An in vivo experimental trial to determine the efficacy of stem-bark extract of Khaya senegalensis A. Juss (Meliaceae) for treating gastric ulcer in rat

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Abstract: Khaya senegalensis A. Juss (Meliaceae) is a plant that has a variety of traditional medical applications and it is used to treat gastroduodenal ulcers and pain. In this study, the antiulcer effect of the plant was evaluated in laboratory animal models. Antiulcer effect of the methanol extract of K. senegalensis was evaluated by measuring gastric acidity, volume, mucus and malondialdehyde concentrations in rats. Similarly, ethanol-induced gastric ulceration was used to assess the antiulcer activity of the extract. When administered orally, the extract at doses of 400 and 800 mg/kg produced significant (p<0.05) dose-dependent decrease in gastric acid concentration and volume. Similarly, the extract caused a significant (p<0.05) increase in gastric mucus and decrease Malondialdehyde concentrations in a dose-dependent fashion. At doses of 200, 400, and 800 mg/kg, the extract also causes remission of gastric ulcer lesions. The pharmacological actions of the extract were comparable to standard drugs (cimetidine and misoprostol) used in this study. The findings in this study suggest that the methanol extract of the stem-bark of K. senegalensis possesses antiulcer and gastric antisecretory effects. Phytochemical investigation of methanol extract of K. senegalensis revealed the presence of alkaloids, carbohydrate, glycosides, flavonoids, steroids, tannins, and triterpenes, which may be involved in the observed pharmacological action of the plant. The results justify the traditional use of the stem-bark of K. senegalensis for the treatment of gastroduodenal ulcers. However, further detailed studies are required in both laboratory and target animal species to fully justify the clinical application of the extract in treating gastroduodenal ulcers.

Keywords: Khaya senegalensis; Antiulcer effect; Methanol extract; Laboratory animals.

Introduction

Gastroduodenal ulcer is a chronic disease of multifactorial aetiology. It is the most prevalent disease among the gastrointestinal tract diseases in most part of the world. The disease is cause mainly as a result of imbalance between offensive (acid, pepsin and the bacterium- Helicobacter pylori) and defensive (mucin, prostaglandin and bicarbonate) factors (Soll 1990). Factors such as stress, smoking among humans, nutritional deficiency and ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) are implicated in altering this balance in favour of the offensive factors and thus causing gastroduodenal ulcers to result. Most gastro-duodenal ulcers result from weakness in the normal gas-

tric mucus barrier against the penetration of hydrochloric acid secreted by the stomach. Free radicals produce as a result of tissue damage have been reported to play a role in the causation of gastric ulcers (Rao, et al. 1999). Modern antiulcer drugs are expensive, toxic and not readily available to the common man (Yusuf et al. 2004).

Several important compounds have been isolated from traditional medicinal plants, and have been shown to have antiulcer activity (Lewis and Hanson 1991). It is therefore pertinent to study the antiulcerogenic effects of Khaya senegalensis A. Juss (Meliaceae) for treating gastro-duodenal ulcerative disease in rats.
Khaya senegalensis A. Juss (Meliaceae) is widely distributed in east, central and west African subregions. It is found in riparian forests and higher-rainfall savannah woodlands; in moist regions it is found on higher ground. Within its first year, the seedling develops a deep root system; an adaptation that makes it the most drought resistant member of its genus. The tree can grow up to 15-30 m in height and 1 m in diameter (Anonymous 2013). The plant has a wide range of applications for treating stomach complaints including gastro-duodenal ulceration (Dalziel 1955).

This study is aimed at evaluating the antiulcer effect of Khaya senegalensis in laboratory rats with a view of finding a safer and cheaper alternative treatment of gastroduodenal ulcers in humans and other domestic animals.

Materials and methods

Plant material

Fresh stem-bark of K. senegalensis was collected from the tree in the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The leaves and seeds were sampled, identified and authenticated by trained botanist in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Thereafter, a specimen of the plant with a voucher number “900181” was deposited for reference purpose. The stem-bark was allowed to dry in the shade and made into a fine powder using porcelain mortar and pestle. The powdered bark (300 g) was then macerated using methanol. The mixture was allowed to stand for 3 days. The liquid extract was then concentrated into a brown-waxy residue. The extract was later kept in a desiccator for two weeks until a constant dry weight of 70 g was obtained.

The methanol extract of K. senegalensis was screened for the presence of carbohydrates, glycosides, tannins, alkaloids, saponins, anthraquinones, flavonoids, sterols and triterpenes using standard procedures (Tiwari et al. 2011).

Animals

Apprently healthy Wistar albino rats of both sexes weighing between 96-123 g were obtained from the Animal House, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (A.B.U.), Zaria, Nigeria. The rats were maintained on standard diet and provided water ad-libitum. Animals were allowed to acclimatize in the laboratory for two weeks. Standard Operating Procedure was employed according to international guidelines for use of animals in experimentation and as approved by the Research Board of Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria (U045VM108882).

Safety evaluation

The method of Lorke (1983) with slight modification was used to determine the median lethal dose (LD$_{50}$) of the extract. In the first part of the trial, nine wistar strains rats were randomly allocated into three groups of three rats each. The rats were deprived of food for 24 hours and water for 12 hours. Groups 1, 2 and 3 received the extract orally at 10, 100 and 1000 mg/kg body weight, respectively. Rats were observed for 48 hours for any sign of toxicity or mortality. The second part of the trial was carried out based on the results obtained during the initial trial. In the second part of the experiment, another nine rats were assigned into three groups of three rats 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 earlier described.

Secretory studies

Fifteen apparently healthy rats were randomly allocated to five groups of three rats each. The rats were fasted for 24 hours prior to commencement of the experiment but water was provided ad libitum. All rats were anaesthetized using ketamine hydrochloride (20 mg/kg) administered intramuscularly. A ventral midline incision was made on each rat and the stomach exteriorized and ligated at the pyloric region. Rats in groups 1, 2 and 3 were treated with the extracts at 200, 400, and 800 mg/kg respectively.
ly, while groups 4 and 5 were given cimetidine (20 mg/kg) and normal saline (5 ml/kg), respectively. All treatments were administered intragastrically. After treatment, the stomach in each rat was returned into the abdominal cavity and the incisions sutured. Four hours after recovery from anaesthesia, all rats were euthanized in a chloroform chamber. The sutured area of individual rats was opened again, the stomach removed and its contents 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 and titrated with NaOH to end-point using 2% phenolphthalein as an indicator. Total acidity was calculated and expressed as mEq/ml (Vogel and Vogel 1995).

Gastroprotective activity

Twenty five rats were randomly allocated into 5 groups of 5 rats each and fasted for 24 hours for food but not water. Groups 1, 2 and 3 were treated with the extract at 200, 400 and 800 mg/kg, respectively, while groups 4 and 5 were given misoprostol (cytotec®) (50 µg/kg) and normal saline (5 ml/kg), respectively. All treatments were administered orally. Thirty minutes after treatment with the extract (groups 1, 2 and 3), misoprostol (group 4), and normal saline (group5), ulcer was induced in each rat using 1ml of 80 % ethanol. All rats were sacrificed three hours after ulcer induction. The stomachs were removed, 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 according to the method of Ohara et al. (1995). 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 ulcer (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 (Yusuf et al. 2004).

Gastric mucus determination

Gastric mucus determination was carried out by the method described by Corne et al. (1974). Briefly, 500 mg of the glandular portion of the stomach of each rat was transferred immediately into 1 % Alcian blue solution (in sucrose solution, buffered with sodium acetate, pH 5.0); the excess dye was removed by rinsing in sucrose solution. The dye complexed with the gastric wall mucus was extracted with 0.5 % 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 supernant was decanted and the absorbance recorded at 580 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 carried out using the double method of Draper and Hadley (1990) as modified by Yavuz et al (2004). The principle of the method is based on the spectrophotometric measurement of the colour developed during reaction of thioarbituric acid (TBA) with malondialdehyde (MDA). About 500 g of the glandular portion of stomach from each rat was weighed and homogenized in ice-cold phosphate buffer to obtain a 10% homogenate. Trichloroacetic acid (TCA) solution (2.5 ml of 100 g/L) was added to 0.5 ml stomach homogenate in a centrifuge tube, placed in boiling water (100 °C) bath for 15 minutes and cooled under tap water to room temperature for another 5 minutes. The mixture was centrifuged at 1000 g for 10 minutes and 2 ml of the supernatant was added to 1 ml of 6.7 g/L of thiobarbituric acid (TBA) solution in a test tube and the absorbance was measured at 532 nm.

Statistical analysis

Values obtained were expressed as mean ± S.E.M and then subjected to one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test using Graphpad Prism version 4.0 for Windows from Graphpad software, San Di-
Results

Extract yield and phytochemical test

The extraction of methanol extract of *K. senegalensis* gave a yield of 14.2%. Glycosides, tannins, flavonoids, triterpenes and reducing sugars were the chemical components shown to be present in the acetone extract of *K. senegalensis*.

Acute toxicity studies

The extract did not produce any apparent toxic effect or mortality when tested at doses of between 10-2900 mg/kg. However, when given at a dose of 5000 mg/kg, the extract caused death in the experimental rats. The LD$_{50}$ was therefore calculated by taking the geometric means of the highest dose that did not cause death (2900 mg/kg) and the lowest dose (5000 mg/kg) that produced mortality (Lorke, 1983) using the equation below:

$$LD_{50} = (2900 \times 5000)^{\frac{1}{2}} = 3807.9 \text{ mg/kg}$$

Effect of the extract on gastric fluid

The extract produced a dose-dependent increase in the volume of gastric fluid in the rats (Figure 1). At doses of 400 and 800 mg/kg, the extract produced a significant (p<0.01) increase in the volume of gastric secretions in rats. There was no significant increase in the amount of gastric secretions in rats treated with the extract (200 mg/kg) and cimetidine (20 mg/kg) when compared with the normal saline treated group.

Effect of the extract on gastric acid

The extract produced a dose-dependent decrease in the concentration of gastric acid in the treated rats (Figure 2). At doses of 400 and 800 mg/kg, the extract produced a significant (p<0.05) decrease in the concentration of gastric HCl in rats. There was also a significant (p<0.01) decrease in the concentration of HCl in rats treated with 800 mg/kg and cimetidine (20 mg/kg) when compared with the normal saline treated group. Similarly, there was a significant (p>0.05) decrease in the concentration of HCl in rats treated with 400 mg/kg when compared with the normal saline treated animals. There is also significant (p<0.01) difference between the concentration of HCl in rats treated with 200 mg/kg of the extract and those that received cimetidine (20 mg/kg).

Figure 1: The effect of different doses of the extract of *K. senegalensis* (200-800 mg/kg) and cimetidine (20 mg/kg) on gastric fluid in rats. Values are mean ± SEM. **p<0.01 show significant difference when compared with normal saline-treated control group.

Figure 2: The effect of different doses of the extract of *K. senegalensis* (200-800 mg/kg) and cimetidine (20 mg/kg) on gastric acid secretion in rats. Values are mean ± SEM. **P<0.01 and *P<0.05 show significant difference when compared with normal saline-treated control group.

Effect of extract on ethanol-induced gastric ulcer

The ulcer indices of rats treated with different doses of the extract and misoprostol
(50µg/kg) are depicted in Figure 3. The ulcer indices of rats treated with the extract at 800 mg/kg and misoprostol (50 µg/kg) were found to be significantly (p<0.05) lower than in rats treated with the extract at 400 and 200 mg/kg. In addition, rats treated with the extract at a dose of 800 mg/kg showed a significant (p<0.01) lower ulcer index than those treated with normal saline (untreated control). However, there was no significant difference (p>0.05) in the ulcer index of rats treated with 800 mg/kg of the extract and those treated with misoprostol (50µg/kg).

Figure 3: The ulcer protective effect of different doses of the extract of K. senegalensis (200-800 mg/kg) and misoprostol (50 µg/kg) against ethanol-induced gastric ulceration in rats. Values are mean ± SEM. **P<0.01 and *P<0.05 show significant difference when compared with normal saline-treated control group.

Effect of extract on gastric mucus production

The effect of the methanol extract of Khaya senegalensis on gastric mucus production is shown in Figure 4. There was a significant (p<0.001) increase in the concentration of gastric mucus in rats treated with the extract (800 mg/kg) and misoprostol (50 µg/kg) when compared with the normal saline treated group. Also rats treated with the extract at a dose of 400 mg/kg showed significant (p<0.01) increase in mucus production compared to normal saline (5 ml/kg) treated rats. Moreover, rats treated with the extract at 200 mg/kg showed significant (p<0.05) increase in gastric mucus production when compared with those treated with 5 ml/kg normal saline.

Figure 4: The effect of K. senegalensis (200-800 mg/kg) and misoprostol (50 µg/kg) on gastric mucus production in rats. Control group received normal saline (5 ml/kg). Values are mean ± SEM. ***p<0.001, **p<0.01 and *p<0.05 show significant difference when compared with normal saline treated control group.

Effect of extract on malondialdehyde concentration

The extract at a dose of 800 mg/kg and misoprostol (50 µg/kg) significantly (p<0.001) reduced the concentration of malondialdehyde in the gastric tissue (Figure 5). However, the extract at doses of 200 and 400 mg/kg did not reduce significantly the level of malondialdehyde in the stomach.

Figure 5: The effect of Khaya senegalensis (200-800 mg/kg) and misoprostol (50µg/kg) on gastric malondialdehyde concentration in rats. Control group was dosed with normal saline (5 ml/kg). Values are mean ± SEM. ***p<0.001 show significant difference when compared with normal saline treated control group.
Discussion

Gastro-duodenal ulcer is a global health problem of the gastrointestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion, which occurs due to an imbalance between “offensive” and “defensive” factors along with the weakness of the mucosal barrier (Aebi 1984). The disorder develops as a result of excessive secretion of HCl and pepsin, and a diminished mucosal defense or a combination of these factors (Rang et al. 2003). Factors that predispose to gastric ulcer diseases include Helicobacter pylori (bacterium) infection, prolonged administration of non-steroidal anti-inflammatory drugs (NSAIDs), cigarette smoking, alcoholism, stress, and, chronic pancreatitis (Tariq et al. 1986). The use of NSAIDs is considered to be the major risk factor in gastric ulcers. The mechanisms suggested for the gastric damage caused by NSAIDs are inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the re-epithelization of the ulcer crater (Levi et al. 1990).

The functional integrity of gastric mucosa depends on equilibrium between the “aggressive and the protective factors”. Thus the success of the pharmacological treatment in the stomach is dependent not only upon inhibition of the acid secretion, but also on the increase in the protective factors in the gastric mucosa (Dajani and Klamut 2000).

Agents that have the ability to reduce acid secretion and or improve microcirculation have been shown to decrease gastric lesions (Takeuchi et al., 2003; Patel et al., 2001; Nakamura et al., 1998; Jayaraj et al., 1988; Padol et al., 1999). For instance, cimetidine (a histamine H2-receptor blocker) causes its antiulcer effect by competitively blocking the H2-receptors on parietal cells, hence decreasing histamine- or gastrin-induced secretion of hydrochloric acid (Rang et al. 2003). Perhaps the extract of K. senegalensis acts in a similar fashion to decrease gastric HCl production. Alternatively, the effect may be due to direct neutralization of HCl in the stomach as it occurs when antacids like aluminium or magnesium salts are administered orally. Given in sufficient quantity for enough periods, they can produce healing of duodenal ulcers (Rang et al. 2003).

The result of the acute toxicity test revealed that the extract at doses of between 10 and 2900 did not produce any apparent toxic effect or mortality. However, when given at a dose of 5000 mg/kg, the extract produced mortality in the tested animal. The LD50 was therefore calculated to be 3807.9 mg/kg. Nwosu et al (2011) showed the oral LD50 of the aqueous leaf extract of the K. senegalensis to be greater than 3000 mg/kg. Based on this finding, doses of 200, 400 and 800 mg/kg that were considered safe were chosen for pharmacological (antulcer) evaluation. The extract, at doses of 200-800 mg/kg, exhibited a dose-dependent gastroprotective activity in all models of gastric ulcer used.

Super oxide anion, hydroxyl radical and other reactive metabolites react with cell components in the gastric mucosa to cause damage. The production of these radicals can be enhanced by ethanol (Bagchi et al. 1998). These effects could cause changes in the structural integrity of the cells and functions that could enhance oxidative damage. Ethanol also causes gastric mucosal injury by causing extensive damage to mucosal capillaries resulting in increased vascular permeability, oedema formation and epithelial lifting (Kato et al. 1990; Nordmann 1994). Gastric ulceration can also enhance the development of gastric cancer in certain individuals (Hansson et al. 1996). It is very possible that substances that protect against ethanol-induced gastric ulceration would be good candidates as cytoprotectives agents (Bighetti et al. 2005) and the ability of the extract of K. senegalensis to attenuate ethanol-induced gastric ulcer may suggest that the extract has cytoprotective effects. Cytoprotection may occur due to the ability of some agents to induce prostaglandin production, which in turn stimulates mucus and bicarbonate synthesis (Robert et al. 1983). Previous studies had shown the antioxidative effect of K. senegalensis (Karou et al. 2005; Atawodi et al. 2009). Gastric mucus (mucin) is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that cover the entire gastrointestinal mucosa (Rang et al. 2003).
antiulcer activity (Yamahara et al. 1987; Yesilada and Takaishi 1999, Morikawa et al. 2006); the effect was suggested to be due to the saponins having protective effect on the mucus membrane (Saito et al. 1977). Triterpenoids have been shown to possess several pharmacological actions (Jung et al. 2005) including antiulcer activities (Arrieta et al. 2003). Similarly, alkaloids extracted from rhizoma Coptis chinensis showed dose-dependent antiulcer effects in the water-immersion stress, intragastric ethanol and pylorus ligation (Li et al. 2005). Flavonoids are other secondary metabolites that have antiulcer, antioxidant and gastroprotective properties (Matsuda et al. 2003; Zayachkivska et al. 2005). This class of secondary compounds is able to protect the gastric mucosa against a variety of antiulcerogenic agents, particularly through scavenging properties on oxygen radicals by inhibition of nitric oxide synthase activity (Di Carlo et al. 1999; Matsuda et al. 2003; Zayachkivska et al. 2005). The presence of these phytochemical constituents in the stem-bark extract of Khaya senegalensis could possibly explain the antiulcer activity produced by the plant.

Conclusion

The results obtained in this study revealed the antiulcer action of the leaves of Khaya senegalensis. These results demonstrated that Khaya senegalensis possesses gastric acid inhibitory properties and also increases the action of mucosal protective factors. The plant has shown promising effect as an antiulcerogenic agent. These findings are important with regards to the future development of an alternative antiulcer agent that is more potent, safer and efficacious. Further studies are required to isolate the bioactive compound(s) and to elucidate their mechanisms of action.

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References


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