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Effects of the methanol extract of the leaves of *Azadirachta indica* on some oxidative stress indices of ethanol-ulcerated rats

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ABSTRACT

Background and Aim: Researches into medicinal plants with potent anti-oxidant activity will continue to receive the attention of researchers due to the role of oxidative stress in many disease conditions and hence, this study was undertaken to investigate the effects of the methanol extract of the leaves of *Azadirachta indica* on oxidative stress indices of ethanol-ulcerated rats. **Methods:** The effects of the methanol extract of the leaves of *A. indica* on the activity of superoxide dismutase (SOD), concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) were determined using standard methods. **Results:** The extract at the administered doses [100, 200 and 400 mg/kg body weight (b.w)] caused significant ($p < 0.05$) and dose-related increases in the activities and amounts of SOD and GSH respectively in the rats of the test groups compared to those of the rats in the ulcer-untreated group (group 2). The extract treatment also led to significant ($p < 0.05$) reductions in the concentrations of MDA in the rats of the test groups compared to that of the rats in the ulcer-untreated group. The 400 mg/kg b.w of the extract exerted the greatest effects which were similar to those of the standard anti-ulcer drug, cimetidine at the dose of 100 mg/kg b.w. **Conclusion:** The methanol extract of the leaves of *A. indica* remarkably ameliorated the gastric ulcer-associated indices in the ulcerated rats and these findings thus support the local application of the leaves of the plant in the cure of gastric ulcer.

KEYWORDS: Medicinal plants, Anti-oxidant, Oxidative stress and Gastric ulcer

1. INTRODUCTION

Azadirachta indica (Linn) (Fig. 1) (Meliaceae) commonly known as neem is native to India and naturalised in most of tropical and sub-tropical countries. It is of great medicinal value and distributed wide-spread in the world[1]. Neem leaf is effective in treating eczema, ring-worm and acne. It has anti-inflammatory and anti-hyperglycaemic properties and is used to heal chronic wounds, diabetic foot and gangrene-developing conditions. It is believed to remove toxins from the body, neutralise free radicals and purify the blood. It is used as anti-cancer agent and possesses hepato-renal protective and hypolipidaemic effects[2].

Fig. 1: *Azadirachta indica* (Linn)

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Peptic ulcer disease (PUD) is a break in the lining of the stomach, the first part of the small intestine or occasionally, the lower oesophagus. An ulcer in the stomach is known as a gastric ulcer while that in the first part of the small intestine is known as a duodenal ulcer. The most common symptoms of peptic ulcer are waking up at night with upper abdominal pains or upper abdominal pains that improve with eating.

The pains are often described as burning or dull aches. Other symptoms include: belching, vomiting, weight loss or poor appetite. About a third of older people have no symptoms. Complications may include: bleeding, perforation and blockage of the stomach[3]. Bleeding occurs in as much as 15 percent of the affected people[4]. Common causes of peptic ulcer include: a bacteria, *Helicobacter pylori* and non-steroidal anti-inflammatory drugs (NSAIDs). Other less common causes include: tobacco smoking, stress due to serious illnesses, Behcet's disease, Zollinger-Ellison syndrome, Crohn's disease and liver cirrhosis among others[3]. Oxidative stress is believed to initiate and aggravate many diseases including PUD. This study was therefore, aimed at evaluating the effects of the methanol extract of the leaves of *A. indica* on some oxidative stress indices of ethanol-ulcerated rats.

2. MATERIALS AND METHODS

2.1 Plant

Fresh leaves of *A. indica* were plucked from their tree at the Botanical

Garden of the University of Nigeria, Nsukka. The leaves were identified by Mr. Alfred Ozioko of Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka where the voucher specimen was deposited in the herbarium.

2.2 Preparation of the Extract

The fresh leaves of *A. indica* were washed with distilled water. The leaves were spread on a clean mat in a well-ventilated room with regular turning to enhance even drying and avoid decaying and allowed to shade-dry for 3 weeks. A known weight (500 g) of the pulverised leaves was macerated in 5 volumes (w/v) of methanol and left for 24 hours. The mixture was thereafter, filtered using Whatman No 1 filter paper and the filtrate concentrated in a rotary evaporator and weighed.

2.3 Animals

Adult male albino Wistar rats of between 3 and 4 months old with average weight of 120 ± 20 g were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were acclimatised to a standard environmental condition for one week with a 12 hour light and dark cycle and maintained on a regular feed and water *ad libitum*. The Principles of Laboratory Animal Care were adhered to.

2.4 Chemicals

The chemicals used in this study were of analytical grade and included: absolute ethanol (BDH Chemicals Ltd., Poole, England), methanol (BDH Chemicals Ltd., Poole, England), cimetidine [standard anti-ulcer drug (Sigma-Aldrich, Inc., St. Louis, USA)] and distilled water.

2.5 Assay of the Activity of Superoxide Dismutase

The activity of superoxide dismutase was assayed using the method described by Misra and Fridovich[5].

2.6 Determination of the Concentration of Reduced Glutathione

The concentration of reduced glutathione was determined according to the method described by Jollow *et al*[6].

2.7 Determination of the Concentration of Malondialdehyde

The concentration of malondialdehyde was determined using the method described by Niki *et al*[7].

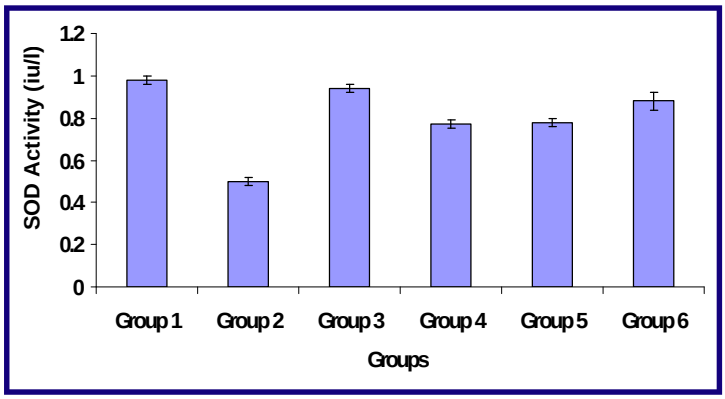
2.8 Statistical Analysis

The data obtained were subjected to one-way Analysis of Variance (ANOVA). The results are expressed as means ± standard errors of the means (SEM). Significant differences are observed at $p < 0.05$. The analysis was done using the computer software known as Statistical Products and Service Solutions (SPSS), Version 18.

3. RESULTS

3.1 Effect of the Methanol Extract of the Leaves of *A. indica* on the Activity of Superoxide Dismutase (SOD)

Fig. 2 shows that the SOD activity (0.98 ± 0.02 iu/l) of the rats in the normal control group (group 1) was significantly ($p < 0.05$) higher than that of the rats (0.50 ± 0.02 iu/l) in the ulcer-untreated group (group 2). The 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) and dose-dependently increased the SOD activities of the rats in groups 4 (0.77 ± 0.02 iu/l), 5 (0.78 ± 0.02 iu/l) and 6 (0.88 ± 0.04 iu/l) when compared to the value obtained for the rats in group 2 (0.50 ± 0.02 iu/l). The effect of the extract at the dose of 400 mg/kg body weight was comparable to that of the standard anti-ulcer drug [cimetidine (100 mg/kg body weight)] as there was no significant ($p > 0.05$) difference between the SOD activity of the rats in group 6 (0.88 ± 0.04 iu/l) and that of the rats in group 3 (0.94 ± 0.02 iu/l).

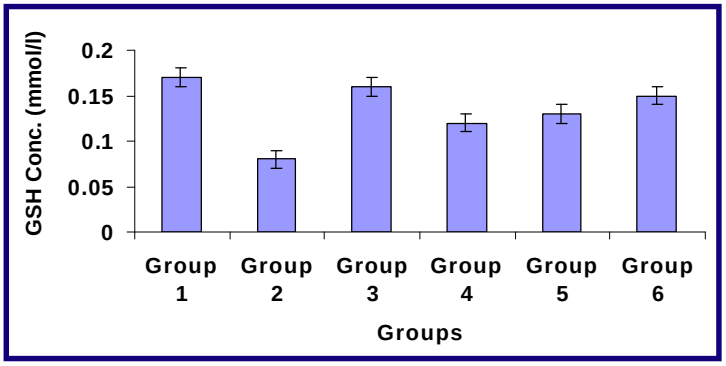


Group 1: 5 ml/kg body weight (b.w) of distilled water only (Normal control)
Group 2: 5 ml/kg b.w of distilled water + 5 ml/kg b.w of absolute ethanol (Positive control)
Group 3: 100 mg/kg b.w of cimetidine + 5 ml/kg b.w of absolute ethanol
Group 4: 100 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 5: 200 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 6: 400 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol

Fig. 2: Effects of the graded doses of the methanol extract of the leaves of *A. indica* on SOD activity

3.2 Effect of the Methanol Extract of the Leaves of *A. indica* on the Concentration of Reduced Glutathione (GSH)

As shown in Fig. 3, the GSH concentration (0.17 ± 0.01 mmol/l) of the rats in the normal control group (group 1) was significantly ($p < 0.05$) higher than that of the rats (0.08 ± 0.01 mmol/l) in the ulcer-untreated group (group 2). The 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) and dose-relatedly increased the GSH concentrations of the rats in groups 4 (0.12 ± 0.01 mmol/l), 5 (0.13 ± 0.01 mmol/l) and 6 (0.15 ± 0.01 mmol/l) when compared to the value obtained for the rats in group 2 (0.08 ± 0.01 mmol/l). The effect of the extract at the dose of 400 mg/kg body weight was comparable to that of the standard anti-ulcer drug, cimetidine at the dose of 100 mg/kg body weight as there was no significant ($p > 0.05$) difference between the GSH concentration of the rats in group 6 (0.15 ± 0.01 mmol/l) and that of the rats in group 3 (0.16 ± 0.01 mmol/l).

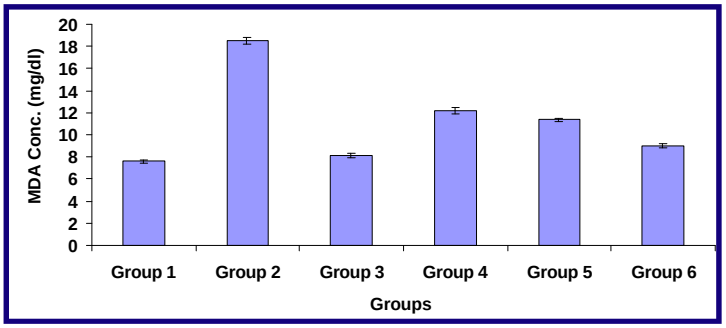


Group 1: 5 ml/kg body weight (b.w) of distilled water only (Normal control)
Group 2: 5 ml/kg b.w of distilled water + 5 ml/kg b.w of absolute ethanol (Positive control)
Group 3: 100 mg/kg b.w of cimetidine + 5 ml/kg b.w of absolute ethanol
Group 4: 100 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 5: 200 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 6: 400 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol

Fig. 3: Effects of the graded doses of the methanol extract of the leaves of *A. indica* on reduced glutathione concentration

3.3 Effect of the Methanol Extract of the Leaves of *A. indica* on the Concentration of Malondialdehyde (MDA)

The MDA concentration (7.59 ± 0.18 mg/dl) of the rats in the normal control group (group 1) was significantly ($p < 0.05$) lower than that of the rats (18 ± 0.31 mg/dl) in the ulcer-untreated group (group 2) as shown in Fig. 4. The 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) and dose-dependently decreased the MDA concentrations of the rats in groups 4 (12.18 ± 0.27 mg/dl), 5 (11.34 ± 0.16 mg/dl) and 6 (9.03 ± 0.17 mg/dl) when compared to the value obtained for the rats in group 2 (18.55 ± 0.31 mg/dl). The effect of the extract at the dose of 400 mg/kg body weight was comparable to that of the standard anti-ulcer drug [cimetidine (100 mg/kg body weight)] as there was no significant ($p > 0.05$) difference between the MDA concentration of the rats in group 6 (9.03 ± 0.17 mg/dl) and that of the rats in group 3 (8.12 ± 0.20 mg/dl).



Group 1: 5 ml/kg body weight (b.w) of distilled water only (Normal control)
Group 2: 5 ml/kg b.w of distilled water + 5 ml/kg b.w of absolute ethanol (Positive control)
Group 3: 100 mg/kg b.w of cimetidine + 5 ml/kg b.w of absolute ethanol
Group 4: 100 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 5: 200 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol
Group 6: 400 mg/kg b.w of the extract + 5 ml/kg b.w of absolute ethanol

Fig. 4: Effects of the graded doses of the methanol extract of the leaves of *A. indica* on MDA concentration

4. DISCUSSION

In this study, the effects of the methanol extract of the leaves of *A. indica* on some oxidative stress indices of ethanol-ulcerated rats were evaluated with a view to elucidating the probable mechanism(s) of the anti-ulcer effect of the leaves of *A. indica*.

Superoxide dismutase (SOD) is an important anti-oxidant defense in nearly all living cells exposed to oxygen[8]. In the present study, there were significant ($p < 0.05$) increases in the activities of SOD in the rats of groups 4, 5 and 6 when compared to that of the rats in the ulcer-untreated group (group 2). This could be as a result of the fact that SOD activity typically decreases with the degree of stress conditions when acclimatising to increased levels of oxidative stress. Treatment of rats with the graded doses of the methanol extract of the leaves of *A. indica* along with ethanol appreciably raised the activity of SOD of rats in the test groups in a dose-dependent manner. The protective potential of the methanol extract of the leaves of *A. indica* to augment the activity of SOD against the ethanol-induced toxicity implies its possible ability in the prevention or amelioration of gastric lesions involving free radical reactions.

Reduced glutathione (GSH) is a remarkable endogenous anti-oxidant whose concentration in the ulcerated rats remarkably decreased in this study possibly due to its excessive utilisation in combating the oxidative stress precipitated by the ulcerogen (ethanol). GSH is used as a cofactor in the removal of hydrogen peroxide and lipoperoxides by the glutathione peroxidase family during which it is converted to the oxidised form (GSSG). Availability of GSH is crucial for the integrity of the mucosa whereas its depletion causes severe ulceration[9]. The protective effect of the methanol extract of the leaves of *A. indica* in maintaining the concentration of GSH towards normalcy might have led to the restoration of GSH and/or its synthesis which in turn increased the efficacy of the endogenous anti-oxidant enzymes against oxidative stress induced by ethanol in the gastric mucosa of the rats.

Ethanol treatment to the rats in the ulcer-untreated group significantly ($p < 0.05$) increased the concentration of malondialdehyde (MDA), an indicator of lipid peroxidation in the rats. The increase in the lipid peroxidation marker could be due to the abundant generation of free radicals and inefficiency of the anti-oxidant system in the ulcerated rats. Exposure of rats to ulcerogen may induce an overwhelming generation of free radicals[10]. The concentrations of MDA of rats in the groups co-treated with ethanol and graded doses of the methanol extract of the leaves of *A. indica* were significantly ($p < 0.05$) lowered when compared to that of the rats in the group administered ethanol only which indicates the protective effect of the methanol extract of the leaves of *A. indica* against ulcer-associated lipid peroxidation and/or its intensification.

In conclusion, the data of this study indicate that the methanol ex-

tract of the leaves of *A. indica* in part, protects against ethanol-induced gastric ulcer in rats by raising the activity and amount of superoxide dismutase and reduced glutathione respectively as well as decreasing the concentration of malondialdehyde.

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