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Research Article

ANTI-ULCER ACTIVITY OF THE STEM BARK OF AFRICAN LOCUST BEAN TREE IN RATS

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ABSTRACT:
Parkiabiglobosa (Jacq.) R.Br. ex G. Don (family fabaceae) popularly called the African locust bean tree have been used traditionally in Nigeria and other West African rural communities to treat a variety of diseases including ulcer related diseases. Methanol extracts and fractions from the stem bark of African locust bean tree were evaluated for their anti-ulcer activity using ethanol and acetyl salicylic acid (ASA) as the ulcerogens. The extract and fraction were administered orally at the doses of 100, 200 and 400 mg/kg b. wt. for the experimental groups while the control and reference groups received distilled water (2 ml/kg, p.o) and omeprazole (20 mg/kg, p.o) respectively. The oral median lethal dose LD50 in mice was estimated to be greater than 5000 mg/kg and the phytochemical analysis revealed the presence of anthraquinones, flavonoids, phenols, saponins, steroids, tannins,and terpenes while flavonoids, tannins and terpenes were present in the fraction. The results show that the extract and fraction significantly ($p < 0.05$) reduced the ulcer index from 13.0 ± 1.25 to 3.00 ± 0.99, 13.0 ± 1.25 to5.20 ± 0.31 and from 12.0 ± 0.68 to 2.81 ± 0.79, 12.0 ± 0.68 to 4.40± 0.67 in the ethanol and ASA induced ulceration respectively. Percentage ulcer inhibitions of extract and fraction at 400 mg/kg for ethanol andASA induced ulcers were 79.9, 60.0, 76.7 and 63.3 % respectively. African locust been tree stem bark is a potential source of new anti-ulcer agent.

Keywords:African locust bean tree,Anti-ulcer activity, Phytochemicals, Stem bark

INTRODUCTION

Peptic ulcer is one of the world's major gastrointestinal disorders and affecting 10% of the world population[1]. The disease is characterized by inflammation of the stomach or duodenal lining, these are ulcers on the digestive tract membrane. The ulcers may be located in the duodenum, these ulcers are called duodenal ulcers while those that are located in the stomach are called gastric ulcers [2].

Peptic ulcer disease is an imbalance between the gastric offensive factors like acid and pepsin and defensive mucosal factors like environmental and host factors [3]. Several factors are also associated in the occurrence of peptic ulcer including stressful lifestyle, alcohol consumption, use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDS), Helicobacter pylori infections, and smoking, lower socio-economic status and

family history[4]. Complications include gastrointestinal bleeding, perforations, penetration of ulcer into adjacent organs and gastric outlet obstruction [5].

Anti-ulcer drugs such as proton pump inhibitors, H2 receptor antagonists, cytoprotectants, demulcents, anti cholinergics, antacids and prostaglandin analogues are used for the treatment of ulceration [6]. Clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy [4].

In the developing countries, 75-80% of the world population still use herbal medicines for primary health care because of better cultural acceptability, better compatibility with human body and lesser side effects [4]. Therefore, medicinal plants have always been the main sources of new drugs candidates for the treatment of gastric ulcer [7].

Parkiabiglobosa (Jacq.) R. Br. Ex G. Don (family Fabaceae) popularly known as African locust bean tree is a perennial, deciduous tree common in West African region. Medicines derived from African locust bean tree are of great value to rural communities that cannot afford or do not have access to "modern medicine" [8]. In Nigeria and other West African countries the stem bark, leaves and seeds of African locust bean tree have been used traditionally for the treatment of ulcers [9]. The result of acute toxicity study of the stem bark, leaf and root of African locust bean tree indicated that LD_{50} fell within the range of 500 – 5000 mg/kg body weight confirming them to be only slightly toxic and hence not potentially dangerous [9].

The aim of this study is to evaluate the gastroprotective activity of the plant and identify the phytochemicals responsible for this activity.

MATERIALS AND METHOD

Collection of plant materials

The fresh stem barks of African locust bean tree were collected in a bush around Chaza, a village near Suleja, Niger State, Nigeria. Identification and authentication were done (Ethno botanist) MallamMuazzamWudil of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

Drugs, chemicals and reagents

All chemicals, drugs and reagents used in this investigation were of analytical grade. All chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, U.S.A.).

Preparation of the plant materials

The plant material (stem bark) was air dried under shade and then ground into coarse powder with a pestle and mortar. 200 g of the powdered bark was evaporated by cold maceration using 1 L of distilled water. The mixture was evaporated in a carefully regulated water bath (maintained at 65°C to yield 20.2 g of a brown solid extract. The extract was stored in a refrigerator at 4°C through the period of the study to preserve the prepared extract[10]. The methanol extract was partitioned with N-hexane, ethylacetate and methanol successively to give 13.0g of a dark brown solid extract of methanol fraction.

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Preliminary phytochemical screening

The methanol stem bark extract and fraction were subjected to various qualitative phytochemical tests, to identify the secondary metabolites using standard phytochemical procedures and tests[10].

Animals

Adult male Swiss mice (20-30 g) and Wistar rats (180-200 g) maintained at Animal Facility Centre (AFC) of the Department of Pharmacology and Therapeutics, Bingham University were used for the study. The animals were fed with commercial pellets with free access to purified drinking water ad libitum, standard conditions of 12h:12h light/dark cycle, and temperature (23˚C-25˚C). All of the applied protocols (BU/105/16) was approved by Bingham University Research Ethics Committee

Acute toxicity test

Acute toxicity study was carried out to determine the median lethal dose (LD_{50}) using the modified method ofLorkes[11].

Experimental design

Ninety animals in total were used for the experiment. The animals were divided into two groups of 45 animals each following two experimental models (ethanol and ASA-induced ulcer models). In each experimental model, animals were further subdivided into nine groups of five animals each for the study. Group 1, 2 and 3 received distilled water (2 ml/kg, negative control) and omeprazole, cimetidine (20 mg/kg, 100mg/kg positive control) respectively. Groups 4, 5, 6, 7, 8 and 9 were given 100, 200 and 400 mg/kg b. wt. of methanol extract and fraction respectively and administration was orally for all groups.

Ethanol-induced ulcer

Ethanol ulcer was induced according to the method described by Aguwa and Ukwe [12]. Ulceration was induced in 24 h fasted rats, after one hour drug treatment, oral administration of 1 mg/kg of 80 % ethanol was givento all animals. The rats were sacrificed with chloroform anesthesia after one hour. The stomachs were isolated, washed gently under clean flowing water and cut open along the greater curvature. The stomachs were then fixed in 10 % formalin and craters observed and ulcer scores were recorded using a standard method [12].

ASA- induced ulcer

Acetyl salicylic acid -induced ulcer was carried according to the method described by Williamson et al [13].Food was withdrawn 24 hours and water one hour before drug treatment. 200 mg/kg of acetyl salicylic acid was given to each rat orally and the rats were sacrificed 4 hrslater as described above. Stomachs were isolated, fixed and ulcers counted using the above mentioned method [13].

Statistical analysis

The data were statistically evaluated by one way ANOVA. Comparison between treatment and control group were made by Student's t- test then followed with Fisher's exact. Differences between groups were considered significant at $P<0.05$

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RESULTS

Phytochemical test

Phytochemical analysis showed that methanol extract and fraction had similar constituents namely flavonoids, tannins and terpenes. Anthraquinones, phenols, saponins and steroids were found in the methanol extract. However, glycosides and alkaloids were absent (Table1).

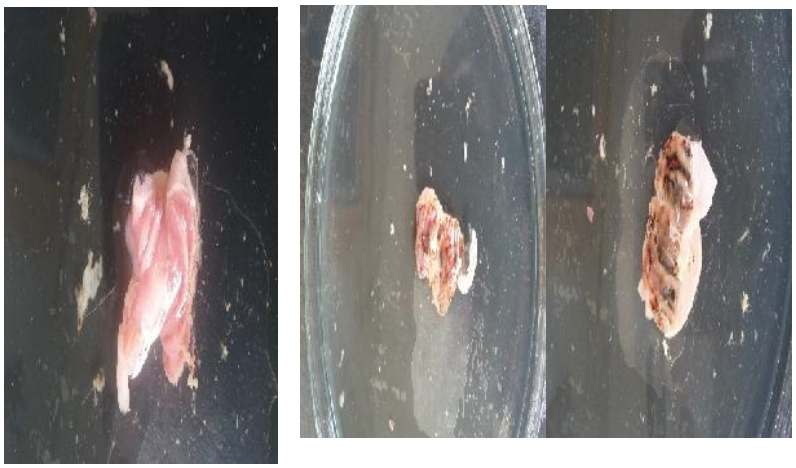
Table 1:Phytochemical analysis of methanol extract and fraction of stem bark of African locust bean tree

Test	African locust bean tree methanol extract	African locust bean tree methanol fraction
Anthraquinones	+	-
Glycosides	-	-
Flavonoids	+	+
Alkaloids	-	-
Phenols	+	-
Saponins	+	-
Steroids	+	-
Tannins	+	+
Terpenes	+	+

+ Presence, - Absence

Acute toxicity study

The behavioral signs of toxicity exhibited by the animals were vigorous paw licking and dullness. No mortality was observed in the different groups of mice that received the extract and fraction, even in the ones treated with (5000mg/kg;b.w, P.O).



A B C

Figure 1: Photomicrographs of ulcerations

A=Normal; B=Ulceration caused by Ethanol; C= Ulceration caused by ASA

Ethanol-induced gastric ulcer model

Table 2 shows effects of methanol extract and fraction of African locust bean tree stem bark on ethanol-induced ulcers in relation to omeprazole and cimetidine. The methanol extract and fraction exhibited significant (($p<0.05$) dose-dependent reduction in ulcer index at doses of 200 and 400 mg/kg. Methanol extract showed higher inhibition (13.0 ±1.25 to 3.00± 0.99) of ulcer index.

Table 2: Effects of Methanol extract and fraction of African locust bean tree stem bark on ethanol-induced ulcers in rats (n=5)

Group	Treatment	Dosage (P.O)	Mean ulcer index ± SEM	% ulcer inhibition
1	Distilled water	2ml/kg	13.0 ±1.25	0.00
2	Omeprazole	20mg/kg	4.20 ± 0.78*	67.7
3	Cimetidine	100mg/kg	3.20± 0.57*	75.4
4	Methanol Extract	100mg/kg	7.81± 0.44	39.9
5	Methanol Extract	200mg/kg	4.42 ± 0.69*	66.0
6	Methanol Extract	400mg/kg	3.00± 0.99*	76.9
7	Methanol fraction	100mg/kg	8.42± 1.05	35.2
8	Methanol fraction	200mg/kg	5.80± 0.85*	55.3
9	Methanol fraction	400mg/kg	5.20± 0.31*	60.0

* $P<0.05$ statistically different from distilled water

ASA- induced gastric ulcer model

Similarly as shown in Table 3, the extract and fraction significantly ($p<0.05$) reduced the ulcer index from 12.0± 0.68 to 2.81± 0.79 and 4.40 ± 0.67 (400mg/kg) in the ASA-induced ulceration group, the inhibition was comparable with that of omeprazole and cimetidine.

Table 3: Effects of Methanol extract and fraction of African locust bean tree stem bark on ASA -induced ulcers in rats (n=5)

Group	Treatment	Dosage (P.O)	Mean ulcer index ± SEM	% Ulcer inhibition
1	Distilled water	2ml/kg	12.0± 0.68	0.00
2	Omeprazole	20mg/kg	3.22±1.00*	73.2
3	Cimetidine	100mg/kg	2.81± 0.24*	76.6
4	Methanol extract	100mg/kg	4.00± 0.56*	66.7
5	Methanol extract	200mg/kg	3.60± 0.88*	70.0
6	Methanol extract	400mg/kg	2.81± 0.79*	76.6
7	Methanol fraction	100mg/kg	9.62± 1.14	19.8
8	Methanol fraction	200mg/kg	4.61± 0.39*	61.6
9	Methanol fraction	400mg/kg	4.40 ± 0.67*	63.3

* $P<0.05$ statistically different from distilled water

DISCUSSION

Phytochemicals refer to a wide-variety of compounds produced by plants with no nutritive value. They are promoted for their protective and disease-preventive properties according to the American Cancer Society website [6]. Phytochemical screening of the methanol extract and fraction of African locust bean tree showed the presence of flavonoids, tannins, and terpenes which are known to have cytoprotectiveactivity for which anti ulcerogenic efficacy has been extensively confirmed [14].

Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants [15, 16]. It has been suggested that these compounds will be able to stimulate mucous, bicarbonate and the prostaglandin secretion. Furthermore, they have been reported to counteract the deleterious effects of reactive oxidants in gastrointestinal lumen [17].

Also other phytoconstituents present in methanol extract like phenols and saponin have been reported in several anti-ulcer literatures as possible gastro protective agents [3]. Phenolic compounds are special metabolites that have significant antioxidant activity [8, 17]. It is reported in the literature that phenolic compounds participate significantly in the gastroprotection of a great number of vegetal extracts [18]. Saponins, are capable of promoting gastric mucosal formation; reduce gastric acid secretion and inhibit pepsinogen production thereby reducing gastric lesions and ulcers [6]. Therefore the presence of these other phytochemicals in the methanol extract of African locust bean tree may be attributed to higher gastroprotective activity evidenced.

The acute toxicity of the crudeextract and methanol fraction were investigated to determine any adverse effect that may arise as a result of a single contact or multiple exposures in a short time within 24 h period.Lorke method was employed for determination of LD_{50} because of some of its attributes which include few numbers of animals to obtain adequate information on the acute toxicity and on the LD_{50}, has no limitation, can be used for crude extract and fraction, and also for oral route of drug administration [11].

Substances with LD_{50} values greater than 5000 mg/kg of body weight are considered to show low toxicity. Thus the crude extract and fraction can be classified in the category of substances with low toxicity [19]. The high safety profile obtained may have been responsible for their wide spread use in the treatment of ulcer, malaria, pain and inflammation.

The anti-ulcer activity was carried out in two different models, the model such as ethanol –induced ulcer and acetyl salicylic acid - induced ulcers. The percentage ulcer protection is been observed in all the models, but the extent of percentage protection is more with crude extract in ethanol induced ulcer and acetyl salicylic acid induced ulcer. The percentage of ulcer protection variance with the standard omeprazole and cimetidineis less when compared tomethanol extract of African locust bean tree.

Ethanol is the most widely used agent in experimental models for the evaluation of the antiulcerative activity in animals [20, 21]. It represents a form of gastric irritation resulting from the inhibition of prostaglandins synthesis, ethanol necroses the superficial

cells of the gastric mucous membrane by precipitation of the cytoplasmatic components, interrupting the function of the cell mucous membranes, with the participation of vasoactive mediators released, such as leukotrienes C4 (LTC4) and histamine [22]. These mediators cause the submucous membranes to constrict with a subsequent blood flow stasis of the microcirculation in the mucous membrane, with the formation of edema, which may contribute to the increase of lesions in this model [23, 24]. Ethanol induces the solubilization of the mucous constituents in the stomach, increases the flow of sodium and potassium in the lumen, increases the pepsin released, and decreases the tissue levels of DNA, RNA and proteins, leaving the mucous membrane unprotected, leading to an injury in the tissue [20].

Since ethanol causes gastric ulcers by lowering protective factors in the gastric mucosa and ulcer induction is characterized by heavy bleeding due to immediate stasis in the blood flow [17]. It is possible that the methanol extract and fraction of African locust bean tree contain compounds that can enhance protective factors and restore gastric blood circulation.

The antioxidant effect of African locust bean tree stem bark may represent another mechanism that contributes to its anti-ulcer activity. Studies have shown that ethanol increases the production of reactive oxygen species (ROS). In ischemia and reperfusion experiments, lesions appear in the cells of the gastric mucous membrane due to the formation of ROS [25]. Builders et al, 2012, demonstrated the antioxidant activity of crude extract and methanol fraction of African locust bean treedue to the presence of flavonoids and tannins[10]. Flavonoids and tannins are phenolic compounds and plant phenolics are major group of compounds that act as primary antioxidants or free radical scanvergers[8, 26].

Acetyl salicylic acid is a non-steroidal anti-inflammatory drug which produces its effects by inhibiting prostaglandins synthesis. Increase in prostaglandins particularly PGE2 and PGI2 has been associated with cytoprotection[27]. Therefore, agents that inhibit the effects of nonsteroidal anti-inflammatory drugs (NSAIDs) such as acetyl salicylic acid will exhibit cytoprotection. The results obtained from using the acetyl salicylic acid ulcer model showed that the extract and fraction may significantly inhibit the aforementioned gastric effect of acetyl salicylic acid and thereby enhance cytoprotection. Usually some substances like the NSAIDS produce gastric mucosal irritation in addition to various degrees of analgesic, anti-inflammatory and antipyretic effects [27].

It is well known that inhibition of prostaglandin synthesis, which is essential for mucosal integrity and regeneration, will trigger the mucosal lining damage. It is also believed that the extract and fraction exert their antiulcer activity by increasing the synthesis of endogenous prostaglandins, which in turn promote mucus secretion and enhance the mucosal barrier against the actions of various damaging agents [7]. African locust bean tree bark has also been reported to possess anti -inflammatory activity [8, 9], therefore gastroprotective effect seen with African locust bean tree could be attributed to its anti-inflammatory activity.

CONCLUSION

African locust bean tree possesses significant anti-ulcer activity in animal models. It has a gastric antisecretory and acid neutralizing effect that are comparable to reference drugs omeprazole and cimetidine. The anti-ulcer activity is probably due to the presence of bioactive compounds like flavanoids, tannins and terpenes. Further studies are required to confirm the exact mechanism underlining the ulcer healing and protecting property of the extract and to identify the chemical constituents responsible for it.

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