Research Article

Investigation of *Heinsia crinata* leaves on laxative activity

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Abstract

Objective: *Heinsia crinata* (Afzel). G. Tayl. (Rubiaceae), is a vegetable used in Ibibio ethnomedicine for the treatment of various diseases such as GIT disorders. The effect of the leaf extract on gastrointestinal tract was therefore studied. **Materials and methods:** The ethanol leaf extract of *H. crinata* (450-1350 mg/kg) was investigated for effect on the gastrointestinal tract using various experimental models. **Results:** The extract was found to significantly ($p < 0.05-0.01$) increased the frequency and output of faecal matters in rats in all the experimental models studied. The leaf extract of *H. crinata* possess laxative activity and thus could be used in the treatment of gastrointestinal disorders. **Keywords:** *Heinsia crinata*, vegetable, laxative, GIT

Introduction

Heinsia crinata (Afzel). G. Tayl. (Rubiaceae) is shrub with woody stems and branches (Hutchinson and Daziel, 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. Ajibesin et al. (2002) reported on the phytochemical constituents of the leaves of the two varieties to be made up of saponins, tannins, flavonoids, cardiac glycosides, terpenes, and alkaloids, with the dark variety having a greater concentration of alkaloids, while saponins were greater in the white variety. Ethnomedically, the decoctions of the leaf are used to treat various diseases and wounds as well as gastrointestinal disorders especially ulcer and spasm (Okokon et al., 2009), while the root is used to treat abscess and hypertension (Ajibesin et al., 2008). Two triterpenoid saponins have been isolated from the leaves of the plants (Babady-Billa et al., 1994). Reports of nutritional values (Etuk et al., 1998; Etuk et al., 2002), antimicrobial (Ajibesin et al., 2002; Andy et al., 2008; Ezech et al., 2014; Morah et al., 2017), antiparasmodial and antidiabetic activities (Okokon et

al., 2009), and antiulcer (Okokon et al., 2010), anti-inflammatory and analgesic (Andrew et al., 2012), antioxidant (Nwanna et al., 2015) and anticonvulsant (Okokon et al., 2021). activities of the leaf extract have been published. The aim of the present study was to evaluate the laxative potential of the dark green variety in rodents.

Materials and methods

Plants collection and identification

The plant material *Heinsia crinata* leaves were collected in a compound in Uyo area, Akwa Ibom State, Nigeria in May 2021. The plant was identified and authenticated by Dr. Margaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

Extraction

The leaves were washed and shade-dried for two weeks. The dried plants' materials were further chopped into small pieces and reduced to powder. The powdered material was macerated in 70% ethanol for 72 h. The liquid ethanol extract obtained by filtration was evaporated to dryness in a rotary evaporator 40°C. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

Animals

Albino wistar rats (120 -135g) of either sex were used for these experiments. The animals were housed in standard

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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.

Effect on castor oil-induced diarrhoea

This was studied in rats using the method of Nwafor et al. (2005). Animals were fasted for 18 h but allowed free access to water. They were randomized into five groups of six rats each. Group 1 (control) received 10% Tween 80 (5 mL/kg) by gavage, Groups 2-4 were treated with *H. crinata* extract (450, 900 or 1350 mg/kg, p.o. respectively); Group 5 was treated with atropine (0.1 mg/kg, i.p.). After 1h, each rat received 2 mL of castor oil (p.o) and was then observed for consistency of faecal matter and frequency of defecation for 3h.

Laxative activity test

Laxative effect was evaluated according to model described by Shankara and Sriram (2014). with slight modification on rats of either sex. Wistar rats of either sex were fasted for 18 hours. The rats were divided into five groups of six animals each. Group I received 10% Tween 80 (5 mL/kg) by gavage, Group 2 received castor oil (2 mL/kg orally), Groups 3-5 received *H. crinata* extract 450, 900 and 1350 mg/kg, orally respectively. The animals were separated in suitable cages for collection and weighing of the faecal output immediately after the dosing. Food and water were given to all rats and faecal output was weighed after a period of 6 hours.

Effect on 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 24 h 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. crinata* extract (450, 900 or 1350 mg/kg, p.o. respectively; Group 5 received Castor oil (2 mg/kg, p.o). After 1h, each rat was administered 1mL charcoal meal (5% activated charcoal suspended in 10% aqueous tragacanth), orally. The animals were killed 30 min 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 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.

Laxative activity on loperamide-induced constipation in rats

The method described by Yadav et al. (2018) was used in this

study. Wistar rats were fasted for 18 hours and were placed individually in cages lined with clean filter paper. The rats were divided into five groups of five animals each. Group I received 5 mL/kg normal saline orally. Group II received castor oil (2 mg/kg orally). Group III and IV received *H. crinata* extract 450, 900 and 1350 mg/kg orally respectively. After one hour, all the animals received loperamide (5 mg/kg orally) by gavage. The faecal outputs in all four groups were monitored for 6 hours.

Castor oil-induced fluid accumulation (enteropooling)

Fluid accumulation was induced in rats according to the method of DiCarlo et al., (1994). Animals were deprived of food for 24 h but allowed free access to water. The rats were divided into five groups of six animals each. Group I received 10% Tween 80 (5 mL/kg) by gavage, Group 2 received castor oil (2 mL/kg orally), Groups 3-5 received *H. crinata* extract 450, 900 and 1350 mg/kg, orally respectively. Group 5 received loperamide (3 mg/kg, p.o). After one hour each rat received 2mL castor oil (p.o), 30 minutes later the rats were killed by cervical dislocation and exanguinated, and the small intestine of each rat ligated at both pyloric sphincter and at 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.

Data and statistical analysis

The results were presented as mean and SEM and comparisons among groups for statistical significant differences were done by analysis of variance (one-way ANOVA) followed by Turkey Kramer's multiple comparison tests using GraphPad Prism 5.3 application software. The p-values of less than 0.05 were considered as indicative of significance.

Results

Effect of castor oil induced diarrhoea

The administration of *H. crinata* extract to rats was observed to cause dose-dependent increases in mean faecal output of the animal. These increases were significant ($p < 0.05-0.001$) when compared to control. The diarrhoeal effect of castor oil was observed to be increased with the administration of the leaf extract (Table 1).

Effect on small intestinal propulsion activity in rats

Administration of leaf extract of *H. crinata* caused a dose-dependent and significant ($p < 0.05-0.01$) increase in the small intestinal propulsion activity of rats. The effect of the highest dose of the extract (1350 mg/kg) was more than that of castor oil (Table 2).

Table 1. Effect on castor oil-induced diarrhoea

Treatment	Dose (mg/kg)	Mean faecal matter
Control (10%Tween 80)	2ml	6.66±0.33
<i>H. crinata</i>	450	5.33± 0.33
	900	6.03±1.00
	1350	4.33±0.55
	0.1	1.66±0.33

Data are expressed as MEAN ± SEM, Significant at ^ap < 0.05, ^cp<0.001, when compared to control. (n=6).

Table 2. Effect on small intestinal propulsion (transit time) activity in rats

Treatment	Dose (mg/kg)	Mean faecal matter
Control (10%Tween 80)	5 mL	52.34±1.20
<i>H. crinata</i>	450	78.56±2.66 ^a
	900	93.10±3.06 ^a
	1350	95.33±2.25 ^b
	2 mL	81.6±3.46 ^c
Castor oil	0.1	39.53±2.56 ^c

ressed as MEAN ± SEM, Significant at ^ap < 0.05, ^cp<0.001, when compared to control. (n=6).

Laxative effect

Administration of leaf extract of *Heinsia crinata* to rats caused significant (p<0.05-0.001) and dose-dependent increase in faecal output of rats. However, the effect of the highest dose of the extract (1350 mg/kg) was found to be less than that of castor oil (Table 3).

Effect on faecal output of Loperamide-induced constipation in rats

Administration of *Heinsia crinata* leaf extract produced a significant (p<0.05-0.001) and dose-dependent increase in mean faecal output. The effect of the highest dose (1350 mg/kg) of the extract was not comparable to that of castor oil (Table 4).

Effect of leaf extract on fluid accumulation (intestinal enteropoolation)

Administration of *Heinsia crinata* leaf extract caused prominent increases in intestinal fluid accumulation especially at the low doses of the extract (450 and 900 mg/kg), while the highest dose (1350 mg/kg) was found to cause a reduction in the volume of intestinal fluid (Table 5).

Discussion

In this work, leaf extract of *H. crinata* was evaluated for effect on

Table 3. Laxative effect of the leaf extract

Treatment	Dose (mg/kg)	Mean faecal matter
Control (10%Tween 80)	5 mL	2.14±1.05
<i>H. crinata</i>	450	3.33±0.45
	900	5.83±1.16 ^a
	1350	8.24±1.28 ^c
	2 mL	21.33±2.96 ^c

Data are expressed as MEAN ± SEM, Significant at ^ap < 0.05, ^cp<0.001, when compared to control. (n=6).

Table 4. Effect on faecal output of Loperamide-induced constipation in rats

Treatment	Dose (mg/kg)	Mean faecal matter
Control (distilled water)	10 mL	1.63±0.68
<i>H. crinata</i>	450	2.46±0.18
	900	3.18±0.42 ^a
	1450	3.76±0.78 ^a
	2 mL	4.02±0.22 ^a

Data are expressed as MEAN ± SEM, Significant at ^ap < 0.001, when compared to control. (n=6).

Table 5. Effect of leaf extract on fluid accumulation (intestinal enteropoolation)

Treatment	Dose (mg/kg)	Mean faecal matter
Control (10%Tween 80)	5 mL	5.36±0.29
<i>H. crinata</i>	450	4.43±0.42
	900	4.66±0.56
	1450	2.20±0.30 ^c
	10	2.16±0.61 ^a

Data are expressed as MEAN ± SEM, Significant at ^ap < 0.05, ^cp<0.001, when compared to control. (n=6).

the gastrointestinal tract of rats. The results showed that ethanol leaf extract of *Heinsia crinata* produced significant and dose-dependent increase in faeces output of rats administered with it. This effect could probably be exerted through the accumulation of water in intestinal loop and the stimulation of gastrointestinal motility as were observed in this study. The effect of the leaf extract was comparable to that of castor oil (standard drug) at highest dose of the extract (1450 mg/kg body weight). 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; Yosho et al., 1999).

Castor oil elicits secretory and motility diarrhea (Rouf et al., 2003). The observed activities therefore suggest that laxative activity of the leaf extract may be mediated through this mechanism.

The results of this study further showed that the leaf extract antagonises the effect of loperamide on the gastrointestinal tract activity to some extent. Loperamide, a synthetic opiate, acts by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluids (Vareinshang and Yadav, 2004). These results suggest that the leaf extract must have acted to stimulate Na⁺, K⁺ and Cl⁻ secretion. Chatsri et al. (2005) had reported that some natural laxative exert their effects on the colonic epithelium by stimulating Cl⁻ secretion and/or inhibiting Na⁺ absorption, resulting in an accumulation of fluid and subsequent increased colonic motility. This could have been the case in this study as the leaf extract was found to promote accumulation of fluid in the small intestine especially at lower doses.

Secondary metabolites of plants such as terpenoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids (Longanga-Otshudi et al., 2000). have been previously found to be responsible for laxative activities in plants. These constituents that have been reported by Okokon et al. (2009) to be present in the leaf extract of *H. crinata* maybe responsible for the laxative activity of the extract.

The results of this study show that the leaf extract of *Heinsia crinata* possesses laxative effect which justifies its use in the treatment of gastrointestinal disorders.

Conflict of interest.

We declare that we have no conflict of interest regarding the publication of this paper.

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