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Research Article

Effect of Aspilia Africana on Necrotizing Agent Induced Gastric Ulcer and Gastric Motility

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ABSTRACT

The antiulcer activity of chloroform leaf extract of Aspilia Africana on necrotizing agent induced gastric ulcer and its effect on gastric motility were investigated using male albino Wistar rats. Phytochemical analysis of the extract revealed the presence alkaloids, flavonoids, saponins, steroids, terprenoid, proteins and tannins. Acute toxicity test showed an oral LD_{50} greater than 5000mg/Kg. The Animals were divided into four groups (A-D); groups A and B were given 250 and 500mg/Kg of the extract respectively, group C was given 200µg/Kg of misoprostol (positive control) while group D was given 5ml/Kg of 3% Tween 80 (negative control). Results showed that the different doses of the extract significantly protected the stomach from ulceration caused by necrotizing agent in a dose-dependent manner. The mean ulcer indices of groups A and B were 3.32 ± 1.52 and 2.90 ± 0.42 respectively while that of group C was 1.64 ±0.25. These values were all significant ($p< 0.05$, $p<0.01$) when compared that of group D (8.94± 1.10). Percentage ulcer inhibition of the misoprostol was higher than that of the extract. Gastrointestinal motility studies showed that the extract significantly inhibited motility but was not potent as atropine, whose percentage inhibition of motility twice that of the extract. Thus, Chloroform extract of Aspilia Africana, demonstrated strong protection against gastric ulcer and moderate inhibition of gastrointestinal motility.

INTRODUCTION

Peptic ulcers are excavations that form in the mucosal wall of the stomach, in the pylorus, duodenum, or oesophagus[1]. They are produced by factors that cause imbalance between the protective factors (mucous and bicarbonate) and aggressive factors (acid and pepsin) in the stomach[2]. Peptic ulcers are usually solitary lesions less than 4cm in diameter located in the following sites in order of decreasing frequency: first portion of duodenum, stomach (usually antrum), gastroesophageal junction, within margins of gastrojejunostomy, in the duodenum and jejunum of patients with zollinger-Ellisons syndrome[3]. The erosion of the gastrointestinal tract by peptic ulcer may involve the entire mucosal thickness, penetrating even the muscularis mucosa[4]. Peptic ulcer has been one the leading causes of gastrointestinal surgery, with high morbidity and mortality rates[5].

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Current treatment of ulcers in developing countries has centered largely on suppression of pain, efforts towards its cure has been minimal or absent. Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower cost, availability, fewer adverse effects and perceived effectiveness[6]. Many tropical herbs have been scientifically reported to possess potent ulcer activity[7-9].

Aspilia Africana is a perennial herb which belongs to the family Asteraceae; it is a semi woody herb occurring throughout savannah regions and tropical Africa wastelands[10]. It is called 'Orangila' in Igbo, 'Tozalin' in Hausa and 'Yunyun' in Yoruba tribes in Nigeria[6]. It has been reported to possess antimicrobial[11], haemostatic[12], antifertility[13] and anti-inflammatory, antiulcer and wound healing[14] activities. In South-East, Nigeria, infusion made from aqueous extract of leaves of this plant is believed to be effective in treating stomach ache and bleeding gastric ulcers.

The study is designed to evaluate the antiulcer potential of chloroform extract of leaves of Aspilia Africana and also its effect on gastric motility.

MATERIALS AND METHODS

Plant Materials

Leaves of Aspilia Africana were collected from Nsukka forest, Enugu State, Nigeria in the month of June, 2010 and authenticated by Mr Ozioko, a taxonomist at the Herbarium section of the Department of Botany, University of Nigeria, Nsukka. A voucher specimen with number UNH/318[c] was kept in the herbarium for future references.

Extract Preparation

The leaves were dried under shade and milled using electric blender. 1000g of the powdered leaves were soaked in 3 litres of chloroform for 72 hours, strained using muslin cloth, thereafter, filtered through Whatman no. 1 filter paper. The filtrate was air dried to produce a dark greenish extract. 20g of the extract was dissolved in 10ml of 3% Tween 80 and made up to 100ml with the same solvent.

Phytochemical Analysis

Phytochemical screening was carried out using the method of Trease and Evans[15].

Animals

Male albino Wistar rats weighing 150-200g were used for the study. They were procured from animal house, University of Nigeria, Enugu Campus, kept in the same place and maintained under standard temperature (28 ± 5^{o} C) condition with 12: 12 hour light and dark cycle. The animals were handled in line with international guideline for handling laboratory animals[16].

Acute Toxicity Test

The method of Lorke[17] was used. Three groups of 3 rats each were administered 10, 100, 1000mg/Kg of the chloroform extract orally. The animals were starved for 24 hours and the number of dead rats in each group noted. No deaths were recorded and the second stage of the test was performed using a similar procedure of three groups of 3 rats per group. They were administered 1600, 2900, 5000mg/Kg of the extract respectively. The rats were observed for 48 hours for the effects of toxicity and the number of deaths in each group recorded.

Induction of Ulcer

Twenty male albino Wistar rats were divided into four groups (A-D) of 5 rats per group. The animals were starved for 24 hours before the commencement of the experiment but had free access to drinking water. Groups A and B received 250 and 500mg/Kg of the extract respectively. Group C received 200µg/Kg of misoprostol (positive control) while group D was given 5ml/Kg of 3% Tween 80 (negative control). One hour after administration, 10ml/kg of necrotizing agent (0.1N HCl in 80% ethanol) was given to all the rats in every group orally. After 4 hours, the animals were euthanized, their stomach removed, cut open through the greater curvature and washed gently in normal saline. Their stomach were spread and pinned flat on a plywood using thumbtacks. With the aid of hand lens, the stomach were observed for ulcers and scored using the method described by Main and Whittle[18] as follows:

<1mm = 1 (pin point)
>1mm <2mm = 2
>2mm <3mm = 3

The total score divided by a factor of 10 was designated as the ulcer index (UI) for that stomach. Percentage ulcer protection was calculated using the method of Suzuki et al[19] as follows:

$$\% \text{ ulcer protection} = 1 - \frac{\text{ulcer index for the test agent}}{\text{ulcer index for negative control}} \times 100$$

Gastrointestinal Motility Studies

Twenty male albino Wistar rats were used for the study. They were divided into four groups (A-D) of 5 rats per group. The animals were fasted for 24 hours prior to the onset of the experiment but drinking water was provided ad libitum. Groups A and B received 250 and 500mg/Kg of the extract respectively. Group C received 10mg/Kg of atropine (positive control) while group D received 5ml/kg of 3 % Tween 80 (negative control). 10 minutes after administration, 0.5ml of 10% charcoal suspension in 3% aqueous solution of Tween 80 was administered to all the animals in each group. All administration was via oral route. After 1 hour, the rats were sacrificed and their abdomen opened. The percentage of the intestine (from pylorus to caecum) travelled by the charcoal plug was determined as described by Akah et al[20]. Percentage inhibition of gastrointestinal motility was also calculated.

Statistical Analysis

Ulcer indices and % distance travelled in the GIT were shown as mean ± standard error of mean. Mean values between test and control groups were calculated at 95% confidence interval using student t-test. Results were considered significant at values of $p<0.05$, $p<0.01$ or $p<0.001$.

RESULTS

Phytochemical screening revealed large amount of alkaloids, flavonoids, sapononis, proteins and polysaccharides; moderate amount of terprenoids, steroids and small quantity of tannins, phlobatannins and reducing sugars (Table 1). Acute toxicity study showed an oral LD50 greater than 5000mg/Kg.

Table 1: Phytochemical composition of leaves of Aspilia Africana

Sl.No.	Phytochemical	Level of Presence
1	Alkaloids	+++
2	Flavonoids	+++
3	Reducing Sugar	+
4	Saponins	+++
5	Tannins	+
6	Terprenoids	++
7	Protein	+++
8	Polysaccharides	+++
9	Steroids	++
10	Phlobatannins	+

+++ = present in large amount
++ = Present in moderate amount
+ = Present in small amount

The mean ulcer indices of the two doses of the extract (groups A and B) were 3.32 ± 1.53 and 2.90 ± 0.45 respectively while that of misoprostol (group C) was 1.64 ± 0.25. These values were all statistically significant ($p<0.05$, $p<0.01$) when compared with that of negative control with a mean ulcer index of 8.94 ± 1.10 (Table 2). Misoprostol had the highest percentage ulcer inhibition of 81.7%, followed by the higher dose of extract with 67.6% while the lower dose had 58.4% (Table 2).

The mean distance travelled by the charcoal plug in the extract treated (groups A and B) were 15.6 ± 1.36 and 14.18 ± 1.02 respectively while that in the atropine treated (group C) was 2.82 ± 1.76. These values were statistically significant ($p<0.01$, $p<0.001$) when compared to that of negative control. Percentage inhibition of GIT motility (91.3%) was highest in the atropine treated group which was more than twice the value obtained in the group treated with 500mg/Kg of the extract (39%) (Table 3).

Table 2: Ulcer indices and % ulcer inhibition in treatment and control groups

Group	Ulcer index	% Ulcer inhibition
A (250mg/Kg) CEAA	3.32 ± 1.53[a]	58.4
B (500mg/Kg) CEAA	2.90 ± 0.45[b]	67.6
C (200µg/Kg) Misoprostol Positive control	1.64 ± 0.25[b]	81.7
D (5ml/Kg) 3% Tween 80 Negative control	8.94 ± 1.10	-

a = $p<0.05$ with respect to control
b = $p<0.01$ with respect to control

Table 3: Showing mean distance travelled by charcoal plug and % inhibition of gastrointestinal motility in treatment and control groups

Group	Distance travelled	% inhibition of GIT motility
A (250mg/Kg) CEAA	15.66 ± 1.36 [a, c]	32.2
B (500mg/Kg) CEAA	14.18 ± 1.02 [b, c]	39
C (10mg/Kg) Atropine positive contro	2.28 ± 1.76 [b]	91.3
D (5ml/Kg) 3% Tween 80 Negative control	29.74 ± 2.49	-

a = $p<0.01$ with respect to negative control
b = $p<0.001$ with respect to negative control
c = $p<0.01$ with respect to positive control

DISCUSSION

Chloroform extract of Aspilia Africana demonstrated a strong and dose-dependent antiulcer potential against necrotizing agent induced ulceration. This effect may be due to the presence of phytochemicals in the leaf extract mainly flavonoids, saponins and tannins. Previous studies have shown that flavonoids are potent water soluble antioxidants and free radical scavangers which prevent oxidative damage to cells and possess antiulcer activity[2][21, 22]. Flavonoids also possess anti-inflammatory activity and prevent gastric mucosal lesion due to ulcer[23]; and have been reported to be the most important plant constituent with antiulcer

activity[24]. Saponins and tannins have also been shown to possess antiulcer and anti-gastric activities[9,25]. Misoprostol, which served as positive control drug is a prostaglandin analogue (methyl PGE_1 ester) and have been shown to inhibit gastric acid production[26]. Therefore, the antiulcer activity of the extract could be due to direct inhibition of acid production or through its antioxidant properties. And yet another possible mechanism for the antiulcer potential of the extract may be due to the fact that it also had a sticky consistency comparable to sucralfate, a sticky gel-like substance that preferentially and strongly adheres, precipitates surface proteins at ulcer base, thus acting as a physical barrier and preventing acid, pepsin and bile from coming in contact with the ulcer base[27].

Result of the study showed that the extract inhibited gastric motility. Agents that inhibit gastric motility have been shown to also reduce its secretion and possess strong antiulcer activities[28]. The extract contain large amount of alkaloids and drugs containing alkaloids, such as Hyosciene-N-methyl bromide (Buscopan®) are potent anti-spasmodic agents[29].

CONCLUSION

The phytochemical compounds present in Aspilia Africana especially flavonoids may be responsible for the observed antiulcer effect. Flavonoids could act by prevention of oxidative damage to cells, direct inhibition of gastric acid production or by acting as a physical barrier that prevents acid and pepsin from coming in contact with ulcer base. Alkaloids are largely responsible for the observed inhibition of gastrointestinal motility.

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