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Research Article

Investigation of the Anti-Ulcer Activity of Chloroform Leaf Extract of Aspilia Africana in Rats

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ABSTRACT

The gastroprotective ability of chloroform leaf extract of Aspilia Africana against ulcer was investigated using male albino wistar rats. Aspirin was used to induce ulcer in the gastric mucosa while omeprazole was the standard anti-ulcer drug used. Phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, steroids, terprenoids, proteins and tannins. Acute toxicity test showed an oral LD50 greater than 5000mg/Kg. Results showed that chloroform leaf extract at different doses of 250mg/Kg and 500mg/Kg demonstrated significant protection against ulcer with mean ulcer indices of 5.04 ± 1.20 and 0.84 ± 0.15 respectively compared with negative control (3% Tween 80) with mean ulcer index of 9.42 ± 0.73. The higher dose of the extract demonstrated greater protective ability with percentage ulcer protection (91.1%) similar to that of omeprazole (92.8%). Thus, chloroform extract of Aspilia Africana was able to protect the stomach against ulceration caused by aspirin.

INTRODUCTION

Peptic ulcers are excavations (hollow out areas) that form in the mucosal wall of the stomach, in the pylorus, in the duodenum or oesophagus [1]. They are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach [2,3]. Such factors could result from natural causes (gastric cancer), infection (H.pylori), or life style (drug: non steroidal anti-inflammatory agent, alcohol, stress and cigarette) [4,5]. Peptic ulcers are usually solitary lesions less than four centimeters (4cm) in diameter, located in the following sites in order of decreasing frequency: duodenum (first portion), stomach (usually antrum), at the gastroesophageal junction (in the setting of gastroesophageal reflux or Barrett oesophagus), within the margins of a gastrojejunostomy, in the duodenum, stomach, and/ or jejunum of patients with Zollinger –Ellison syndrome, within or adjacent to ileal Meckel diverticulum that contains ectopic gastric mucosa [6].

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Gastric ulceris an excoriated area in the lining of the stomach. It occurs at the lesser curvature of the stomach and is caused by gastric secretion and is sometimes located at the junction of the antrum and fundus within 6cm to the pylorus [7]. The riddle of etiopathogenesis of peptic ulcer remains unsolved. Its worldwide distribution with varying prevalence and epidemiological and clinical behaviour makes it a unique geopathological problem [8]. The incidence of this malady may vary even within the same country, which may be due to different epidemiological factors [9]. Its pathogenesis is influenced by factors such as acid-pepsin secretion, mucosal barrier, mucous secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermal growth factor [10].

Medicinal plants are sources of several chemical compounds that have played dominant roles in maintenance of human health since antiquity. Over fifty percent (50%) of all modern chemical drugs are of natural plant origin [11]. Thus, plants are essential in drug development; moreover, plant based drugs have been observed to be non-toxic and have little or no side effects [12].

Extracts from the leaves, seeds, fruits, barks and roots of medicinal plants have been used in the preparation of infusions in traditional medicine. These infusions have been used to treat ailments ranging from common cold to malaria, liver cirrhosis, hypertension and so on. The active constituents contributing to the protective effects are the phytochemicals, vitamins and minerals [13]. One of such medicinal plants is Aspilia africana which has a folk line reputation as an anti ulcer remedy.

Aspilia africana is a perennial herb varying in height from 60cm to about 1.5m depending on rainfall. It is a common weed of field crops in West Africa and sometimes found in fallow land especially the forest zones [14]. In Nigeria, it is variously known as "Oranjila" in Igbo, "Tozalin" in Hausa, "Yunyun" in Yoruba and "Edemedong" in Efik [15]. The plant is a weed grazed by cattle and sheep and is mostly used in the South-West, Nigeria, as food for rabbits and hares [16]. *Aspilia africana* is widely used in African folk medicine to stop bleeding, remove corneal opacities, induce delivery and in the treatment of anaemia and various stomachs complains. Infusion of the leaves is taken by children and can also be mixed with clay as a medicine for stomach trouble [17]. It has been reported that the plant is effective against malaria infection [18]. The plant has been reported to possess anti-microbial [19] and anti-haemostatic, antifertility [20] and anti-inflammatory activity [21].

Its phytochemical analysis revealed that it is rich in flavonoids, saponin, tannins, alkaloid, sterols, terpens. The medicinal plant also contains vitamins: ascorbic acid, riboflavin, thiamine, and niacin [17].

Allopathic drugs have been used to treat or manage gastric ulcer. Some of these agents like proton pump inhibitors (e.g. esomeprazole, omeprazole), H2-receptor antagonists e.g. cimetidine have side effects and there is a high incidence of ulcer relapse and drug interaction following the use of these drug [22, 23]. Therefore, there is need to evaluate medicinal plants with potential antiulcer properties since plant based drugs have been observed to be non-toxic and have little or no side effects12. The search has yielded good results as the antiulcer activity of some medicinal plants has been scientifically validated [24-27]. Moreover, pharmacoeconomic considerations make it impossible for patients in low socioeconomic background to afford these allopathic drugs. Consequently, there is tendency to seek traditional medical care. We, therefore, decided to embark on this study in order to evaluate the gastroprotective activity of leaf extract of Aspilia Africana in experimentally induced gastric ulcer and contribute the on-going effort aimed at providing cheaper and better remedy for this disease.

MATERIALS AND METHODS

Animals

Thirty-three male albino wistar rats weighing 160-240g were used for the study. They were procured from the animal house, Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus. The rats were kept in the same environment under standard temperature condition (28±3^{0}C) with12:12 hour light/dark cycle. They were fed with commercial grower's feed (Guinea feed®Nig. PLC) and had free access to clean drinking water.

Plant Materials

Leaves of Aspilia Africana were collected from the premises of University of Nigeria, Nsukka and authenticated by a taxonomist at the herbarium section of Department of Botany, University of Nigeria. A voucher specimen with number UNH/315c was kept in the herbarium for future references. The leaves were dried under shade and milled using an electric blender.

Extract Preparation

1000g of powdered leaves of Aspilia Africana were soaked in 3 litres of chloroform for 72hours and strained with muslin cloth, then filtered using Whatman no. 1 filter paper. The filtrate was allowed to dry in open air and a dark greenish extract was formed. 20g of the extract was dissolved in 10ml of 3% Tween 80 and made up to 100ml with chloroform.

Phytochemical Analysis

Phytochemical screening was carried out using the method of Trease and Evans [28]. This method is useful in determine the various constituents of the plant and their pharmacological and toxicological effects [29].

Acute Toxicity Test

13 male albino wistar rats were used for determination of LD50 as described by Lorke [30].

Aspirin induced ulceration

20 male albino wistar rats were divided into four groups (A-D) of 5 rats each. The rats were starved for 24 hours prior to the commencement

of the experiment but had free access to clean water. 250mg/Kg of Chloroform extract (CE) was given to rats in group A; 500mg/Kg was given to rats in group B while those in group C were given 20mg/Kg of omeprazole. Group D served as the negative control and rats in this group were given 5ml/Kg of 3% Tween 80. All administration was via oral route using gavage. Thirty minutes after administration, 200mg/Kg of aspirin were given orally to all the rats in every group. The animals were euthanized after 4 hours, their stomach removed, cut open through the greater curvature and then washed in normal saline. Their stomach were spread and pinned flat on a plywood using thumb-tacks. With the aid of a hand lens, their stomach were observed for ulcers and scored as described by Main and Whittle [31] 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. The percentage ulcer protection was calculated using the formula of Suzuki et al [32] as follows:

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

RESULTS

Phytochemical analysis of Aspilia Africana showed the presence of large amount of alkaloids, saponins, flavonoids, proteins and polysaccharide; moderate amount of Terprenoids and steroids and little amount of reducing sugar, tannins, cardiac glycosides and phlobatannins (Table 1). Fats and oil were absent.

The means ulcer indices of the different doses (250mg/Kg and 500mg/Kg) of Aspilia Africana were 5.04 ± 0.02 and 0.84 ± 0.15 respectively. These values were statistically significant when compared that of negative control with a mean ulcer index of 9.42 ± 0.73. However, group B (500mg/Kg CEAA) produced a greater protection against ulcer (91.1%) compared to the group A (250mg/Kg CEAA) whose ulcer protection was 46.5% (Table 2). The ulcer index of the 500mg/Kg CEAA was not statistically significant ($p>0.05$) when compared to that of omeprazole, in fact, both groups showed similar protective potency against ulcer (Table 2).

Table 1: Phytochemical Analysis of Aspilia Africana

Sl/No	Phytochemical	Degree of presence
1	Alkaloids	+++
2	Flavonoids	+++
3	Fats and Oil	-
4	Reducing sugar	+
5	Saponins	+++
6	Tannins	+
7	Terprenoids	++
8	Proteins	+++
9	Polysaccharides	+++
10	Steroids	++
11	Cardiac glycosides	+
12	Phlobatanins	+

- Absent
+ Present in small concentration
++ Moderately high concentration
+++ Very high concentration

Result of acute toxicity study showed an oral LD_{50} greater than 5000mg/Kg

Table 2: Comparison of the ulcer indices in treatment groups with control groups

Groups	Ulcer index Mean ± SEM	% Ulcer protection
A (250mg/Kg) CEAA	$5.04 \pm 1.02^{a,c}$	46.5
B (500mg/Kg) CEAA	$0.84 \pm 0.15^{b,d}$	91.1
C (20mg/Kg) Omeprazole	0.68 ± 0.15^{b}	92.8
D (5ml/Kg of 3% Tween 80)	9.42 ± 0.73	-

DISCUSSION

We evaluated the antiulcer activity of chloroform extract of leaves of Aspilia Africana against aspirin induced ulceration. Phytochemical analysis and acute toxicity study were also carried out. An oral LD50 is greater than 5000mg/Kg indicates that the extract is safe.

Aspirin is a weak organic acid that reversibly inactivates cycloxygenase (COX I) required for the synthesis of prostaglandins. This inhibits gastric cytoprotective action of prostaglandins [33]. Another possible mechanism of ulcer induction by aspirin is by the process of 'ion trapping'. Aspirin (pKa 3.5) becomes ionized and non-diffusible on entering mucosal cells (pH 7.1) causing focal necrosis of the cells, acute ulcers and microscopic haemorrhage [23]. Aspirin may also cause inhibition of gastric mucous secretion and mucosal blood flow [34].

Omeprazole is a proton pump inhibitor which forms a covalent disulphide bond with H+- K+ ATPase (proton pump) and irreversibly inactivates the enzyme, thus blocking the final common pathway for acid secretion [35]. Omeprazole also has anti-secretory and anti-ulcer effects [3].

Chloroform leaf extract of Aspilia Africana demonstrated gastroprotective activity against ulcer induced by aspirin in a dose-dependent manner; the higher dose of the extract (500mg/Kg) showed similar potency as omeprazole. The protection effect of the extract may be due its high content of flavonoids, alkaloids and saponins, phytochemicals that have demonstrated strong antioxidant properties [36]. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and ulceration [36-38]. Flavonoids also prevent gastric mucosal lesion and was identified as the most important plant constituents associated with anti-ulcer activity [39]. Flavonoids also possess anti-inflammatory activity [37]. Thus, the presence of large amount of flavonoids in Aspilia Africana may justify its use in folk medicine for the treatment of ulcers, wounds and burns. Flavonoids, Saponins and tannins were reported to possess anti-ulcerogenic and anti-gastric activity [40, 41]. Therefore, the presence of these of these compounds in the extract may be responsible for the observed anti-ulcer activity.

CONCLUSION

The leaf extract of Aspilia Africana demonstrated considerable anti-ulcer activity comparable to that of omeprazole in aspirin induced ulceration in rats. This action may be due to its phytochemical constituents especially flavonoids.

REFERENCES

[1] Adreoli T, Chan PD, Cowell JC, Gilbert DM, Green G, Johnson M. et al. Management of patients with Gastric and Duodenal disorders In Brunner and Suddarth's Textbook of Medical Nursing. 11th edition, Elsevier, Philaldephia, USA. pp 1203-1279; 2008.

[2] Ojewole EB. Peptic ulcer disease. In Therapeutic Basis of clinical pharmacy in the tropics. 3rd edition, SNAAP press, Enugu, Nigeria. pp 541-564; 2004.

[3] Del Valle J, Cheg W, Scherman J. Acid Peptic Disorder in Textbook of gastroenteroloy. 4th edition,Philaldephia, Lippincott William and Wilkins pp: 1321-1376; 2003.

[4] Berandi RR, Welage S. Peptic Ulcer Disease In Pharmacotherapy: A pathophysiologic approach. 6th edition McGraw-Hill pp: 629-648; 2005.

[5] Suerbaum S, Michetti P. Helicobacter pylori infection. New English Journal of Medicine 2002; 347: 1175-1186.

[6] Chan FK, Leung WK. Peptic ulcer disease. Lancet 2002; 360: 933-939

[7] Ojewole EB. Peptic ulcer disease In: Therapeutic basis of clinical pharmacy in the tropic. 2nd edition, optimal publishers Enugu, Nigeria. Pp 401-437; 1996.

[8] Watkins G. Geographical aspects of peptic ulcer. Modern trends in Gastroenterology, third edition by W.L. Card Butterworths, London; 1961.

[9] Dogra JR. Studies on peptic ulcer in South India, part I: Introduction and Clinical study of 258 cases. Indian JL Med. Res. 1940; 28:145.

[10] Hadey GG. A study of peptic ulcer as found in South India: In proceedings of the group discussion on peptic ulcer, p 31, I.C.M.R. Publication, New Delhi, India; 1959.

[11] Burton GW, Joyce D, Ingolk KU. Is vitamin E the only lipid soluble chain having antioxidant activity in human blood, plasma and erythrocyte? Archives of Biochemistry and Biophsiology. 1993; 222: 228-232.

[12] Momin A. Role of indigenous medicine in primary health care. 1st International Seminar on Unani Medicine, New Delhi, India. 1987; p 54.

[13] Okwu DE, Ekeke O. Phytochemical Screening and Mineral Composition of Chewing Sticks in South eastern Nigeria. Global Journal of Pure and Applied Sciences 2003; 9: 235-238.

[14] Eweka AO. Histological Studies of the effect of Oral administration of Aspilia africana (Asteraceae) leaf extract on Ovaries of female Wistar rats. African Journal of Traditional Complementary and Alternative Medicine. 2009; 6(1): 57-61.

[15] Singha SC. Medicinal Plants of Nigeria. National Press, Lagos pp: 24-30; 1965.

[16] Burkill HM. The useful plants of West Africa. African Royal Botanical Garden 1985; 1: 446-447.

[17] Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian

medicinal plants. African Journal of Biotechnology 2006; 5(4): 357-361.

[18] Okokon JE, Nwidu LI, Essiet GA. (2006). Evaluation of in vivo Antiplasmodial Activity of Aspilia africana. International Journal of Pharmacology 2006; 2(3): 348-351.

[19] Macfoy CA, Cline EI. In vitro antibacterial activities of three plants used in traditional medicine in Sierra Leone. Journal of ethnopharmacology 1990; 28: 323-327.

[20] Eweka A. Histological Studies of the effect of Oral administration of Aspilia africana (Asteraceae) leaf extract on Ovaries of female Wistar rats. Internet Journal of Alternative Medicine. 2007; 4(2): 120-124.

[21] Okoli CO, Akah P, Okoli A. Potential of leaves of Aspilia africana (Composite) in wound care: an experimental evaluation. Boimedical Centre Complimentary and Alternative Medicine. 2007; 7(24): 101-109.

[22] Dharmani P, Palit G. Exploring Indian medicinal plants for antiulcer activity. Indian Journal of Pharmacology 2006; 38: 95-99.

[23] Tripathi RD. "Drugs for Peptic Ulcer", Essenticals of Medical Pharmacology. 6th edition, Jaypee Brothers Medical Publisher Ltd New Delhi, India pp: 627-636; 2005.

[24] Garg GP, Nigam SS, Ogle CW. The gastric antiulcer effect of the leaves of Neem tree. Planta Medica. 1993; 59: 215-217.

[25] Anoop A, Jegadeesan M. Studies on the antiulcerogenic potential of Hemidesmus indicus. Journal of Ethnopharmacology 2003; 84: 149-156.

[26] Okwuosa CN, Unekwe PC, Nwobodo EW, Chilaka K. The antiulcer activities of the Leaf extracts of Combretum racemoum. Journal of Biomedical Investigation 2006; 4(1): 8-14.

[27] Al-mofleh IA, Al-haider AA, Mossa JS, Al-sohaibai MO, Al-yahya MA, Rafatullah S. et al. Gastroprotective effect of Nigella sativa on necrotising agent induced gastric injury in experimental animals. Saudi Journal of Gastroenterology 2008; 14(30): 128-134

[28] Trease GE, Evans WC. Textbook of pharmacognosy. 12th edition, Balliese Tindall and Company Publisher, London. pp 343-383; 1983.

[29] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian Medicial Plants. African Journal of Biotechnology 2005; 4(7): 685-688.

[30] Lorke D. New approach to practical acute toxicity. Archives of Toxicology 1983; 54: 275-287.

[31] Main G, Whittle NB. Investigation of vasodilator and antisecretor role of prostaglandin in the rat mucosa by use of NSAIDs. British Pharmacology 1975; 53: 217-224.

[32] Suzuki Y, Hamagani M, Ito M, Yamagani T. Anti-ulcer effect of cetraxate on various experimental gastric ulcers in rats. Japanese Journal of Pharmacology 1976; 26: 471-474.

[33] Harvey RA, Champe PC, Finkel R, Cubedu LX, Clark M.A. "Anti-inflammatory drugs and autocoids" Lippincott's illustrated reviews: pharmachology. 4th edition, Lippincott Williams and Wilkins Pp 500-518; 2009.

[34] Wang GZ, Huang GP, Zhou G, Yin GL, Guo CJ, Xie CG, et al. Aspirin can elicit the occurrence of gastric ulcer induced with acetic acid in rats. Cell Physiol. Biochem. 2007; 20(1-4): 205- 212.

[35] McQuid RK. Drugs used in treatment of gastrointestinal diseases In Basic and Clinical Pharmacology. 11th edition, McGraw-Hill companies New York, USA pp 1068-1098; 2009.

[36] Salah N, Miller NJ, Payange G, Bolwell GP, Rice E, Evans C. Polyphenol flavonoids as scavenger of aqueous phase radicals as chain breaking antioxidant. Archives of Biochemistry and Biophsiology. 1995;

[37] Okwu DE. Phytochemicals and Vitamin content of indigenous species of South-Eastern Nigeria. Journal of Sustainable Agriculture and Environment. 2004; 6(1):30-37.

[38] Del Rio A, Obudulu BG, Casfillo J, Marin FG, Ortuno A. Use and prosperties of citrus flavonoids. Journal of Agricultural food Chemistry. 1997; 45: 4505-4515.

[39] Martin MJ, Motilva V, Alarcon de la lastra C. Quecertin and naringenin effect of ulcer formation and gastric secretion in rats. Phytotherapy Research. 1993; 7: 150-153.

[40] Carlo GD, Mascolo N, Capasso F, Autore G. Effect of quercetin on gastrointestinal tract of rats and mice. Phtotherapy research 1994; 8: 179-185.

[41] Aguwa CN, Ukwe C. Gastrointestinal activities of Steculia tragacantha leaf extracts. Fitoterapia. 1997; 68(2): 127-131