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Gastroprotective effect of the aqueous leaf extract of *Guiera senegalensis* in Albino rats

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ABSTRACT

Objective: To evaluate the effect of aqueous leaf extract of *Guiera senegalensis* (*G. senegalensis*) on gastric mucosal damage using different ulcer models. **Methods:** Considering the above claims, the present study was undertaken to validate the gastroprotective potential of the aqueous leaf extract of this plant against ethanol, water immersion and Aspirin induced ulcer models. **Results:** The leaf extract (50, 100 and 200 mg/kg, *p.o.*) significantly ($P < 0.05$) decreased the ulcer index in all assays used. **Conclusions:** The results obtained, provide strong evidence of antiulcer activity of the leaf extract of *G. senegalensis* and support the traditional uses of the plant for the treatment of ulcer.

1. Introduction

Stress has been found to be involved in the pathogenesis of variety of states which includes muscle pain, hypertension, endocrine disorder, male infertility, peptic ulcer and gastritis. Peptic ulcer is a benign lesion of gastric or duodenal mucosa occurring at the site where the mucosal epithelium is exposed to acid and pepsin. There is always confrontation in the stomach and small intestine between acid–pepsin aggression and mucosal defense. Usually, the mucosa can withstand the acid–pepsin attack and remain healthy. That is, a mucosal ‘barrier’ to back diffusion of acid is maintained[1]. However, an excess of acid production

or an intrinsic defect in the barrier function of the mucosa can allow the defense mechanism to fail, then result into ulcer. Moreover, treatment of peptic is generally based on inhibition of gastric acid secretion by H2 antagonists and proton pump inhibitors such as omeprazole and anti–muscarinics as well as acid–independent treatment by sucralfat and bismuth[2].

Considering the several side effects of modern medicine, indigenous drug with fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. Recent studies found that different substances from plant sources such as polyphenols, tocopherols, alkaloids and flavonoids[3], not only afford gastroprotection but also accelerate ulcer healing.

Guiera senegalensis J.F. Gmel (Combretaceae) (*G. senegalensis*) is a shrub that grows to about 3 m high. In Northern Nigeria, the Hausas call it ‘sabara’, while the Fulanis call it ‘geloki’. The plant is indigenous to Nigeria,

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Mauritania and Sudan. The leaves, fruits and roots are used medicinally. The leaf has a bitter taste and is widely acknowledged as a 'cure-all' medicine.

G. senegalensis is one of such medicinal plants whose therapeutic application no doubt has a folkloric background. The present study was designed to evaluate the effect of aqueous leaf extract of this plant on gastric mucosal damage using different ulcer models.

2. Materials and methods

2.1. Collection and preparation of plant material

The plant material was collected in April, 2010 at Chaza, Suleja, Niger State, Nigeria. The plant was identified by Mallam Ibrahim Muazzam and Mrs. Jemilat A. Ibrahim at the Department of Medicinal Plant Research and Traditional Medicine, NIPRD (Herbarium voucher number: 6457). The international plant number index is Combretaceae *G. senegalensis* J.F. Gmel. Syst. Nat. ed. 13 (bis). 2 (1): 675. 1791 (late Sept–Nov, 1791 (IK).

The leaves were cleaned and air-dried at room temperature for 7 day and ground to fine powder using mortar and pestle. Three hundred and fifty grams of the powdered material was macerated in 1.5 L of distilled water and left for 24 h after which it was filtered. The filtrate was dried on water bath to give a yield of 38 g (10.86% w/w) of the aqueous leaf extract used for the study.

2.2. Phytochemical screening

The phytochemical composition on the aqueous leaf extract of *G. senegalensis* was determined using standard procedures^[4] for detecting the presence of secondary metabolites; alkaloids, tannins, saponins, flavonoides, sterols, terpenes, carbohydrates and glycosides.

2.3. Acute toxicity test

The LD₅₀ of the leaf extract was tested to determine the safety of the agent as described by OECD^[5, 6]. The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice per group and administered 10, 100 and 1 000 mg/kg of the extract orally. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality. The results of this phase informed the choice of doses for the second phase, in which 1 600, 2 900 and 5 000 mg/kg were administered to another set of three mice per group. The mice were also observed for signs of toxicity and mortality.

2.4. Animals

Adult Wistar rats (180–250 g) of both sexes were used for the study. The animals were kept in cages and housed under the same condition of 12:12 h light cycle and had water ad libitum. Permission and approval for animal studies were obtained from College of Medical Sciences Animal Ethics committee, University of Calabar.

2.5. Ethanol-induced gastric ulceration in rats

The experimental rats were fasted for 48 h prior to the study. They were randomly divided into five groups of six per group. Group 1 and 2 were administered normal saline (10 mL/kg) and ranitidine (20 mg/kg) respectively. Group 3, 4 and 5 were administered (50, 100 and 200 mg/kg) of the aqueous leaf extract of *G. senegalensis*. All administered orally. One hour later, ulceration was induced by intragastric instillation of 1 mL of 90% ethanol. One hour after, rats were sacrificed under ether anaesthesia and their stomachs removed and opened along the greater curvature to macroscopically examine the lesions^[7]. The number, length and severity of the erosions were noted and scored^[8].

2.6. Water immersion stress-induced ulceration in rats

The animals were fasted for 48 h prior to the experiment but had water ad libitum. They were randomly divided into five groups of six per group. Group 1 and 2 were administered normal saline (10 mL/kg) and ranitidine (20 mg/kg) respectively. Group 3, 4 and 5 were administered (50, 100 and 200 mg/kg) of the aqueous leaf extract of *G. senegalensis*. All administered orally. Stress ulcers were induced by forcing the rats to swim for 1 h in a cylinder with a height of 45 cm and diameter of 25 cm containing water to the height of 35 cm maintained at (30±1) °C. After swimming, rats were removed, dried and injected intravenously via the tail vein with 30 mg Evans blue. Ten min later, animals were sacrificed under ether anaesthesia and their stomachs removed. Formosaline (2% v/v) was then injected into the ligated stomachs for storage overnight. The following day, each stomach was opened along the greater curvature, washed in warm water; macroscopically and microscopically examine the lesions. The number of erosions was noted and the severity recorded. Mean scores for each group were expressed ulcer index (UI). From the data, the percentage inhibition of ulceration was determined.

2.7. Aspirin-induced gastric ulcer in rats

The rats employed for the study were deprived of food for 48 h but had access to water ad libitum. They were divided

into five of six in each cage. They were randomly divided into five groups of six per group. Group 1 and 2 were administered normal saline (10 mL/kg) and ranitidine (20 mg/kg) respectively. Group 3, 4 and 5 were administered (50, 100 and 200 mg/kg) of the aqueous leaf extract of *G. senegalensis*. All administered Thirty min later ulcer was induced by oral administration of 150 mg/kg of Aspirin to all the groups. The animals were sacrificed 5 h after and their stomachs opened along the greater curvature and washed to remove gastric contents and examined under a dissecting microscope with square-grid eye piece to assess the formation of ulcers. For each stomach, ulcerated and total areas were measured as mm².

2.8. Statistical analysis

Results were analyzed as mean ± S.E.M. The significance of difference between the control and treated groups were determined using one way ANOVA, followed by student’s *t*-test. *P*<0.05 was considered to be statistically significance.

3. Results

3.1. Phytochemical test

Phytochemical screening of the aqueous leaf extract of *G. senegalensis* revealed the presence of alkaloids, tannins, flavonoids, sterols, terpenoids, and saponins. All these classes of compounds are reported to show important biological activities[9, 10].

3.2. Acute toxicity test

There was no mortality observed in mice upon oral administration of the aqueous extract, even at doses as high as 5 000 mg/kg signifying that the oral LD₅₀ was greater than 5 000 mg/kg.

3.3. Effect of *G. senegalensis* aqueous leaf extract on ethanol-induced gastric ulcers

Pre-treatment of rats with the aqueous extract at doses employed (50, 100 and 200 mg/kg) produced 56.12%, 60.19% and 76.00% protection against gastric mucosal damage, while the standard, ranitidine 20 mg/kg exhibited 88.00% protection under the same condition (Table 1).

3.4. Effect of *G. senegalensis* aqueous leaf extract on water immersion stress induced ulcers

In water immersion stress-induced ulcer model,

G. senegalensis aqueous leaf extract (50, 100 and 200 mg/kg) showed protection index of 70.44%, 74.00% and 81.55% respectively, whereas Ranitidine, exhibited 85.11% protection (Table 2).

3.5. Effect of *G. senegalensis* aqueous leaf extract on aspirin induced ulcers

The results obtained with the extract in this experimental model are shown in Table 3. *G. senegalensis* provided marked protection at the doses of 50, 100 and 200 mg/kg with protection in ulcer index of 53.81%, 61.43% and 76.91%, respectively. The standard drug, Ranitidine, showed a protection of 92.38%.

Table 1

Effect of aqueous leaf extract of *G. senegalensis* on ethanol induced ulcers in rats.

Group	Dose	Ulcer index (UI)	% maximal protection of ulceration
Normal saline	0.2 mL/kg	4.17±0.31	–
Ranitidine	20 mg/kg	0.50±0.34	88.00*
<i>G. senegalensis</i>	50 mg/kg	1.83±0.40	56.12*
	100 mg/kg	1.66±0.55	60.19*
	200 mg/kg	1.00±0.36	76.01*

Results are expressed as mean ± S.E.M, *significantly different from control at *P*< 0.05.

Table 2

Effect of aqueous leaf extract of *G. senegalensis* on water immersion stress induced ulcers in rats.

Group	Dose	Ulcer index (UI)	% maximal protection of ulceration
Normal saline	0.2 mL/kg	4.50±0.34	–
Ranitidine	20 mg/kg	0.67±0.21	85.11*
<i>G. senegalensis</i>	50 mg/kg	1.33±0.33	70.44*
	100 mg/kg	1.17±0.31	74.00*
	200 mg/kg	0.83±0.31	81.55*

Results are expressed as mean±S.E.M, *significantly different from control at *P*< 0.05.

Table 3

Effect of aqueous leaf extract of *G. senegalensis* on aspirin induced ulcers in rats.

Group	Dose	Ulcer index (UI)	% maximal protection of ulceration
Normal saline	0.2 mL/kg	4.33±0.21	–
Ranitidine	20 mg/kg	0.33±0.21	92.38*
<i>G. senegalensis</i>	50 mg/kg	2.00±0.26	53.81*
	100 mg/kg	1.67±0.33	61.43*
	200 mg/kg	1.00±0.45	76.91*

Results are expressed as mean±S.E.M, *significantly different from control at *P*< 0.05.

4. Discussion

The data presented here provided scientific evidence that aqueous leaf extract of *G. senegalensis* may contain biologically active substances with potential anti ulcer properties.

Due to the reported side effects of available anti ulcer drugs, focus has been shifted towards natural products as the new sources of anti ulcer agents. With the growing interest in natural medicine, various plants have been studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing ulcer^[11]. In addition, medicinal plants have been known to be amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of various diseases including gastric and duodenal ulcers^[12].

The pathogenesis of ethanol-induced gastric damage in rats involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leucotriene C4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage. The pre-treatments with *G. senegalensis* significantly inhibited gastric lesions produced by acidified ethanol. The fact that the extract protected the gastric mucosa of rats against ethanol-induced acute mucosal damage with a reduction of the ulcer index indicates that *G. senegalensis* could be an effective gastroprotective agent. The mechanism for the mucosal protective action of this extract may be due partly to the stimulation of PG synthesis since endogenous PGs play a crucial role in gastroprotection^[13]. It is also possible that an increase in gastric mucus or a possible leukotriene antagonism may contribute to the gastroprotective effect of this plant extract.

Physiologic stress stimulate adenohipophyical axis and causes a concomitant release of endogenous opiates. Stress also produces severe gastric erosion through the activation of central vagal discharge. The endogenous opiates released during stress can cause mucosal congestion by a peripheral mechanism, leading to the development of gastric ulcers. Stress induced ulcers are as a result of autodigestion of gastric mucosal barrier, accumulation of HCL and generation of free radicals^[14]. *G. senegalensis* leaf extract showed a dose dependent ulcer curative ratio in stress induced ulcers. The ulcer inhibition percentage of the extract was closer to the standard drug ranitidine. Therefore, it may be concluded that the plant extract may follow ranitidine inhibitory mechanism.

Aspirin is a potent cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow^[14]. Aspirin increases acid secretion and produce microvasculature damage by generation of free radicals. It is well known that inhibition of prostaglandin synthesis which is essential for mucosal integrity and regeneration will trigger the mucosal lining damage. However, it is believed that *G. senegalensis* leaf extract exert its 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. The gastroprotective effect exhibited by *G. senegalensis* may be attributed to the presence of flavonoids and polyphenolic compounds, saponins and tannins^[15, 16]. These compounds most likely inhibit gastric mucosal injury by scavenging the stress-generated oxygen metabolites^[17].

Flavonoids have anti-inflammatory activity and protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species^[18]. Hence, many studies have examined the antiulcerogenic activities of plants containing flavonoids. Plants containing flavonoids were found to be effective in preventing this kind of lesion, mainly because of their antioxidant properties. Recently, the antioxidant activity of flavonoids has attracted interest because of the strong evidence that oxidation processes are involved in the mechanisms of several gastric disorders, including ulcerogenesis^[19].

Extensive damage to gastric mucosa by stress leads to increase neutrophil, which are a major source of inflammatory mediators, inhibit gastric ulcer healing by mediating lipid peroxidation through the release of highly cytotoxic and tissue damaging reactive oxygen species such as superoxide, hydrogen peroxide and myeloperoxidase derived oxidants. Suppression of neutrophil infiltration during inflammation enhances gastric ulcer healing.

Furthermore, leukotrienes antagonist and 5-lipoxygenase inhibitors have been demonstrated to inhibit NSAIDs-induced gastric ulceration in rats^[20]. Hence, the observed antiulcer activity of aqueous leaf extract of *G. senegalensis* could also be suggested to be due to inhibition of 5-lipoxygenase partway or to leukotriene's antagonistic activity.

In conclusion, the present study has shown that the leaf extract of *G. senegalensis* possesses significant antiulcer activity, thus justifying its wide spread use by local population in the treatment of gastric ulcer. Further studies are required to confirm the exact mechanism of underlining

the ulcer healing and protecting properties of the extract.

Conflict of interest statement

We declare that we have no conflict of interest.

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