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Evaluation of Anti-Ulcer Properties of the Stem-Bark Fractions of *Khaya senegalensis* (Desr.) A. Jussin Albino Rats

Mohammed M. Suleiman[1,2], Ramatu Umar[1,3], Hudu G. Mika'il[1,4], Abdullah M. Tauheed[1]*, Mohammed Mamman[1]

[1]*Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.*
[2]*College of Agriculture and Animal Science, Mando, Ahmadu Bello University, Kaduna State, Nigeria.*
[3]*Federal Capital Territory Administration, Agriculture and Rural Development Secretariat, Kapital Road, Area 11, Gariki, Abuja.*
[4]*Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria.*

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ABSTRACT

Ulcers are lesions in the mucosa of gastrointestinal tract, characterized by different stages of necrosis. Ulcer management has been challenging because of increasing antimicrobial resistance and indiscriminate use of complex antithrombotic therapy. Anti-ulcer activities of the fractions of the crude methanol extract of *Khaya senegalensis* was evaluated against ethanol-induced gastric ulcer. The powdered bark of *K. senegalensis* was extracted using absolute methanol and the crude extract was serially partitioned with n-hexane and ethyl acetate. The aqueous methanol (AMF), hexane (HEF) and ethyl acetate (EAF) fractions of *K. senegalensis* were tested for acute toxicity and further evaluated for gastric anti-secretory, gastroprotective and antioxidant effects in rat. The EAF and AMF significantly ($P < 0.05$) decreased the concentration of gastric HCl at 400 and 800 mg/kg. All the fractions significantly lowered ($P < 0.05$) gastric ulcer indices when compared with the normal saline control group (NS). Furthermore, the fractions significantly ($P < 0.05$) increased the concentration of gastric mucus compared to NS. The AMF and EAF fractions significantly ($P < 0.05$) reduced the concentration of malondialdehyde in the gastric tissue relative to NSgroup. The three fractions (AMF, EAF and HEF) significantly ($P < 0.05$) increased the level of superoxide dismutase activity when compared with NS. However, the activity of catalase was significantly ($P < 0.05$) decreased in the groups treated with AMF and EAF when compared with the NS. Findings in this study strongly suggest that *Khaya senegalensis* has anti-ulcerative properties, which could be due to its antisecretory and antioxidant activities.

Keywords: Antioxidant activities, anti-ulcer, Gastric mucus, *Khaya senegalensis*, plant extract

Introduction

Peptic ulcer is defined as a break in the continuity of the mucosa of stomach or few centimeters of duodenum (duodenal ulcer).[1]It is a gastro-intestinal disorder due to an imbalance between the stomach aggressive acid, pepsin, non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori* infection, and defensive factors like bicarbonate ion, prostaglandins, gastric mucus, and innate resistance of the mucosal cell factors.[2] Peptic ulcer usually develops when there is a shift in balance in favour of the aggressive factors.[3] The major factors that disrupt the equilibrium of the integrity of the stomach mucosa are *H. pylori*, acid–pepsin hyper secretion, NSAIDs, and sometimes idiopathic, due to usage of tobacco, psychological stress, rapid gastric emptying and Zollinger-Ellison syndrome (ZES).[4] The pathogenesis of ulcer is not clearly defined because of complexity of confounding and predisposing factors. It is however, well established that chronic use of NSAIDs predisposes to the gastric ulcer.[5]

*Corresponding author. E mail: mtauheed@abu.edu.ng; amtauheed40@gmail.com
Tel: +2348033190292

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Hydrochloric acid is primarily responsible for the ulcers in the distal oesophagus and nonglandular region of the stomach; while breakdown in the mucosal defence mechanisms is responsible for the ulcers in the glandular stomach and proximal duodenum.[6] In ZES, a gastrin secreting tumor of the pancreas stimulates the parietal cells in stomach to increase the acidity, resulting in gastrointestinal mucosal ulceration. This ulcer is refractory to proton pump inhibitor therapy.[7] Tobacco smoking aggravates gastric ulcer by inhibiting bicarbonate production from the pancreas, resulting in increased acidity in the duodenum and delayed healing.[8] The prevalence of *H. pylori* in gastric ulcer has decreased in developed countries due to improved hygiene and reduced transmission in early childhood.[9]

Triple therapy comprising proton pump inhibitors (e.g, omeprazole), clarithromycin and amoxicillin or metronidazole twice daily has been recommended as the gold standard first choice of therapy for the eradication of *H. pylori*.[10] This has largely replaced the earlier triple therapy of tetracycline (2 g), metronidazole (750 mg) and bismuth subsalicylate (5-8 tablets).[11] Bismuth-containing quadruple therapy has also been recommended first line therapy for the eradication of *H. pylori*.[10]

Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for the treatment of ulcer, possibly due to lower costs and lesser side effects. Many tropical herbs have been scientifically reported to possess potent anti-ulcer activity.[12,13] Various chemical compounds have been isolated from medicinal plants with anti-ulcer activity.[14, 15]*K. senegalensis* is a medicinal plant with many medicinal applications. Stem-bark extract of *K. senegalensis* has been reported to possess antihyperglycemic,[16] antiprotozoal,[17] antimicrobial,[18] anthelmintic[19] and anticancer effects.[20] Furthermore,

preliminary evaluation of crude methanol stem-bark extract of the plant revealed potent antiulcer properties.[21]

Although much progress has been made in the management of *H. pylori* infection in gastric ulcer due to the widespread use of antisecretory drugs and antibiotics, increasing antimicrobial resistance has posed a great threat to its eradication.[22] Furthermore, emerging causes of peptic ulcers other than *H. pylori* and NSAIDs are imposing diagnostic and therapeutic challenges. Therefore, the aim of this study was to investigate the anti-ulcer effects of the stem-bark fractions of *K. senegalensis* in rats.

Materials and Methods

Plant material

The stem-bark of *K. senegalensis* was collected in August 2016 from the main campus of Ahmadu Bello University, Zaria, Nigeria. The leaves and seeds samples of the plant were sent to the Herbarium, Department of Biological Sciences, A.B.U, Zaria, for identification. The plant was identified by Mal Namadi Sunusi. A voucher specimen number 90081 was deposited at the Herbarium for reference purpose. The stem-bark was dried in the laboratory at room temperature, and the dried plant part was made into powdered. One kilogramme of the powdered stem-bark was extracted exhaustively with methanol (3 litres) in a soxhlet apparatus and concentrated *in vacuo* at 40ºC. The crude methanol extract (CME) was dissolved in distilled water and serially partitioned with *n*-hexane and ethyl acetate. The solvents (JHD®) were obtained from Gunsgdong Guandgua Chemical Factory Co. Ltd., China

Phytochemical Test

Phytochemical screening of the fractions of *K. senegalensis* stem-bark was carried out using standard method.[23]

Experimental Animals

Rats of both sexes weighing between 150 g and 180 g were obtained from Animal House, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were acclimatized to the laboratory condition in plastic cages for two weeks before the commencement of the experiment. All animals were maintained on standard rat diet and water was provided *ad-libitum.* All animal experimentation procedures were conducted according to the Ahmadu Bello University Animal Use and Care Guidelines (P13VTPP8004) which conformto International Guidelines for Animal Experimentation.

Acute toxicity studies

The method of Lorke[24] was used to determine the median lethal dose (LD_{50}) of the extracts in the rats. In the first part of the trial, nine albino rats were randomly allocated into three groups of three rats each. Rats in groups 1, 2 and 3 were treated with the extract orally at 10, 100 and 1000 mg/kg body weight, respectively. All treated animals were observed for 48 hours for any sign of toxicity or mortality. In the second part of the experiment, three rats were assigned into three groups of 1 rat each. Rats in groups 1, 2 and 3 were given the extract at 1600, 2900 and 5000 mg/kg, respectively. All animals were observed as in the first trial.

Experimental anti-secretory studies

Twenty five rats were randomly allocated into five groups of five rats each. The animals were fasted for food 24 hours prior to surgery. All the rats were anaesthetized using ketamine HCl at 20 mg/kg given by the intramuscular route. A ventral midline incision was made on each rat and the stomach exteriorized and ligated at the pyloric region. Groups 1, 2 and 3 were treated with the aqueous fraction at 200, 400, and 800 mg/kg respectively, while groups 4 and 5 were given cimetidine at 20 mg/kg and normal saline at 5 mL/kg, respectively. The treatments were given intra-gastrically. After treatment, the stomach in each rat was returned into the abdominal cavity and the incisions sutured. Animals were allowed to recover from anaesthesia. Four hours after recovery from anaesthesia, all animals were euthanized in a chloroform chamber. The sutured area of individual animals was opened again, the stomach removed and its contents were emptied into sample container and measured. Thereafter, the content of each stomach was centrifuged at 3000 g for 5 minutes and the supernatant was decanted[17]. Similarly, the n-hexane and ethyl acetate fractions of *K. senegalensis* obtained during partitioning were evaluated for anti-secretory effect in the same fashion. The supernatant collected was titrated with 0.01 M sodium hydroxide (NaOH) to end point using 2 % phenolphthalein as an indicator. Total acidity was calculated and expressed as mEq/ml in each sample.[25]

Gastroprotective activity

Twenty five rats were randomly allocated into 5 groups of 5 rats each and were fasted for 24 hours for food but not water. Groups 1, 2 and 3 were treated with the aqueous fraction at 200, 400 and 800 mg/kg, respectively, while groups 4 and 5 were given misoprostol at 5 µg/kg and normal saline at 5 ml/kg, respectively. All treatments were administered orally. Thirty minutes after treatment with the extract, misoprostol and normal saline, ulcer was induced using 1ml of 80 % ethanol. All animals were sacrificed after three hours in chloroform chamber. The stomachs were removed and opened along the lesser curvature, rinsed, laid out on a flat surface, and examined for the presence of mucosal lesions. A × 2 hand lens was used to locate and score the lesions.[26] Severity of the gastric mucosal damage was graded as follows: grade 0, no lesion; grade 1, haemorrhagic erosions (less than five); grade 2, haemorrhagic erosions (more than five); grade 3, many small linear ulcers (shorter than 2 mm) or single linear ulcer of marked size (larger than 2 mm); grade 4, multiple linear ulcer of marked size. The ulcer index for each group was calculated by multiplying the number of rats in each grade by the number of grade divided by the number of rats in each group.[27] Similarly, the n-hexane and the ethyl acetate fractions of *K. senegalensis* obtained during partitioning were evaluated for gastroprotective activity in the same fashion, while still maintaining the control groups.

Gastric mucus determination

Gastric mucus determination was done by the method described by Corne.[28] Five hundred milligrams of the glandular portion of the stomach of each rat in the gastroprotective study was transferred immediately into 1% Alcian blue solution (in 0.1 M sucrose solution, buffered with 0.05 M sodium acetate, pH 5.8); the excess dye was removed by rinsing in sucrose solution. The dye-gastric mucus complex was extracted with 0.5 M magnesium chloride and 4 ml sample of the blue extract was shaken with an equal volume of diethyl ether and the resulting emulsion was centrifuged at 3000 *g* for 10 minutes. The supernatant was decanted and the absorbance was recorded at 560 nm. The quantity of Alcian blue extracted/g of the glandular tissue was calculated.

Gastric malondialdehyde determination

Determination of malondialdehyde (MDA) concentration as an index of lipid peroxidation was done using the double method of Draper and Hadley[29] modified by Yavuz.[30] The principle of the method is based on the spectrophotometric measurement of the colour developed during reaction of thiobarbituric acid (TBA) with MDA. Five hundred milligrams of the glandular portion of stomach from each animal was weighed and homogenized in ice-cold phosphate buffer to obtain a 10% homogenate. Trichloroacetic acid solution (2.5 mL of 100 g/L) was added to 0.5 ml stomach homogenate in a centrifuge tube, placed in boiling water bath for 15 minutes and cooled under tap water for another 5 minutes. The mixture was centrifuge at 1000 *g* for 10 minutes and 2 ml of the supernatant was added to 1 mL of 6.7 g/L of TBA solution in a test tube. The mixture was boiled in a water bath (100ºC) for 15 minutes, the solution was cooled under tap water and the absorbance was measured at 532 nm using UV spectrophotometer (T80t UV-VIS Spectrophotometer, Leicestershire, United Kingdom).

Catalase Activity (CAT)

Catalase activity was measured using the method of Abebi.[31] Exactly 10 µl of serum was added to a test tube containing 2.8 mL of 50 mM

potassium phosphate buffer (pH 7.0). The reaction initiated by adding 0.1ml of freshly prepared 30 mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of 0.041 $mM^{-1}cm^{-1}$ was used to calculate the catalase activity.

Catalase Concentration = Absorbance/E
Catalase Activity = Catalase concentration/protein concentration (mg/mL)

Superoxide Dismutase (SOD)
An indirect method of inhibiting auto- oxidation of epinephrine to its adrenochrome was used to assay SOD activity in the blood plasma.[32] The auto-oxidation was monitored in a UV spectrophotometer at 480 nm every 30 seconds for 5 minutes. A graph of absorbance against time was plotted for each absorbance, and the initial rate of auto-oxidation calculated. One unit of SOD activity was defined as the concentration of the enzyme (mg protein/ml) in the plasma that caused 50% reduction in the auto-oxidation of epinephrine.[33]

Histopathology
Tissue samples obtained from the stomach of rats after the gastroprotective testing were preserved in 10% formalin and processed for histological examination as described by Luna.[34]

Statistical analysis
Data obtained was expressed as mean ± standard error of mean (S.E.M.) and was subjected to one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using Graphpad prism version 5.0. Values of $P < 0.05$ were considered significant.

Results and Discussion

Gastric ulcer is a defect in the mucosa of the stomach that penetrates the muscularis mucosa. The ulcer of the stomach and the proximal duodenum are also called peptic ulcers, because these ulcers are bathed by acid and pepsin.[35]Gastroduodenal ulcers can occur independently or as a complication of many systemic diseases or following administration of various drugs to treat many diseases. Thus, it is important to understand its aetiological factors, pathophysiology for their effective treatment and early prophylaxis.[36]
Phytochemical analysis revealed the presence of carbohydrate, cardiac glycosides, saponins, flavonoids, steroids/triterpenes, alkaloids and tannins in the crude methanol extract (CME) and aqueous methanol fraction (AMF). The ethyl acetate fraction (EAF) contains all the metabolites except alkaloids, while the n-hexane fraction (HEF) contains only steroids/triterpenes and flavonoids. *Khaya senegalensis* is a plant used in traditional medicine to treat and protect against gastric ulceration.[37] In our previous studies, the crude methanol extract of the stem-bark of *Khaya senegalensis* showed anti-ulcer activity against ethanol induced gastric ulceration in rats.[21] In this study, the crude methanol extract was partitioned by solvent-solvent extraction into different extracts based on the polarities of the compounds they contain.
The HEF, ethyl acetate EAF and the AMFfractions did not produce any apparent toxic effect or mortality when tested at doses between 10 and 5000 mg/kg and the median lethal doses of the extracts were assumed to be ≥ 5000 mg/kg. According to expert opinions, any substance administered to an animal and is not lethal acutely at a dose of 5000 mg/kg is considered non-toxic.[38, 39]
There was a significant decrease in the concentration of gastric HCl in rats treated with AMF (800 mg/kg) and EAF (400 and 800 mg/kg) relative to rats in the untreated control group (figure 1). Although not significant ($P > 0.05$), rats treated with AMF (200 and 400 mg/kg), EAF (200 mg/kg) and cimetidine (20 mg/kg) showed decreased levels of gastric HCl production as well. The extracts of *K. senegalensis* afforded a significant protection against ulcers induced by pylorus ligation in rats. Pylorus ligation is one of the most widely used models for studying the effect of drugs on gastric acid and mucus secretion.[40] Ulcers developed by ligating the pyloric end of the stomach are caused by an increase in gastric HCl secretion and/or stasis of acid, leading to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier.[41] Parietal or oxyntic cells are the principal cells in gastric glands, which secrete gastric HCl to promote proteolytic digestion of foodstuffs, iron absorption, and killing of pathogens.[42] The three most important mediators, namely acetylcholine, gastrin, and histamine, interact with specific receptors located at the basolateral membrane of the parietal cells that stimulate gastric acid secretion.[43] The regulation of gastric acid secretion by the parietal cells is an important factor in the pathogenesis of peptic ulcer. Therefore, the inhibition of gastric acid secretion is a key therapeutic target for the ulcer diseases.[44] Findings in this study demonstrated that the extracts of *Khaya senegalensis* significantly reduced the total acidity in the rats. Perhaps this could be due to the anti-secretory property of the plant. The HEF was tested at only 800 mg/kg in contrast to AMF and EAF that were evaluated at three different doses (200, 400 and 800 mg/kg). This was done because during the partitioning process we obtained very little quantity of HEF and therefore the maximum dose used for the other extracts was chosen for evaluating the anti-ulcer activity of the fraction.
There was no significant difference in the volume of gastric fluid in rats treated with the AMF and EAF all tested doses when compared with the normal saline untreated group (figure 2). Similarly, no significant difference was observed in the volume of gastric fluid in rats treated with cimetidine (20 mg/kg) relative to the normal saline untreated group. Gastric mucus plays an important role in the gastric ulcer defense mechanism, whereby it forms a continuous mucus gel-like protective barrier coating the entire gastric mucosa that maintains the mucosal surface at a pH of 6–7 in the acidic environment (pH 1–2). In gastric ulcers, in spite of low acid secretion, weakening of mucosal defenses can also lead to severe injury to the gastric mucosa.[44]
The ulcer indices of rats treated with the AMF, EAF and HEF were statistically significant ($P < 0.05$) when compared with the normal saline untreated group (figure 3). Rats treated with the AMF and EAF at 400 and 800 mg/kg had significantly ($P < 0.01$) lower ulcer index than those treated with the HEF and misoprostol (standard drug).There were significant ($P < 0.05$) increases in the concentration of gastric mucus in rats treated with the fractions (AMF, EAF and HEF) of *K. senegalensis* when compared with the normal saline untreated group. In a similar manner, rats treated with EAF (200 and 400 mg/kg) had a more significant ($P < 0.01$) increase in mucus production when compared to all treated rats (figure 4). The important criteria that determine the status of mucosal defense barrier against the unpleasant attack of acid and pepsin are the quality and quantity of gastric mucus secretion.[45] According to Venables (1986),[46] rise in amount of mucus secreted by the gastric mucosal cells prevents ulcer formation by acting as an effective barrier to the back diffusion of hydrogen ions, improving the buffering of gastric acid juice and reducing stomach wall friction during peristalsis. The mucus comprises mucin-type glycoproteins that can be detected by amounts of alcian blue binding.[28] The study revealed that administration of the extracts of *K. senegalensis* increased the amount of mucus in the stomach which is an indication of the potential mechanism of the gastroprotective effect of the plant as a result of enhancement of the gastric mucosal defense action. This finding agrees with finding of Rodrigues *et al*. (2017),[47] who reported increased gastric mucous production by aqueous fraction of hydroacetonic leaf extract of pitanga in mice. Treatment with the extracts obtained from the crude extract of *K. senegalensis* appears to significantly reduce one of the gastric aggressive factors (HCl) by decreasing the amount of gastric secretion and total acidity as well as enhancing the cytoprotective effect of the gastric mucosal barrier. This perhaps explains some of the possible anti-ulcer effects of the extracts of *Khaya senegalensis*. The AMF, EAF and HEF significantly ($P < 0.05$) reduced the concentration of malondialdehyde in the gastric tissues of treated rats (figure 5).

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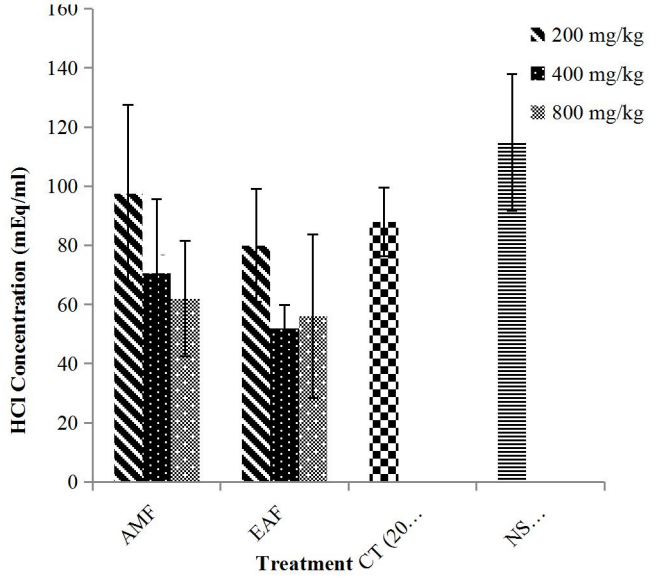

Figure 1: The effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* on gastric acid secretion in rats. Cimetidine (CT) and normal saline (NS) are used as treated and untreated controls, respectively. $^{a}P < 0.05$ show significant difference when compared with the normal saline untreated control group.

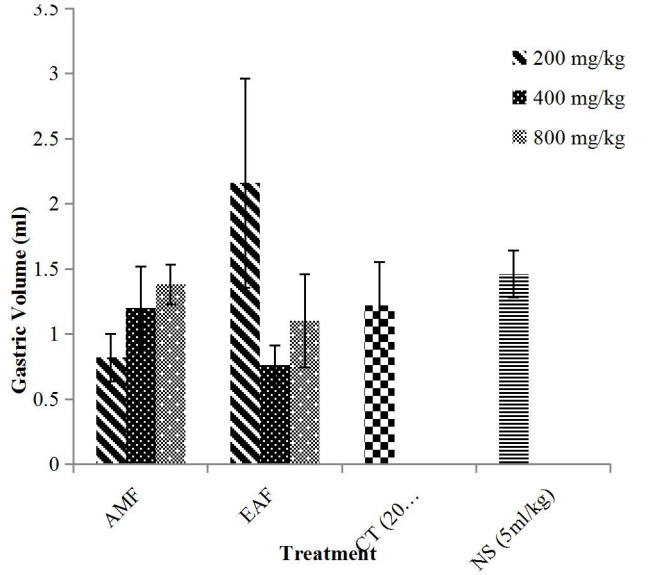

Figure 2: The effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* on gastric fluid in rats.
Cimetidine (CT) and normal saline (NS) are used as treated and untreated controls, respectively.

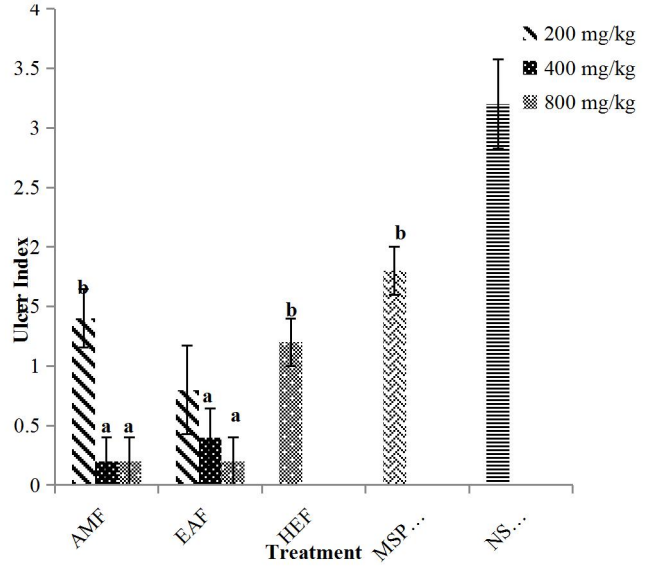

Figure 3: The ulcer protective effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* against ethanol-induced gastric ulceration in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls, respectively. $^{a}P < 0.01$, $^{b}P < 0.05$ show significant difference when compared with the normal saline untreated control group.

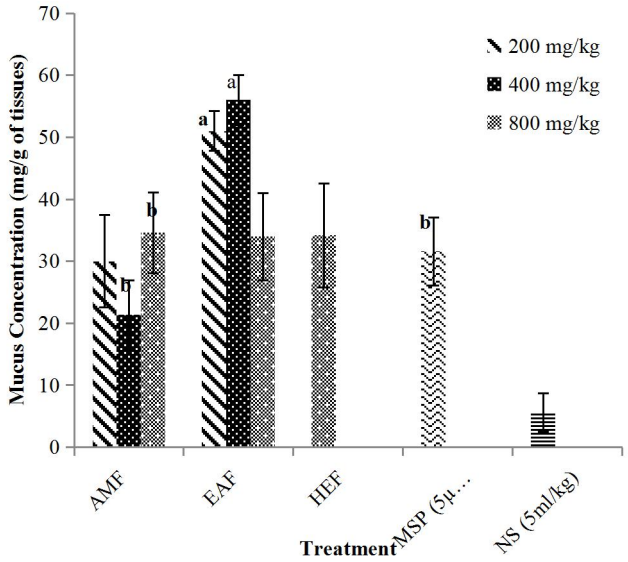

Figure 4: The effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* on gastric mucus production in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls, respectively.$^{a}P< 0.01$, $^{b}P< 0.05$ show significant difference when compared with the normal saline untreated control group.

The AMF (400 and 800 mg/kg) and EAF (200 mg/kg) reduced significantly ($P < 0.05$) the level of malondialdehyde in the stomach tissue of treated rats. Similarly, EAF (400 and 800 mg/kg) also reduced significantly ($P < 0.01$) the level of malondialdehyde in the stomach tissue of rats when compared with the normal saline untreated group. Although, there was a decrease in the level of malondialdehyde in rats treated with HEF (800 mg/kg) it was not statistically significant. Gastric ulcer could result due to increase in free radical and oxidative processes generation.[48]Similarly, it has been shown that pathogenesis of gastric ulcer involves oxidative stress and antioxidants that play a very important role in mucosal gastroprotection and repair of gastric damage.[49]

Body inflammatory processes are also responsible for producing various mediators, which are involved in the production of reactive oxygen species (ROS) and nitric oxide (NO) that contribute to the pathogenesis of ulcer disease. Nitric oxide is a mediator that plays an important role as an endogenous modulator of numerous physiological functions. In the gastrointestinal tract (GIT), NO participates in the

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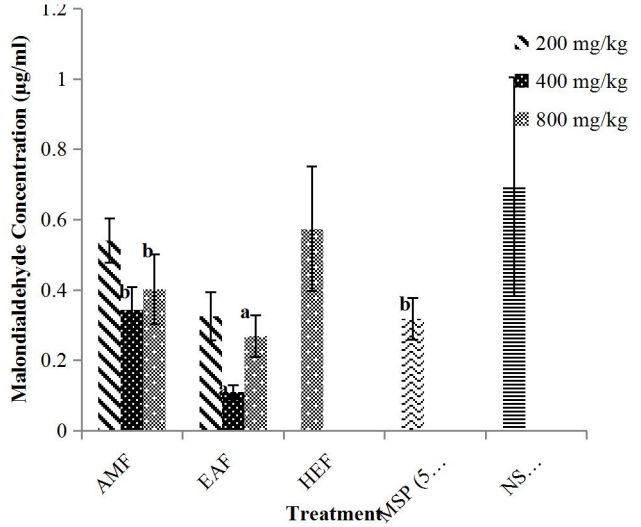

Figure 5: The effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* on gastric malondialdehyde concentration in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls, respectively.[a]P< 0.01, [b]P< 0.05 show significant difference when compared with the normal saline untreated control group.

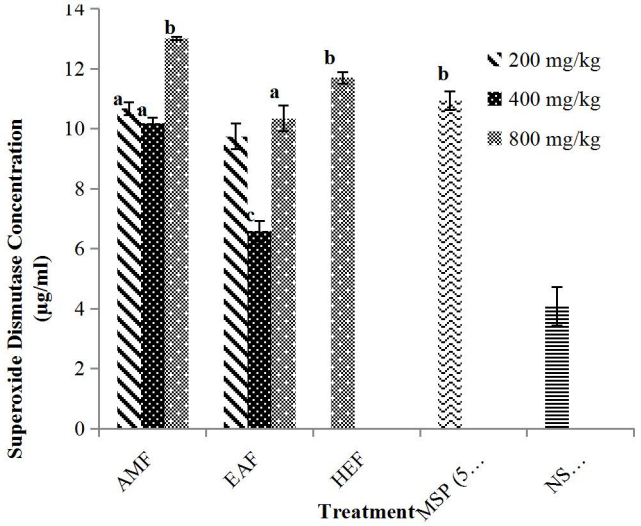

Figure 6: The effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* onstomach superoxide dismutase activity in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls, respectively.[a]P< 0.05, [b]P< 0.01, [c]p<0.001 show significant difference when compared with the normal saline untreated control group.

modulation of the smooth musculature tone, such as the regulation of intestinal peristalsis, gastric emptying, and antral motor activity.[50] It also helps in maintaining the gastric mucosal blood flow, barrier function, alkaline production, and regulates gastric mucus and acid secretion.[51] In physiological conditions, NO modulates both the integrity and repair of the tissues.[50] However, overproduction of NO is associated with tissue injury in the gut during inflammatory reactions such as peptic ulcer, chronic gastritis, gastrointestinal cancer, bacterial gastro-enteritis, celiac or chronic inflammatory bowel diseases.[52] This shows the double-edged role played by NO in gastrointestinal ulcerative and inflammatory diseases. The extracts of *K. senegalensis* may contain bioactive compounds that have gastroprotective activity via modulation of NO.

Xanthine oxidase (XO) is an enzyme that generates hydrogen peroxide (H_2O_2) by consumption of molecular oxygen in metabolic reactions it catalyzes.[53] H_2O_2 reacts with cellular lipids, causing the formation of lipid peroxides, which are metabolized to malondialdehyde, a major product of lipid peroxidation.[54] Huh *et al*. (1996)[55] also reported that alcohol-induced gastric mucosal damage may be, in part, due to the increased activity of xanthine oxidase and the type of conversion rate of the enzyme, leading to oxidative stress. An endogenous anti-oxidant defense mechanism may constantly remove the continuous production of ROS during normal metabolism.[56]Thus, the decrease level of malondialdehyde observed in this study in groups treated with the extracts of *K. senegalensis* could be due to inhibition either partially or in whole of xanthine oxidase by the extracts which may have contributed to their anti-ulcerative effect.

Flavonoids have been reported to act in the gastrointestinal tract, exhibiting antispasmodic,[57] anti-secretory, antidiarrhoeal[58] and anti-ulcer properties.[59] Flavonoids were also reported to exhibit antioxidant[60, 61] and anti-inflammatory[62] activities. Flavonoids are able to activate the mucosal defense system through stimulation of gastric mucus secretion and scavenge for the ROS and free radicals produced by ethanol.[63] In addition, flavonoids are able to decrease ulcerogenic lesions by promoting the formation of gastric mucosa inhibit the production of pepsinogen and diminish acid mucosal secretion.[59] Kelly *et al*. (2009)[64] also reported that flavonoids have antihistaminic properties, thus, decrease histamine levels, as well as preventing the release of histamine from gastric mast cells, and inhibiting the gastric H^+/K^+ proton pump and diminishing gastric acid secretion. Mota*et al*. (2009)[65] have summarized the literature on 95 flavonoids with varying degrees of anti-ulcerogenic activity, confirming that flavonoids have a therapeutic potential for a more effective treatment of peptic ulcers. Alkaloids have been shown to possess anticholinergic properties and some alkaloids (atropine, scopolamine) are used clinically to block the muscarinic activity of acetylcholine thereby showing antisecretory effects in the treatment of peptic ulcer[66]. On the other hand, tannins are known to protect the outermost layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation.[67] Tannins form a protective pellicle by promoting precipitation of protein on the ulcer in order to prevent ulcer development. This pellicle helps in preventing toxic substance absorption and combat the attack of proteolytic enzymes.[68] Furthermore, saponins may exert protective activities in gastric ulceration by the activation of mucous membrane protective factors.[69]

The fractions of *Khaya senegalensis* and misoprostol significantly ($P < 0.05$) increased the activity of superoxide dismutase (SOD) (figure 6). The activities of SOD in rats treated with AMF (200 and 400 mg/kg) and EAF (200 and 800 mg/kg) were significantly ($P < 0.05$) higher when compared with the normal saline untreated group. Also HEF (800 mg/kg) and AMF (800 mg/kg) produced a more significant ($P < 0.01$) higher SOD activity in the treated rats. In addition, rats treated with EAF (400 mg/kg) had significant ($P<0.001$) increased in the level of SOD in their stomach tissue. SOD represents one aspect of protection against free radical mediated organ toxicity. Decreased SOD activity may cause many deleterious effect due to accumulation of superoxide radicals.[70] Flavonoids have been shown to increase the activity of SOD and other antioxidant enzymes. In addition they cause a decrease in activity of MDA.[71]The increase observed in the SOD activity of the groups treated with the extracts of *Khaya Senegalensis* may be due to the antioxidant properties of the stem-bark extracts of the plant.

The effect of the fractions of *Khaya senegalensis* on the activity of catalase (CAT) is shown in figure 7. The CAT activity for the AMF group was significantly ($P < 0.01$) decreased when compared with the normal saline untreated group. The EAF treated group at the dose of 400 mg/kg also significantly ($P < 0.05$) decreased the CAT activity when compared with the normal saline untreated group. The decrease in CAT activity in rats treated with the extracts of *Khaya senegalensis* may be attributed to the possible binding of the enzymes to flavonoid

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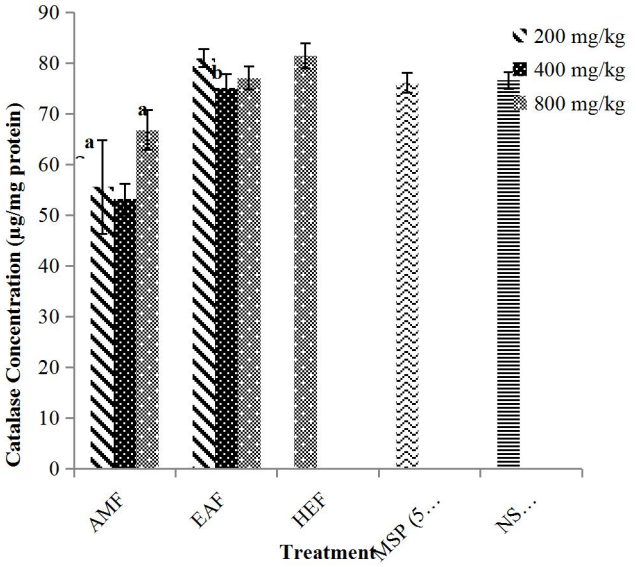

Figure 7: The effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* onstomach catalase activity in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls, respectively.[a]P< 0.01, [b]P< 0.05 show significant difference when compared with the normal saline untreated control group.

components of the extracts. Flavonoids such as quercetin and myricetin bind to CAT and alter conformation of the enzyme.[72] Krych and Gebicka (2013) [73] also reported that flavonoids inhibit CAT activity, due to the formation of hydrogen bonds between catalase and flavonoids to form an unreactive catalase compound.

Histopathological changes were observed in the stomach of rats treated with AMF at the dose of 200 and 400 mg/kg (figure 8A and B), but no changes were observed in the stomach of rats treated with AMF at the dose of 800 mg/kg (figure 8C). Rats treated with EAF (200, 400 and 800 mg/kg) showed milder lesions to the surface epithelium (figure 9A, B and C). Mild disruptions of the surface epithelium were also observed in rats treated with HEF 800 mg/kg (Figure 10A) and misoprostol (standard drug) at 5 µg/kg (figure 10B). The stomachs of rats treated with normal saline (untreated control group) had severe necrosis and loss of epithelium lining (figure 10C).

Conclusion

In conclusion, this study has shown that the stem-bark extracts of *Khaya senegalensis* inhibit gastric acid secretions, increase the action of mucosal protective factors, possess antioxidant and cytoprotective properties in rats experimentally induced with gastric ulceration. Further studies are required to isolate, identify and elucidate the mechanisms of action of compounds responsible for the anti-ulcer properties from the stem-bark of *K. senegalensis*.

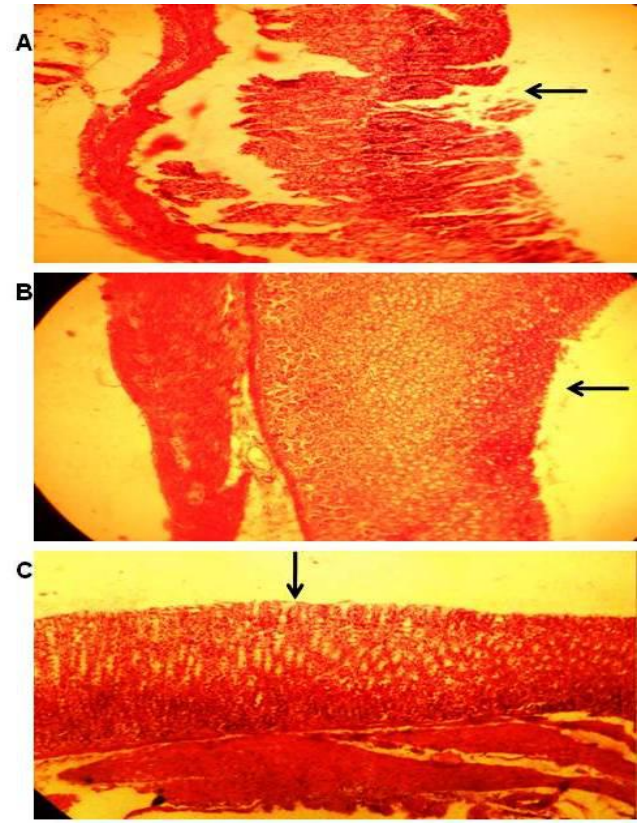

Figure 8: Photomicrograph of sections of the stomach of rats induced with gastric ulceration using 80% ethanol and treated with different doses of aqueous methanol (AMF) fraction of *Khaya senegalensis*. Necrosis and loss of epithelial lining were observed on the stomach of rats treated with 200 m/kg (arrow; **A**), mild disruption on the surface epithelium was observed on stomach of rats treated with 400 mg/kg (arrow; **B**). Rats treated with 800 mg/kg (arrow; **C**) of the extract had normal stomach epithelium (H and E × 100).

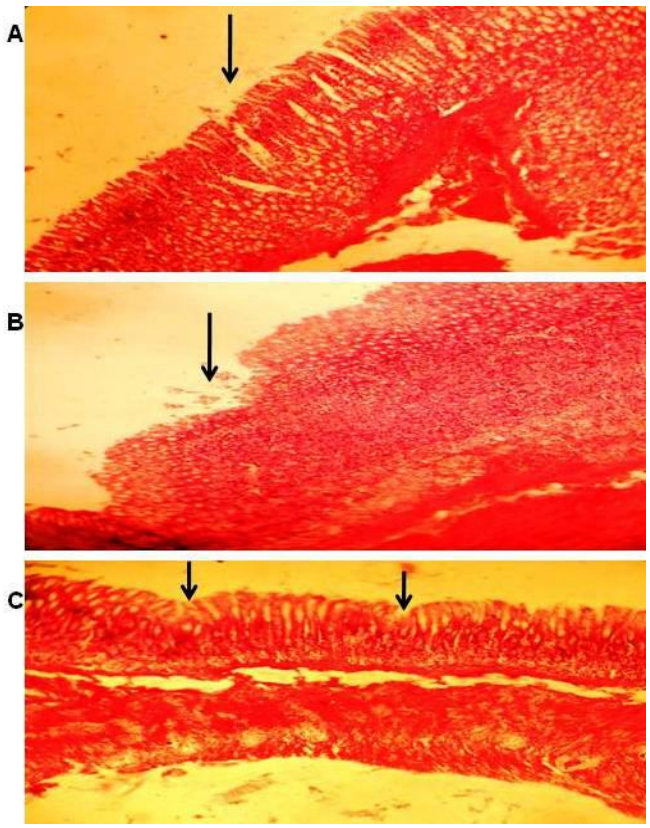

Figure 9: Photomicrograph of sections of the stomach of rats induced with gastric ulceration using 80% ethanol and treated with different doses of ethyl acetate (EAF) fraction of *Khaya senegalensis*. Mild excoriation of the epithelial lining was observed on the stomach of rats treated with 200 m/kg (arrow; **A**), while partial loss of epithelial lining was observed on stomach of rats treated with 400 mg/kg (arrow; **B**). Rats treated with 800 mg/kg (arrow; **C**) of the extract had mild loss of epithelial lining (H and E × 100).

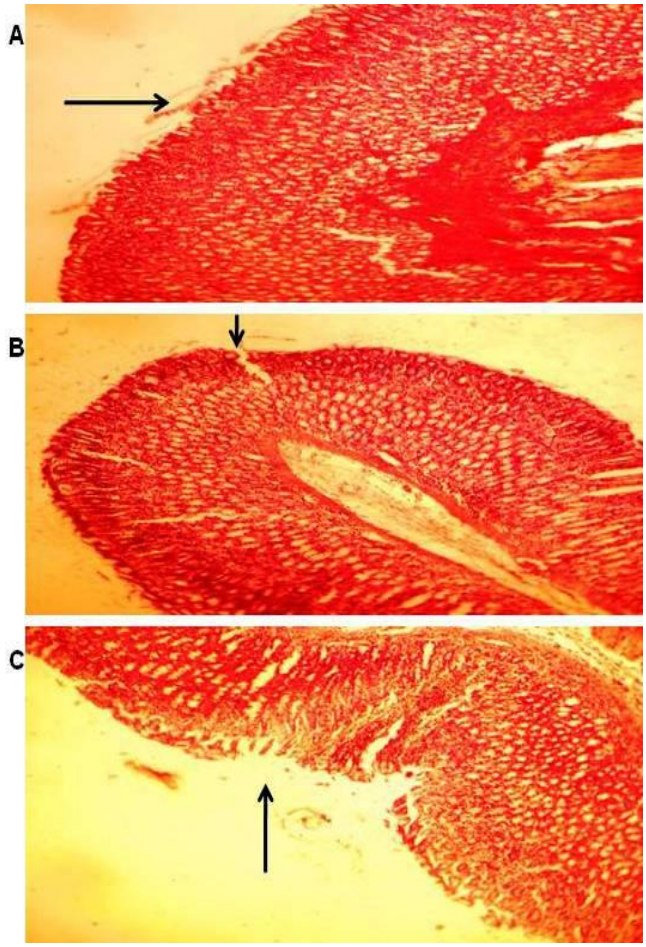

Figure 10: Photomicrograph of sections of the stomach of rats induced with gastric ulceration using 80% ethanol and treated with hexane (HEF) fraction of *Khaya senegalensis* (800 mg/kg) showing mild disruption of the surface epithelium (arrow; **A**). Similarly, mild excoriation of the surface epithelium was observed on the stomach of rats treated with misoprostol (5 µg/kg; treated control) (arrow; **B**). Stomachs of rats treated with normal saline (5 ml/kg; untreated control) (arrow; **C**) had severe necrosis and loss of epithelium lining (H and E × 100).

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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