Andrographis paniculata as Promising novel protective therapy of oxidative stress in INDOMETHACIN-INDUCED gastric ulcer in rats

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, 20mg/kg indomethacin plus omeprazole at 20mg/kg and 20mg/kg indomethacin plus *AP* at 16.7mg/kg respectively. After treatment period, estimation of oxidative stress parameters was carried out on the animals. The LD_{50} 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.

Key words: peptic ulcer disease; oxidative stress; antioxidants; indomethacin; *Andrographis paniculata;* LD_{50} , 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 E_2 (PGE_2), 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, H_2 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):
Group1 (Normal Control) - (fed with normal chow + water)
Group2 (positive Control) – Induced with ulcer using indomethacin at dose of 20mg/kg bw
Group3 (Ulcer+Om) - Induced with ulcer using indomethacin at dose of 20mg/kg bw and treated with omeprazole.
Group4 (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 (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 [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].

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

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

TABLE 1: Acute lethal effect of extract of *Andrographis 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

3.2 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. Moreso, 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).

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).

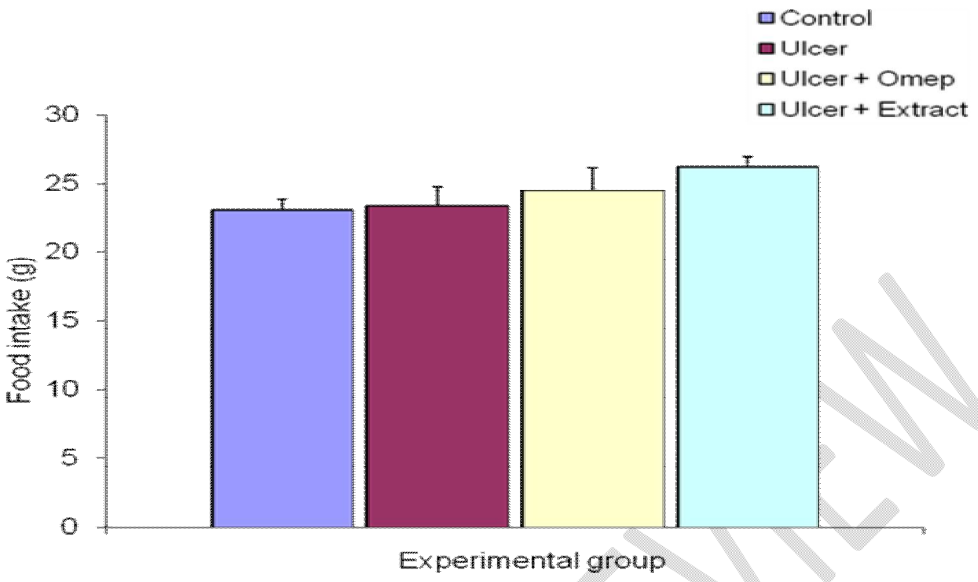

FIG. 1: Comparison of mean food intake of control, ulcer and ulcer treated groups.

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

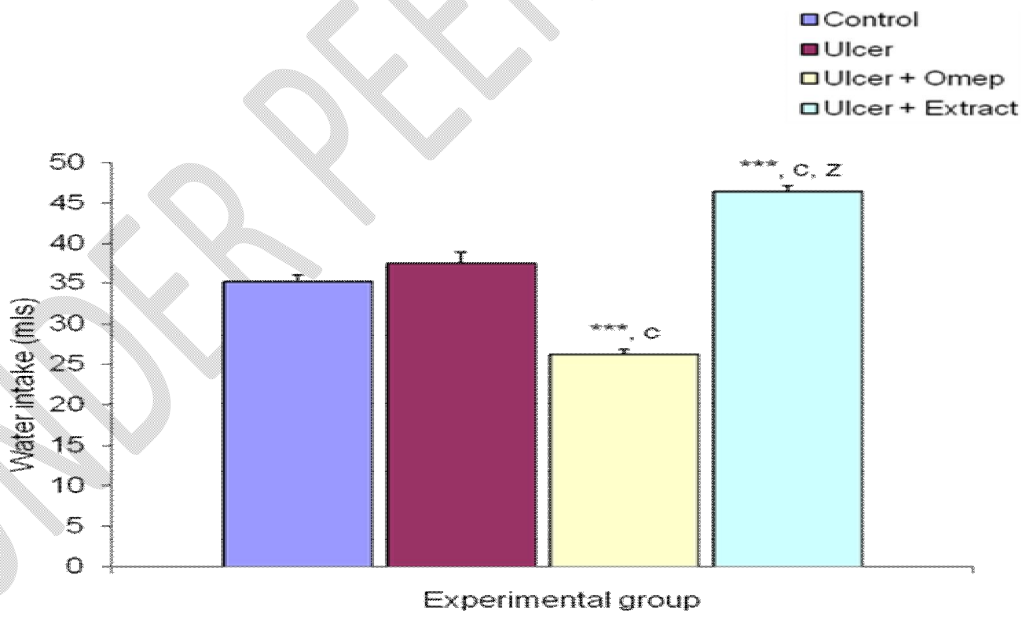

FIG. 2: Comparison of mean water intake of control, ulcer and ulcer treated groups.

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

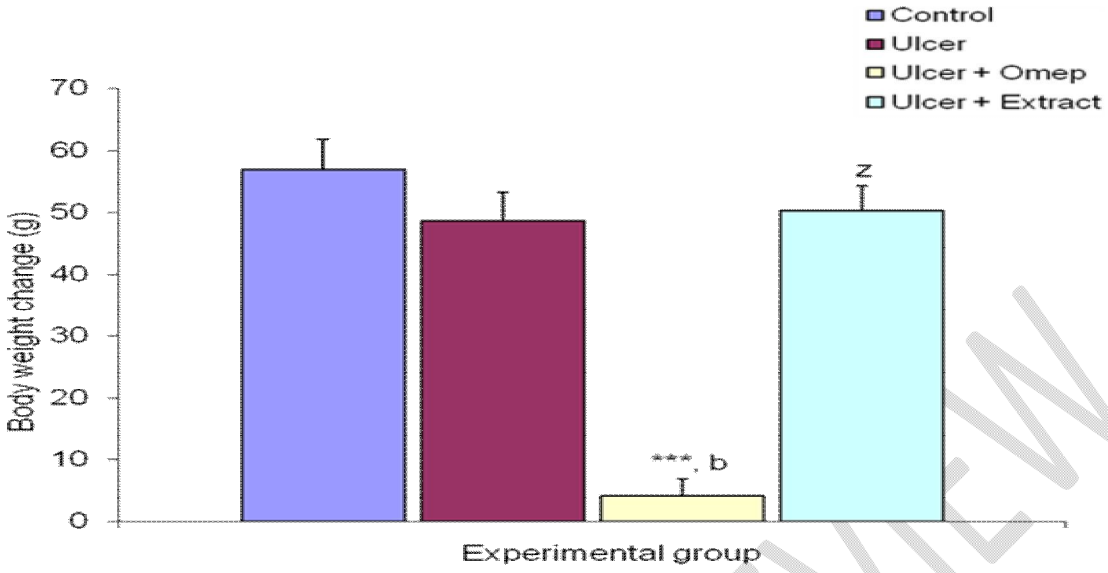

FIG. 3: Comparison of body weight change of control, ulcer and ulcer treated groups.

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

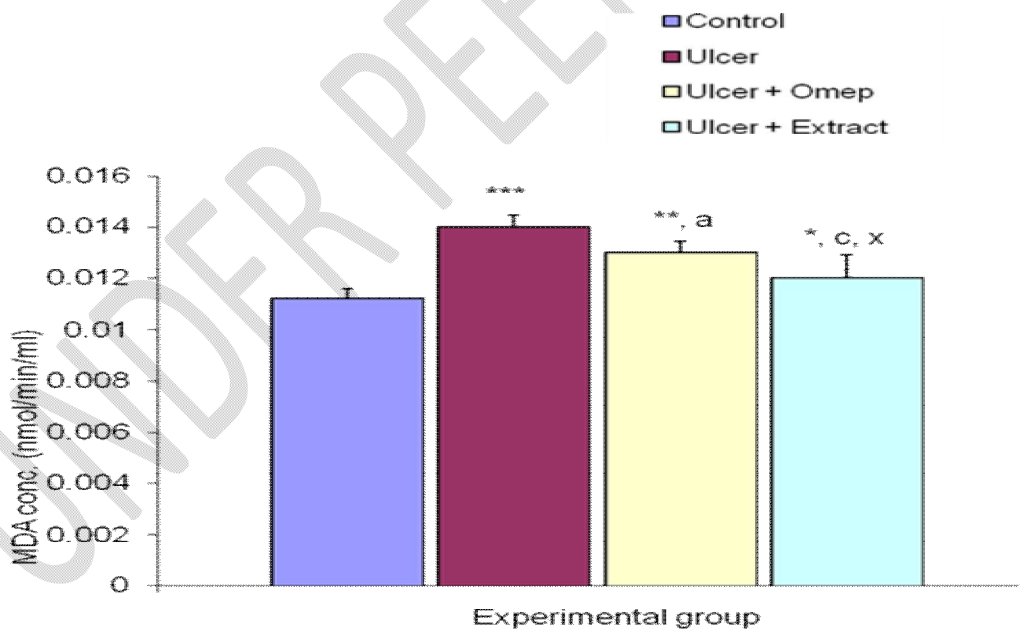

FIG. 4 : Comparison of malondialdehyde concentration in control, ulcer and ulcer treated groups.

Values are expressed as mean ± 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 + omep at p<0.05

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 (µmol/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. Moreso, the extract treated group had significantly increased ($P<0.001$) SOD concentration when compared to omeprazole treated group (Fig. 6)

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).

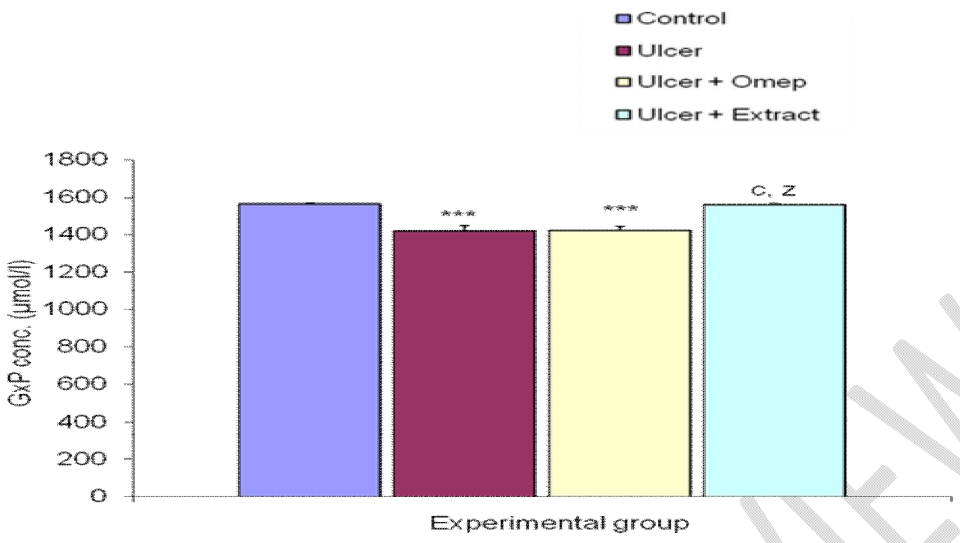

FIG. 5 Comparison of glutathion peroxidase concentration in control, ulcer and ulcer treated groups.

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;
z = significantly different from ulcer + omep at p<0.001

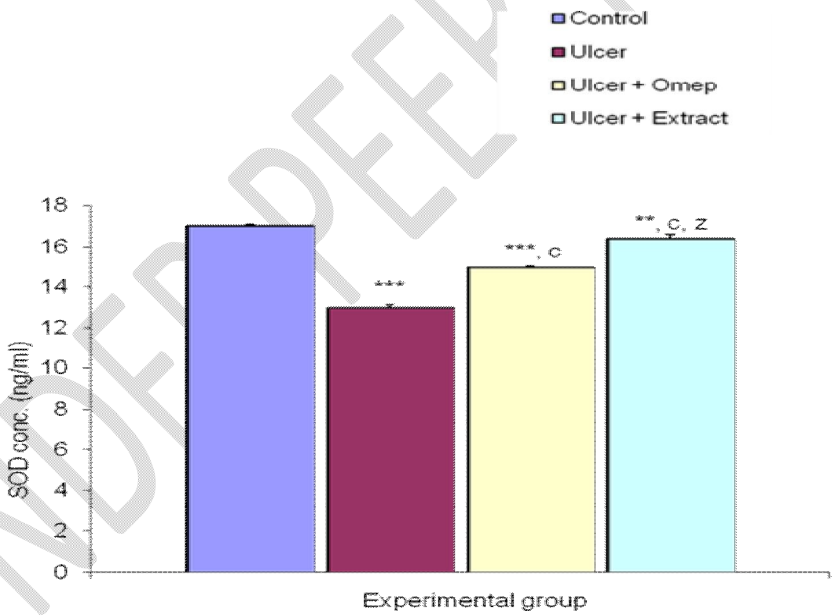

FIG. 6 Comparison of superoxide dismutase concentration in control, ulcer and ulcer treated groups.

Values are expressed as mean ± 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 + omep at p<0.001

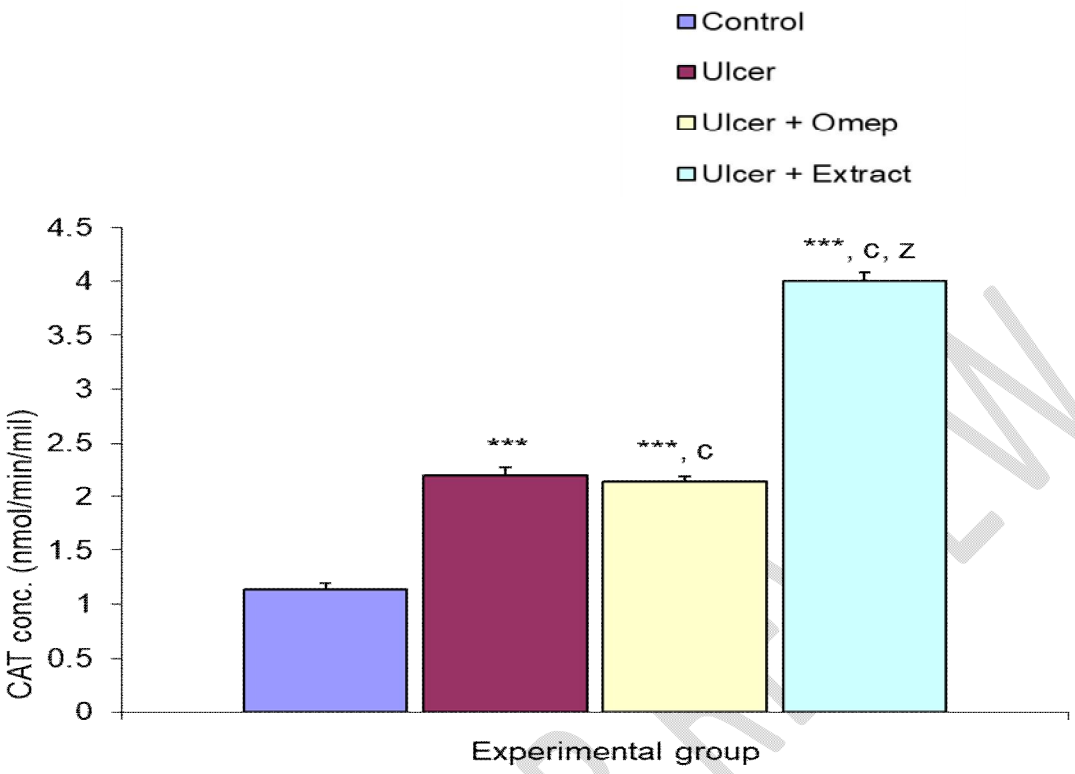

FIG. 7: Comparison of catalase concentration in control, ulcer and ulcer treated groups.

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;
z = significantly different from ulcer + omep at p<0.001

4. DISCUSSION

From the acute toxicity study, the LD_{50} 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 LD_{50} of 5000mg/kg or more should be considered as practically harmless. Due to the low LD_{50} 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 Niranjan *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 in 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 (O_2^-) radical into either ordinary molecular oxygen (O_2) 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 [45] (Ighodaro and Akinloye, 2018). 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 *Andrgraphis 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.

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.

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