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Investigation into the Anti-ulcer Activity of the Aqueous Leaf Extract of *Aspilia africana* C.D. Adams

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Abstract: The objective of this study was to investigate the antiulcer activity of leaves of *Aspilia africana* C.D. Adams (Compositae) in rats. Fresh dried leaves of *A. africana* were extracted by hot water maceration and the yield freeze dried. Antiulcer effects of the aqueous extract at 25, 50 and 100 mg/kg were evaluated in rats using ethanol, indomethacin and aspirin induced ulcer methods. Phytochemical analysis and lethality tests (LD_{50}) were carried out using standard methods. Results showed that the aqueous extract exhibited significant ($p<0.05$) and dose dependent anti-ulcer activity in all ulcer models. Percentage ulcer inhibitions of extract at 100 mg/kg for ethanol, indomethacin and aspirin induced ulcers were 73.0, 60.9 and 87.6%, respectively. Ulcer protections in all the models by the extract were dose-dependent. The ulcer inhibitory effects of the extract were comparable with those of standard drugs especially in the drug-induced ulcers. Oral LD_{50} value greater than 5000 mg/kg was obtained indicating the safety of the plant for consumption. Phytochemical analysis showed the presence of glycosides, tannins, alkaloids, saponins and flavonoids. Therefore results of our study suggest the aqueous extract of *A. africana* possesses antiulcer activity as claimed by its folkloric use.

Key words: *Aspilia africana*, anti-ulcer activity, necrotizing agents, rats, sucralfate

INTRODUCTION

The exact pathogenesis of ulcer continues to elude scientists and medical researchers, but a common ground has been proposed. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach (Ojewole, 2004; Del Valle *et al*., 2003). Such factors could range from natural causes (gastric cancer), infections (*H. pylori*), lifestyle (drugs-non steriodal antiinflammatory agents, alcohol, stress and cigarette smoking) (Berardi and Welage, 2005; Suerbaum and Michetti, 2002). Current treatment of ulcers in developing countries has been largely suppression of pain, with little or no strategy aimed at a cure. Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness. Many tropical herbs have been scientifically reported to possess potent antiulcer activity with examples as (Vela *et al*., 1997; Goulart *et al*., 2005; Singh *et al*., 2008; Aguwa and Ukwe, 1997).

Aspilia africana (Orangila in Igbo, Tozalin in Hausa and Yunyun in Yoruba) is a semi woody herb occurring throughout the regions of the savannah and tropical Africa on wastelands (Burkill, 1985; Hutchinson, 1962). The plant has been reported in literature to possess anti-microbial (Macfoy and Cline, 1990), haemostatic (Achonye, 1976), antifertility (Eweka, 2007) and antiinflammatory activity (Okoli *et al*., 2006). Also earlier studies in West Africa have reported the wound healing and antiulcer activity of its n-hexane and methanolic extracts (Okoli *et al*., 2007; Nguelefack *et al*., 2005). In South-eastern Nigeria, leaves of this plant is claimed to be effective in the treatment of stomach ache and bleeding gastric ulcers especially when taken as an aqueous decoction. This study was designed to evaluate the anti-ulcer activity of the plant and to identify phytochemically the constituents of the extract responsible for the observed activity.

MATERIALS AND METHODS

Plant material: The leaves of *A. africana* were collected in large quantities form Nsukka forests, Enugu State, Nigeria in February, 2008 and were identified by Mr. A. Ozioko of the Bioresources Development and Conservation Project in Nsukka. A voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy, University of Nigeria, Nsukka for future reference.

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Preparation of extracts: A weighed amount of shade-dried coarse powdered leaves was macerated overnight in hot distilled water. The mixture was filtered and air-dried to produce an amber green solution. The solution was sent for lyophilization (freeze-drying) at the National Institute of Pharmaceutical Research and Development (NIPRID), Abuja, Nigeria. The dried greenish extract was stored in a refrigerator till its use.

Phytochemical screening: The aqueous extract was tested for the presence or absence of secondary metabolites using standard phytochemical procedures and tests (Harbourne, 1984).

Animals: Mice of both sex (16-30 g), and male rats (120-200 g) supplied by the staff of the Department of Veterinary Pathology of the University of Nigeria, Nsukka were used. They were housed in steel cages, placed on standard pellet feed (Nigerfeed, Nigeria) and were given free access to clean water. They were kept in well ventilated rooms with a 12/12 h light/dark conditions and ambient room temperature. Animals were procured two weeks before the experiments to acclimatize with the laboratory environment. Animal experiments were done in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub. No. 85-23, revised 1985).

Acute toxicity and lethality tests: Lorke's (1983) method was used to ascertain the acute toxicity of the aqueous extract of *A. africana*. Three groups of 3 mice each were administered 10, 100 and 1000 mg/kg of the aqueous extract orally. The mice were observed for 24 h for effects of toxicity and the number dying in each group within the period noted. When no deaths were recorded, another three groups of 3 mice each were administered 1600, 2900 and 5000 mg/kg of the extract orally. The animals were observed for 48 h for effects of toxicity and the number dying in each group within the period was recorded.

Anti-ulcer activity: Three models (Ethanol, Aspirin, and Indomethacin) with effective induction of ulcer experimentally in rats were employed to evaluate the anti-ulcer activity of the aqueous extract of *A. africana*. All the rats used were fasted for eighteen hours but were given water *ad libitum* till the start of the experiment.

Ethanol-induced ulcer: Thirty fasted animals were used in five grous of six animals each. Groups A and B received 2 ml/kg distilled water (negative control) and 100 mg/kg p.o. sucralfate (Antepsin®) while rats in groups C, D and E were given 25, 50 and 100 mg/kg of MEAA orally (p.o) respectively. After one hour all animals received 1 ml/kg of 80% ethanol (Sigma-Aldrich, Germany) orally. The rats were sacrificed with chloroform (Sigma-Aldrich, Germany) anesthesia after one hour. The stomachs were isolated, washed gently under clean flowing water and cut open along the greater curvature. The stomachs were then fixed in 10% formalin and craters observed and ulcer scores were recorded using the method by Aguwa and Ukwe (1997).

Aspirin-induced ulcer: Thirty fasted rats were also used this model as five groups of six rats each. Groups A and B of this model received distilled water (2 ml/kg) and omeprazole 20 mg/kg p.o (Meprasil®) respectively, while groups C, D and E received 25 mg/kg, 50 mg/kg and 100 mg/kg p.o of the extract. After one hour, 200 mg/kg p.o of aspirin was given to each rat, and was scarified 4 h later (Williamson *et al.*, 1986) as described above. Stomachs were isolated, fixed and ulcers counted using the above mentioned method.

Indomethacin-induced ulcer: Animals (five groups of six rats each) in groups A, B, C, D and E received distilled water 2 ml/kg p.o., omeprazole 20 mg/kg p.o, 25, 50 and 100 mg/kg p.o of extract respectively. After 30 min, indomethacin 40 mg/kg p.o was administered to each rat. After 8 h of drug treatment (Urushidani *et al.*, 1979), stomachs were isolated, cut and ulcers counted as before.

Statistical analysis: Ulcer indices were shown as the mean±standard error of mean and level of ulcer protection presented as percentage inhibition. The significance of the differences in mean ulcer indices between extract and negative control was calculated at 95% confidence interval using Student's t-test.

RESULTS

Phytochemical screening showed that the extract contains alkaloids, glycosides, saponins, tannins, flavonoids and resins. Acute toxicity results showed that the LD_{50} was greater than 5000 mg/kg.

Ethanol-induced ulcer: In Table 1, ulcer inhibition was evident in all treatment of the aqueous extract of *A. africana* compared to the negative control. However, statistically significant ulcer inhibition (59.5 and 73.5%, $p<0.05$) could be seen only at doses of 50 and 100 mg/kg of the aqueous extract. The protection from ulcer was dose dependent even as ulcer was produced in all rats in this model.

Aspirin-induced ulcer: The aqueous extract at all the doses provided protection from ulcer and the protection was dose dependent. The aqueous extract at doses of 50 mg/kg and 100 mg/kg provided statistically significant protection (74.6% and 87.6%, $p<0.05$) when compared with the negative control (Table 2).

Table 1: Effects of aqueous leaf extract of *A. africana* on Ethanol Induced Ulcers in Rats (n = 6)

Treatments	Dose mg/kg p.o	Quantal ulcer incidence	Ulcer index	Ulcer inhibition (%)
Distilled water	2 ml/kg	6/6	1.90 ± 0.15	
Sucralfate	100	6/6	1.52 ± 0.14	20.00
Extract	25	6/6	1.30 ± 0.24	31.50
Extract	50	5/6	0.77 ± 0.09*	59.50
Extract	100	4/6	0.50 ± 0.09*	73.50

Ulcer indices are expressed as mean ± SEM: n = number of animals in each group.*: p<0.05 vs negative control (Students t-test)

Table 2: Effects of aqueous leaf extract of *A. africana* on Aspirin Induced Ulcers in Rats (n = 6)

Treatments	Dose mg/kg p.o	Quantal ulcer incidence	Ulcer index	Ulcer inhibition (%)
Distilled water	2 ml/kg	6/6	0.67 ± 0.10	
Omeprazole	20	5/6	0.11 ± 0.03	83.40
Extract	25	6/6	0.25 ± 0.06*	62.70
Extract	50	6/6	0.17 ± 0.02*	74.60
Extract	100	2/6	0.08 ± 0.03*	87.60

Values are mean ± SEM: n = number of animals in each group.*: p<0.05 vs. negative control (Students t-test)

Table 3: Effects of aqueous leaf extract of *A. africana* on Indomethacin Induced Ulcers in Rats (n = 6)

Treatments	Dose mg/kg p.o	Quantal ulcer incidence	Ulcer index	Ulcer inhibition (%)
Distilled water	2 ml/kg	6/6	3.45 ± 0.43	
Omeprazole	20	6/6	0.55 ± 0.12	84.00
Extract	25	6/6	3.17 ± 0.4	38.00
Extract	50	6/6	2.60 ± 0.25	24.60
Extract	100	6/6	1.35 ± 0.15*	60.90

Values are mean ± SEM: n = number of animals in each group.*: p<0.05 vs. negative control (Students t-test)

Indomethacin-induced ulcer: The aqueous extract protected the rats from experimentally-induced ulcers at all dose levels but the lesions produced in this model were noticeably more severe than the aspirin model (Table 3). However, the percentage ulcer inhibition was the least when compared to values obtained in the other two models. The dose of 100 mg/kg proved to be the only dose with statistically significant protection (60.9%, p<0.05).

DISCUSSION

The anti-ulcer activity of the aqueous of *A. africana* against ethanol-, aspirin- and indomethacin-induced ulcers was established in this study. Results of acute toxicity showed that the plant is safe as exemplified by its use as food in domestic and wild animals. The extract protected the stomach against ethanol's necrotic damage and its effect was more pronounced than sucralfate, a cytoprotective agent. Ethanol challenge induces gastric injury due to production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane (Pihan *et al*., 1987) presenting as red streaks of sores. The protection by the extract of this type may suggest a possible cytoprotective mechanism of action. An earlier study has suggested the plant's ability to protect against HCl/Ethanol challenge (Nguelefack *et al*., 2005) by prostaglandin-like cytoprotection. However, an antisecretory effect might be indicated as the extract protected the stomach mucosa from NSAIDS (aspirin and indomethacin) induced damage. This damage is elicited by the inhibition of prostaglandin synthesis, which is essential for mucosal integrity and regeneration (Lanza, 1998). This results to a sustained reduction in mucosal blood flow and a subsequent generation of ulcer. Sucralfate and omeprazole were employed in this study for the latter's cytoprotective but not anti-secretory effect and its effectiveness against experimentally induced ethanol ulcers (Hollander *et al*., 1984) and omeprazole exhibits an anti-secretory and protective effect (Del Valle *et al*., 2003) against ulcers and agents providing ulcer healing against NSAID induced ulcers may provide similar effect.

The presence of saponins, tannins, glucosides and alkaloids in this extract as seen in this study has also been reported by earlier studies (Adeniyi and Odufowora, 2000; Iwu, 1993). Ulcer protection may be attributed to any of these phytochemical constituents as flavonoids, tannins and saponins which have been shown to produce anti-ulcerogenic and anti-gastric activity (Carlo and Mascolo, 1994; Aguwa and Ukwe, 1997). However, until specific constituents are isolated and characterized, exact mechanism of action cannot be ascertained. Studies on the sub-acute and chronic toxicity of the extract are however in progress.

We have demonstrated in this study that the aqueous leaf extract of *Aspilia africana* has an ulcer healing property against experimentally induced ulcers in rats and this study confirms folkloric claims of the benefits of *Aspilia africana* in treatment of ulcer.

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