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Antiulcerogenic activity of *Panicum maximum* root extract

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Abstract
Root extract of *Panicum maximum* is used in Ibibio traditional medicine for the treatment of various ailments including diabetes mellitus, malaria and gastrointestinal disorders. The study aimed to evaluate the antiulcerogenic activity of the root extract against experimentally-induced ulcer in rats. Ethanol root extract of *Panicum maximum* (137-547 mg/kg) was investigated for antiulcerogenic activity against indomethacin, ethanol and histamine-induced ulcers 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. The root extract (137-547 mg/kg) was found to exert significant (p<0.05-0.001) and dose-dependent activity against indomethacin, ethanol and histamine-induced ulcers. These results suggest that the root extract of *Panicum maximum* possess antiulcerogenic potential which is due to the activities of the phytochemical constituents which can be explored for the treatment of ulcers.

Keywords: *Panicum maximum*, root, antiulcer

1. Introduction
Panicum maximum Jacq (Poacace) is a perennial grass which is distributed widely in Africa and other tropical regions of the world (Van Oudtshoorn, 1999). The leaves have been employed ethnomedically by the Ibibios of Akwa Ibom State, Nigeria in the treatment of various ailments such as malaria, microbial infections, rheumatism pain, inflammation and diabetes.
Antidiabetic (Antia *et al.*, 2010) [3], antimalarial and analgesic (Okokon *et al.*, 2012) [24], antibacterial (Gothandam *et al.*, 2010; Doss *et al.*, 2011a; Doss *et al.*, 2011b) [11, 8, 9] anti-inflammatory and antipyretic (Okokon *et al.*, 2011) [23], antifungal (Kanife, 2012) [14], anticancer, antioxidative burst and antileishmanial (Okokon *et al.*, 2014) [21] activities of the leaf extract have been reported. Also, *Panicum maximum* root extract has been reported to possess analgesic and antimalarial properties (Okokon *et al*., 2016) [20] with LD_{50} value of 2738.1 mg/kg, antidepressant and anticonvulsant (Okokon *et al.*, 2018) and anti-inflammatory activities (Udobang *et al.*, 2020) [28]. Phytochemical constituents of the root such as alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides (Okokon *et al.*, 2016) have been reported. In this study, we investigated the antiulcerogenic activity of ethanol root extract of *Panicum maximum.*

2. Materials and Methods
2.1 Plants collection
The plant material *Panicum maximum* (root) was collected in compounds in a farmland in Uyo area, Akwa Ibom State, Nigeria in August, 2018. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

2.2 Extraction
The plant parts (root) were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder using mortar and pestle. The powdered material was soaked in 50% ethanol. The liquid filtrate was concentrated and evaporated to dryness in vacuo 40 ˚C using rotary evaporator and stored in a refrigerator at – 4 ˚C.

2.3 Animals
Swiss albino male rats (145 – 170g) used for these experiments were gotten from Animal house of Department of Pharmacology and Toxicology, University of Uyo. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea Feed) and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.4. Indomethacin-induced ulcer
Male adult albino rats used for the experiment were randomly divided into five groups of six rats each. The animals were deprived of food 24 h and water 2 h prior to the experiment (Alphin and Ward, 1967) [2]. Group 1 (control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na_2CO_3); groups 2 - 4 were pretreated with *Panicum maximum* root extract (137, 273 and 547 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 post administration of the extract, groups 2-5 were administered with indomethacin.
Four hours after indomethacin administration, animals were killed 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 (Nwafor *et al*., 1996) [18]. Ulcer index (UI) and preventive ratio (PR) of each of the groups pretreated with extract was calculated using standard methods (Zaidi and Mukerji, 1985; Nwafor *et al*., 2000) [19]. Ulcer index represents the degree of lesion or ulceration caused by the ulcerogen, while preventive ratio is the protective potential of the extract/drug.

2.5 Ethanol-induced gastric ulceration
The procedure was similar to that used in indomethacin induced ulceration. The rats randomly 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 (Alphin and Ward, 1967) [2]. Group 1 (control) received only ethanol (2.5 mL/kg p.o), groups 2-4 were pretreated with *Panicum maximum* root extract (137, 273 and 547 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 killed 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 (Nwafor *et al*., 2000) [19].

2.6 Histamine-induced gastric ulceration in rats
Adult albino rats of both sexes weighing 125- 150 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 experiment (Alphin and Ward, 1967) [2]. Group 1 (control) received only histamine acid phosphate (Sigma, 100 mg/kg i.p. dissolved in distilled water) (Maity *et al*., 1995); groups 2 - 4 were pretreated with *Panicum maximum* root extract (137, 273 and 547 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 killed 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 (Nwafor *et al*., 1996) [18]. Ulcer indexes (UI) and preventive ratio (PR) of each of the groups pretreated with the extract were calculated using standard methods (Zaidi and Mukerji, 1985; Nwafor *et al*., 2000) [19].

2.7 Statistical Analysis
Data are reported as mean ± standard error of the mean (SEM) and were analyzed statistically using One-way ANOVA followed by Turkey-Kramer multiple comparison. test and values of $p<0.01$ were considered significant.

3. Results and Discussion

Table 1: Effect of ethanol root extract of *Panicum maximum* on indomethacin-induced ulcer

Treatment	Dose (mg/kg)	Ulcer Indices	Preventive Ratio
Control normal Indomethacin	60	13.33±1.66	-
Cimetidine	100	0.50 ±0.16^c	96.24
Crude extract	137	5.83±2.20 ^{c}	56.26
	273	3.33 ±0.83^b	75.01
	547	1.16 ±0.66^c	91.29

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 2: Effect of ethanol root extract of *Panicum maximum* on ethanol-induced ulcer

Treatment	Dose (mg/kg)	Ulcer Indices	Preventive Ratio
Control normal	60	4.66 ±0.33	-
Propranolol	40	0.66 ±0.33^c	85.83
Crude extract	137	2.33±0.33^a	50.00
	273	1.66 ± 0.33^c	64.37
	547	1.33 ± 0.33^c	71.45

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

Table 3: Effect of ethanol root extract of *Panicum maximum* on histamine-induced ulcer

Treatment	Dose (Mg/Kg)	Ulcer Indices	Preventive Ratio
Control normal	60	5.28 ±0.46	-
Cimetidine	100	0.72 ±0.18^c	86.36
Crude extract	137	3.22±0.22	39.01
	273	2.80± 0.54	46.96
	547	1.84 ± 0.63	65.15

Data are expressed as MEAN ± SEM, Significant at ^{a}pc0.05,$^b p<0.01$,$^c p<0.001$, when compared to control. (n=6).

4. Discussion
The root extract (p.o.) pretreatment on indomethacin-induced gastric ulceration showed dose-dependent reduction

in ulcer indices in pretreated groups relative to control. These reductions were significant (p<0.05-0.001) when compared to control. 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 standard drug, cimetidine, was the most effective with preventive ration of 91.29% (Table 1). Pretreatment of rats with root extract offered considerable protection to the animals from ethanol–induced ulcer (Table 2). This protection was significant (p<0.05-0.01) and dose-dependent as shown in the reduction of ulcer indices relative to control.

The ulcerative wounds observed in the stomachs of the extract-pretreated groups were not as severe wound as those present in the stomachs of the control animals. The standard drug, propranolol, gave a preventive ratio of 85.83% (Table 2). Administration of the root extract exerted considerable dose-dependent reductions in histamine-induced gastric ulceration at all doses (137-547 mg/kg) administered (Table 3). These reductions were statistically significant (p<0.05-0.01) when compared to control. Animals in the extract-treated groups had less severe wounds on their stomachs compared to those present on the stomachs of animals in the control group. However, the standard drug, cimetidine produced a preventive ratio of 86.36% (Table 3). Root of *Panicum maximum* is used traditionally to treat various gastrointestinal disorders. For this reason, the antiulcer activity of the root extract was evaluated using indomethacin, ethanol and histamine–induced ulcer models. Indomethacin is known to cause ulcer especially in an empty stomach (Bhargava *et al*., 1973) [4] and mostly on the glandular (mucosal) part of the stomach (Evbuonwa and Bolarinwa, 1990; Nwafor *et al*., 1996) [10, 18] by inhibiting prostaglandin synthetase through the cycloxygenase pathway (Rainsford, 1987) [26]. 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 (Hayllar and Bjarnason, 1995; Hiruma-Lima *et al*., 2006) [12, 13]. Suppression of prostaglandins synthesis by indomethacin results in increase 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 (Salim, 1990) [27]. This is attributed to the release of superoxide anion and hydroperoxy free radicals during 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 (Pihan *et al*., 1987) [25]. It was observed in this study that the extract significantly reduced ethanol induced ulcer. This may be due to the cytoprotective and antioxidant effects of the extract. Ethanol is also reported to cause gastric mucosal damage by stimulating the formation of leukotriene C4 (LTC4) (Whittle *et al*., 1985) [30]. The extract probably could have caused significant suppression of lipoxygenase activity (Nwafor *et al*., 1996) [18]. Histamine-induced ulceration is known to be mediated by enhanced gastric acid secretion as well as by vasospastic action of histamine (Cho and Pfeiffer, 1981) [6]. The extract was found to have considerable effect suggesting its potential in inhibiting gastric acid secretion and maybe vasospastic activity of histamine. *P. maximum* root extract has been reported to be rich in flavonoids and other phenolic compounds (Okokon *et al*., 2011; 2014) [23, 21]. Flavonoids such as quercetin have been reported to prevent gastric mucosal lesions in various experimental models (Di Carlo *et al*., 1999; Zayachkivska, 2005) [7, 32] by increasing the amount of neutral glycoproteins (Di Carlo *et al*., 1999) [7]. Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion. The antiulcer activity observed in this study maybe due to the activities of these phenolic compounds.

5. Conclusion

The results of the present study show that *Panicum maximum* root extract displays gastro protective activity as demonstrated by inhibition of the formation of ulcers induced through the three different ulcer models. This supports its use in the treatment of gastrointestinal disorders in traditional medicine.

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