Anti-Ulcer Effect Of Aspilia Africana (Asteraceae) Leaf Extract On Induced Duodenal Ulcer Of Adult Wistar Rats (Rattus Norvegicus) – A Histological Study

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Citation

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Abstract

Histological studies of the effects of oral administration of extract of Aspilia africana, used in ethno medical practice in Africa for the management of various ailments, on the duodenum of adult Wistar rats previously exposed to varied concentration of hydrochloric acid were carefully studied. The rats (n=30), average weight of 189g were randomly assigned into three treatments (n=24), 'A', 'B', 'C', each (n=8) and control (n=6), D groups. The experimental rats each received 0.5mls of 50% dilute Hydrochloric acid 48hrs prior to administration of the extract. The rats in the treatment groups 'A' and 'B' received 0.5g/kg and 1g/kg respectively of aqueous extract of Aspilia africana orally through orogastric tube for fourteen days, while the control rats (group D) received equal volume of distilled water without the extract of Aspilia added. The rats in group 'C' received only the hydrochloric acid and were sacrificed 72hrs after administration. The rats were fed with growers' mash purchased from Edo feeds and Flour Mill Ltd, Ewu, Edo state and were given water liberally. The rats were sacrificed on day fifteen of the experiment. The small intestine was carefully dissected out and quickly fixed in 10% formal saline for routine histological study after H&E method. The histological findings indicated that the treated sections of the small intestine showed varying degrees of cellular proliferation and epithelia regeneration. These findings indicate that Aspilia africana consumption may probably have anti-ulcer effects on the duodenal ulcer by its healing effects on the Brunnals gland and epithelia cells of the small intestine of adult Wistar rats. It is recommended that further studies aimed at corroborating these observations be carried out.

INTRODUCTION

Plant materials as sources of medical compounds continue to play a dominant role in the maintenance of human health since antiquity. Over 50% of all modern chemical drugs are of natural plant product origin, and is essential in drug development programs of the pharmaceutical industry [1] .Like any therapeutic agent, when overdosed or incorrectly used they also have the potential to induce adverse effects. The historic role of medicinal herbs in the treatment and prevention of disease, and their role as catalysts in the development of pharmacology do not, however, assure their safety for uncontrolled use by an uninformed public [2] .

There has been minimal research to address possible adverse reproductive, immunologic, or neurological effects or even systemic toxicity and/or carcinogenicity that might be associated with high doses or prolonged use of these products [3] . This concern was frequently expressed at the International Workshop to Evaluate Research Needs on the Use and Safety of Medicinal herbs could not be assumed safe because they are "natural" [4] .

In Benin City, Nigeria, many plants are used in herbal medicine to cure diseases and heal injuries. Such medicinal plants include Aspilia africana (Asteraceae), 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 [5] . It is ligneous at the base, its fruit quadrangular akenes and leaves opposite and hairy. The plant is a weed grazed by cattle and sheep and is mostly used in the western state of Nigeria as food for rabbits and hares [6] .

Aspilia africana is widely used in ethno medical practice in Africa for its ability to stop bleeding, even from a severed artery, as well as promote rapid healing of wounds and sores and for the management of problems related to cardiovascular diseases [7] . It has also been established that Aspillia africana has an anticoagulant activities [8] . Infusion of the leaves is taken by children and can also be mixed with

clay as a medicine for stomach trouble [9] . It has been reported that the plant is effective against malaria infection [10] . It has been classified among substances with low toxicity, with an LD_{50} averaging 6.6g/Kg body weight [11] . The methanolic and aqueous extracts of the leaves of Aspilia africana has exhibited differential anti-bacterial activities on both Gram-positive and Gram-negative bacterial species [12-13] .

The small intestine functions in the digestion and absorption of food materials in the body. It also prevents duodenal ulceration due to the presence of the Brunner's gland. The small intestine consists of the duodenum, jejunum and ileum.

This work is carried out to investigate and corroborate the previous work done on the efficacy of Aspilia africana leave extract in the management of peptic ulcer disease [14] , by studying the histological effects of the extract on previously induced peptic ulcer in adult Wistar rats.

MATERIALS AND METHODS

PLANT MATERIALS: Fresh leaves of Aspilia africana were collected in December, 2008 at Iduwunomwina Community in Ovia North-East local government area of Edo State. The plant was identified and authenticated at the Botany department of the University of Benin, Benin City. The harvested fresh leaves were sun dried and ground into a fine powder. The dried material (300g) was macerated in 6 liters of distilled water for 48hrs at 4oC in a refrigerator. The extract was sieved and the juice was filtered using Whatman No 1 filter paper. The filtrate was put in a stainless-steel tray, and concentrated in an air-circulating oven at 42oC until total dryness. The resultant extract was put into small glass dishes and stored at 28oC in an incubator for further studies.

ANIMALS: Thirty,(30) adult Wistar rats of both sexes, with average weight of 189g were randomly assigned into four groups A, B, C of (n=8) and D of (n=6) in each group. Groups A, B and C of (n=24) serves as treatments groups while Group D (n=6) was the control. The rats were obtained and maintained in the Animal Holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Nigeria. They were fed with growers' mash obtained from Edo feed and flour mill limited, Ewu, Edo state and given water liberally. The rats gained maximum acclimatization within two weeks before actual commencement of the experiment.

ASPILIA AFRICANA ADMINISTRATION: The experimental rats each received 0.5mls of 50% dilute Hcl acid 48hrs prior to administration of the extract. The rats in the treatment groups 'A' and 'B' received 0.5g/kg and 1g/kg respectively of aqueous extract of Aspilia africana orally through orogastric tube for fourteen days, while the control rats (group D) received equal volume of distilled water without the extract of Aspilia added. The rats in group 'C' received only the hydrochloric acid without the extract of Aspilia added, and were sacrificed 72hrs after administration. The remaining rats (groups A, B and D) were sacrificed on day fifteen of the experiment. The small intestine was carefully dissected out and quickly fixed in 10% formal saline for routine histological study after H&E method [15] . The 0.5g/kg and 1g/kg extract of Aspilia africana doses were chosen and extrapolated in this experiment based on the indiscriminate use of the plant here in Nigeria and on previous work done with this plant [11-14] .

HISTOLOGICAL STUDY

The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffinised sections were stained routinely with haematoxylin and eosin. Photomicrographs of the desired results were obtained using digital research photographic microscope in the University of Benin research laboratory.

RESULTS

The small intestine of the control group (D) showed normal histological features of the duodenum; illustrating well defined and distinct long villi, tall columnar epithelium, and numerous Brunner's glands (Fig.1).

The small intestine of the treated group (C) showed marked distortions in the epithelia and obvious histological changes in Brunner's glands of the duodenum; while that in groups (A) and (B) showed varying degrees of cellular proliferation and epithelia regeneration, and evidence of increased basophilia in the nucleus; with the animals in the group receiving 1g/kg extract of Aspilia africana more marked.

Figure 1

Fig.1: Photomicrograph of the duodenum of control animals (Group D) (Mag. x400)

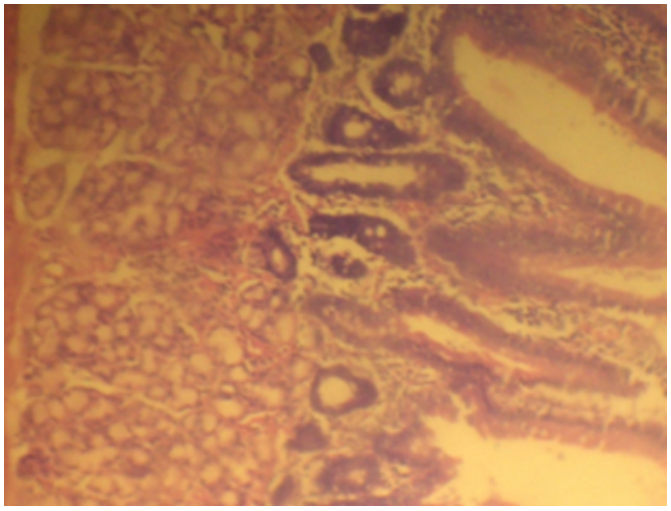


Figure 2

Fig.2: Photomicrograph of the duodenum of animals in group "C" rats treated with 0.5mls of 50% dilute Hcl acid 72hrs prior to sacrifice

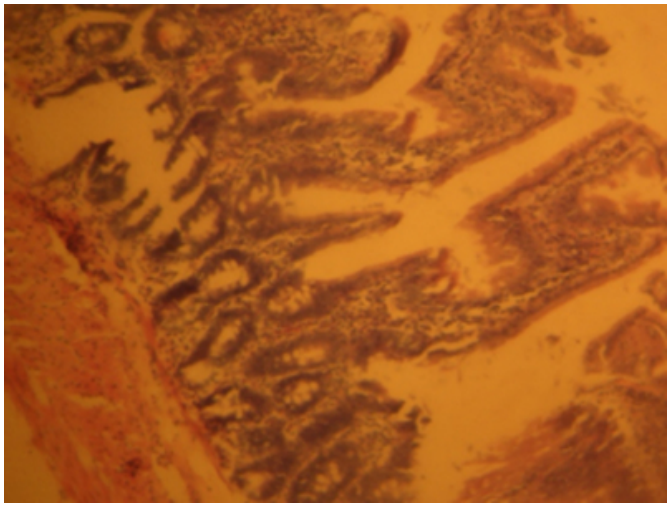


Figure 3

Fig.3: Photomicrograph of the duodenum of animals treated with 0.5g/kg of extract. (Group A) (Mag. x400)

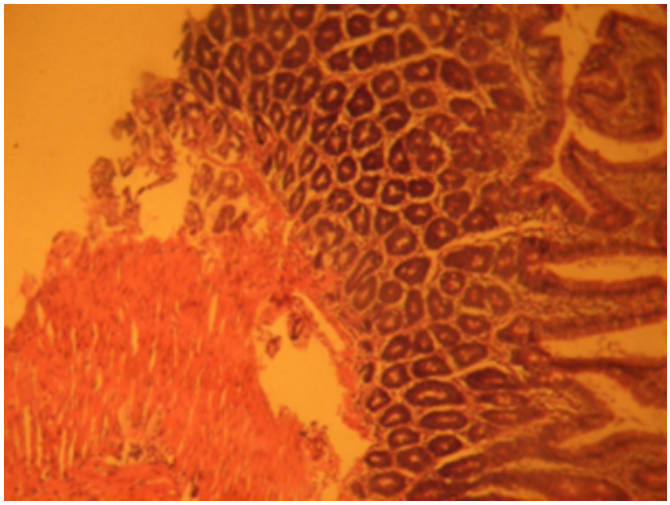
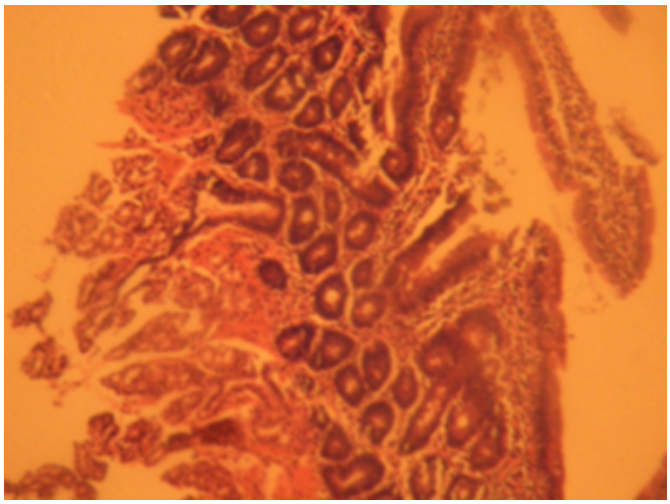


Figure 4

Fig.4: Photomicrograph of the duodenum of animals treated with 1g/kg of extract. (Group B) (Mag. x400)



DISCUSSION

The results of the haematoxylin and eosin staining (H & E) reactions showed marked distortions in the epithelia and obvious histological changes in Brunner's glands in group "C" rats that received only the dilute Hcl, and varying degrees of cellular proliferation and epithelia regeneration with evidence of increased basophilia in the nucleus of groups "A" and "B" rats; with the animals in group "B" that received 1g/kg extract of Aspilia africana more marked.

The increase in cellular proliferation and epithelial regeneration in the duodenum in the treatment groups (A & B) as reported in this study may have been as a result of the

effects of constituents of Aspilia africana as earlier reported by Okoli et al in 2007 [16] . Evaluation of the potentials of A. africana in wound care showed that the leaf extract and fractions exhibited haemostatic, antimicrobial and wound healing activities suggesting that the constituents of the leaves may play a useful role in wound care. The extract and fractions arrested bleeding from fresh wounds by reducing bleeding/clotting and whole blood coagulation time which are important indices of haemostatic activity [16] . In separate studies elsewhere, A. africana leaf extracts demonstrated haemostatic activity [17, 18] . Haemostasis involves the spontaneous arrest of bleeding from damaged blood vessels [19] , which is important for initiation of tissue repair processes and prevention of tissue death through haemorrhage. The haemostatic process proceeds through a cascade of reactions, which starts with vascular spasm of the ruptured vessels [20, 21] , formation of platelet plug through platelet aggregation, and coagulation of the blood [20] . Leaf extracts of A. africana have been shown to increase vascular tone [7] which is a measure of vasoconstriction and suggestive of the possibility of the leaf extracts arresting bleeding from fresh wounds through this mechanism. However, blood clotting and coagulation also involve other mechanisms such as prothrombin activation with its subsequent conversion to thrombin and which in turn converts fibrinogen to insoluble fibrin [21] . The reduction of coagulation time of whole blood by the leaf extracts is an indication that the extracts may also interfere with the blood coagulation pathways. Thus, the haemostatic effect of the extract may derive from acceleration of the coagulation process with the consequent reduction in clotting time as well as vasoconstriction which are necessary in limiting blood loss from damaged vessels.

Wounds provide environments conducive for the growth of microbial organisms. Usually, microbial contaminations of wounds involve a variety of organisms such as Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Clostridium perfringens, Clostridium tetani, Coliform bacilli and enterococcus [22, 23] . Evaluation of the effect of the extractives on clinically isolated microbial contaminants of wounds showed varying levels of inhibitory activity against species of Pseudomonas and Staphylococcus. Microbial infection of wounds delays healing [23, 24] and causes a more pronounced acute inflammatory reaction [25] which can lead to further tissue injury and damage. Thus, the antimicrobial activity of the extract and fractions on these wound isolates may partly contribute to the wound healing effect by eliminating infection thus allowing the natural tissue repair processes to start. It also suggests that the leaf extracts may also play a useful role in accelerating the healing of old wounds by eradicating already established infection. The antimicrobial activity of honey and the essential oil of Melaleuca alternifolia is believed to underlie their usefulness as alternative therapy in wound healing [23, 26, 27] .

In addition to inhibiting the growth of these micro-organisms, the extract and fractions effectively reduced the epithelialisation period of experimentally-induced wounds which is an index of pro-healing activity. The precise aspect as well as the exact mechanism of wound healing affected by the extract and fractions is yet to be elucidated. In the tissue repair process, inflammatory cells promote the migration and proliferation of endothelial cells, leading to neovascularisation of connective tissue cells which synthesize extracellular matrices including collagen, and of keratinocytes resulting to re-epithelialisation of the wounded tissue [28] . In the wound healing process, collagen formation peaks at day 7 and epithelialisation occurs in 48 h under optimal conditions [29] . The extent to which the extractives interact with these processes is not known [16] .

Documented literature reports of Phytochemical analysis of the extract and fractions of A. africana indicated the presence of typical plant constituents such as alkaloids, saponins, sterols, terpenoids, carbohydrates, glycosides and tannins [14, 16] . These metabolites are usually responsible for the pharmacological activities of medicinal plants. -pinene, one of the terpenoids in A. africana leaves, is known to possess anti-inflammatory activity [30] and may contribute to the wound healing activity by suppressing inflammatory reactions invoked by the injured tissues. In addition to this, the documented identification of the abundant presence of saponins and tannins in the leaves of this plant [18] is implicating for these constituents in the activities of the leaf extracts, especially tannins, which have been implicated in the haemostatic activity of plants where they arrest bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs. To a reasonable extent, going by the quantified relative presence in the leaves of this plant and documented role in haemostatic activity, we may safely assume that the tannins in the extracts partly contribute to the activity since mechanisms other than vascular plugs formation are likely involved.

It may be inferred from the present results that higher dose and prolonged administration of Aspilia africana extract

resulted in rapid healing of the ulcer crater.

CONCLUSION AND RECOMMENDATION

In conclusion, our study revealed that Aspilia africana extract caused varying degrees of cellular proliferation and epithelia regeneration, and evidence of increased basophilia in the nucleus; with the animals in the group receiving 1g/kg extract of Aspilia africana more marked. The results of this study indicate that extracts of leaves of A. africana have good potentials for use in peptic ulcer disease and further provide a rationale for the use of the leaves of this plant in peptic ulcer management by alternative medical practitioners and rural dwellers. It also corroborates the findings of previous researchers in this field [14, 16] . It is recommended that further studies be carried out to examine these findings.

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