

Full Length Research Paper

Studies on gastrointestinal properties of ethanolic leaf extract of *Salacia lehmbachii* in Wistar rats

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***Salacia lehmbachii* Loes, is used traditionally in Nigeria for the treatment of gastrointestinal disorders. The aim of this study was to investigate the anti-ulcer and anti-diarrhoeal activities of the ethanolic leaf extract of *S. lehmbachii*. The ethanolic leaf extract was evaluated for castor oil-induced diarrhoeal, intestinal transit as well as intestinal fluid accumulation in rats, while indomethacin, water immersion stress-induced and histamine were used for anti-ulcer tests. The extract at the doses used significantly ($P<0.05$) decreased castor oil-induced diarrhoea in rats as judged by a decrease in the number of wet faeces in the extract treated rats. Moreso, the leaf extract inhibited the propulsive movement of intestinal contents. *S. lehmbachii* also showed a dose-related inhibitory activity on castor oil-induced intestinal fluid accumulation in rats. The leaf extract of *S. lehmbachii* significantly ($P<0.05$) reduced the ulcer index in all assays used. The results of the current study support the folkloric usage of *S. lehmbachii* leaf extract in the management of gastrointestinal disorders in Nigerian herbal traditional medicine.**

Key words: *Salacia lehmbachii*, leaves, herbal medicine, antidiarrhoea, antiulcer, rats.

INTRODUCTION

Medicinal plants are known to possess a variety of substances and are used in the treatment of many kinds of ailments in traditional medicine. The beneficial effects of medicinal plant materials result from the combinations of secondary products present in the plant. These constituents are capable of producing definite

physiological action on the human body (Erdogru, 2002). Natural products from plant materials are being continuously used to treat different diseases. Investigation of natural and synthetic compounds has been the source of many therapeutic agents (Mahesh and Satish, 2008). Plant products play essential role in

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drug development programs of the pharmaceutical industry (Parke and Sapota, 1996). In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders. These agents have fewer side effects, easily accessible and also affordable (Edeoga et al., 2005).

Salacia lehmbachii, which belongs to the family Celastraceae is commonly found in the tropical rain forest region of West, Central and East Africa. The decoction of the leaves and roots are used traditionally in the folk medicine in Nigeria for the treatment of a number of diseases including renal dysfunction, pain, fever, and gastrointestinal disorders (Essien et al., 2015a, b; Lapah et al., 2014). In present study, the ethanolic leaf extract of *S. lehmbachii* was investigated for its antidiarrhoeal and antiulcer activities to ascertain the folkloric claim made by the indigenes.

MATERIALS AND METHODS

Plant collection

The leaves of *S. lehmbachii* were collected from Akwa Ibom State, Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Botany, University of Calabar, Nigeria, where a voucher specimen (No.688) was deposited at the herbarium for reference.

Extraction

The leaves were cleaned and taken to the laboratory, where they were cut into pieces and air-dried at room temperature for 7 days and ground to powder using mortar and pestle. 500 g of the ground leaf powder was then macerated in 1.5 L of ethanol for 24 h and was filtered. The filtrate was dried on a water bath at reduced temperature to recover the extract and the yield was calculated to be 12.5% w/w. The leaf extract was subsequently reconstituted in normal saline at appropriate concentration for the experiment.

Phytochemical screening

Phytochemical analysis of the ethanolic leaf extract was carried out employing standard procedures to determine the following compounds: flavonoids, tannins, saponins, terpenoids, alkaloids, cardiac glycosides, steroids, resins, anthraquinones and phlobatanins (Mukherjee, 2006; Sumitra et al., 2006).

Animals

Adult Wistar rats (180 to 220 g) of both sexes obtained from Animal House, Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria, were used for the study. The animals were housed in cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/light cycle. The animals were fed on standard pellets and had free access to water. NIH guide for the care and use of Laboratory Animal was employed in this study (NIH, 1985).

Acute toxicity study of the extract

The LD₅₀ of the leaf extract was tested to determine the safety of

the agent according to the guidelines set by OECD (2010). These studies were done in two phases. Nine rats, randomized and divided into three were used in the first phase. The rats were orally administered with 100, 600 and 1000 mg/kg of the leaf extract, respectively. The animals were observed for the first 4 and 24 h for signs of toxicity and mortality. This was followed by the second phase in which 2000, 3000 and 5000 mg/kg of the extract was administered to the next three groups of three rats per cage. The signs of toxicity and mortality were observed for 24, 48 and 72 h, respectively.

Induction of diarrhoea with castor oil

Anti-diarrhoea activity of the extract was evaluated using the castor oil-induced diarrhoea model in rats (Akuodor et al., 2011; Capasso et al., 2008). Thirty rats fasted for 24 h were randomly grouped into five with six rats in each. Normal saline (20 ml/kg) was given to rats in group 1. The extract (100, 200, and 400 mg/kg) was given rats in groups 2, 3, and 4, while group 5 received 4 mg/kg loperamide. All were administered orally via cannula. One hour after the treatment, rats in all the groups were orally challenged with 1 ml castor oil. The rats in each group were then placed singly in cages with adsorbent paper on their floors. The diarrhoea episodes were observed for 4 h and the cumulative frequency of wet and formed stools was noted. Percentage protection was calculated using the mean stool frequency and anti-diarrhoea activity.

Intestinal transit test

The effect of the extract on gastrointestinal motility was determined as previously described (Akuodor et al., 2012). Thirty rats were randomly divided into five groups of six rats each and they fasted for 24 h prior to the test and had water *ad libitum*. Group 1 (control) was treated with 10 ml/kg normal saline. Group 2, 3 and 4 received 100, 200 and 400 mg/kg of ethanol leaf extract of *S. lehmbachii* orally, respectively. Group 5 was treated with 5 mg/kg Atropine (standard drug) orally. Thirty minutes after, 1 ml of charcoal (5% deactivated charcoal suspension in 10% tragacanth) was orally given to all animals and thirty minutes later, all were sacrificed. The distance travelled by the marker (charcoal) was then measured and expressed as a percentage of the total length of the small intestine (pylorus to caecum).

Intestinal fluid accumulation test

Intestinal fluid accumulation was determined by the method as described by Robert et al. (1976) with slight modification. Thirty rats of both sexes were divided into five groups of six each. Normal saline (20 ml/kg) was administered to group 1, while the extract group (2, 3 and 4) was treated with 100, 200 and 400 mg/kg, respectively. Group 5 was given 4 mg/kg loperamide. All administered orally. One hour later, 1 ml castor oil was orally given to all the test animals and 1 h after, they were sacrificed and their small intestines were removed after ligating the ends. Intestinal contents were collected by milking into a graduated tube and the measured volumes were recorded. Percentage inhibition was then determined by calculating the mean volume of intestinal contents and comparing it with values obtained from control group.

Indomethacin-induced gastric ulcer in rats

The method as described by Anosike and Ofoegbu (2013) was adopted with slight modification. Rats used for this experiment were fasted for 48 h having access to water *ad libitum*. They were

Table 1. Effect of the ethanolic leaf extract of *S. lehmbachii* on castor oil-induced diarrhoea in rats.

Treatment	Dose (mg/kg)	Frequency of diarrhoea in 4 h	% Inhibition
Normal saline	20 ml/kg	13.5±0.81	-
<i>S. lehmbachii</i>	100	5.5±0.67*	59
	200	3.67±0.21*	72
	400	2.5±0.67*	81
Loperamide	4	1.17±0.48*	91

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

grouped into five of six rats per cage. Group 1 was treated with 20 ml/kg normal saline. Groups 2, 3 and 4 were treated with 100, 200 and 400 mg/kg of the ethanolic leaf extract, while the standard drug ranitidine (20 mg/kg) was administered to group 5, respectively. All drugs were given orally. In this model, gastric ulcers were induced by indomethacin after 1 h of drug treatment. The animals were sacrificed by ether anaesthesia after 5 h for the determination of ulcerative index.

Water immersion stress-induced ulceration in rats

The experiment was performed according to the method of Akuodor et al. (2013). In this model, animals were fasted for 48 h prior to the study. The animals under study were grouped into 5 with 6 rats in each and treated with normal saline (20 ml/kg) in group 1. Those in groups 2, 3 and 4 were treated with the leaf extract (100, 200 and 400 mg/kg), respectively. Standard drug ranitidine (20 mg/kg) was administered to group 5. All administered by oral route. One hour after drug administration, rats were made to swim in a cylinder containing water to the height of 35 cm and maintained at 30±1°C for 1 h. Thereafter, animals were removed, dried and injected intravenously via the tail vein with 30 mg of Evans blue. Ten minutes later, they were all sacrificed under ether anaesthesia and their stomachs removed. Formolsaline (2% v/v) was then injected into the ligated stomachs for storage overnight. The next day, each stomach was opened, washed in warm water and examined under a dissection microscope.

Histamine-induced ulceration in rats

The effect of *S. lehmbachii* leaf extract on histamine-induced ulcers was carried out following the method of Bodhankar et al. (2006) with slight modification. Animals were fasted for 48 h prior to the experiment. After the fasting period, they were divided into five groups of six rats per cage. The control (group 1) was treated with 20 ml/kg of normal saline. The ethanolic leaf extract of *S. lehmbachii* (100, 200 and 400 mg/kg) was administered to groups 2, 3 and 4. Group 5 was treated with standard drug, ranitidine (20 mg/kg). The drugs were all given by oral route. Gastric ulcers were induced after 1 h by subcutaneous administration of 100 mg/kg of histamine. All the animals were sacrificed by ether anaesthesia 4 h later for the determination of ulcerative lesion index.

Measurement of ulcer index

Ulcer index of each rat was calculated following the methods as described by Malairajan et al. (2007). Ulcer index of the experimental rats were calculated by adding the values and their

mean values were determined as follows: (i) 0 = no ulcer, (ii) 1 = hemorrhagic and slightly dispersed ulcers less than 2 mm length, (iii) 2 = 1 ulcer, hemorrhagic and up to 5 mm length, (iv) 3 = more than 1 ulcer, each up to 5 mm length, (v) 4 = 1 ulcer above 5 mm in length, (vi) 5 = more than 1 ulcer above 5 mm in length. Percentage of ulcer protection index was calculated by adopting the following formula: % Protection = $(Uc - Ut/Uc) \times 100$, where Uc is the ulcer index in control group, and Ut is the ulcer index in treated groups.

Statistical analysis

The data are expressed as the mean ± standard error of mean (SEM) for each group. The results were statistically analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test. Differences were considered significant at $P < 0.05$.

RESULTS

Phytochemical screening

Phytochemical screening of the leaf extract revealed the presence of tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, resins and cardiac glycosides, while phlobatannins and anthraquinones were absent.

Acute toxicity test

The acute oral toxicity test showed normal behaviour of the treated rats. There were no lethality or toxic reactions observed. However, the experimental doses used (100, 200 and 400 mg/kg) were orally within safe margin.

Effect of *S. lehmbachii* on castor oil-induced diarrhoea

The ethanolic leaf extract of *S. lehmbachii* exhibited dose-dependent anti-diarrhoea activity in the study. The extract significantly ($P < 0.05$) decreased both the frequency of defaecation as well as the wetness of faecal dropping in rats. However, the effect of the leaf extract was less potent in comparison to the standard drug, loperamide (Table 1).

Table 2. Effect of the ethanolic leaf extract of *S. lehmbachii* on intestinal motility in rats.

Treatment	Dose (mg/kg)	Mean intestinal length (cm)	Mean distance travelled by marker(cm)	% Inhibition
Normal saline	20 ml/kg	88.17±0.79	85.17±1.19	-
<i>S. lehmbachii</i>	100	87.5±1.3.45	45.83±2.71*	46
	200	80.17±2.36	35.17± 1.70*	59
	400	82.0±1.48	31.33±2.27*	63
Atropine	5	88.67±1.26	29.17±1.47*	66

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

Table 3. Effect of the ethanolic leaf extract of *S. lehmbachii* on castor oil-induced enteropooling in rats.

Treatment	Dose (mg/kg)	Volume of intestinal content (ml)	% Inhibition
Normal saline	20 ml/kg	4.33±0.13	-
<i>S.lehmbachii</i>	100	1.58±0.14*	41
	200	1.42±0.09*	47
	400	1.18±0.09*	56
Loperamide	4	0.47±0.05*	83

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

Effect of *S. lehmbachii* on intestinal transit

S. lehmbachii leaf extract significantly ($P<0.05$) reduced distance travelled in the intestine by the marker in a dose-dependent manner. The standard drug, atropine at 5 mg/kg compared favourably with the extract at 400 mg/kg (Table 2).

Effect of *S. lehmbachii* on castor oil-induced enteropooling

The ethanolic leaf extract of *S. lehmbachii* was found to possess anti-enteropooling activity. The extract significantly ($P<0.05$) decreased intestinal fluid volume in rats. However, this effect was less potent in comparison with the standard drug, loperamide (Table 3).

Effect of *S. lehmbachii* on indomethacin-induced ulcers

Oral administration of ethanol leaf extract of *S. lehmbachii* at a dose of 100, 200 and 400 mg/kg exhibited dose dependent ulcer protection of 57, 72 and 82%, whereas the standard drug, ranitidine (20 mg/kg) had 89% inhibition (Table 4).

Effect of *S. lehmbachii* on water immersion stress-induced ulcers

Pre-treatment of ethanolic leaf extract of *S. lehmbachii* 1 h before water immersion stress-induced ulcers showed dose dependent protection of 59, 74 and 84% at doses of 100, 200 and 400 mg/kg, respectively. Ranitidine (20 mg/kg) showed an inhibition of 88% (Table 5).

Effect of *S. lehmbachii* on histamine-induced ulcers

The ethanolic leaf extract of *S. lehmbachii* significantly protected gastric mucosal against damage induced by histamine. The extract (100, 200 and 400 mg/kg) was found to possess remarkable ulcer protective properties of 52, 73 and 77%, respectively, whereas ranitidine (20 mg/kg) exhibited 88% protection (Table 6).

DISCUSSION

The phytochemical evaluation of the ethanolic leaf extract of *S. lehmbachii* revealed the presence of alkaloids, tannins, saponins, terpenoides, flavonoids, steroids and resins which showed that the plant is of high pharmacological importance. These classes of

Table 4. Effect of ethanolic leaf extract of *S. lehmbachii* on indomethacin-induced ulcers in rats

Treatment	Dose	Ulcer index (UI)	% protection of ulceration
Normal saline	20 ml/kg	4.25±0.36	-
<i>S. lehmbachii</i>	100 mg/kg	1.83±0.32*	57
	200 mg/kg	1.18±0.24*	72
	400 mg/kg	0.78±0.35*	82
Ranitidine	20 mg/kg	0.43±0.28*	89

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

Table 5. Effect of ethanolic leaf extract of *S. lehmbachii* in water immersion stress- induced ulcers in rats

Treatment	Dose	Ulcer index (UI)	% protection of ulceration
Normal saline	20 ml/kg	4.38±0.31	-
<i>S. lehmbachii</i>	100 mg/kg	1.80±0.27*	59
	200 mg/kg	1.15±0.24*	74
	400 mg/kg	0.72±0.32*	84
Ranitidine	20 mg/kg	0.53±0.34*	88

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

Table 6. Effect of ethanolic leaf extract of *S. lehmbachii* on histamine-induced ulcers in rats.

Treatment	Dose	Ulcer index (UI)	% protection of ulceration
Normal saline	20 ml/kg	4.28±0.29	-
<i>S. lehmbachii</i>	100 mg/kg	2.07±0.39*	52
	200 mg/kg	1.17±0.24*	73
	400 mg/kg	0.97± 0.31*	77
Ranitidine	20 mg/kg	0.52±0.23*	88

Data are mean ± SEM (n=6). *P < 0.05 compared to control group (ANOVA, Tukey's test).

compounds have been reported to show important biological effects (Longanga-Otshudi et al., 2000; Ghoghari and Rajan, 2006; Panda and Kar, 2007) and the presence of these constituents may be responsible for the anti-diarrhoeal properties seen in the extract. Earlier studies have shown that antidiarrhoeal potential of medicinal plants were due to its secondary metabolites (Kouitcheu et al., 2006). The anti-diarrhoeal effect of the plant extract may also be due the precipitation of proteins in enterocyte and production of protein tannates which result in decreased secretion and peristaltic movement (Salawu et al., 2007). The remarkable dose-dependent reduction in castor oil-induced diarrhoea in rats is a

demonstration of the effect of *S. lehmbachii* leaf extract as anti-diarrhoeal agent. The absence of death at oral treatment of over 5000 mg/kg suggests that ethanol leaf extract of *S. lehmbachii* is non-toxic acutely. It is therefore safe for oral use in the therapeutic treatment of diarrhoea.

The high safety profile observed may be responsible for its wide spread use in gastrointestinal disorders. The leaf extract has shown significant activity in reducing the frequency of castor oil-induced diarrhoea, a feat comparable to loperamide. Loperamide is an anti-diarrhoeal agent whose action is by increasing colonic phasic segmenting effects which inhibits presynaptic

cholinergic nerves in submucosal and myenteric plexuses. These activities lead to increased colonic transit time and faecal water absorption, hence reducing the frequency of defaecation (Yu et al., 2000; Cammilleri, 2004). Atropine and various doses of the leaf extract of *S. lehmbachii* reduced the propulsive movement in the charcoal meal study, atropine being more potent than the leaf extract at the doses used. The observed effect of castor oil-induced enteropooling suggests that the ethanolic leaf extract of *S. lehmbachii* exhibited significant antidiarrhoea by spasmolytic activity and also anti-enteropooling effects. However, *S. lehmbachii* may have supported Geiger's criteria for the classification of a drug as an antidiarrhoeal (Aniagu et al., 2005).

Ulcer formation induced by indomethacin is known to be related with inhibition of cyclooxygenase which prevent prostaglandin biosynthesis and this in turn inhibits the release of mucus, which is a defensive factor against gastrointestinal damage (Dengiz and Gursan, 2005). However, it is believed that *S. lehmbachii* exert its antiulcer activity by increasing the formation of endogenous prostaglandin synthesis, which in turn promote mucus secretion and enhance the mucosal barrier against the actions of various damaging agents (Malairanjan et al., 2007).

Stress induced ulcers are due to autodigestion of gastric mucosal barrier, presence of HCL and production of free radicals (Olaleye and Farombi, 2006). The ulcers are generated due to the release of histamine which results in increase in acid secretion and reduction in mucus production (Dengiz and Gursan, 2005). Stress may also cause an increase in gastrointestinal motility resulting in stomach folds which are more susceptible to damage when they come in contact with acid (Demirbilek et al., 2004). The ethanolic leaf extract of *S. lehmbachii* showed a dose dependent activity in stress induced ulcers. The reduction in ulcer index of the leaf extract was comparable to the standard drug ranitidine, which suggest that the ethanolic leaf extract may follow ranitidine inhibitory mechanism.

Histamine-induced gastric ulcers have long been recognized and mediated through stimulation of H₂ receptors and may result in enhanced gastric acid secretion and vasodilatation (Adinortey et al., 2013). Histamine does not only increase gastric acid secretion, but also causes disturbances of the gastric mucosa, abnormal motility and reduction in mucus production (Ghodekar et al., 2010). The leaf extract of *S. lehmbachii* significantly reduced histamine induced ulcers by probably blocking H₂ receptors, thus inhibiting gastric acid secretion.

In conclusion, the present study has shown that the ethanolic leaf extract of *S. lehmbachii* possesses significant gastrointestinal activities, thus justifying the wide spread use of this plant in traditional medicine for the treatment of gastrointestinal disorders. The bioassay-guided fractionation, identification and characterization of

the active principle(s) responsible for the gastrointestinal potential of the plant are in progress in our laboratory.

Conflict of interest

The authors have not declared any conflict of interest.

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