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RESEARCH ARTICLE

ANTI-ULCER STUDIES OF ETHANOL STEM BARK EXTRACTS OF *ISOBERLINIA DOKA* CRAIB & STAPF AND *ISOBERLINIA TOMENTOSA* (HARMS) CRAIB & STAPF IN MALE WISTAR RATS

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ABSTRACT

Background and aim: *Isoberlinia* species (Fabaceae) are used in ethno medicines for the treatment of mostly gastric ulcer and stomach pain with no scientific validation on their toxicity and anti-ulcer potentials. This study aimed to evaluate the anti-ulcer property of *Isoberlinia doka* Craib & Stapf and *I. tomentosa* (Harms) Craib & Stapf.

Methods: Acute toxicity study was carried out as per OECD 425 guidelines. The ethanol (70%) extracts of stem bark of both plants were prepared by maceration and anti-ulcer effects of various doses (150, 250 and 500 mg/kg) of aqueous EtOH extracts, and ethyl acetate, butanol and aqueous fractions determined using ethanol-induced gastric ulcer (EIGU) and the pylorus ligation models.

Results: LD_{50} values of crude extract and fractions of both plants were found to be greater than 5000 mg/kg body weight. EIGU model produced significant ($p<0.05$) dose-dependent gastro-protective activity for ethyl acetate fraction of *I. doka* at 150, 250 and 500 mg/kg and ulcer inhibition data of 80%, 88% and 96% respectively. The corresponding ulcer inhibition for the less effective ethyl acetate fraction of *I. tomentosa* were: 60, 68 and 88% respectively. Omeprazole (30 mg/kg) showed 72% inhibition of ulcer score which was higher than all *I. tomentosa*-treated groups, except for 500 mg/kg of butanol (84%) and ethylacetate (88%) fractions. With pyrolic ligation model, significant ($p<0.05$) reduction in ulcer score with the crude extracts of *I. doka* (0.8±0.49) and *I. tomentosa* (1.7 ± 0.39) at 500 mg/kg, were recorded. Total acidities were higher in the control group.

Conclusion: There was a significant P (<0.05) reduction in ulcer with the ethyl acetate fraction at 250 mg/kg and 500 mg/kg of *I. doka* compared to *I. tomentosa*, indicating that *I. doka* is a better potential source of new anti-ulcer drugs.

Key words: *Isoberlinia doka, Isoberlinia tomentosa*, stem bark, peptic ulcer, acute toxicity, ethanol extract

INTRODUCTION

Isoberlinia is a genus with high economic and pharmacological values, the wood having been exploited as commercial timbers from time immemorial. *Isoberlinia doka* and *I. tomentosa* are naturalised in the woodland belt in Northern Nigeria and Guinea Savanna [1]. *Isoberlinia* species are characterised by pinnate and paripinnate compound leaves, 2–5 pairs of leaflets per leaf, opposite or sub-opposite in arrangement and petiolated with short stalk [2]. Qualitatively, flavonoids, tannins, terpenoid/steroid, saponin and alkaloids without anthraquinones have been reported in *I. doka* and *I. tomentosa* [3]. According to traditional medical practitioners, a preparation from the roots and leaves of *I. doka* and *I. tomentosa* is used to cure jaundice and headache in Nigeria [4-6]. *I. doka* is employed for the treatment of diabetes, ulcer, wounds and cough in Northern Nigeria and its roots in the treatment of hepatitis, against nausea [7]. The bark of *I. doka* is also a vermifuge and has healing, medico-religious uses (against curses) while the stem and leaves are used in combating convulsion in Nigeria [8].

Peptic ulcer disease (PUD) is a break in the lining of the gastrointestinal tract, including the stomach and/or the first part of the small intestine. It is mostly caused by bacterial infection with *Helicobacter pylori* or nonsteroidal anti-inflammatory drugs [9]. Peptic ulcer disease follows gastric mucosal injuries as a result of an imbalance between defensive and aggressive factors affecting the mucosa. Anti-ulcer drugs such as H_2 receptor blockers, proton pump inhibitors, and anti-muscarinic drugs produce adverse reactions such as hypersensitivity, arrhythmia, and hematopoietic changes with a possibility of increased rate of ulcer recurrence within one year after cessation of the treatment [9]. Peptic ulcer is one of the world's major gastrointestinal disorders, affecting 10% of the world population and affects about 4.6 million people annually [10, 11]. The mortality rate of peptic ulcer disease is approximately one death per 10,000 cases. The occurrence of peptic ulcer disease is similar in men and women and approximately 11-14% of men and 8-11% of women develop peptic ulcer disease in their lifetime. Recent reports have revealed that Africa has the highest rates of global prevalence (70.1%) of *Helicobacter pylori* infection worldwide. Nigeria has the highest global prevalence (87.7%) among Africa [11].

Peptic ulcers can be life-threatening, with symptoms such as bloody stools, sharp stomach pain, cramps, and vomiting. Owing to the persistent problem of ulcer recurrence after medication and attendant adverse reactions, a new approach is constantly being pursued. Some plant extracts with potential for the treatment of gastric ulcers have been documented [12, 13]. The modern medicines have their limitations, especially against ulcers with serious pathology indicating the need to substitute medication from alternative systems of medicine [14]. Many medicinal plants have enjoyed the confidence of different populations for the treatment of different types of ulcers. The aqueous extract of the leaves of *Azadirachta indica* contains some flavonoids and tannins that can potentially be used in the treatment of indomethacin-induced gastric ulcer [15]. The potential of *Asparagus* roots in the treatment of indomethacin-induced gastric ulcer has been reported [16], and different therapeutic agents, especially plant extracts, are currently being investigated in various studies to evaluate their anti-ulcerogenic potential. The use of *I. doka* and *I. tomentosa* in traditional medicine in Nigeria in the management of

stomach ulcer has not been scientifically validated. Therefore, *I. doka* and *I. tomentosa* stem bark aqueous ethanol extracts and fractions were investigated for anti-ulcer properties in male Wistar rats.

MATERIALS AND METHODS

Solutions, chemicals and reagents: Freshly prepared solutions and analytical grade chemicals used in all the experiments were sourced as follows: acetic acid, diethylether, n-butanol, sulphuric acid (JHD, AR; Lobal Chem, India); chloroform, sodium hydroxide, dimethylsulphoxide (Sigma-Aldrich, St. Lous, MO, USA); ethanol (Park Scientific Ltd, Northampton, UK); ethylacetate (Qualikems Fine Chem., Nandesari, India); Omeprazole (Actavis Labs, Apotex, Aurobindo Pharma, Breckenridge); phenolphthalein (Fine Chemicals, Mumbai, India) were used.

Collection, authentication and preparation of plants: Fresh *I. doka* and *I. tomentosa* were first identified in the field using their morphological features, and stem bark samples were collected from man-made forests in Shika Village, Giwa Local Government, Kaduna State in December, 2019. The plants were taxonomically authenticated with voucher specimen numbers ABU 022478 and ABU 016280 for *I. doka* and *I. tomentosa*, respectively at the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria, Nigeria. Sufficient quantity of each bark was obtained by making longitudinal and transverse incisions through the outer layer of the stems of the plants, followed by peeling. Bark samples were dusted, cleaned, and washed with water to remove any foreign matter. They were then sliced into pieces and air-dried in the shade until a constant weight was obtained. Each sample was pulverized into a coarse powder with a mortar and pestle and stored in cellophane bags at room temperature until required for the experiment.

Extraction and fractionation of plant materials: Air-dried powdered stem barks of *I. doka* and *I. tomentosa* (1.5 kg each) were separately macerated with 5 L of 70% w/v aqueous ethanol for three days and filtered. The filtrates were collected and concentrated using a Rotary Evaporator at 60^{o}C, and yields recorded. Fifty grams of the crude ethanol extracts of *I. doka* and *I. tomentosa* were suspended separately in distilled water (500 ml) and aqueous portion fractionated sequentially with ethyl acetate (5x 400 ml) and n-butanol (5 × 400 ml). The mixture was partitioned into different solvent layers; each solvent layer was evaporated to yield the respective dried fractions as earlier described by Cho *et al.* [17] and Bello *et al.* [18].

Experimental animals: Wistar rats (260) weighing 125 – 200 g were used for anti-ulcer studies, and 40 rats for toxicity studies. The rats were bred in the laboratory animal unit of the Faculty of Pharmaceutical Science A.B.U., Zaria. The experiment was conducted under similar conditions of temperature, relative humidity, and light and dark cycles. The animals were maintained with *ad libitum* access to food and water and kept in stainless steel wire mesh cages that separated them from their feces. Ethical rules guiding the use of animals for experimentation strictly adhered to the ethical approval number (ABUCAUC/2020/69) obtained from the ABU Committee on Animal Use and Care.

Oral acute toxicity study of ethanol extracts: Acute toxicity studies were performed according to the Organization for Economic Cooperation and Development

(OECD/OCDE) guidelines 425 [19]. A total of forty (40) Wistar rats were used, five female Wistar rats each for crude extract, and each fraction (ethyl acetate, butanol and aqueous) of *I. doka* and *I. tomentosa*, and a limit test dose of 5000 mg/kg was administered after fasting for 24 h but allowed free access to water. Animals were observed individually for behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response, and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and morbidity or mortality, after dosing continuously for 2 h, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 days.

Evaluation of the anti-ulcer activity using ethanol-induced ulcer (EIU) model: Ethanol-induced ulcers were evaluated in rats, as described previously by Choudhary *et al.* [20], with slight modifications. The rats were fasted for 24 h to induce a significant effect of the drug, the separated groups were orally administered distilled water (1 ml), standard drug as positive control (omeprazole, 30 mg/kg), ethanol extract, ethyl acetate, butanol, and aqueous fractions (150, 350, 500 mg/kg). After 30 min of administration, 1 ml of absolute ethanol was administered orally to all rats that were later sacrificed by cervical dislocation and dissected after one hour. Their stomachs were carefully removed, cut open through the greater curvature with scissors, rinsed, lightly stretched, and spread on filter paper for proper viewing and assessment of ulcers. Stomachs were examined macroscopically. The extent of mucosal damage was measured using a calibrated meter rule (in millimeters), and ulcer indices were measured from the left to the right of each tissue.

The ulcer score was calculated for each animal according to the scale by Kulkami [21], where 0 = no lesion, 0.5 = red coloration, 1.5 = hemorrhagic streak, 2 = ulcer ≥3≤5, and 3 = ulcer ≥5. Ulcer index (U.Idx) was calculated as the mean ulcer score (U.S). The average mucosal damage was determined and calculated. Effectiveness of all tested agents was calculated using the following formula of Bello *et al*. [18].

$$\text{Percentage Ulcer inhibition (\% U.I)} = \left[\frac{\text{U.Idx (negative control} - \text{tested agent)}}{\text{U.Idx (negative control)}}\right] \times 100$$

Tissues were then kept in air tight containers and preserved with formalin for reference and further study.

Evaluation of the anti-ulcer activity using the pylorus ligation-induced ulcer model: Pylorus ligation was performed in all groups of rats to induce gastric ulcers, as previously described by Choudhary *et al* [20]. Male rats (130) were weighed, marked, and randomly sorted into 14 groups, each consisting of 5 rats. The groups were treated as follows: Group 1 (control): received distilled water (1 ml per body weight) orally, Group 2: Omeprazole (30 mg/kg), Groups 3 to 14 were administered various fractions of crude ethanol extract, ethyl acetate, butanol, and aqueous fractions from *I. doka* and *I. tomentosa* at doses of 150, 350, and 500 mg/kg.

Male rats were deprived of food for 18 h. before pyloric ligation but had free access to water. The experimental rats were anesthetized with ether, the abdomen of each rat was opened using a small incision below the xiphoid process. The pyloric position of

the stomach was slightly lifted and ligated to avoid traction into the pylorus or damage to the blood supply. Rats were sacrificed after three hours of treatment, their stomachs were removed, and the contents were drained into tubes and centrifuged at 1000 rpm for 10 min. The supernatants were analyzed for gastric volume, pH, and total acidity. The stomachs were cut along the greater curvature, the inner surface was examined for ulceration, and the ulcer index (U.Idx) calculated as described in the EIU model. An aliquot of 1 ml of gastric juice was diluted with 1 ml distilled water, and pH measured using pH meter

Determination of total acidity: An aliquot of 1 ml of gastric juice was diluted with 1 ml distilled water and transferred into 50 ml conical flask. Phenolphthalein indicator (2 drops) was added and titrated with 0.01N NaOH until a permanent pink color was observed. The volume of 0.01N NaOH used was noted. Total acidity was expressed as mEq/L and calculated by the following formula [20]

Acidity = [VNaOH × N × 100 mEq]/ L
Where V is volume, L: litre and N is normality

Statistical analysis: The results were given as mean ±standard error mean (SEM). One-way Analysis of variance (ANOVA) was used to test for significant differences between the means of the treated and control A difference was considered statistically significant when $p < 0.05$. Means were separated by Duncan multiple range test.

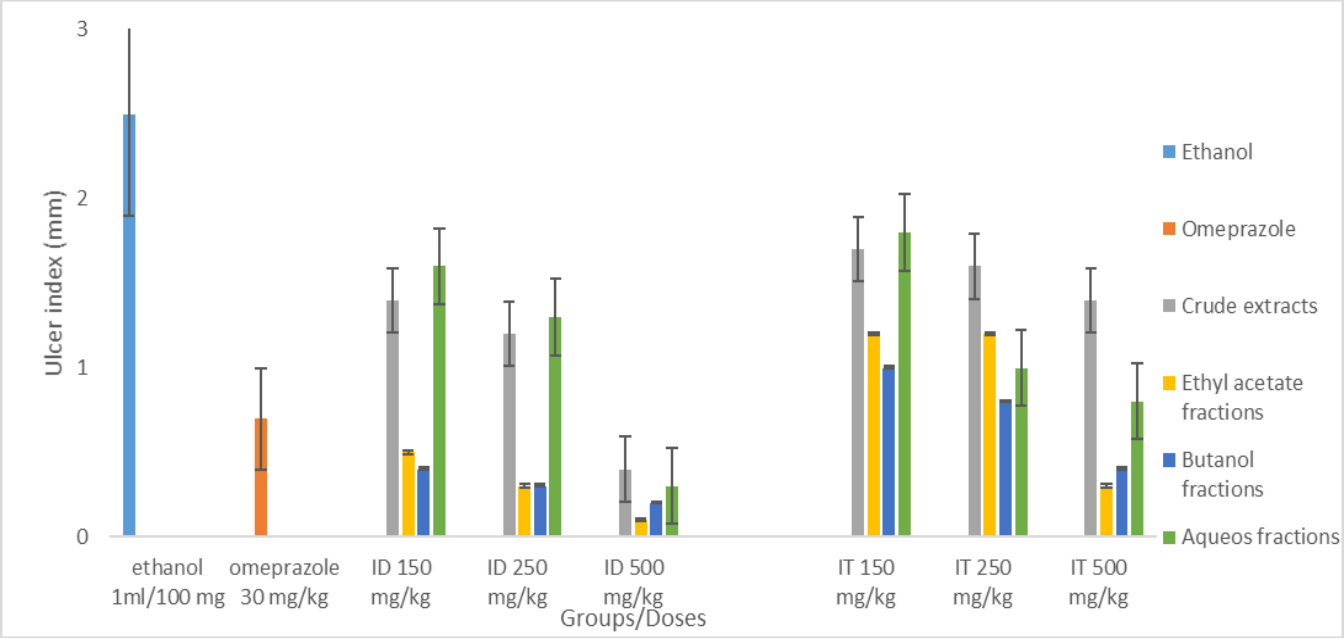
RESULTS

All the experimental rats did not show any sign of toxicity for the period of 14 days in both *I. doka* and *I. tomentosa* treated-crude extracts and fractions. LD_{50} > 5000 mg/kg suggesting safety during oral administration. The results of the ulcer studies showed a decrease in the standard drug omeprazole (U.Idx, 0.7±0.37 mm^2). Additionally, there was dose-dependent reductions in ulcer effect with the ethanol crude extracts of *I. doka* (U.Idx, 1.4-0.2 mm^2) and *I. tomentosa* (U.Idx, 1.7-1.4 mm^2). Notably, the ethyl acetate fraction of *I. doka* (U.Idx, 0.5-0.1 mm^2) was the most effective (Figure 1).

From Figure 2 data on ulcer inhibition, the negative control (0%) was inactive as expected. As regards *I. doka*, dose-dependent ($p<0.05$) increases in gastro protective activity at 150, 250 and 500 mg/kg expressed by ulcer inhibition (U.I) were observed as: butanol fraction (36%, 88% and 92%, respectively); ethylacetate fraction (84%, 88% and 96%); crude ethanol extract (52%, 80% and 84%), and Omeprazole (72%).

However, in the case of *I. tomentosa* tested at lower doses of 150 and 250 mg/kg, the positive control, Omeprazole (30 mg/kg) was more effective (UI, 72%) butanol fraction (52% each), ethylacetate fraction (60% and 68%, respectively). Highest dose of 500 mg/kg of butanol (U.I, 84%) and ethylacetate (U.I, 88%) fractions were more effective than standard drug. Comparatively, *I. doka* was more effective than *I. tomentosa* as an anti-ulcer agent.

The pyrolic ligation model showed a significant ulcer effect in the treated groups of *I. doka* and *I. tomentosa*. The U.Idx (12.3±2.4 mm^2) and ulcer score (U.S, 3.0±0.78 mm^2) for the negative control group were the highest among the groups. Omeprazole (U.Idx, 1.8±0.13 mm^2; U.S (2.0±0.49 mm^2).



* ID= *I. doka*, IT= *I. tomentosa.* Different letters indicate statistically significant differences between groups (mean ± SEM, N = 5, one-way analysis of variance (ANOVA) followed by Duncan multiple range test, $p<0.05$)

Figure 1: Ulcer index of the stem bark extracts of *I. doka* and *I. tomentosa* on male rats in ethanol-induced model

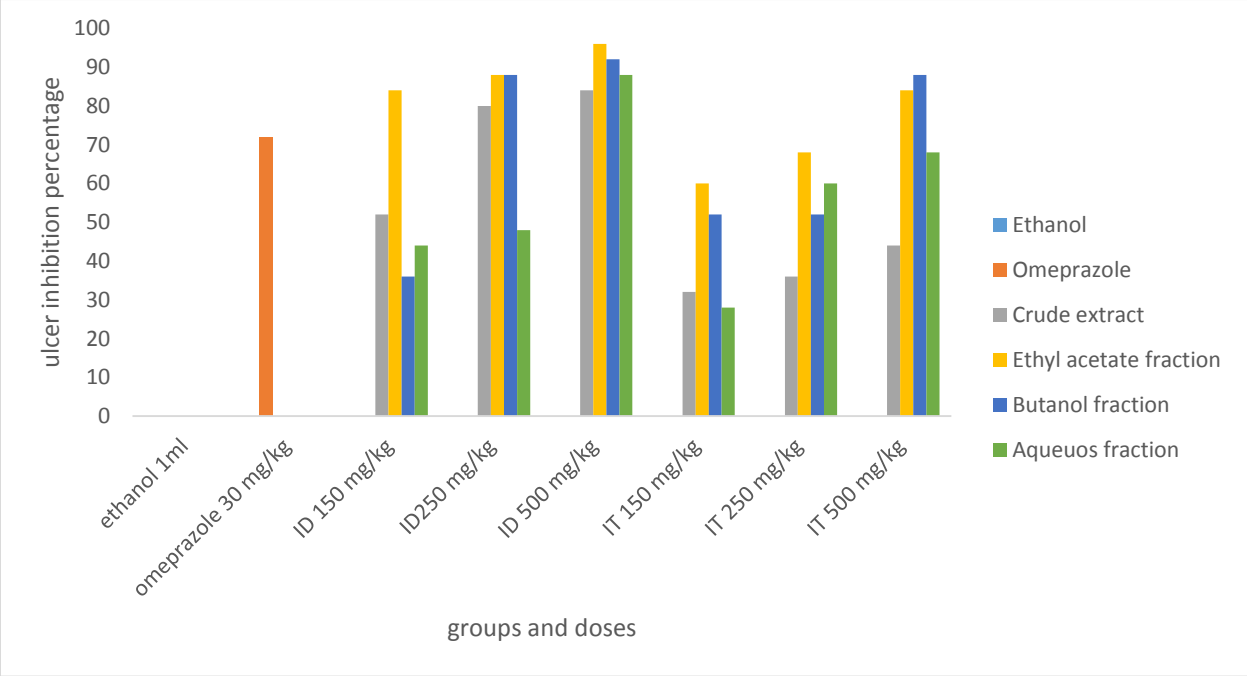


Figure 2: Anti-ulcer effect of stem bark ethanol extract and fractions of *I. doka* and *I. tomentosa* on male rats in ethanol-ulcer model (* ID= *I. doka*, IT= *I. tomentosa*)

The crude extract of *I. doka* at 150, 250 and 500 mg/kg gave the data: (U.Idx, 2±0.63 mm^2, 2.6±1.78 mm^2 and 0.8±0.49 mm^2, respectively; ethyl acetate fraction (U.Idx, 6.6-4.2 mm^2); butanol fraction (U.Idx, 7.6-4.0 mm^2) and aqueous fraction (U.Idx, 7.6-5.2 mm^2). There were gastric volume reductions in all the dose levels of *I. doka* crude (1.4-1.2 mm^2); ethyl acetate (1.6-2.8 mm^2); butanol fractions (1.8-2.4 mm^2) and aqueous fractions (3.0-1.8 mm^2) when compared to the control group. The pH of omeprazole (5.3); and for *I. doka* ethyl acetate (6.6-4.2); butanol (5.76 -6.44) and aqueous fraction (6.9 -6.8).

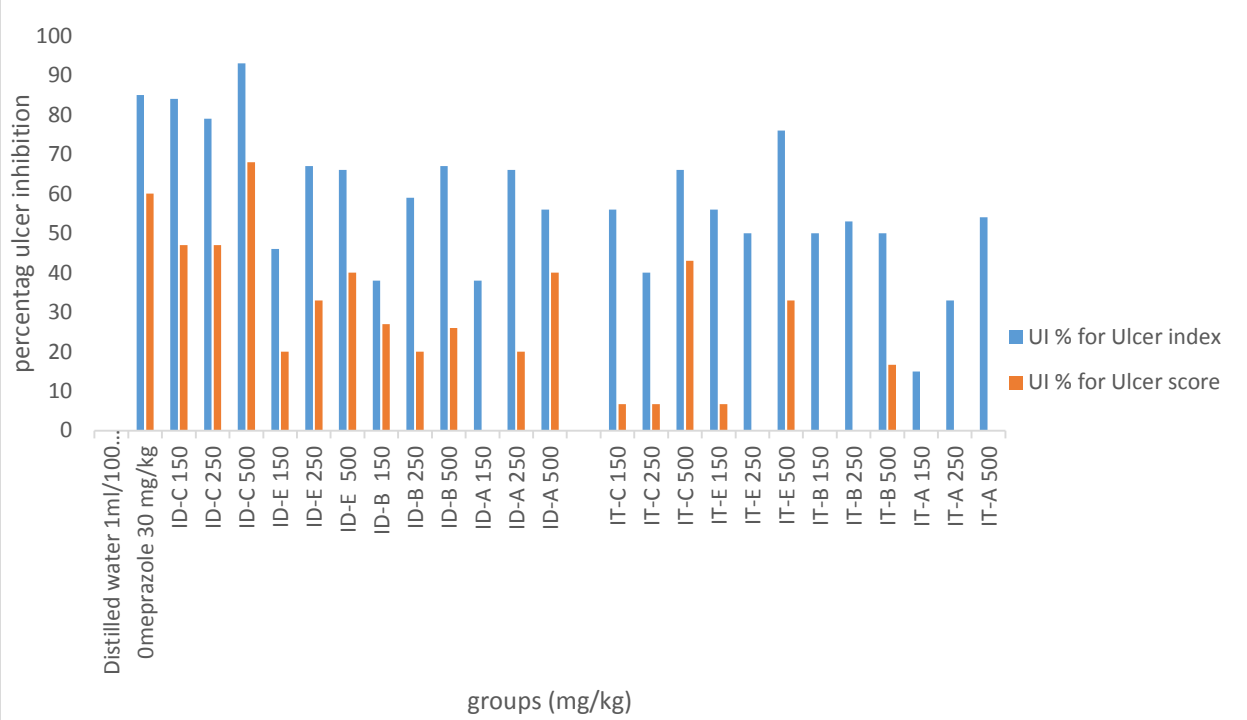
Table 1. Pyloric ligation model parameters of *Isoberlinia doka* and *Isoberlinia tomentosa* on male Wistar rats

Group/ doses (mg/kg)	**Ulcer index (mm^2)**	**Ulcer score**	**Gastric volume (cm^3)**	**pH**
Distilled H_2O (1 ml/bw)	12.3±2.4^a	3.0±0.78^a	3.1±0.78^a	4.0±0.13^b
Omeprazole - 30	1.8±1.3^c	1.2±0.13^b	1.4±0.19^b	5.3±0.29^{ab}
I. doka				
ID-C 150	2.0±0.63^c	1.6±0.40^a	1.4±0.29^b	6.0±0.11^a
ID-C 250	2.6±1.17^c	1.0±0.63^b	1.2±0.20^b	6.0±0.29^a
ID-C 500	0.8±0.49^c	0.8±0.49^b	1.6±0.23^b	6.1±0.27^a
ID-E 150	6.6±0.20^b	2.4±0.60^a	1.6±0.29^b	6.1±0.10^a
ID-E 250	4.0±1.7^c	2.0±0.55^a	2.5±0.28^a	6.1±0.10^a
ID-E 500	4.2±0.8^c	1.8±0.23^a	2.8±0.24^a	6.0±0.21^a
ID-B 150	7.6±2.7^b	2.2±0.23^a	2.8±0.30^a	5.8±0.07^{ab}
ID-B 250	5.0±1.58^b	2.4±0.6^a	1.7±0.21^b	6.3±0.17^a
ID-B 500	4.0±1.3^c	2.2±0.23^a	1.8±0.19^b	6.4±0.08^a
ID-A 150	7.6±1.36^b	3.0±0.00^a	2.9±0.37^a	6.9±0.03^a
ID-A 250	4.2±1.5^c	2.4±0.6^a	2.8±0.34^a	6.8±0.03^a
ID-A 500	5.2±2.3^b	1.8±0.73^a	3.0±0.06^a	6.8±0.23^a
I. tomentosa				
IT-C 150	5.2±0.49^b	2.8±0.02^a	1.4±0.17^b	6.2±0.18^a
IT-C 250	7.4±1.33^b	2.8±0.02^a	1.3±0.03^b	6.7±0.02^a
IT-C 500	4.2±0.23^c	1.7±0.40^a	1.5±0.23^b	7.2±0.02^a
IT-E 150	5.4±0.12^b	2.8±0.20^a	1.6±0.22^b	5.6±0.12^{ab}
IT-E 250	6.2±0.23^b	3.0±0.00^a	2.1±0.22^a	6.3±0.03^a
IT-E 500	3.0±1.36^c	2.0±0.23^a	3.0±0.00^a	6.4±0.06^a
IT-B 150	6.2±0.70^a	3.0±0.00^a	2.0±0.29^a	5.8±0.21^{ab}
IT-B 250	5.8±0.20^b	3.0±0.00^a	2.1±0.20^a	6.2±0.20^a
IT-B 500	6.2±0.20^b	2.5±0.25^a	2.0±0.23^a	6.2±0.24^a
IT-A 150	10.2±1.5^a	3.0±0.23^a	3.0±0.17^a	6.6±0.06
IT-A 250	8.2±0.20^a	3.0±0.00^a	2.9±0.14^a	6.6±0.06
IT-A 500	5.7±0.92^b	3.0±0.00^a	3.6±0.07^a	6.8±0.04^a

Mean ± SEM; N = 5, ID-C: *I. doka* crude extract; ID-E: *I. doka* ethyl acetate fraction; ID-B: *I. doka* butanol fraction; ID-A: *I. doka* aqueous fraction; IT-C: *I. tomentosa* crude extract; IT-E: *I. tomentosa* ethyl acetate fraction; IT-A: *I. tomentosa* aqueous fraction. Different letters indicate statistically significant differences ($p<0.05$) between groups.

There was gastric volume reduction at all doses omeprazole (1.4± 0.19 mm^3); *I. tomentosa* ethyl acetate (1.6-3.0 mm^3) and *I. doka* butanol (2.0-2.1 mm^3) when compared to the negative control (3.05 ± 0.78 mm^3). The pH values for *I. tomentosa* were: crude extract (6.2-7.2); ethyl acetate fraction (5.56-6.4); butanol fraction (5.8 - 6.2) and aqueous fraction (6.6 - 6.8) (Table 1).

The inhibition percentage for Omeprazole (U.Idx 85%: U.S 60%); *I. doka* crude extract (U.Idx 84-93%; U.S, 47-68%); *I. tomentosa* crude extract (U.Idx, 56- 66%; U.S 6.7-46%); *I. doka* aqueous fraction (U.Idx, 38-56%; U.S 0-40%); *I. tomentosa* aqueous fraction (U.Idx, 15-54%; U.S 0-0%).



ID-C: *I. doka* crude extract; ID-E: *I. doka* ethyl acetate fraction; ID-B: *I. doka* butanol fraction; ID-A: *I. doka* aqueous fraction; IT-C: *I. tomentosa* crude extract; IT-E: *I. tomentosa* ethyl acetate fraction; IT-A: *I. tomentosa* aqueous fraction

Figure 3: Pyloric ligation model; ulcer inhibition percentage of *I. doka* and *I. tomentosa* on male Wistar rats.

I. tomentosa ethyl acetate fraction (U.Idx, 56-76%; U.S 6.7- 46%); *I. doka* ethyl acetate fraction (U.Idx, 46- 66%; U.S 20- 40%); *I. tomentosa* butanol fraction (U.Idx, 50- 50%; U.S 0- 16.7%); *I. doka* butanol fraction (U.Idx, 38- 67%; U.S 27- 27%). (Figure 3)

Total acidity for negative control (110 meq/l); Omeprazole (60 meq/l); *I. doka* crude extract (14-98 meq/l; ethyl acetate (10-82 meq/l) butanol (12-90 meq/l) and aqueous (10-52 meq/l). While the total acidity for *I. tomentosa* crude extract (44-19 meq/l); ethyl acetate fraction (14-8 meq/l); butanol (12- 24 meq/l) and aqueous fraction (3-22 meq/l) (Figure 4).

DISCUSSION

The results of the toxicity study in ethanol crude extract and fractions of *I. doka* and *I. tomentosa* stem bark revealed that both plant extracts were safe in the tested rats at a limit

dose of 5000 mg/kg since there were no clinical signs of toxicity observed on the tested rats.

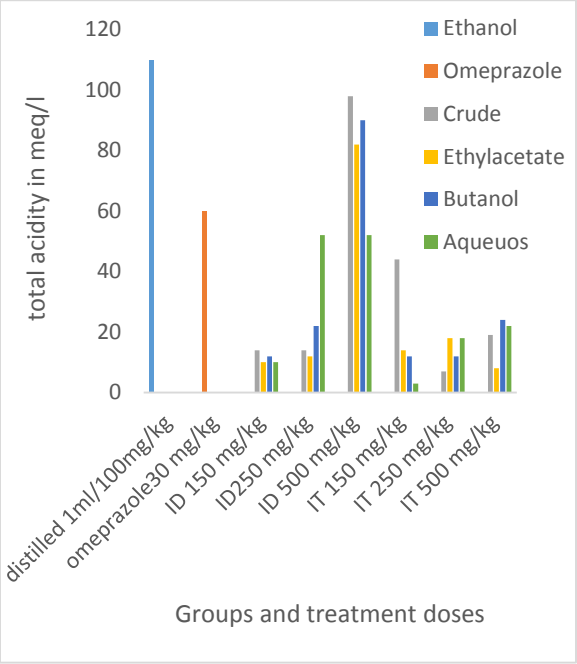


Figure 4: Pyloric ligation ulcer model; total acidity of *I. doka* and *I. tomentosa* on male Wistar rats

These results are in contrast with the findings of Abdu *et al.* [6] who reported that the Brine shrimp LC_{50} values for the stem bark extracts of *I. doka* is cytotoxic (LC_{50} < 1000 µg/ml).

The crude extracts, fractions and standard drug-treated groups showed ~~a~~ significant reduction ($P<0.05$) in both the ulcer score and ulcer index. This was in support of the findings of Bello *et al.*, [18] and Paguigan *et al.*, [22]. The percentage ulcer inhibition of extract and fractions increase~~s~~d dose-dependently as shown in Figure 1. The findings of the present study demonstrated that stem bark ethanolic extracts of *I. doka* and *I. tomentosa* significantly and dose-dependently possess a cytoprotective action against mucosal damage, a form of gastric irritation resulting from the inhibition of prostaglandins synthesis and free radical productions by ethanol via anti-oxidant actions. This study corroborated the effectiveness of ethanol in inducing ulcers by lowering protective factors in the gastric mucosa [18]. The mechanisms involved in the reduction of ulcers induced ethanol has been linked to regeneration of the glandular epithelium, formation of collagen, increased capillary density, increased pH, and increased free-radical scavenging action [23]. Remarkably, extract and fractions of *I. doka* at 500 mg/kg produced greater protection than omeprazole (30 mg/kg) in the ethanol-induced ulcer models. These findings possibly indicate that *I. doka* extracts produced a significant anti-ulcer activity when compared to omeprazole 30 mg/kg (positive control) via anti-oxidant properties as proposed by earlier reports of Abdulkadir *et al.* [7] and Ahmed *et al.* [24].

The pyloric ligation ulcer model as presented in Figure 2 showed ~~a~~ decreases in gastric volume as the dose of extract~~s~~ increased. But the pH ranged from 5.7 -6.9 which showed a dose-dependent increase in all the treated groups. This is consistent with the findings of Bhajoni *et al*, [25], and may be attributed to increase in the secretions of the acids around the internal organs following ligation of pyloric end of the abdomen [26]. The negative control group (U.Idx, 12.3 mm^2) showed higher inductions of gastric ulcers due to increased levels of gastric juice (3.1 cm^3) in the rat's stomachs. The values of the pH for the gastric contents were lower than other treated groups with omeprazole (5.7) showed a significant ($p < 0.05$) produced an anti-secretory effect via inhibition of gastric secretion and pepsin activity when compared to the ulcers produced in the control group. *Isoberlinia doka* and *I. tomentosa* crude extract and fractions showed significant ($p < 0.05$) decreases in the Ulcers index compared to the control group, by reduction in gastric acid secretion, proving its anti-secretory

effect. This study is in support of the findings of Manchala *et al.* [27].

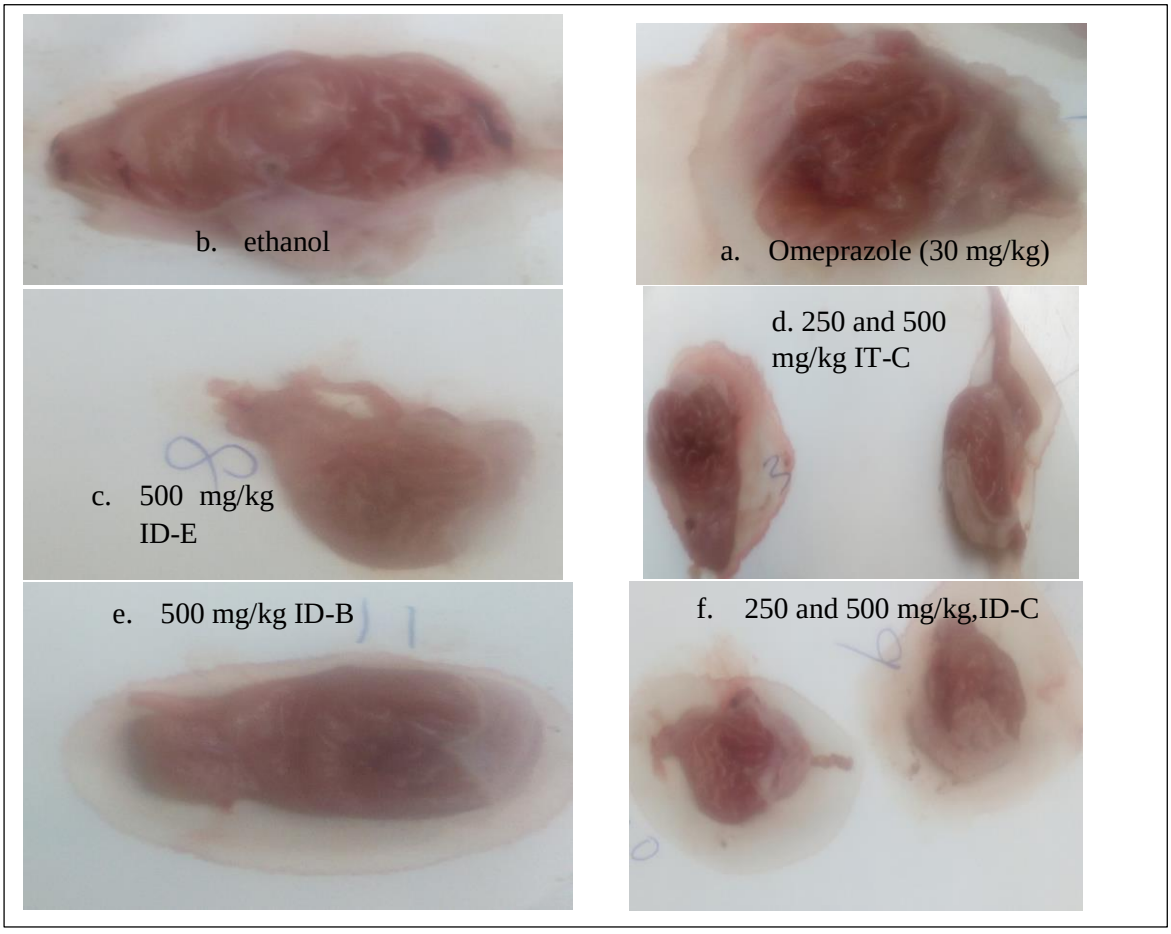


Plate I: Ethanol-induced ulcer of the stem bark extract of *I. doka* and *I. tomentosa* on male rats. ID-E: *I. doka* ethyl acetate fraction; IT-C: *I. tomentosa* crude extract; ID-B: *I. doka* butanol fraction; ID-C: *I. doka* crude extract.

The pyloric-induced ulcer model experiments involving *I. tomentosa* crude extract (500 mg/kg) were characterised by heavy bleeding [28, 29], implying immediate stasis in blood flow,

Figure 3 showed that the inhibition percentage of standard drugs (U.Idx, 85%) was found to be higher than all the treated groups of *I. doka* and *I. tomentosa* except for *I. doka* crude extracts (U.Idx 93%) at 500 mg/kg. This figure also showed a dose-dependent increase in U.I percentage in *I. doka* crude extracts, *I. doka* butanol fractions and *I. tomentosa* aqueous fractions which was supported by the findings of Sahoo *et al.*, [30], the rest of the treated groups did not show a dose-dependent U.I percentage which was not in conformity with Sahoo *et al.* [31]. There were variations in the values of U.I. percentage of ulcer score and index. All the values obtained for U.I percentage of ulcer score were lower than that of ulcer index with 0% in control, *I. doka* aqueous fractions at 150, *I. tomentosa* ethyl acetate at 250, *I. tomentosa* butanol fractions at 150, *I. tomentosa* aqueous at 150, 250 and 500 mg/kg but significant higher values were obtained for their respective UI% of ulcer

indices, this implies that UI percentage of ulcer score does not show the extend of mucosal damage and a true representative of data. Hence, once the ulcer index can be measured for all groups, their percentage inhibition/preventive index should be calculated as well as using the scoring method.

High phenolic compounds in *I. doka* and *I. tomentosa* as reported by Hadiza *et al.* [5] and Abdulkadir *et al.* [7] are known to have anti-oxidant activities thus enhancing the protective factors capable of promoting gastric mucosal formation; reduce gastric acid secretion, inhibit pepsinogen production and restore gastric blood [31]. Tannins are used in medicine primarily because of their astringent properties, which are attributable to their reaction with proteins in the layers of tissue on contact. Tannins are known to "tan" the outermost layer of the mucosa and render it less permeable and more resistant to chemical and mechanical injury or irritation [32]. Saponins, especially triterpenes type have been implicated in antiulcer activity mediated by the formation of protective mucous on the gastric mucosa [27]. The presence of polyphenolic compounds like flavonoids, saponins and tannins has been reported by several studies to have an anti-ulcer effect via anti-oxidant properties that inhibit gastric mucosal damage [27].

CONCLUSION

The acute toxicity study revealed that *I. doka* and *I. tomentosa* stem bark are safe for therapeutic purposes. The results of this study being reported for the first time, indicated *Isoberlinia* species may possess anti-ulcer activity, and the ethyl acetate fractions of *I. doka* showed better anti-ulcer potential in ethanol and pyloric ligation ulcer models than *I. tomentosa*.

Conflict of interest

The authors declare no conflicting interest

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