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Effect of the methanol extract of the leaves of *Azadirachta indica* on ethanol-induced gastric ulcer in rats

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ABSTRACT

Background and Aim: Plants have been the bases for medicinal treatments through much of human history and such medicine however, traditional, is still widely in use today such as the application of the leaves of *Azadirachta indica* in the treatment of gastric ulcer. The thrust of this work was therefore, to evaluate the effect of the methanol extract of the leaves of *A. indica* on ethanol-induced gastric ulcer in rats. **Methods:** The phytochemical constituents, acute toxicity and lethality and effects of the extract on ulcer index, gastric juice volume and gastric juice pH were assessed using standard methods. **Results:** The phytochemical screening revealed that the plant extract contained alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides, proteins, steroids, terpenoids and fats and oil but resins, reducing sugars and acidic compounds were not detected. The extract at a dose even as high as 5000 mg/kg body weight (b.w) was safe for administration. The extract at the tested doses [100, 200 and 400 mg/kg b.w] caused significant ($p < 0.05$) and dose-dependent decreases in ulcer indices (gastric lesions) and gastric juice volumes of the rats in the test groups compared to the values obtained for the rats in the positive control group (group 2). Significant ($p < 0.05$) and dose-related increases in gastric juice pH of the rats in the test groups compared to that of the rats in the positive control group were also brought about by the extract. The effects of the 400 mg/kg b.w of the extract were similar to those of the standard anti-ulcer drug, cimetidine at the dose of 100 mg/kg b.w. **Conclusion:** These observations show that the methanol extract of the leaves of *A. indica* possesses notable anti-ulcer effect and contains some pharmacologically active principles which might be cynosures of future modern anti-ulcer drugs.

KEYWORDS: Traditional medicine, *Azadirachta indica*, Gastric ulcer and Cimetidine

1. INTRODUCTION

Ulcers are open sores of the skin or mucus membrane characterised by sloughing of inflamed dead tissue. There are many types of ulcer such as mouth ulcer, oesophageal ulcer, peptic ulcer and genital ulcer. Peptic ulcer is basically an inflamed break in the skin or mucus lining the alimentary tract that can cause stomach upset. It is an erosion of the lining of the stomach or duodenum[1]. The two most common types of peptic ulcer are gastric and duodenal ulcers. The names refer to the sites of ulcerations. Gastric ulcer is located in the stomach, characterised by pains and is common in older age group. Generally, pains occur when the stomach is empty and are relieved after eating. Eating may increase pains rather than relieve them. Although, patients with gastric ulcer have normal or increased acid production, gastric ulcer may occur even in the complete absence of acid. In the duodenum, ulcer (duodenal ulcer) appears both in the anterior and posterior walls and is more common in younger individuals. It predominantly affects males. Other symptoms of peptic ulcer may include: nausea, vomiting and weight loss[2].

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Azadirachta indica (Linn) (Fig. 1) also known as neem, neem tree or Indian lilac is a tree in the mahogany family, Meliaceae. It is also referred to as Indian tree of life. It is one of the two species in the genus, *Azadirachta* which is in India and Indian subcontinents including Nepal, Pakistan, Bangladesh and Sri Lanka and typically growing in tropical and semi-tropical regions. Neem trees now also grow in islands in the Southern part of Iran. Its fruits and seeds are the sources of neem oil. Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), rarely 35–40 metres (115–131 ft). It is evergreen but in severe drought, it may shed most or nearly all of its leaves. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 15–20 metres (49–66 ft) in old and free-

standing specimens. The neem tree is very similar in appearance to its relative, chinaberry (Melia azedarach)[3]. The plant has many medicinal properties such as anti-viral, anti-inflammatory, anti-fungal, anti-bacterial, anti-plasmodial, anti-pyretic and anti-diabetic properties[4]. *A. indica* leaves are used in treatment of gastric ulcer and hence, the aim of this study was to investigate the effect of the methanol extract of its leaves on ethanol-induced gastric ulcer in rats.

Fig. 1: ***Azadirachta indica*** **(Linn)**

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 and albino mice weighing 25 ± 5 g were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals 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 and Reagents

The chemicals and reagents used in this study were of analytical grade and included: absolute ethanol (BDH Chemicals Ltd., Poole, England), methanol (BDH Chemicals Ltd., Poole, England), chloroform (BDH Chemicals Ltd., Poole, England), ethyl acetate (BDH Chemicals Ltd., Poole, England), cimetidine [standard anti-ulcer drug (Sigma-Aldrich, Inc., St. Louis, USA)], dilute tetraoxosulphate (vi) acid, 2% (v/v) hydrochloric acid, 1% (w/v) picric acid, methyl orange, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Fehling's solution, 5% (w/v) ferric chloride solution, aluminium chloride solution, lead sub acetate solution, ammonium solution and distilled water.

2.5 Phytochemical Analyses

Qualitative phytochemical analyses were carried out on the extract according to the procedures described by[5,6].

2.6 Acute Toxicity and Lethality

The acute toxicity and lethality (LD_{50}) of the extract was determined using mice according to slightly modified method of[7].

2.7 Ulcer Studies

Evaluation of the effects of the extract on ulcer index, gastric juice volume and gastric juice pH were by the methods described by[8].

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 Qualitative Phytochemical Composition of the Methanol Extract of the Leaves of *A. indica*

As shown in Table 1, the qualitative phytochemical analyses showed

the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates, glycosides, proteins, steroids, terpenoids and fats and oil in the extract. Resins, reducing sugars and acidic compounds were not detected in the extracts.

Table 1: Qualitative phytochemical constituents of the methanol extract of the leaves of *A. indica*

Phytochemical constituents	Methanol extract
Alkaloids	+
Flavonoids	+
Tannins	+
Resins	ND
Saponins	+
Carbohydrates	+
Reducing sugars	ND
Glycosides	+
Proteins	+
Steroids	+
Terpenoids	+
Fats and oil	+
Acidic compounds	ND

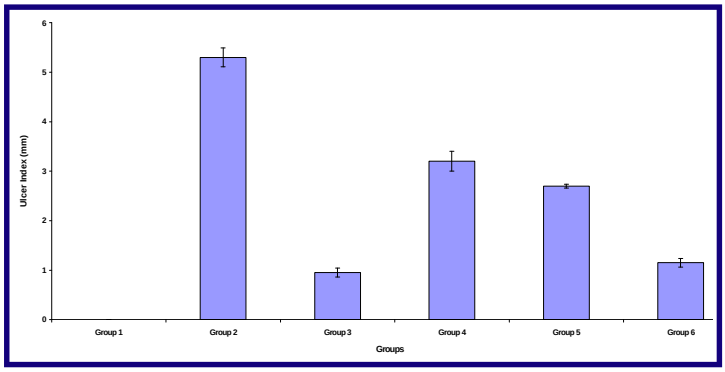
+ = present, ND = not detected

3.2 Acute Toxicity and Lethality (LD_{50}) of the Methanol Extract of the Leaves of *A. indica*

There was neither lethality nor any sign of toxicity in the four groups of three mice each that received 10, 100, 1000 mg/kg body weight of the methanol extract of the leaves of *A. indica* and 5 ml/kg body weight of distilled water respectively at the end of the first phase of the study. At the end of the second phase of the study, there was not death or obvious sign of toxicity in the groups of mice that received 1900, 2600 and 5000 mg/kg body weight of the extract.

3.3 Effect of the Methanol Extract of the Leaves of *A. indica* on Ulcer Index

Fig. 2 shows that the ulcer index (0.00 ± 0.00 mm) of the rats in the normal control group (group 1) was significantly ($p < 0.05$) lower than that of the rats (5.30 ± 0.19 mm) in the positive control group (group 2). The 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) and dose-dependently decreased the ulcer indices of the rats in groups 4 (3.20 ± 0.20 mm), 5 (2.70 ± 0.04 mm) and 6 (1.15 ± 0.09 mm) when compared to the value obtained for the rats in group 2 (5.30 ± 0.19 mm). 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 ulcer index of the rats in group 6 (1.15 ± 0.09 mm) and that of the rats in group 3 (0.95 ± 0.09 mm).

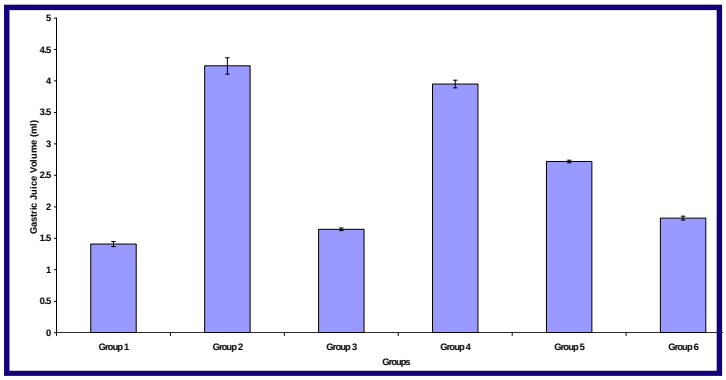


Group 1: 5 ml/kg body weight (b.w) of distilled water only (Normal control)
Group 2: 5 ml/kg b.w of distilled water + 5 ml/kg b.w of absolute ethanol (Positive control)
Group 3: 100 mg/kg b.w of cimetidine + 5 ml/kg b.w of absolute ethanol
Group 4: 100 mg/kg b.w of the extract + 5 ml/kg b.w of absolute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

3.4 Effect of the Methanol Extract of the Leaves of *A. indica* on Gastric Juice Volume

As shown in Fig. 3, the gastric juice volume (1.41 ± 0.04 ml) of the rats in the normal control group (group 1) was significantly ($p < 0.05$) lower than that of the rats (4.24 ± 0.13 ml) in the positive control group (group 2). The 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) and dose-dependently decreased the gastric juice volumes of the rats in groups 4 (3.95 ± 0.06 ml), 5 (2.72 ± 0.02 ml) and 6 (1.82 ± 0.03 ml) when compared to the value obtained for the rats in group 2 (4.24 ± 0.13 ml). 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 a dose of 100 mg/kg body weight as there was no significant ($p > 0.05$) difference between the gastric juice volume of the rats in the group 6 (1.82 ± 0.03 ml) and that of the rats in group 3 (1.64 ± 0.02 ml).

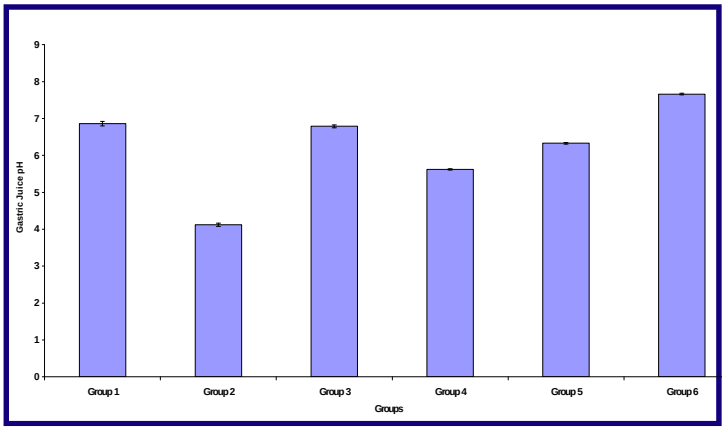


Group 1: 5 ml/kg body weight (b.w) of distilled water only (Normal control)
Group 2: 5 ml/kg b.w of distilled water + 5 ml/kg b.w of absolute ethanol (Positive control)
Group 3: 100 mg/kg b.w of cimetidine + 5 ml/kg b.w of absolute ethanol
Group 4: 100 mg/kg b.w of the extract + 5 ml/kg b.w of absolute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

3.5 Effect of the Methanol Extract of the Leaves of *A. indica* on Gastric Juice pH

The gastric juice pH (6.86 ± 0.06) of the rats in the normal control group (group 1) was significantly ($p < 0.05$) higher than that of the rats (4.12 ± 0.04) in the positive control group (group 2). The 100, 200 and 400 mg/kg body weight of the extract significantly ($p < 0.05$) and dose-dependently increased the gastric juice pH of the rats in groups 4 (5.62 ± 0.02), 5 (6.33 ± 0.02) and 6 (7.66 ± 0.02) when compared to the value obtained for the rats in group 2 (4.12 ± 0.04). 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 gastric juice pH of the rats in group 6 (7.66 ± 0.02) and that of the rats in group 3 (6.79 ± 0.04) as shown in Fig. 4.



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 gastric juice pH

4. DISCUSSION

The phytochemical composition, acute toxicity and lethality as well as effect on ethanol-induced gastric ulceration in rats of the methanol extract of the leaves of *A. indica* were investigated in this study with a view to substantiating their (*A. indica* leaves) traditional use in the treatment of gastric ulcer.

That there was no death or any sign of toxicity in the groups of mice fed the methanol extract of the leaves of *A. indica* even at a dose as high as 5000 mg/kg body weight implies that the leaves of *A. indica* have low toxicity when taken orally.

The presence of certain bioactive compounds in the methanol extract of the leaves of *A. indica* as shown in the present study might have been responsible for the observed anti-ulcer effect of the extract. It is possible that flavonoids and tannins, acting dually or in combination with other phytochemical constituents caused the extract effects. Flavonoids and tannins are among the cytoprotective active compounds for which ulcer-counteracting property has been extensively confirmed. While flavonoids are suggested to be able to stimulate the secretions of mucus, bicarbonate and prostaglandins and counter the deteriorating effects of reactive oxidants in the gastro-intestinal lumen, tannins are known to "tan" the outermost layer of the mucosa and render it less permeable and more resistant to chemical and mechanical injuries[9,10].

Gastric cytoprotection was exhibited by the methanol extract of the leaves of *A. indica* against ulcer experimentally induced with ethanol as it remarkably decreased the ulcer index and gastric juice volume and raised the gastric juice pH in the treated rats. The significant ($p < 0.05$) decreases in the ulcer index and gastric juice volume and increase in the gastric juice pH in the rats of the positive control group (group 2) in each of the parameters supra imply that the ulcerogen (ethanol), induced ulcer in all the ethanol-administered rats. Ethanol-induced gastric damage has been associated with depletion of gastric mucus, back diffusion of acid, increased gastric mucosal permeability, increasing leak of hydrogen ion from the lumen, decrease in the transmucosal electrical potential difference, changes in the mucosal blood flow, destruction of microvascular and non vascular types of cells, mast cell degranulation, neutrophil-mediated mucosal injury (release of oxygen free radicals, proteases and lysosomal enzymes, digestion of proteins and lipid peroxidation in cell membrane) and depletion of certain oxygen free radical scavengers[11]. It is therefore, presumed that the extract might have exerted its anti-ulcer effect by interfering with any of the outlined pathologic processes. Similar findings had been reported[12].

In conclusion, it is evident that the methanol extract of the leaves of *A. indica* protects against gastric ulcer by stimulating the decreases in ulcer index and gastric juice volume and increase in gastric juice pH. This study thus, justifies the use of *A. indica* leaves in traditional medicine for the treatment of gastric ulcer.

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