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Original Research Article

Anti-ulcer Activity of Crude Extract and Fractions of *Acanthus montanus* (Nees) T. Anderson on Indomethacin-Induced Ulcer

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ABSTRACT

Peptic ulcer disease has remained a global threat due to several complications such as perforation, gastrointestinal obstruction and organ penetration associated with the disease. This study evaluated the phytochemical composition and anti-ulcer activity of crude extract and fractions of *Acanthus montanus* on an indomethacin-induced ulcer in a mouse model. The plant was extracted using cold maceration in methanol and fractionated using solvents of varying polarities into their corresponding fractions. The phytochemical screening was conducted using a standard procedure. The acute toxicity test employed Lorke's method and the anti-ulcer activity was conducted using the indomethacin-induced method. The phytochemical composition revealed the abundance of tannins, saponins, steroids/terpenoids and flavonoids in the crude extract and fractions. An acute toxicity study showed that the extract is safe even at a high dose of 5000 mg/kg. The anti-ulcer study revealed that *A. montanus* extract and fractions elicited a dose-dependent activity against peptic ulcer with strong curative indices of 79, 93, and 71% for the 400 mg/kg doses of methanol extract aqueous, and n-hexane fractions respectively compared with 42% index of the control. There was a dose-dependent ulcer healing with 400 mg/kg doses eliciting higher curative indices than the 200 mg/kg doses. The 200 mg/kg doses of aqueous fraction and 400 mg/kg doses of methanol extract, aqueous, and n-hexane fractions elicited ulcer indices significantly different ($p<0.05$) from those of controls. The rich presence of biologically active constituents with anti-ulcerogenic properties in the methanol extract and fractions of *A. montanus* supports its use in ethnomedicine.

Keywords: *Acanthus montanus*, phytochemical screening, gastro-protective activity, acute toxicity test

Introduction

A peptic ulcer disease (PUD) is a collection of gastrointestinal disorders known to cause lesions (ulcers) in any portion of the gastrointestinal tract exposed to sufficiently high concentrations of acid for a longer duration.[1] It results from the imbalance between aggressive predisposing factors like *Helicobacter pylori*, low pH, and pepsin and some protective factors such as the mucosal cells' innate resistance, gastric mucus, prostaglandins (PGs) and bicarbonate ions.[1] There is an estimated prevalence rate of 5 to 10% with a yearly incidence of 0.1 to 0.3% in developed countries which may be worse in developing countries.[2] The available epidemiological data suggest a decreased mortality in the 19th century due to the therapeutic strategies (proton pump inhibitors) and enhanced environmental hygiene (*H. pylori* eradication). However, uncontrolled use or abuse of non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, physiological stress, H-2 receptor blockers and selective serotonin reuptake inhibitors have reversed the gains made in the 19th century.[3]

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The suggested mechanisms of indomethacin-induced gastrointestinal ulceration were the generation of lipid peroxides and oxygen free radicals[2]. Several plants of the Acanthaceae family have shown promising potential in the cure and management of diseases in sub-Saharan Africa.[3]

The vital roles of plants in the ethnomedicine of communicable and non-communicable diseases are well documented.[4-7] *Acanthus montanus* (Nees) T. Anderson, commonly known as Bear's breech, alligator plant, mountain thistle, or ahgam-evu or ogwu_ahga in Agbani, Enugu State Nigeria, belongs to the Acanthaceae family. It is an angiosperm, with documented uses in ethnomedicine.[3] The leaves, combined with *Ananas comosus* and *Costus species* are pounded with water and used to treat urinary tract infections in the Democratic Republic of Congo.[8] The leaf decoction is frequently used as a folk remedy to treat gastrointestinal disorders while the root is used as an analgesic.[9] The root is claimed to be an effective remedy for abscesses while the leaf extract had been used to treat cases of threatened abortion, abdominal pains, and pains of acute gastritis and it is also believed to be an antacid.[10] The phytochemical analysis of the leaves shows that it contains saponins, alkaloids, flavonoids, phenols, tannins, and glycosides (3-*O*-β-D-glucopyranoside). Some micro-elements including calcium, magnesium, zinc, potassium, lead, iron, nickel, copper, cadmium and chromium have been detected.[11] Traditionally, aqueous and other solvents extracts of *A. montanus* are used for several stomach upsets which are related to peptic ulcers in Agbani, Nkanu West LGA, Enugu State.

Ulcer healing has remained one of the major health challenges in the world among all age groups. The persistence of gastrointestinal ulcers especially peptic ulcers despite numerous interventions already existing is of great concern and has raised many questions, especially about the efficacy and ultimately the safety of existing interventions including

their adverse reactions and effects.[12] Hence, the continuous search for safe, more efficacious and economical therapeutic agents is a worthwhile adventure. Currently, all the available therapeutic agents possess unfavorable pharmacokinetic properties. There is a need, therefore, to source for newer, safer, more efficacious and economical molecules. Several medicinal plants with anti-ulcer potential have been reported and many developing populations still rely on these plants for health-related challenges[13--15]. So far, no study has reported the anti-ulcer potential of *A. montanus*. The study, therefore, investigated the potential anti-ulcer effect of *A. montanus* against indomethacin-induced gastric ulcers in a mouse model.

Materials and Methods

Materials

Plant material

Fresh leaves of *A. montanus* were collected in the morning hours (8-10 am) between October and November 2021 from Agbani, Enugu State, Nigeria. They were authenticated by Mr. Alfred Ozioko of the Bioresources Development and Conservation Program Centre, Nigeria. A voucher specimen (ID: BDCP No. 308) is maintained at the centre. The leaves were dried under a shade and maintained at room temperature for 10 days. The dried leaves were ground to a coarse powder using a blender.

Experimental mice

Ninety-six (96) mice weighing between 12-30 g were purchased and acclimatized in the Pharmacology laboratory of the Faculty of Pharmaceutical Sciences ESUT for 2 weeks under the environmental conditions and fed with a prescribed locally prepared animal feed mix by the name "chukun". The feed mix was necessary to get all the required nutrients needed by the animals. The mice were fasted for 24 h with free access to water for up to 1 h before ulcer induction. The protocol for the use of mice was reviewed, approved and granted by the ethical committee of ESUT, where the ethical code of ESUT/FPS/PHA/2022/010 was obtained.

Methods

Extraction and Fractionation

A weighed quantity (647 g) of the dry powder was cold macerated with 5.7 L of methanol for 72 h, sieved with a filter cloth and further filtered using a cotton wool clogged glass funnel as previously reported.[16] .The filtrate was concentrated to dryness using a rotary evaporator at a temperature of 40 °C. Thereafter, the extract was exposed for 48 h to obtain a dark semi-solid extract. It was stored in the refrigerator pending further analysis. The extract was subjected to successive fractionation using a separating funnel. A 22 g of extract (CMe) was dissolved in 250 ml of 10% methanol aqueous solvent and partitioned with 3 x 250 ml of *n*-hexane (nHex), 2 x 250 ml of ethyl acetate (EtOAc), 1 x 250 ml of *n*-butanol (nBut) and water (Aqu). Each of the filtrates was concentrated using a rotary evaporator to yield nHex, EtOAc, nBut and Aqu fractions respectively. The resulting fractions were stored in the refrigerator at 4 °C.

Qualitative phytochemical screening

Phytochemical analysis was conducted using the standard procedure of phytochemical screening[17].

Animal studies

Acute toxicity studies

Phases 1 and 2 of Lorke's method of toxicity studies were employed.[18] In phase 1, nine mice were divided randomly into three groups (n = 3). Each group of mice received 10, 100 and 1000 mg/kg of extract. They were observed for 24 h for unusual behavior and/or mortality.

In Phase 2, the three mice were randomly distributed into three groups (n = 1). The mice were administered with doses of 1600, 2900 and 5000 mg/kg of *A. montanus* and were observed for 24 h for unusual behavior and/or mortality.[18] Thereafter, the LD_{50} was calculated by equation 1:

$$LD_{50} = \sqrt{(D_0 \times D_{100})} - \quad \text{Equation 1}$$

Where D_0 = highest dose that resulted in no mortality while D_{100} = lowest dose that caused mortality.

Induction of gastric ulcer

For the induction of gastric lesions with indomethacin, all fasted animals were administered indomethacin 30 mg/kg orally. Four hours later, two animals each were picked at random from each group, sacrificed, and their stomach removed to ensure that an ulcer has been induced. Each of the remaining mice in their groups was treated with the corresponding dose of extract once daily for seven days.

Anti-ulcer studies

The mice were divided into 12 groups (1-12) of seven mice each and the treatments were as follows:

Group 1, Positive control (omeprazole 20 mg/kg)
Group 2, Negative control, untreated (received water)
Groups 3 and 4: 200 and 400 mg/kg CMe respectively
Groups 5 and 6, 200 and 400 mg/kg Aqu respectively
Groups 7 and 8, 200 and 400 mg/kg *n*Hex respectively
Groups 9 and 10, 200 and 400 mg/kg *n*But respectively
Groups 11 and 12, 200 and 400 mg/kg EtOAc respectively

A 30 mg/kg dose of indomethacin was used to induce gastric ulceration in the mice. The parameters obtainable included statistical variables (ulcer score, ulcer Index (UI) and percentage curative) using methods described by Tokagi and Okabe[19]. The ulcer score was subjectively assigned a score of 0 to 5 representing no lesions, mucosal edema, 1-5 small lesions (1-2 mm in size), > 5 small or intermediate (3-4 mm in size) lesions, ≥ 2 intermediate lesions or 1 gross (> 4 mm in size) lesion and perforated lesions respectively. The ulcer index (UI) and curative (%) were calculated using equations 2 and 3.

$$Ulcer\ index\ (UI) = \frac{Total\ ulcer\ score}{Number\ of\ ulcerated\ mice} \quad \text{Equation 2}$$

$$Curative\ (\%) = \frac{UI\ of\ treated - UI\ of\ untreated}{UI\ of\ untreated} \quad \text{Equation 3}$$

Data analysis

The result was presented as mean + SEM (n=7). A One-way (ANOVA) was used for the statistical analysis, using SPSS version 23. Post-hoc was done using Turkey's test. Differences between mean were considered significant at $p < 0.05$.

Results and Discussion

Extraction and phytochemical constituents of *A. montanus*

Methanol extraction of *A. montanus* yielded 26.8 g of extract equivalent to 4.14 %w/w of coarse powder. Among the solvent fractions, the aqueous fraction gave the highest yield (43.6 %w/w), while ethyl acetate gave the lowest yield (4.4 %w/w). Both n-hexane and n-butanol yielded 41.2 and 9 % w/w of CMe (Table 1). The choice of extraction and fractionation solvents affects the yield of phytochemicals from natural sources due to the relative polarity differences of secondary metabolic products of plants.[20] In this study, the high yield of nHex could be a result of the higher pigment content noticed in the extract which was also evident in the moderate abundance of steroids and terpenoids in the nHex fraction (Table 2). It could also be that the *A. montanus* plant contains volatile or non-polar components.[21] Phytochemical studies on *A. montanus* revealed the presence of tannins, saponins, steroids/terpenoids and flavonoids at different concentrations, with tannins and saponins showing more abundance in the CMe, Aqu, EtOAc and nBut fraction of *A. montamus* (Table 2).

The secondary metabolic products of plants represent an important biomarker for the biological activity of the plant.[22] The abundance of phytochemicals was mostly in the polar fraction and consisted mainly of tannins and saponins. While non-polar solvents like n-hexane also revealed the presence of steroids and terpenoids. The phytochemical constituent of *A. montanus* in this study agrees with the work previously reported on the same plant.[11,23] The high presence of tannins and flavonoids in the plant also justified it claimed traditional use for healing abdominal pains.[24,25] Saponins form a defensive mucus material on the gastric mucosa and thus are effective in ulcer healing.[20,26] They are shown, from the result, to be abundantly present in the plant and could also be responsible for its traditional use as an antiulcer.

Table 1: Percentage yields of *A. montanus* extracts

Samples	Yield (g)	Yield (%)
Methanol extract (CMe)	26.8	4.14*
n-hexane fraction (nHex)	9.08	41.27
Ethyl acetate fraction (EtOAc)	0.97	4.41
n-butanol fraction (nBut)	1.98	9.00
Aqueous fraction (Aqu)	9.60	43.64

*Calculated based on 647 g of coarse powder. Others were based on the 22 g of CMe

Table 2: Phytochemical constituents *A. montanus*.

Phytochemicals	CMe	Aqu	nBut	EtOAc	nHex
Alkaloids	-	-	-	-	-
Tannins	+	+	+	+	-
Flavonoids	+	+	+	+	-
Saponin	+	+	+	+	-
Steroids/terpenoids	+	-	-	+	+
Cardiac Glycosides	-	-	-	-	-
Reducing sugar	-	-	-	-	-

+ =present; - = absent

Table 3: Acute toxicity of *A. montanus*

Phases	Groups	Mice(g)	Doses (mg/kg)	Response
		1 (28)		0
	1	1 (15)	10	0
		1 (20)		0
		1 (17)		0
1	2	1 (13)	100	0
		1 (30)		0
		1 (19)		0
	3	1 (18)	1000	0
		1 (18)		0
	1	1 (26)	1600	0
2	2	1 (20)	2900	0
	3	1 (22)	5000	0

Zero (0) = no toxicity response like behavioral changes or death

Acute toxicity test

The safety of herbs and herbal products constitutes a vital parameter for the use and translation of the useful activities of the plant. Lorke's acute toxicity study is aimed at identifying lethality for any given substance. In this study, all the mice used survived the treatments, even those treated with the highest dose (5000 mg/kg) after 24 h of close monitoring. There were also no behavioral changes associated with the treatments (Table 3). The safety profile in this study also justified the vital roles of the plant in folk medicine for the treatment and prevention of various ailments. Previous studies had attributed the widespread use of plants in ethnomedicine to its activity and safety.[27]

Anti-ulcer activity

The antiulcer activity of *A. montanus* crude extract and its fractions expressed by their ulcer and percentage curative indices are shown in Table 4. The result showed that the antiulcer activity resides more in the polar fraction; highest in the aqueous fraction (UI of 0.6– 0.2) and curative index of 79 and 93% and lowest in the moderately non-polar (EtOAc) fraction (UI of 2.4) and curative index of 14 % as illustrated in Figure 1. All the treatments caused variable degrees of ulcer healing. Generally, there was a dose-dependent ulcer healing with 400 mg/kg doses eliciting higher curative indices than the 200 mg/kg doses. The 200 mg/kg doses of Aqu and 400 mg/kg doses of CMe, Aqu, nHex elicited ulcer indices significantly different ($p < 0.05$) from those of both controls. Only the 200 mg/kg of nHex and nBut as well as the 400 mg/kg caused a significant decrease ($p < 0.05$) in ulcer indices compared with the untreated control group (Table 4).

Indomethacin is an NSAIDs well known for its ability to cause gastric mucosal erosion by suppressing PG synthesis via cyclooxygenase inhibition. The PGs play a vital role in stabilizing the integrity of the gastric mucosal by stimulating bicarbonate and mucus production, enhancing mucosal perfusion, reducing the acidity and increasing the gastric epithelial production.[28,29] The suppression of PG promotes the damage of gastric mucosa by causing gastric hyper-motility, gastric blood flow disruption, reactive oxygen species stimulation, lipid peroxidation as well as neutrophil infiltration. The anti-ulcerogenic property of *A. montanus* extract could be attributed to its ability to suppress the synthesis of PGs and leukotrienes. It has been reported that inhibitors of leukotriene and 5-lipoxygenase are known to inhibit NSAIDs- and alcohol-induced gastric erosion in rats.[20,30,31] The gastric mucosa integrity is maintained by the counter effects of protective and aggressive factors. Thus, the effectiveness of the *A. montanus* extract treatments in this study is dependent on both the inhibition of gastric acid secretion and also on the enhanced protective factors such as bicarbonates and PGs in the gastric mucosa.[32]

The treatments with the extract and aqueous and n-hexane fractions were found to protect the mice's gastric mucosa against indomethacin-induced ulcers. There was evidence that the phytoconstituents of the extract and fractions could significantly inhibit the production or the activities of some aggressive factors facilitating the production of excess acid or enhancing the increased production of protective factors such as mucus and bicarbonate. This is further confirmed by the presence of gastrointestinal protective tannins in abundance. The astringent, soothing and emollient properties of tannins on the mucosal membrane of the gastrointestinal tract may precipitate micro-proteins at the ulcerated site resulting in the formation of an impervious protective matrix lining that prevents the attack of proteolytic enzymes.[15] The anti-ulcer activity of *A. montanus* confirmed its use in folk remedies to treat leading symptoms of ulcers such as indigestion constipation and nausea as described in previous work.[8] The anti-ulcer activity findings in this work agrees with the previous report on the leaf of *A. montanus* being used to treat cases of abdominal pains, pains of acute gastritis and its believed to be an antacid.[10]

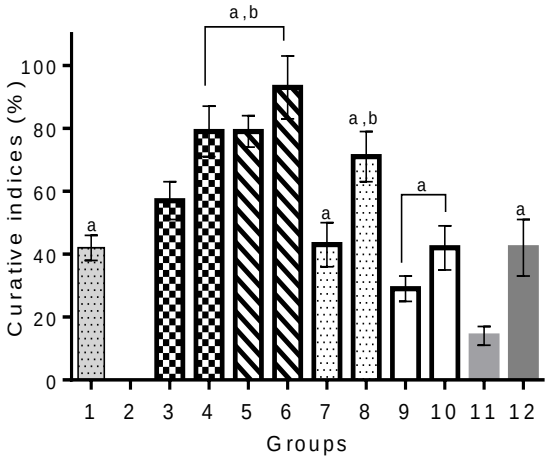

Figure. 1: Curative indices of extract and fractions; Data are CI ± SEM; the mean difference is significant at $p < 0.05$ compared to [a]untreated and [b]standard control

Table 4: Anti-ulcer activity of *A. montanus*

Groups	Dose (mg/kg)	UI	CI (%)
1	20	1.6 ± 0.5[a]	42
2	-	2.8 ± 0.4	-
3	200	1.2 ± 0.4	57
4	400	0.6 ± 0.2[a,b]	79
5	200	0.6 ± 0.4[a,b]	79
6	400	0.2 ± 0.2[a,b]	93
7	200	1.6 ± 0.2[a]	42
8	400	0.8 ± 0.4[a,b]	71
9	200	2.0 ± 0.3[a]	29
10	400	1.6 ± 0.5[a]	42
11	200	2.4 ± 0.2	14
12	400	1.6 ± 0.5[a]	42

Data represented as mean UI ± SEM; The mean difference is significant at $p < 0.05$ compared to *group 2 and [b]group 1

Conclusion

The study revealed that the xtract and fractions of *A. montanus* were rich in biologically active phytoconstituents with strong anti-ulcerogenic properties. The saponins and tannins constituents of the plant could be responsible for the anti-ulcer activity. The study justified the use of *A. montanus* leaves extract as an herbal remedy for the treatment of gastric ailments in South-eastern Nigeria.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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