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Anti-ulcerogenic activity of ethanolic leaf extract of *Heinsia crinata*

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Abstract
The anti-ulcer activity of *Heinsia crinata*, a vegetable, used ethnomedicinally in the treatment of ulcer was evaluated to confirm this claim.The crude ethanolic leaf extract (450 – 1350mg/kg) was investigated against indomethacin, ethanol and histamine – induced ulcer models in rats.The crude leaf extract demonstrated significant ($p<0.001$) inhibition of ulcer produced by the ulcerogens used; indomethacin, ethanol and histamine.The results of this work confirms the folkloric use of this plant in the treatment of ulcer.

Keywords: Heinsia crinata; *Anti-ulcer; Vegetable*

Introduction

Vegetables and leaves of some domesticated and wild plants are commonly use by the *Ibibios* of Niger Delta region of Nigeria in the preparation of soups. Some of these edible plants are equally medicinal and are use ethnomedically in the therapy of some diseases such as malaria, diabetes, ulcer, diarrhea and other gastrointestinal disorders. Some of these vegetables eaten by the Ibibios like *Telfairia occidentalis* and *Lasianthera africana* contain vital chemical compounds of medicinal importance and have been reported to have antiplasmodial (Okokon *et al*., 2007a, 2007b) and antidiabetic (Eseyin *et al*., 2000) activities. *Heinsia crinata* (Rubiaceae) is shrub with woody stems and branches (Hutchinson and Dalziel, 1954). It is indigenous to West Africa, especially eastern part of Nigeria, but it is now cultivated in Central Africa, south of Sahara and Francophone Africa (Babady-Billa *et al*.,1994). *Heinsia crinata* is casually classified as white and dark by indigenes of Akwa Ibom State in southern Nigeria. The white variety is cultivated by Annang tribe of the State, while the dark variety is cultivated by the Ibibios. Both varieties are readily available in the market and are cultivated for their nutritious values. The leaf juice is used to treat various diseases and wounds as well as to treat gastrointestinal disorders (Okokon *et al*., 2009). Two triterpenoid saponins have been isolated from the leaves of the plants (Babady-Billa *et al*., 1994). The two varieties resemble each other morphologically and are only different from one another in terms of their taste. The dark variety is bitter while that of the white variety is only slightly bitter. A nutritional study of *H. crinata* leaves has been

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reported by Etuk *et al*., (1998) and Etuk *et al.*, (2002). Antimicrobial activity of the leaves of the plant have been reported (Ajibesin *et al*.,2003; Andy *et al.*, 2008). Antiplasmodial and antidiabetic activities of the leaf extract have also been reported (Okokon *et al*.,2009) Ajibesin *et al*.,(2003) also reported on the phytochemical constituents of the leaves of the two varieties to be made up of saponins, tannins, cardiac glycosides, terpenes, and alkaloids, with the dark variety having a greater concentration of alkaloids, while saponins were greater in the white variety. Reports of scientific studies on *H. crinata* are few and there is no information regarding the anti-ulcer activity of *H. crinata* leaf extract in rats.

The aim of the present study was to evaluate the anti-ulcer potential of the dark green variety on some experimentally induced–ulcer models in rats to confirm its folkloric claims in the treatment of gastrointestinal disorders

Experimental

Plant materials. Fresh leaves of *H. crinata* were procured from Uyo market, Akwa Ibom State, Nigeria, in January, 2009. The plant was identified and authenticated by Dr Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy Herbarium (voucher no. FPHUU. 225). The fresh leaves (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^{o}C and all the ethanol was completely removed. The yield was 1.17% w/w. The extract was stored in a refrigerator at 4^{o}C until used for experiment reported in this study.

Animals. Albino 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.

Evaluation of anti-ulcer 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 (Alphin and Ward, 1967). Group 1 (control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na_2Co_3); Groups 2 - 4 were pretreated with *Heinsia crinata* (HCE) extract (450, 900 and 1350 mg/kg p.o. respectively); Group 5 received cimetidine (100mg/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 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) and preventive ratio (PR) of each of the groups pretreated with extract were calculated using standard methods (Zaidi and Mukerji, 1985; Nwafor *et al*., 2000).

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). Group 1 (control) received only ethanol (2.5 ml/kg p.o), Groups 2-4 were pretreated with *H. crinata* extract (450, 900 and 1350 mg/kg p.o. respectively); 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).

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 experiment (Alphin and Ward, 1967). Group 1 (control) received only histamine acid phosphate (Sigma, 100mg/kg i.p. dissolved in distilled water) (Maity *et al*.,1995); Groups 2 - 4 were pretreated with *H. crinata* extract (450, 900 and 1350 mg/kg p.o. respectively); 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 (100mg/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). 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).

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.

Results

Indomethacin-induced gastric ulceration. The extract (p.o.) pretreatment on indomethacin induced gastric ulceration showed a dose dependent reduction in ulcer indices in pretreated groups relative to control. The reduction was statistically significant ($P<0.05$) compared to control. (Table 1).The effect was comparable to that of the standard drug, cimetidine.

Ethanol-induced gastric ulceration. The extract significantly protected rats from ethanol–induced ulcer (Table 2). There was a significant ($P<0.01$) dose-dependent reduction in the ulcer indices relative to control.

Histamine–induced ulceration.
Administration of the extract significantly ($P<0.001$) reduced histamine-induced gastric ulceration in a dose dependent fashion compared to control (Table 3).

Discussion

Heinsia crinata leaves though used as a vegetable have been reported by Okokon *et al.*, (2009), to be used traditionally in the treatment of gastrointestinal disorders. For this reason, the anti-ulcer activity of the leaf extract was evaluated using indomethacin, ethanol and histamine–induced ulcer models.

Table 1: Effect of *H. crinata* extract on indomethacin- induced ulcer

Treatment	Dose (mg/kg)	Ulcer Indices	Preventive Ratio
Control (indomethacin)	60	18.66 ± 1.17	-
H. crinata extract (p.o.)	450	8.50 ± 1.91*	54.44
H. crinata extract (p.o.)	900	2.66 ± 0.83*	85.74
H. crinata extract (p.o.)	1350	0.83 ± 0.10*	95.55
Cimetidine	100	0.76 ± 0.47*	95.92

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

Table 2: Effect of *H. crinata* extract on ethanol- induced ulcer

Treatment	Dose (mg/kg)	Ulcer indices	Preventive ratio
Control (ethanol)	-	4.33 ± 0.47	-
H. crinata extract (p.o.)	450	2.0 ± 0.00*	46.19
H. crinata extract (p.o.)	900	1.66 ± 0.21*	61.66
H. crinata extract (p.o.)	1350	0.66 ± 0.21*	84.75
Propranolol	40	1.66 ± 0.21*	61.66

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

Table 3: Effect of *Heinsia crinata* extract on histamine- induced ulceration in rats

Treatment	Dose (mg/kg)	Ulcer index	Preventive ratio
Control (Histamine)	100	15.33 ± 0.45	-
H. crinata extract (p.o.)	450	3.33 ± 0.73*	78.27
H. crinata extract (p.o.)	900	1.0 ± 0.63*	93.47
H. crinata extract (p.o.)	1350	0.00 ± 0.00*	100
Cimetidine	100	0.33 ± 0.21*	97.84

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

Indomethacin is known to cause ulcer especially in an empty stomach (Bhargava *et al*., 1973) and mostly on the glandular (mucosal) part of the stomach (Evbuonwa and Bolarinwa, 1990; Nwafor *et al*., 1996) by inhibiting prostaglandin synthetase through the cycloxygenase 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 prostaglandins synthesis by indomethacin result 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 (Salim, 1990). 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). 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 C_4 (LTC_4) (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). 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). The inhibition of ulcer due to histamine by the extract may be due to its suppression of histamine-induced vasospastic effect and gastric secretion. Ajibesin *et al*., (2003) reported that the leaf extract contains flavonoids, terpenes, saponins, alkaloids and cardiac glycosides among others. Flavonoids such as quercetin have 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 anti-ulcer activity mediated by formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting $PGF_{2\alpha}$ (Agwu and Okunji, 1986; Lewis and Hanson, 1991).

In conclusion, the results of the present study show that *Heinsia crinata* leaf extract displays gastroprotective activity as demonstrated by significant inhibition of the formation of ulcers induced through the three different ulcer models studied. The anti-ulcer activity of the extract may be due to the action of the chemical compounds present in the extract. The observation justifies the ethnomedical uses of the plants as anti-ulcer agent and as antacid in addition to its nutritional values.

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