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Preliminary study on the ulcerogenic effect of the crude extract of aloe vera administered to ulcer-induced albino rats

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Abstract
Introduction: Ulcer serious health issue associated with high risk of mortality and morbidity. This study was carried out to determine protective effects of Aloe vera in indomethacin induced ulcers in albino rats.
Materials and Methods: Albino rats were divided into four group five rats per group. Group I: control group, placed on food and water only. Group II: Indomethacin induced gastric ulcer. Group III: *Aloe vera* pre-treated. Group IV: Omeprazole pre-treated group. Liver enzymes, ulcer index and histological changes on the stomach were studied using standard methods.
Results: There was an increased in the serum levels of AST and ALT while ALP levels decreased in gastric ulcer untreated. These enzymes were observed to be normal in Aloe vera pre-treated group and omeprazole pre-treated group. There was also a decreased level of serum bicarbonate and an increased level of serum chloride in the gastric ulcer untreated group when compared to Aloe vera pre-treated group and omeprazole pre-treated group. The mean ulcer indexes of the Aloe vera pre-treated and Omeprazole pre-treated were (0.36±0.074, ±0.074mm) respectively. Aloe vera showed statistical significant gastro-protective activity ($P < 0.05$) similar to conventional drug (omeprazole).
Conclusion: The results revealed that Aloe vera extracts can be used to prevent ulcer.

Keywords: Omeprazole, histological changes, Indomethacin, Gastric ulcer, liver enzymes.

Introduction
Ulcer is a chronic disease which impairs the quality of life, associated with increased morbidity and mortality [1]. The aetiology of peptic ulcers is not clearly known, it results probably due to an in- balance between the aggressive (acid, pepsin and *Helicobacter pylori*) and defensive (gastric mucus, bicarbonate secretions, prostaglandins, nitric oxide, and innate resistance of mucosa cells factor [2]. Pepetic ulcer is a worldwide problem, Statistics from all sources indicate 10% or more of adult population are affected within their life time and 50% of healthy individuals complain of dyspepsia [1]. Peptic ulcer affects individuals from 20 to 60 years of age with males being predominantly affected [1]. A small but important percentage of patients have adverse gastrointestinal effects associated with NSAIDS usage which results in substantial morbidity and mortality. Risk factors for the development of NSAIDS-associated gastric and duodenal ulcers include advanced age, history of previous ulcer disease, concomitant use of corticosteroids and anticoagulants, higher doses of NSAIDS, and serious systemic disorders [3].
Aloe vera is a stemless or very short stemmed succulent plant growing to 60- 100 cm (24-39) in tall, spreading by offsets. The leaves are thick and fleshy, green to grey with some varieties showing white flecks on their upper and lower stem surface the margin of the leaf is serrated and has small white teeth [4]. *Aloe barbadensis miller* (1, 8-Dihydroxy-3-hydroxymethyl-10-(6- hydroxymethyl-3, 4, 5-trihydroxy-2-pyranyl anthrone) commonly called Aloe vera. The plant is one of the most widely used healing plants in the history of mankind. Two distinct preparations of *Aloe* plants are most used medicinally. The leaf exudates (*Aloe*) is used as a laxative and the mucilaginous gel (*A. vera*) extracted from the leaf parenchyma is used as a remedy against a variety of skin disorders [5]. It is used on facial tissues where it is promoted as a moisturiser, anti –irritant to reduce chafing of the nose however, cosmetic companies commonly add sap or other derivatives from Aloe vera to product such as makeup, soaps, sunscreens, shaving cream or shampoos and as ingredient yogurts [6]. In addition to that Aloe vera is also used in dilution of semen for the artificial

fertilization of sheep, as fresh food preservatives [7, 8]. This study was aimed at evaluating the "anti-ulcer activity of *A. vera* in non- steroidal anti-inflammatory drug induced gastric ulcers in albino rats".

Materials and Method

Aloe vera Gel Preparation

Fresh Aloe vera plant was purchased from Biological Science Garden, Usmanu Danfodiyo University Sokoto. The plant was washed thoroughly with clean water in order to remove dirties and insects. The gel was then extracted and blended in order to archive a smoother version of the extracts with help of filter paper the residues were discarded. Then 5g of the pure extracts was measured and placed in a measuring cylinder. The volume was made up to 50ml with distilled water. The pre-treated was in the dosage of 20mg/kg and was continued for five days [9].

Experimental Animals

Twenty albino rats of both sexes weighing between 200-300g were obtained from Pre-clinical house College of Faculty of Veterinary Medicine Teaching Hospital, Usmanu Danfodiyo University Sokoto, Nigeria. Rats were then acclimatised for two weeks before experimentation, they were maintained on synthetic pellet feed (purchased from Rahusa viatal feeds Sokoto) and clean water *ad libitum*. Animals were housed in controlled conditions with temperature of 27oc and 12/12 h light dark cycle environment; they were randomly allocated to different experimental group and placed in cages with mesh bottom to prevent coprophagy.

Experimental Design

Four groups of albino Wister rats five per each group were selected for the present study.

- **Control Group:** First group was taken as a normal control group. In this group; rats were allowed free to constant feed and water supply throughout the experiment.
- **Indomethacin induced gastric ulcer group:** Second group was taken as an indomethacin induced gastric ulcer control group. In this group, rats were fasted 24 h and then indomethacin was administrated orally through the gastric gavages (20 mg/kg). The rats were sacrificed 6 h after indomethacin administration [10].
- **Aloe vera pre-treated group:** Third group was taken as a test (*A. vera*) group. *A. vera* gel was diluted with distilled water and the prepared solution was administered orally through the gavages to rats. The pre-treatment of *A. vera* gel was given in the dose of 200 mg/kg to albino rats [9], for five days. At the fifth day, rats were kept fasted for 24hrs and then indomethacin was given orally, and after 6 h rats were sacrificed.
- **Omeprazole pre-treated group:** Fourth group was taken as a standard (omeprazole) group. Omeprazole was encapsulated and the powder was mixed with distilled water and administered orally. This treatment was continued for five days, at the end of the last day rats were kept fasted for 24 h, after which indomethacin was administered orally, rats were killed after 6 hours in which stomach were opened and washed with normal saline and stored in 10% formalin solution.

Collection of Blood Samples

At end of the experimental period sacrificing the rats they were kept unconscious using chloroform. Blood was collected and placed in a plane sample bottle to clot. After clotting the blood was centrifuge 3000rpm and serum was separated in different bottles and kept in the freezer prior to use.

Assay of biochemical parameters

The serum obtained was used for the assay of biochemical parameters. AST and ALT activities were determined using Reitman and Frankel method [11]. ALP activity was determine using methods of Roy [12]. Bicarbonate concentration was determined using titration method12 and the chloride was estimated using titration method [13].

Ulcer number

Total numbers of ulcers in each stomach were noted and petechial haemorrhage congestion, etc. was also noted.

Ulcer length

With the help of magnifying lens the length of each ulcer was noted. The lesions were visible and were also counted, each five petechial lesions were considered as 1mm ulceration [14].

Ulcer indexing (UI/)

The sum of the total length of the ulcers in each group of the animals were divided by its number to calculate the ulcer index [14].

%Curative ratio

100-(ulcer index in test group × 100/ulcer index in control group) [15]

Histological studies

A portion of the ulcer region in the stomach was dissected out and fixed in 10% buffered neutral formalin solution for histological observations. After fixations, tissues were embedded in paraffin; solid sections were cut at 5µm and stained with haematoxylin and eosin (H and E). The sections were examined with the help of a light microscope and photomicrographs were taken [16].

Data Analysis

Differences between control and treated groups (Mean±SEM) were tested for significance using a one-way analysis of variance (ANOVA) followed by Dunnett test. Differences were considered significant at a level of ($P < 0.05$) using SPSS version 10 computer program.

Results

Table 1: The Serum Levels of Liver Marker Enzymes of Controls and Ulcerative Albino Rats Administered with Aqueous *Aloe vera* Extract.

Groups		ALT activity(U/l)	AST activity(U/l)	ALP activity(IU/L)
1	Normal (Negative control)	14.2±1.020	24.2±6.028	28.384±6.003
2	Gastric Ulcer Untreated (Positive control)	30.6±3.530*	42.6±2.542	19.672±2.948
3	*Aloe vera* pre-treated	23.6±2.249	37.4±6.816	25.802±5.802
4	Omeprazole pre-treated	20.2±1.530	34.4±3.970	27.414±4.446

Data are expressed as Mean±SEM. The different parameters in each group, (*) denotes significant difference at ($P<0.05$)

Table 2: The Serum Levels of Electrolytes of Controls and Ulcerative Albino Rats Administered with Aqueous *Aloe vera* Extract for Five Days

S/N		Bicarbonate (mmol/l)	Chloride (mg/dl)
1	Normal (Negative control)	68.00±2.000	24.95±0.918
2	Gastric Ulcer Untreated (Positive control)	28.00±3.742*	35.38±1.024*
3	*Aloe vera* pre-treated	58.00±3.742*	26.82±0.863*
4	Omeprazole pre-treated	66.00±2.449*	25.46±0.681*

Data are expressed as Mean ± SEM. The different parameters in each group, (*) denotes significant difference at (P<0.05)

Table 3: The ulcerative Indexes of Controls and Ulcerative Albino Rats Administered with *Aloe vera*.

S/N		Ulcer index (UI) mm	% Curative ratio
1	Gastric Ulcer Untreated (Positive control)	1.12±0.102	------
2	*Aloe vera* pre-treated	0.36±0.074*	67.85
3	Omeprazole pre-treated	0.24±0.074*	82.14

Data is expressed as Mean±SEM. The different parameters in each group, (*) denotes significant difference at (P<0.05)

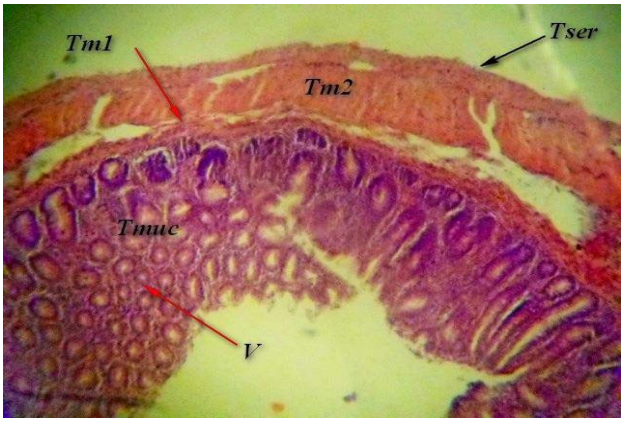

Plate 1: Photomicrograph of monogastric stomach (Group A) showings normal orientation of Tunica Serosa (Tser), Tunica muscularis: outer longitudinal (Tm2) and inner circular (Tm1), and Tunica mucosa (Tmuc) having short villi (Red arrow) H&E x200.

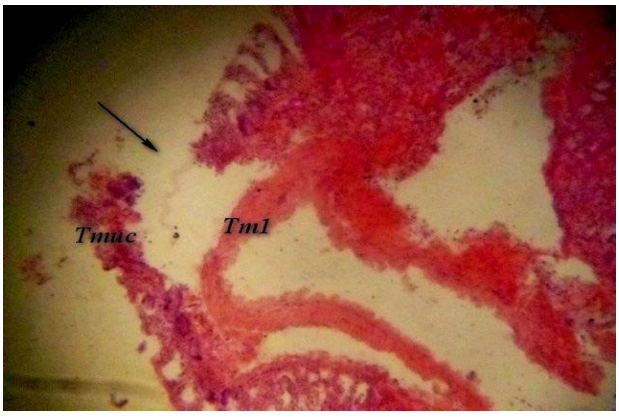

Plate 2: Photomicrograph of monogastric stomach (Group B) showing PERFORATED lumen with discontinuity in Tunica Serosa (Tser), Tunica muscularis (Tm1), Tunica sub mucosa (Tsub), Tunica mucosa (Tmuc) and very short villi (Red V) H&E x200.

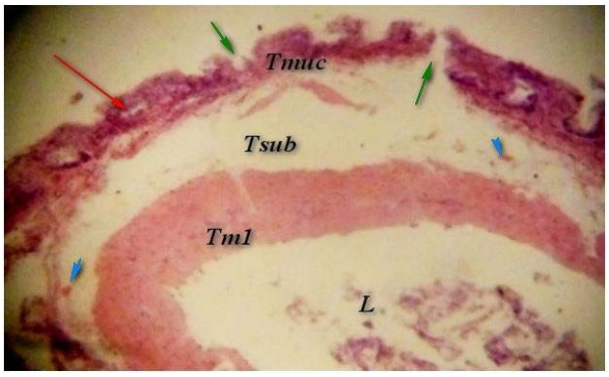

Plate 3: Photomicrograph of monogastric stomach(Group C) showing absence Tunica Serosa (Tser), Highly congested Tunica muscularis with poorly developed layers (Tm1), Tunica sub mucosa (Tsub) with poorly developed blood vessels (blue arrow) and Tunica mucosa (Tmuc) having interdigitating discontinuity (green arrow) H&E x200.

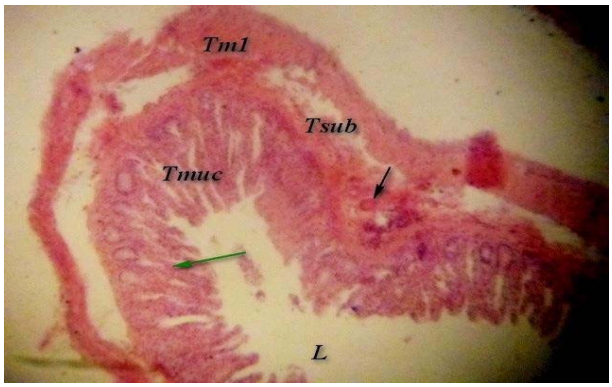

Plate 3: Photomicrograph of monogastric stomach (Group D) poorly developed Tunica Serosa (Tser) and Tunica muscularis (Tm1), Tunica sub mucosa (Tsub) with few blood vessels (black arrow) and well developed Tunica mucosa (Tmuc) having short villi (green arrow) H&E x200.

Discussion

Aspartate transaminase is mainly found in the liver, heart, skeletal muscle, kidneys, brain and the red blood cells while Alanine transaminase is more specific to the liver. The mechanisms of cell injury by hepatotoxins may result from specific organelle damage such as alteration in the smooth endoplasmic reticulum with bile secretes failure. The presentation, symptomatology, clinical features, and laboratory abnormalities of drug –induced liver damage are highly variable.

In the present study, serum AST and ALT increases in indomethacin induced ulcer group which be as a result of hepato-cellular injury caused by the dosage of Indomethacin, and shows a normal level of these enzymes in Aloe vera and omeprazole pre-treated groups when compared with subsequently negative control groups. Thus, this reflects a hepato-protective effects of Aloe vera gel [17]. Studied the side effect of *B. prionitis* on liver in Ulcer induced Albino rats, and serum AST and ALT also increases significantly.

Alkaline phosphatase enzyme have maximum activity in the pH range 9.6-10.0, their exact biochemical role in the tissue is not known but because they are often found attached to cell membranes, they are thought to be concerned with transport of phosphate across the cell membranes. They are mainly found in tissues particularly in the liver and bone, and also in the small intestine, kidneys, and placental tissues.

The level of serum ALP in both treated and standard drug group were significantly increased when compared with the positive control group suggesting a reversal of ulcerogenic effect which is in accordance with earlier report of Glew and Rosenthal [19] that effect of NSAIDS could either be on the liver which leads to an increased in ALP activity or on the intestine which leads to a significant decreased in the level of ALP [18].

Decreased level of Bicarbonate and an increased level of chloride in the indomethacin induced ulcer group may be as a result of higher buffering capacity of bicarbonate. However in the other groups bicarbonate and chloride levels tend to be normal. The mean ulcer index of *Aloe vera* pre-treated and omeprazole pre-treated groups explained the severity of the ulcer, with %curative ratio of 67.85% and 82.14% respectively. A normal gastric stomach of Albino rats consist of four layers namely; the Epithelium or the mucosa, the Sub mucosa, the Muscular and the Serosa. In the indomethacin induced ulcer group the first three layers were perforated as a results of the gastric injury caused by Indomethacin (NSAIDS), ulcers of these category are regarded as third degree ulcers. While pre-treatment with Aloe vera resulted in cytoprotective effect of the gastric stomach similar to the effect provided by the standard drug Omeprazole.

Comparable non-steroidal anti-inflammatory drugs like indomethacin and aspirin are known to induce numerous gastric ulcers during the course of anti-inflammatory therapy and hence, indomethacin induced model was used in the present study. Although, the mechanisms underlying the ulcero-genecity of indomethacin are not completely understood, but inhibition of prostaglandin synthesis may be important [19]. This is supported by an earlier report [20], stating prostaglandins normally have protective function in the stomach by maintaining gastric micro- circulation. It was earlier reported that prostaglandin promotes mucus and bicarbonate secretions [21]. Indomethacin induced gastric damage to rat is markedly dependent on luminal Ph [22]. Indomethacin induced mucosal damage were reported to be in: (1) enhancing gastric absorption of these drugs or (2) amplifying mucosal injury [23].

The antiulcer activity of *A. vera* may be due its anti-inflammatory [24], cytoprotective [25, 26] antioxidant activity and mucus stimulatory effects [27, 5]. *A. vera has* anti-inflammatory effects of leukocyte-endothelium interaction in the gastric microcirculation of *H. pylori* infected rats [28]. The observation that *A. vera* extract inhibits acid secretion may be due to the presence of lectins in the plant [29]. Lectins are proteins/glycoproteins which are capable of recognizing and binding to carbohydrate moieties [30]. It has been shown that lectins inhibit aminopyrine uptake by parietal cells [31]. Thus, the ability of the extract to inhibit gastric acid output maybe as a result of direct action on the acid producing cells.

Administration of *Aloe vera* enhance mucous resistance and resulted in decrease of ulcer index and ulcerated surfaces. *Aloe buettneri* extract increased gastric mucus production [32]. It was reported in an earlier reported that, gastric mucus is a viscous, elastic, adherent and transparent gel formed by water and glycoproteins covering the entire gastrointestinal mucosa [33]. Earlier report suggest that the protective properties of the mucus barrier depends not only on its gel-like structure, but are also related to the amount or thickness of the layer covering the mucosal surface. Mucus protects the gastric mucosa against irritants, such as ethanol, HCl and acetyl acid. The cytoprotective action of *A. vera* may be due to its active ingredients like tannins, saponins and flavonoids [33]. The proton pump inhibitor, omeprazole which has a mechanism of action on the development of acute ulcers and accelerate the healing of pre-existing ulcers appeared to be mainly due to its' potent and long lasting anti-secretory activities [34]. This study suggests that *A. vera* possess cytoprotective effects and acid reducing effects like omeprazole.

Conclusion

Aloe vera produces anti-ulcer activity Similar to a standard drug omeprazole, the mean ulcer indexes of two drugs were found to be statistically significant ($P < 0.05$). Therefore, the results were suggestive of anti ulcerogenic activity of aqueous Aloe vera gel extract. Aloe vera also possess hepato-protective effect in drug induced liver damage. Aloe vera showed a statistical significant decrease in AST activity ($P<0.05$), a decrease in ALT activity and an increase in ALP activity when compared to normal rats.

Conflict of Interest

Authors have no conflict of interest

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