ± 0.05 and 4.00 ± 0.07 for control, ulcer untreated, ulcer+Om and Ulcer+AP respectively. From this result, all three groups had significantly increased ($P < 0.001$)

catalase concentration when compared to control. The two treated groups also had significantly increased ($P < 0.001$) catalase concentration when compared to ulcer untreated group. The extract treated group also had significantly increased ($P < 0.001$) catalase concentration compared to omeprazole treated group (Fig. 7).

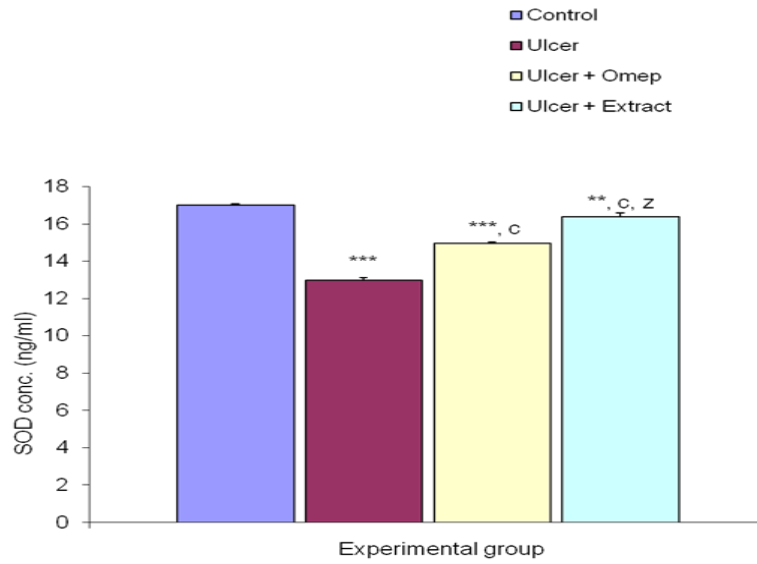


Fig. 6. Comparison of superoxide dismutase concentration in control, ulcer and ulcer treated groups.

Values are expressed as mean \pm SEM, n = 5.
 ** = $p < 0.01$, *** = $p < 0.001$ vs control;
 c = significantly different from ulcer at $p < 0.001$;
 z = significantly different from ulcer + omeprazole at $p < 0.001$

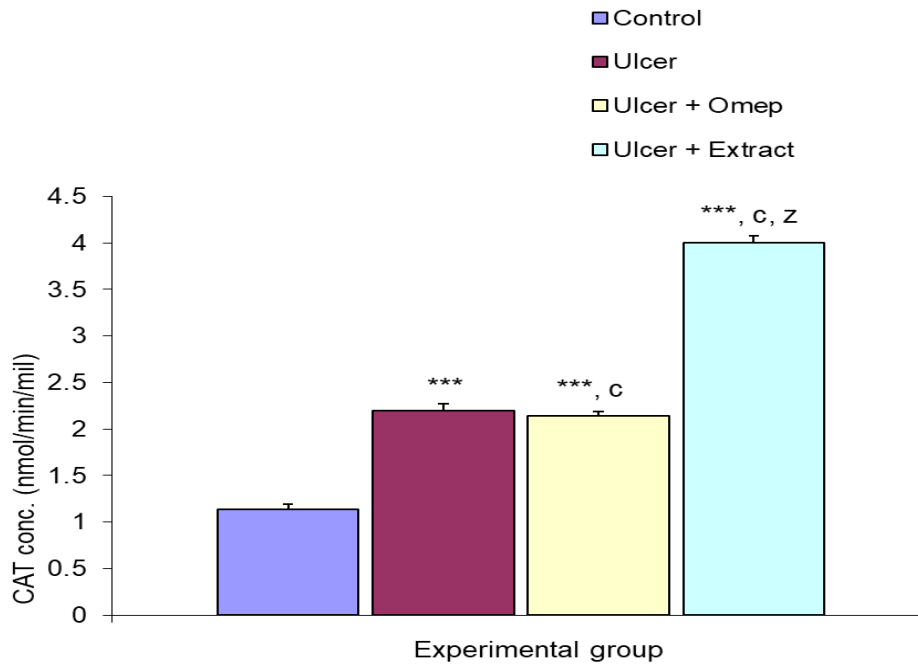


FIG. 7: Comparison of catalase concentration in control, ulcer and ulcer treated groups.

Values are expressed as mean \pm 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 + omeprazole at $p < 0.001$

4. DISCUSSION

From the acute toxicity study, the LD₅₀ was arrived at 50mg/kg body weight and 1/3 of the LD50 value was used as the safe dose in line with the method as reported by Ime et al. [20]. According to Ahmed [31], any compound (in rat) with LD₅₀ of 5000mg/kg or more should be considered as practically harmless. Due to the low LD₅₀ value, there is serious concern for herbal medicine practitioners who prescribe this extract indiscriminately without having the knowledge of the lethal dose and the effective dose.

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 [32,33]. This may greatly increase extracellular fluid volume and thus set 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. Food intake across all the groups showed no significant difference. Meanwhile, 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 [34,35,36] 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 Niranjana et al. [37] who reported that AP had no effect on body weight.

There was a significant increase in MDA levels of ulcer untreated group when compared to control. MDA is a product of lipid peroxidation [38] and its increase in this group shows possible increase of peroxidation of the gastric cell membranes which could be attributable to ulcer induced increase in the level of free radicals that caused lipid peroxidation. Meanwhile, there was a significant decrease in MDA levels of omeprazole and extract treated groups when compared to ulcer untreated

group. The extract treated group showed a significant decrease in MDA level when compared to omeprazole treated group. In this study, the extract treated group has shown to increase the level of glutathione peroxidase, SOD and catalase which is consistent with a study by Verma and Vinayak [39]. These antioxidants may have helped reduce the level of lipid oxidation, hence MDA as seen in this study.

Glutathione peroxidase is an intracellular antioxidant enzyme that enzymatically reduces hydrogen peroxide to water and oxygen to limit its harmful effects on the cell [40,41]. From the result of the study, there was an increase in glutathione peroxidase level in extract treated group compared to ulcer untreated. This means that there is increased activity of these enzymes resulting in conversion of hydrogen peroxide as well as other reactive oxygen species that is formed during lipid peroxidation or oxidative stress. Moreso, the level of Glutathione peroxidase in extract treated group and that of control is almost the same unlike the level in omeprazole treated group. *Andrographis paniculata* has a better effect than the conventional proton pump inhibitor (omeprazole) in terms of its ability to degrade hydrogen peroxide to water and oxygen. Increased activity of Glutathione peroxidase (as seen in extract treated group) may result in regulation and removal of hydrogen peroxide and also prevents the formation of highly reactive/damaging hydroxyl radical. This hydroxyl radical can be formed by reaction of hydrogen peroxide and iron (Fenton reaction). However, there is a need for further study to ascertain possible mechanism(s) of action.

Superoxide dismutase is an enzyme that alternately catalyzes the dismutation (partitioning) of the superoxide (O₂⁻) radical into either ordinary molecular oxygen (O₂) or hydrogen peroxide [42,43]. From the result, there is marked reduction in the level of superoxide dismutase in extract as well as omeprazole treated group compared to control. Catalase is an enzymatic antioxidant found in nearly all cells exposed to oxygen. These enzymes catalyze the decomposition of hydrogen peroxide to water and oxygen [41]. It protects the cell against the effects of the reactive oxygen species produced by the cell during cellular metabolism. In the result, there is a markedly increased level of catalase in extract treated group compared to control. There is also

a marked increase of catalase level in extract treated group compared to ulcer untreated and omeprazole treated group. This shows that in an ulcer that is treated with *Andrographis Paniculata*, there is a high level of catalase activity which mops up the hydrogen peroxide that is being produced during cellular metabolism. The catalase activity in male rats treated with *Andrographis Paniculata* is twice that produced by proton pump inhibitor (omeprazole).

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.

CONSENT

It is not applicable.

ETHICS 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yegen BC. Lifestyle and peptic ulcer disease. *Current Pharmaceutical Design* 2018;24(18):2034-2040.
2. Mahmoud YI, Abd El-Ghffar EA. Spirulina ameliorates aspirin-induced gastric ulcer in albino mice by alleviating oxidative stress and inflammation. *Biomedicine & Pharmacotherapy*. 2019; 109:314-321.
3. Hossen MA, Reza AA, Ahmed AA, Islam MK, Jahan I, Hossain R, Rahman MA. Pretreatment of *Blumea lacera* leaves ameliorate acute ulcer and oxidative stress in ethanol-induced Long-Evan rat: A combined experimental and chemico-biological interaction. *Biomedicine & Pharmacotherapy*. 2021;135.
4. Mizokami Y, Oda K, Funao N, Nishimura A, Soen S, Kawai T, Sugano K. Vonoprazan prevents ulcer recurrence during long-term NSAID therapy: randomised, lansoprazole-controlled non-inferiority and single-blind extension study. *Gut*. 2018;67(6):1042-1051.
5. Tai FWD, McAlindon ME. NSAIDs and the small bowel. *Current Opinion in Gastroenterology*. 2018;34(3):175-182.
6. Kamada T, Satoh K, Itoh T, Ito M, Iwamoto J, Okimoto T, Koike K. Evidence-based clinical practice guidelines for peptic ulcer disease. *Journal of gastroenterology*. 2021;56:303-322.
7. Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical Pharmacology*. 2020;180:114147.
8. Maziero Alves G, Aires R, de Souza Santos V, Zambom Côco L, Peters B, de Leone Evangelista Monteiro Assis A. Sildenafil attenuates nonsteroidal anti-inflammatory-induced gastric ulceration in mice via antioxidant and antigenotoxic mechanisms. *Clinical and Experimental Pharmacology and Physiology*. 2021; 48:401-411.
9. Traoré O, Diarra AS, Kassogué O, Abu T, Maïga A, Kanté M. The clinical and endoscopic aspects of peptic ulcers secondary to the use of nonsteroidal anti-inflammatory drugs of various origins. *The Pan African Medical Journal*. 2021;38.
10. Lee SW, Chang CS, Lee TY, Yeh HZ, Tung CF, Peng YC. Risk factors and therapeutic response in Chinese patients with peptic ulcer disease. *World Journal of Gastroenterology*. 2010;16(16):2017-2022.
11. Melcarne L, García-Iglesias P, Calvet X. Management of NSAID-associated peptic ulcer disease. *Expert Review of Gastroenterology & Hepatology*. 2016; 10(6):723-733.
12. Satoh K, Yoshino J, Akamatsu T, Itoh T, Kato M, Kamada T, Shimosegawa T.

- Evidence-based clinical practice guidelines for peptic ulcer disease. *Journal of Gastroenterology*. 2016;51: 177-194.
13. Lanas A, Chan FK. Peptic ulcer disease. *The Lancet*. 2017;390:613-624.
 14. García-Rayado G, Navarro M, Lanas A. NSAID induced gastrointestinal damage and designing GI-sparing NSAIDs. *Expert Review of Clinical Pharmacology*. 2018; 11:1031-1043.
 15. Liu J, Sun D, He J, Yang C, Hu T, Zhang L. Gastroprotective effects of several H2RAs on ibuprofen-induced gastric ulcer in rats. *Life Sciences* 2016;149:65-71.
 16. Athaydes BR, Alves GM, de Assis ALEM, Gomes JVD, Rodrigues RP, Campagnaro BP, Gonçalves RDCR. Avocado seeds (*Persea americana* Mill.) prevents indomethacin-induced gastric ulcer in mice. *Food Research International* 2019; 119:751-760.
 17. Tamaddonfard E, Erfanparast A, Farshid AA, Imani M, Mirzakhani N, Salighedar R, Tamaddonfard S. Safranal, a constituent of saffron, exerts gastro-protective effects against indomethacin-induced gastric ulcer. *Life Sciences*. 2019; 224: 88-94.
 18. Ugan RA, Un H. The protective roles of butein on indomethacin induced gastric ulcer in mice. *The Eurasian Journal of Medicine*. 2020; 52(3):265.
 19. Suleyman H, Albayrak A, Bilici M, Cadirci E, Halici Z. Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers. *Inflammation*. 2010;33(4):224-234.
 20. Ime AU, Ani EJ, Nna VU. Aloe vera and Garlic ameliorate deleterious consequences of thermoxidized palm oil diet on liver function and histology in rat. *Journal of Nutrition and Food Science*. 2016;46(6):803-815.
 21. Ime AU, Ani EJ, Nna VU, Obeten CE. Aloe vera and garlic ameliorate thermoxidized palm oil-induced haemostatic derangement in albino Wistar rats. *Micro Medicine*. 2017;5(2):53-59.
 22. Ofem OE, Ani EJ, Archibong AN, Ufford JM. Variations in blood parameters of high salt loaded rats following administration of *Moringa oleifera* leaf extract. *Trends in Medical Research*, 2015;10(4):97-105.
 23. Ani EJ, Ibu JO, Ofem OE, Onwuelingo S. Gastric acid secretion induced by Aloe barbadensis (*Aloe vera*) Gel in rats. *West African Journal of Biological Sciences* 2005;16:15-24.
 24. Uduak OA, Ani EJ, Etoh ECI, Macstephen AO. Comparative effect of *Citrus sinensis* and carbimazole on serum T4, T3 and TSH levels. *Nigerian Medical Journal: journal of the Nigeria Medical Association*. 2014;55(3):230.
 25. Lorke D. A new approach to practical acute toxicity testing. *Archives of Toxicology*. 1983;54:275-287.
 26. Satoh H, Inada I, Hirata T, Maki Y. Indomethacin produces gastric antral ulcers in the refed rat. *Gastroenterology*. 1981;81:719-725.
 27. Omodanisi EI, Aboua YG, Oguntibeju OO. Assessment of the anti-hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of *Moringa oleifera* in diabetes-induced nephrotoxic male wistar rats. *Molecules*. 2017;22(4):439.
 28. Abarikwu SO, Olufemi PD, Lawrence CJ, Wekere FC, Ochulor AC, Barikuma AM. Rutin, an antioxidant flavonoid, induces glutathione and glutathione peroxidase activities to protect against ethanol effects in cadmium-induced oxidative stress in the testis of adult rats. *Andrologia*. 2017;49(7): e12696.
 29. Gorzi A, Asadi M. Useful effects of curcumin supplementation on gastric superoxide dismutase activity and serum malondialdehyde level during endurance training in male wistar rats. *Zahedan Journal of Research in Medical Sciences*. 2020;22(2).
 30. Safaeian L, Emami R, Hajhashemi V, Haghghatian Z. Antihypertensive and antioxidant effects of protocatechuic acid in deoxycorticosterone acetate-salt hypertensive rats. *Biomedicine & Pharmacotherapy*. 2018;100:147-155.
 31. Ahmed M. Acute toxicity (lethal dose 50 calculation) of herbal drug somina in rats and mice. *Pharmacology & Pharmacy*. 2015;6:185.
 32. Jeppesen PB, Staun M, Tjellesen L, Mortensen PB. Effect of intravenous ranitidine and omeprazole on intestinal absorption of water, sodium, and macronutrients in patients with intestinal resection. *Gut*. 1998;43:763-769.
 33. Suksridechacin N, Kulwong P, Chamniansawat S, Thongon N. Effect of prolonged omeprazole administration on segmental intestinal Mg²⁺ absorption in

- male Sprague-Dawley rats. World Journal of Gastroenterology. 2020; 26:1142.
34. Elaine WY, Blackwell T, Ensrud KE, Hillier TA, Lane NE, Orwoll E, Bauer DC. Acid-suppressive medications and risk of bone loss and fracture in older adults. *Calcified Tissue International*. 2008;83:251-259.
 35. Khalili H, Huang ES, Jacobson BC, Camargo CA, Feskanich D, Chan AT. Use of proton pump inhibitors and risk of hip fracture in relation to dietary and lifestyle factors: a prospective cohort study. *BMJ*. 2012;344:e372.
 36. Ursomanno BL, Cohen RE, Levine MJ, Yerke LM. Effect of Proton Pump Inhibitors on Bone Loss at Dental Implants. *International Journal of Oral & Maxillofacial Implants*. 2020;35.
 37. Niranjana A, Tewari SK, Lehri A. Biological activities of kalmegh (*Andrographis paniculata* Nees); 2020.
 38. Gęgotek A, Skrzydlewska E. Biological effect of protein modifications by lipid peroxidation products. *Chemistry and Physics of Lipids*. 2019;221:46-52.
 39. Verma N, Vinayak M. Antioxidant action of *Andrographis paniculata* on lymphoma. *Molecular Biology Reports*. 2008;35(4): 535-540.
 40. Benhar M. Roles of mammalian glutathione peroxidase and thioredoxin reductase enzymes in the cellular response to nitrosative stress. *Free Radical Biology and Medicine*. 2018; 127: 160-164.
 41. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*. 2018;54(4):287-293.
 42. Borgstahl GE, Oberley-Deegan RE. Superoxide dismutases (SODs) and SOD mimetics. *Antioxidants* 2018;7(11):156.
 43. Zhao H, Zhang R, Yan X, Fan K. Superoxide dismutase nanozymes: an emerging star for anti-oxidation. *Journal of Materials Chemistry B*. 2021;9(35):6939-6957.

© 2023 Kokelu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<https://www.sdiarticle5.com/review-history/99110>