

ANTIDIARRHOEAL AND ANTIULCER ACTIVITIES OF HIPPOCRATEA AFRICANA ROOT EXTRACT

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ABSTRACT

The effect of ethanol root extract of *H. africana* on experimentally induced diarrhoea and ulcer was studied in rodents. The extract (200 – 600mg/kg) considerably inhibited castor oil induced diarrhoea, small intestine transit time and castor oil induced fluid accumulation as well as indomethacin and ethanol induced ulcer models. The effect of the extract in these models was comparable to the various standard drugs used. The root extract of *H. africana* has antidiarrhoeal and antiulcer properties which justify its uses in ethnomedicine.

Keywords: *Hippocratea africana*, antidiarrhoea, antiulcer, rodents.

INTRODUCTION

Hippocratea africana Willd.) Loes. (Hippocrateaceae) is a green forest perennial climber without hairs (glabrous) and reproducing from seeds (Dalziel, 1956). The plant is widely distributed in tropical Africa. The root of the plant is used traditionally by the Ibibios of the Niger Delta region of Nigeria in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea (Okokon *et al*, 2006). The plant (root) has been reported by Okokon *et al*, (2006) to possess *in vivo* antiplasmodial activity with LD₅₀ of 2.45 g/kg as well as anti-inflammatory and analgesic activities (Okokon *et al*, 2008). Reports of scientific studies on *Hippocratea africana* are few and there is no data on the antidiarrhoeal and antiulcer activities of the extract. So we investigated the antidiarrhoeal and antiulcer activities of the root extract of *Hippocratea africana*

MATERIALS AND METHODS

Plant materials

Fresh roots of *H. africana* were collected in November, 2006 at Nyan forest in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy Herbarium. The fresh roots (2kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder 100g was macerated in 95% ethanol (300ml) for 72 hours. The liquid filtrate obtained was concentrated in vacuo at 40°C. The yield was 2.08% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

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Animals

Albino wistar rats (105-165g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

Castor oil induced diarrhoea

Diarrhoea was induced in rats using a modified method developed by Sunil *et al*. (2001) and Nwafor *et al*. (2005). Animals were fasted for 24h but allowed free access to water. They were randomized into five groups of six rats each. Group 1(control) received 10% Tween 80 (5ml/kg) by gavage, Groups 2-4 were treated with *H. africana* extract (200,400 or 600mg/kg, p.o. respectively); Group 5 was treated with atropine (0.1mg/kg,i.p). After 1h, each rat received 2ml of castor oil (p.o) and was then observed for consistency of faecal matter and frequency of defecation for 3h.

Small intestinal propulsion

The effect of the extract on intestinal propulsion in unanaesthetized rats was tested using charcoal method of Nwafor and Okwuasaba (2001). Animals were fasted for 24h but allowed free access to water only and were further randomized into six groups of six mice each. Group 1(control) received 10% Tween 80 (5ml/kg) by orogastric gavage; groups 2-4 were treated with *H. africana* extract (200,400 or 600 mg/kg, p.o. respectively); Group 5 received atropine (0.1mg/kg, i.p). After 1h, each rat was administered 1ml charcoal meal (5% activated charcoal suspended in 10% aqueous tragacanth), orally. The animals were killed 30min later by cervical dislocation and bled, and the small intestines were rapidly dissected out and placed on a clean surface. The small intestine of each animal was carefully inspected

Table 1: Effect of *Hippocratea africana* extract on castor oil -induced diarrhea in rats

Treatment	Dose(mg/kg)	Mean faecal matter	%inhibition
Control(10%Tween 80)	-	6.87 ±0.15	-
<i>H. africana</i> extract	200	6.00 ±0.15*	12.66
	400	3.66±0.19*	46.72
	600	3.66 ± 0.76*	46.72
<i>H. africana</i> +atropine	400+0.1	2.33 ± 0.68*	66.08
Atropine	0.1	3.66± 0.50*	46.72

Table 2: Effect of *Hippocratea africana* extract on small intestinal propulsion in rats

Treatment	Dose(mg/kg)	Intestinal transit%	%inhibition
Control(10%Tween80)	-	74.62±2.42	-
<i>H. africana</i> extract	200	72.06± 1.09*	3.43
	400	53.20 ± 5.49*	28.70
	600	54.37 ± 1.46*	27.13
<i>H. africana</i> +atropine	400+0.1	47.00 ± 4.56*	37.01
Atropine	0.1	46.01 ± 3.55*	38.34

Table 3: Effect of *Hippocratea africana* extract on castor oil induced fluid accumulation in rats

Treatment	Dose(mg/kg)	Mean volume of intestinal fluid(ml)	% reduction
Control(10%Tween80)	-	1.16 ± 0.53	-
		4.73 ± 0.82	
<i>H. africana</i> extract	200	2.43 ± 0.71*	48.62
	400	1.90 ± 0.94*	59.83
	600	2.20 ± 0.21*	54.48
<i>H. africana</i> + loperamide	400+ 3.0	2.00 ± 0.16*	57.71
Loperamide	3.0	2.03 ± 0.53*	57.08

Data were expressed as mean ± SEM. significant at *P < 0.001, when compared to control n = 6.

and the distance traversed by the charcoal meal from the pylorus was measured. The length of the whole small intestine was also measured. The distance traversed by the charcoal meal from the pylorus was expressed as a percentage of the distance from the pylorus to the ileocaecal junction.

Castor oil induced fluid accumulation

Fluid accumulation was induced in rats according to the method of DiCarlo *et al.* (1994). Animals were deprived of food for 24h but allowed free access to water. They were randomized into seven groups of six rats each. Group1 (control) received 10% Tween 80 (5ml/kg) by gavage, Group 2 received castor oil (2ml/rat), Groups 3-5 were administered with *H. africana* (200, 400 or 600 mg/kg, p.o), Group 6 received loperamide (3.0mg/kg,p.o), and group 7 received loperamide (3.0mg/kg,p.o), 10min later *H. africana* extract (400mg/kg,p.o). After 1h, each rat received 2ml castor oil (p.o.), 30 minutes later, the rats were killed by cervical dislocation and exsanguinated, and the small intestine of each rat was ligated at both pyloric sphincter and at the ileocaecal junctions. The entire small intestine was dissected out, its contents were expelled into a graduated measuring cylinder and the volume of the contents recorded.

Indomethacin induced ulcer

Male adult albino rats were used for the experiment. They were randomized into six groups of six rats each. Food was withdrawn 24 hours and water 2h before the commencement of experiment (Alphin and Ward, 1967). Group 1 (control) received only indomethacin (Sigma, 60mg/kg p.o. dissolved in 5% Na₂CO₃); Groups 2-4 were pretreated with *Hippocratea africana* extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received cimetidine (100mg/kg p.o. dissolved in 50% Tween 80), while Group 6 received cimetidine (100mg/kg,p.o), 10 minutes later, extract of *Hippocratea africana* (400mg/kg,p.o) was given. One hour later, groups 2 -6 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). Ulcer index (UI), preventive ratio (PR) and degree of ulceration (DU) of each of the groups pretreated with extract were calculated using standard methods (Zaidi and Mukerji 1985; Nwafor *et al.*, 2000).

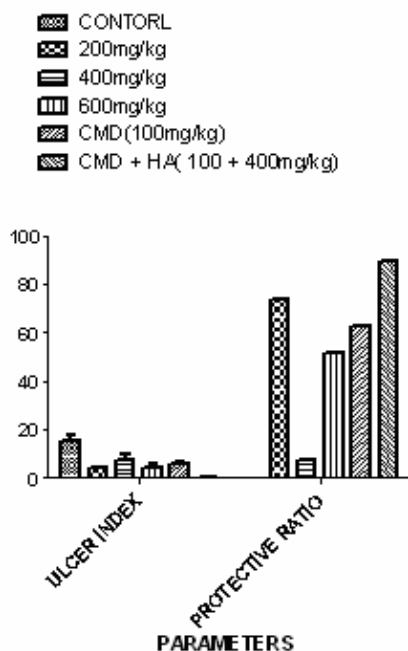


Fig. 1: Effect of *H. africana* extract on indomethacin induced ulcer

Ethanol induced gastric ulceration

The procedure was similar to that used in indomethacin induced ulceration. The rats were randomly assigned into six groups of six rats each. Food was withdrawn 24 hours and water 2h before the commencement of experiment (Alphin and Ward, 1967). Group 1 (control) received only ethanol (2.5 ml/kg p.o), Groups 2- 4 were pretreated with *Hippocratea africana* extract (200, 400, and 600mg/kg p.o. respectively); Group 5 received propranolol (40 mg/kg p.o. dissolved in distilled water), while Group 6 received propranolol (40mg/kg.p.o dissolved in distilled water), 10 minutes later, extract (400mg/kg.p.o) was given. One hour later, groups 2-6 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).

STATISTICAL ANALYSIS

All the group data were statistically analysed with one-way ANOVA, followed by Tukey-Kramer multiple comparison post test. Values of $p < 0.01$ were considered significant.

RESULTS

Castor oil-induced diarrhoea

Hippocratea africana extract (200-600mg/kg) reduced the castor oil-induced diarrhea in a dose-dependent manner

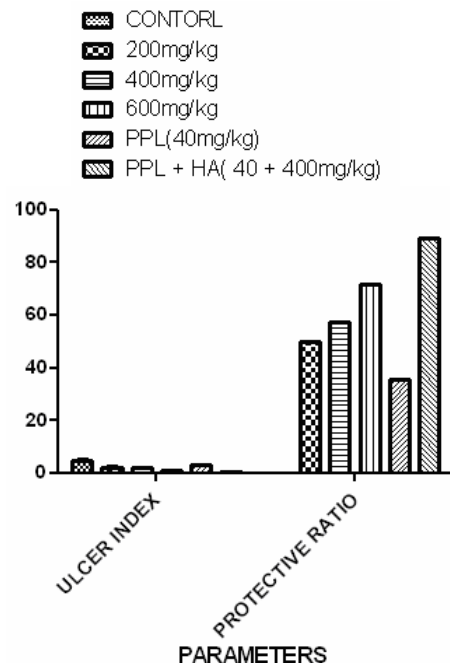


Fig. 2: Effect of *Hippocratea africana* extract on ethanol induced ulcer

significantly ($p < 0.01$). The effect of the extract was comparable to that of the standard drug, atropine and was also enhanced in the presence of the standard drug, atropine (table 1).

Castor oil induced intestinal propulsion

Hippocratea africana root extract inhibited the intestinal propulsion from 3.43 to 28.70% relative to control. The effect was enhanced in the presence of atropine, a muscarinic blocker (table 2).

Castor oil-induced intestinal fluid accumulation

The extract (200-600mg/kg) demonstrated a significant reduction in intestinal fluid accumulation due to castor oil administration relative to control. The reduction, though non dose-dependent was comparable to that of the standard drug, loperamide, a synthetic opiate analogue.

Indomethacin-induced gastric ulceration

The extract pretreatment on indomethacin-induced gastric ulceration demonstrated a significant decrease ($P < 0.01$) in ulcer indices relative to control. The effect of the lowest dose of the extract was comparable to that of the standard drug, cimetidine. The effect however, reduced with increasing doses of the extract (fig. 1).

Ethanol-induced gastric ulceration

The root extract pretreatment significantly ($P < 0.001$) reduced the ulcer indices of ethanol -induced ulceration relative to control. The effect of the extract was more than that of the standard drug, propranolol (fig. 2).

DISCUSSION

In this study, ethanolic root extract of *H. africana* exhibited a significant antidiarrhoeal and antiulcer activities in the models tested. Castor oil induces diarrhea due to active ingredient, ricinoleic acid, which is liberated as a resulting action of lipases on castor oil. This stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. It also stimulates the release of endogenous prostaglandins (Galvez *et al.*, 1994; Yoshio *et al.*, 1999). Castor oil elicits secretory and motility diarrhea (Rouf *et al.*, 2003). Inhibitors of prostaglandin synthesis are known to delay diarrhea induced with castor oil (Sunil *et al.*, 2001). The observation suggests that the antidiarrhoeal effect of the extract may be due to inhibition of prostaglandin synthesis. Also since the effect was enhanced in the presence of atropine, the extract may have acted also potentiated antimuscarinic activity to reduce castor oil induced diarrhea. The extract exhibited also a significant inhibition of the small intestine propulsive movement, but the effect was not comparable to that of the standard drug, atropine, used in the study. Moreso, when the extract was given with atropine, an anticholinergic, enhanced activity was not observed, perhaps due to the crude nature of the extract. Conversely, the extract may be inhibiting the small intestinal movement through some other mechanism such as antagonism of α_2 adrenoceptor stimulation. This extract also inhibited significantly ($p < 0.001$) castor oil induced intestinal fluid accumulation (enteropooling). This effect was not comparable to that of the standard drug, loperamide, a synthetic opiate, which act by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluids (Vareinshang and Yadav, 2004). Also, the effect of the extract was not enhanced in the presence of loperamide suggesting that the extract may be acting through a different mechanism such as anticholinergic activity as enhanced activity was observed earlier with atropine. Antidiarrhoeal and antidyseric properties of medicinal plants were found to be due to the presence of tannins, alkaloids, saponins, flavonoids, steroids and or terpenoids (Havagiray *et al.*, 2004). These constituents which have been reported by Okokon *et al.* (2006) to be present in the root extract of the *H. africana* maybe responsible for the in vivo antidiarrhoeal activity of *H. africana* extract.

Hippocratea africana root extract was also evaluated for antiulcer activity using indomethacin and ethanol – induced ulcer models. Indomethacin, a known ulcerogen especially in an empty stomach (Bhargava *et al.*, 1973) causes ulcer mostly on the glandular (mucosal) part of the stomach (Evbuonwa and Bolarinwa, 1990; Nwafor *et al.*, 1996) by inhibiting prostaglandin synthetase through the cyclooxygenase pathway (Rainsford, 1987). 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). Suppression of prostaglandin synthesis by indomethacin results in increased susceptibility of the stomach to mucosal injury and gastro-duodenal 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 antiulcer 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). This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals have been found to be involved in the mechanism of acute and chronic ulcerations in the gastric mucosa (Pihan *et al.*, 1987). It was observed in this study that the extract reduced significantly 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) (Whittle *et al.*, 1985). The gastroprotective effect of the extract may in part be due to the suppression, by the extract of lipoxygenase activity (Nwafor *et al.*, 1996). Okokon *et al.*, (2006) reported that the root extract contains flavonoids, terpenes, saponins, alkaloids and cardiac glycosides among others. Flavonoid such as quercetin has been reported to prevent gastric mucosal lesions in various experimental models (Di carlo *et al.*, 1999; Zayachkivska, 2005) by increasing the amount of neutral glycoproteins (Di carlo *et al.*, 1999). 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 (Borrelli and Izzo, 2000). 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 PGF_{2 α} (Agwu and Okunji, 1986; Lewis and Hanson, 1991).

CONCLUSION

The results of this study show that the ethanolic root extract of *H. africana* possess antidiarrhoeal and antiulcer properties which justify the ethnobotanical uses of this plant to treat various gastrointestinal problems.

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