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Antiulcerogenic activity of leaf extract of *Saccharum officinarum* (Poaceae) in rodents

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ABSTRACT

Background:*Saccharum officinarum* (Family-Poaceae) is used in Ibibio ethnomedicine for the treatment of various diseases such as malaria and gastrointestinal disorders. The study aimed to evaluate the antiulcerogenic activity of the leaf extract against experimentally-induced ulcer in rats.

Methods:The leaf extract of *S. officinarum* (170-510 mg/kg) was investigated for antiulcer activity in rodents using indomethacin, ethanol and histamine-induced ulcer models in overnight fasted Wistar rats weighing 125-150 g. The rats were randomized into different groups and treated with extract and ulcerogens. The stomachs of the animals were removed, examined for ulcerations and scored accordingly.

Results: The leaf extract (170-510 mg/kg) was found to exert significant ($p<0.05$-0.001) and dose-dependent activity against indomethacin, ethanol and histamine-induced ulcers.

Conclusion: These results suggest that the leaf extract of *Saccharum officinarum* possesses antiulcerogenic potential which is due to the activities of the phytochemical constituents which can be explored for the treatment of ulcers.

Keywords: Antiulcerogenic, gastrointestinal disorders, *Saccharum officinarum*, ulcer

1.INTRODUCTION

Saccharum officinarum (Family-Poaceae) also called sugarcane thrives throughout tropical and subtropical regions worldwide. In traditional medicine, it is used in the treatment of diarrhoea, dysentery, eyes infirmities, fever, arthritis, bedsores, boils, cancer, colds, cough, opacity, skin sores, sore throat, hiccups, inflammation, laryngitis, splenic diseases, tumors, and wounds[1]. the leaf extract possesses some biological activities such as antibacterial and anthelmintic[2], anti-hyperglycaemic, anti-hyperlipidaemic[3], antioxidant [3,4], diuretic and antiurolithiatic[5], antidepressant and anticonvulsant[6], analgesic[7] and antimalarial[8], antioxidative stress and hepatoprotective [9], anti-inflammatory and antipyretic[10] activities. SAABMAL®: a polyherbal preparation containing *S. officinarum* is utilised as malarial remedy in Nigeria [11]. The leaves are employed in Ghana for the treatment of malaria locally[12]. Phytochemical screening of leaf extract of *Saccharum officinarum* revealed the presence of glycosides, phytosterols, saponins, tannins, flavonoids[2,13]. Some flavones and phenolics as well as their derivatives from the leaves of *S. officinarum* have been identified[8,14]. The medicinal potentials of the plant have been widely reported, but there is paucity of information on its toxicological potentials. We report in this paper the antiulcer activity of the leaf of *S. officinarum*.

2.MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

Fresh leaves of *Saccharum officinarum* were collected in June, 2020 from residential quarters in Uyo village in Uyo LGA, Akwa Ibom State, Nigeria. The leaves were identified and authenticated as

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Saccharum officinarum by Prof Margaret Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria and a voucher specimen (UUPH 215b) was prepared and deposited at the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

2.2 Methods

2.2.1 Extraction

Fresh leaves of *S. officinarum* were washed, cut into smaller pieces and dried under shade for two weeks. The leaves were further pulverized to powder using an electric grinder. The powdered leaf material (2 kg) was soaked in 50% ethanol (7.5 L) at room temperature (28 ± 2 °C) for 72 hours. It was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in vacuo at 40 °C using a rotary evaporator (BuchiLab Switzerland). The dry extract was stored in a refrigerator at -4 °C, until used for the proposed experiments.

2.2.2 Animals

In this study, male albino Wistar rats (135-170 g) were used. The animals were sourced from University of Uyo Animal house and sheltered in plastic cages. The rats were fed with pelleted standard Feed (Guinea feed) and given unlimited access to water. The study was approved by Faculty of Pharmacy Animal Ethics Committee, University of Uyo.

2.2.3 Evaluation of antiulcer activity

Indomethacin-induced ulcer: Male adult albino rats were used for the experiment. They were randomly divided into five groups of six rats each. Food was withdrawn 24 hours and water 2h before the commencement of experiment [15]. Group 1 (control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na_2CO_3); Groups 2 - 4 were pretreated with *S. officinarum* leaf extract (170, 340 and 510 mg/kg p.o. respectively) dissolved in distilled water and administered as aqueous suspension; Group 5 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80). One hour later, groups 2-5 were administered with indomethacin. Four hours after indomethacin administration, animals were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored [16]. The ulcer index (UI) and preventive ratio (PR) of each of the groups pretreated with extract were calculated using standard methods [17,18]. Ulcer index represents the degree of lesion or ulceration caused by the ulcerogen, while the preventive ratio is the protective potential of the extract/drug.

Ethanol-induced gastric ulceration: The procedure for this experiment was similar to that used in indomethacin induced ulceration. The rats randomly were assigned into five groups of six rats each based on their body weight. Food was withdrawn 24 hours and water 2h before the commencement of experiment [15]. Group 1 (control) received only ethanol (2.5 mL/kg p.o), Groups 2-4 were pretreated with *S. officinarum* leaf extract (170, 340 and 510 mg/kg p.o. respectively) dissolved in distilled water and administered as aqueous suspension; Group 5 received propranolol (40 mg/kg p.o. dissolved in distilled water). One hour later, groups 2- 5 were administered with ethanol. Four hours after ethanol administration, animals were sacriiced by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored [18].

Histamine-induced gastric ulceration in rats: Adult albino rats of both sexes weighing 120– 170 g were used for the experiment. They were randomized into five groups of six rats each. Food was withdrawn 24 hours and water 2 h before the commencement of the experiment [15]. Group 1 (control) received only histamine acid phosphate (Sigma, 100 mg/kg i.p. dissolved in distilled water)[19]; Groups 2 - 4 were pretreated with *S. officinarum* leaf extract (170, 340 and 510 mg/kg p.o. respectively) dissolved in distilled water and administered as aqueous suspension; Group 5 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80), 1 hour prior to histamine administration. One hour later, groups 2-5 were administered with histamine acid phosphate (100 mg/kg, i.p). 18 hours after histamine administration, animals were sacriiced by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored [16]. The ulcer indexes (UI) and preventive ratio (PR) of each of the groups pretreated with the extract were calculated using standard methods [17,18].

2.3 Statistical analysis

Data collected were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean ± SEM and significance relative to control were considered at $p<0.001$ and $p<0.05$.

RESULTS

3.1 Evaluation of antiulcer activity of the leaf extract

Indomethacin-induced gastric ulceration: The extract (170-510 mg/kg, p.o.) pretreatment on indomethacin induced gastric ulceration showed dose-dependent reductions in ulcer indices in pretreated groups relative to control. These reductions were statistically significant ($p<0.05$) compared to control. (Table 1). The ulcerations observed in the stomachs of the extract-pretreated groups were majorly pinpoint wounds and no severe wound was present in the stomach of the animals. Severe wounds were observed in the stomach of the animals in the control group. The effect was lower when compared to that of the standard drug, cimetidine. The preventive ratio was 09.10, 51.67, 72.13 and 91.67 % respectively for cimetidine, 170, 340 and 520 mg/kg of the extract (Table 1).

Table 1: Effect of ethanol leaf extract of *Saccharum officinarum* on indomethacin-induced ulcer

Treatment	Dose (mg/kg)	Ulcer indices	Preventive ratio
Control normal	60	13.10 ±1.12	-
Indomethacin			
Cimetidine	100	0.51 ±0.01^{c}	96.10
Crude extract	170	6.33±1.57^{a}	51.67
	340	3.65 ± 0.98^{b}	72.13
	510	1.09 ± 0.16^{c}	91.67

Data are expressed as MEAN ± SEM, Significant at $^{a}p < 0.05$,$^{b}p<0.01$,$^{c}p<0.001$, when compared to control. (n=6).

Ethanol-induced gastric ulceration: Pretreatment of rats with leaf extract of *S. officinarum* (170- 510 mg/kg) significantly protected the animals from ethanol–induced ulcer (Table 2). This protection was significant ($p<0.01$) and dose-dependent as shown in the reduction of ulcer indices relative to control especially in the group treated with the highest dose (510 mg/kg) of the extract with preventive ratio of 70.30%. The ulcerative wounds observed in the stomachs of the extract-pretreated groups were majorly pinpoint wounds without any severe wound in the stomachs of the animals. However, there were severe wounds in the stomachs of the control animals. The standard drug, propranolol, exhibited the highest protection (78.51%)(Table 2). Histamine–induced ulceration: Administration of the leaf extract of *S. officinarum* (170- 510 mg/kg) significantly ($p<0.001$) reduced histamine-induced gastric ulceration in a dose-dependent fashion compared to the control (Table 3). There was absence of severe wound in the stomachs of the extract/drug pretreated groups. However, severe wounds were observed in the stomachs of animals in the control group The preventive ratios were 43.70,79.25 and 92.40 mg/kg for 170, 340 and 510 mg/kg of the extract respectively (Table 3).

Table 2: Effect of leaf extract of *Sacharum officinarum* on ethanol-induced ulcer

Treatment	Dose (mg/kg)	Ulcer indices	Preventive ratio
Control normal	60	6.33 ±0.57	-
Propranolol	40	1.36 ±0.23^{c}	78.51
Crude extract	170	3.21±1.22^{a}	49.28
	340	2.56 ± 0.83^{b}	59.55
	510	1.88 ± 0.55^{c}	70.30

Data are expressed as MEAN ± SEM, Significant at $^{a}p < 0.05$,$^{b}p<0.01$,$^{c}p<0.001$, when compared to control. (n=6).

Table 3: Effect of leaf extract of *Saccharum officinarum* on histamine-induced ulcer

Treatment	Dose (mg/kg)	Ulcer indices	Preventive ratio
Control normal	60	5.40±0.08	-
Cimetidine	100	0.00 ±0.00^{c}	100
Crude extract	170	3.04±0.15^{a}	43.70

340	1.12 ± 0.33[c]	79.25
510	0.41 ± 0.14[c]	92.40

Data are expressed as MEAN ± SEM, Significant at [a]p < 0.05,[b]p<0.01,[c]p<0.001, when compared to control. (n=6).

4. DISCUSSION

The antiulcer activity of the leaf extract of *S. officinarum* was evaluated using indomethacin, ethanol and histamine–induced ulcer models. Indomethacin inhibits prostaglandin synthetase through the cycloxygenase pathway [20] and caused ulcer especially in an empty stomach [21] and particularly on the glandular (mucosal) part of the stomach [16,22). Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair [22,23]. Suppression of prostaglandin synthesis by indomethacin results in increased susceptibility of the stomach to mucosal injury and gastroduodenal ulceration. The extract was observed to significantly reduce mucosal damage in the indomethacin–induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti- ulcer effect of the extract. Administration of ethanol has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production [24]. This is attributed to the release of superoxide anion and hydroperoxy free radicals during the metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa [25]. It was observed in this study that the extract significantly reduced ethanol-induced ulcer. This may be due to cytoprotective effect of the extract via antioxidant effects. Ethanol is also reported to cause gastric mucosal damage by stimulating the formation of leukotriene C4 (LTC4) [26]. The gastroprotective effect of the extract may in part be due to the suppression, by the extract of lipoxygenase activity [16].

Histamine-induced ulceration is known to be mediated by enhanced gastric acid secretion as well as by vasospastic action of histamine [27]. The inhibition of ulcer due to histamine by the extract may be due to its suppression of histamine-induced vasospastic effect and gastric secretion. The leaf extract was found to contain various chemical constituents such as tannins, flavonoids, alkaloids, terpenes, triterpenes like squalene, phenolics, β-sitosterol and polyunsaturated fatty acids (PUFAs), 3,4,5-trihydroxy benzoic acid (gallic acid), β-sitosterol, p-coumaric acid (4-hydroxycinnamic acid) and tricin-7-O-eohesperoside among others[8,14]. Flavonoids such as quercetin have been reported to prevent gastric mucosal lesions in various experimental models [28,29] by increasing the amount of neutral glycoproteins [28]. Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion [30]. Saponins, especially triterpenes type have been implicated in antiulcer activity mediated by formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting PGF2α [31,32]. However, squalene, a triterpene, hexadecanoic acid , 3,4,5-trihydroxy benzoic acid (gallic acid), β-sitosterol, p-coumaric acid (4-hydroxycinnamic acid) are potent active antioxidant compounds [33,34,35] and their presence in the extract may have contributed to the observed activity. The antiulcer activity observed in this study is due to the antioxidant activities of these phenolic compounds.

5. CONCLUSION

The results of the study suggest that ethanol leaf extract of *Saccharum officinarum* possesses antiulcer activity which is attributable to the activities of its phytochemical constituents.

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Conflict of Interest

There is no conflict of interest associated with this work.

Contribution of the Authors

JEO, JAU,UAE, - Research concept and design; JEO,AIL,UAE- Animal studies, ULI,JAU-Data analysis and interpretation; JEO,ULI, JAU- Writing the article. All authors read and approved the manuscript for publication.

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