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Original Research Article

Ethanolic Stem Bark Extract of *Boswellia dalzielli H* Promotes the Healing of Acetic Acid Induced Chronic Gastric Ulcer by Down-Regulation of Tumor Necrosis Factor (TNF) -Alpha in Wistar Albino Rats

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Abstract

The present study determines the effects of the ethanolic stem bark extract of Boswellia dalzielii H on gastric homogenate and serum levels of TNF-α and vascular endothelial growth factor (VEGF), during the healing of chronic gastric ulcer induced by acetic acid in Wistar albino rats. Round and deep chronic gastric ulcers were induced by a high concentration of acetic acid in Wistar albino rats. 75 rats with gastric ulcers were randomly assigned to five treatment groups (2-5). Rats in group 1 had no gastric ulcer and were administered with vehicle 5ml/kg. Rats in group 2, negative control had gastric ulcers and were treated with vehicle 5ml/kg. Rats in group 3 were treated with ranitidine (50mg/kg) while rats in groups 4, 5, and 6 were treated daily with 100, 200, and 400mg/kg of ethanolic stem bark extract of Boswellia dalzielli H respectively. Treatment in all the groups was by intragastric administration. Five rats from groups (2-6) were euthanized on days 3, 7, and 9 along with the control group (1). Blood was collected, serum prepared, and assayed for TNFα and VEGF using ELISA. The stomach of each animal was evaluated for gastric ulcer, assessed for histopathologic changes, and preparation of gastric tissue homogenates. Intragastric administration of graded doses of ethanolic stem bark extract of Boswellia dalzielli H and ranitidine (50mg/kg) respectively, down-regulated serum and gastric tissue levels of TNF–α significantly ($p<0.05$), up-regulated VEGF significantly ($p<0.05$), and increased gastric ulcer healing rate in a significant manner ($p<0.05$). The increase was time and Dose and time dependent manner when compared to control. Attenuation of TNFα and up-regulation of VEGF by graded intragastric administration of the ethanolic stem bark extract of Boswellia dalzielli promotes the healing of chronic gastric ulcer induced by acetic acid in Wister albino rats.

Keywords: Healing, gastric, ulcer, VEGF, TNF-α.

1.0 INTRODUCTION

Gastric ulcer is a breach in the continuity of the mucosal lining to the level of muscularis mucosae. The incidence, prevalence, morbidity and mortality associated with gastric ulcer have geographic and seasonal disparity [1-4]. Gastric ulcer is a debilitating disorder with enormous economic and health care burden. It decreases labor turnover, diminish income and reduce quality of life. Endogenous and environmental risk factors responsible for the formation of gastric ulcer include Helicobacter *pylori* [4-6], the routine utilization of Non-steroidal anti-inflammatory drugs, NSAIDs, nicotine/ cigarette smoking and alcohol [7]. Excessive secretion of pepsin, hydrochloric acid, pancreatic juice, and bile acids [8] also aggravate gastric ulcers. Neurotransmitter like acetylcholine, paracrine- histamine, hormones like gastrin, cholecystokinin and second messengers (Ca^{2+}) when over stimulated may cause the parietal cells to release gastric acid in excess which promote gastric ulcer [6, 9, 10]. Dietary habits, psychogenic factors and stress may also contribute in various ways to cause gastric mucosal damage [11]. Some of these risk factors disrupt gastric mucosal defense, which is arranged in hierarchy analogous to the anatomic organization of the gastrointestinal tract [12]. They alter the stability of the. Mucus- bicarbonate - lipid layer that is superimposed on epithelial cells, coupled by tight junctions and

surrounded by adequate microvascular circulation to prevent gastric mucosal damage. In addition, down regulation of epidermal growth factor, trefoil, calcitonin gene related peptide (CGRP), nitric oxide, and restitution regulated by growth factors can adversely undermine the gastric mucosal barrier. Furthermore, the variety of phytochemical stimuli elicited by gastric luminal contents and products of digestion can stress the gastric mucosa and ultimately subverts its stability and overwhelm the gastric mucosal defense to cause gastric ulcer in certain instances [13-17].

Tumor necrosis factor (TNF) α is a strategic mediator in host immunity with diverse and opposing actions. Excess amount of gastric mucosal TNF α undermine gastric microcirculation, gastric cell proliferation and angiogenesis, which leads to formation of gastric ulcer. Over expression of tumor necrosis factor alpha may lead to gastric cell apoptosis through caspase-3 pathway and eventual disruption of gastric mucosal integrity [18]. The exaggerated cytokines cause the gastric macrophages and endothelial cells to activate a cascade of secondary cytokines such as IL-8, monocyte chemoattractant protein (MCP-10) and increase their adherence to the endothelium through expression of intercellular adhesion molecule, subsequently resulting in the extravasation and recruitment of neutrophils to the gastric mucosal lamina propria [19, 20]. These inflammatory cocktail activates macrophages and monocyte to release TNF α and other cytokines [21].This neutrophil dependent inflammatory response help in the generation of reactive oxygen species, acute gastric mucosal damage and perpetuation of the chronic inflammatory phases of gastric ulcer.

Gastric ulcer healing is a robust mitotic activity that restocks damaged gastric mucosal architecture. The gastric repair/healing process regulate proliferation, migration, differentiation, tissue remodeling and regeneration [22]. Growth factors such as vascular endothelial growth factor and their receptors are essential for angiogenesis gastric ulcer healing. Angiogenesis is an important requirement for oxygen and nutrient supply to maintain the gastric mucosal integrity [23]. The main regulators of gastric ulcer healing include the products of arachidonic metabolites cyclooxygenases (COX), prostaglandins (PGE), free radicals, proteases proinflammatory cytokine such a tumor necrosis factor (TNF) -α, interleukin (IL) -1β.

Boswellia dalzielii H is a tree in the Burseracceae family and is found in the Savannah areas of West Africa, hot regions of Africa and Asia [25]. *Boswellia dalzielii* Hutch contains tannins, saponins, flavonoids, cardiac glycosides, sterols, terpenes. Others are boswellic, oleanoic, triterpenoic, ursolic, and glycrrhetic acids [26]. The stem bark of the plant is used to treat fever, rheumatism and gastrointestinal disorders. It is also used as anticonvulsant, anti-asthmaic and antiinflammation agent [27]. The ability of *Boswellia dalzielii* Hutch to reduce inflammation related disorders, arthritis, rheumatism and diarrhoea, is due to the presence of pentacyclic triterpenics acids such as boswellic acid. It also reduces pain, act against certain cancers and microbes [28, 29].

In this study, the effects of an ethanolic stem bark extract of *Boswellia dalzielii* H on TNF-α and VEGF in serum and gastric tissue homogenate were investigated during the healing of acetic acid-chronic ulcer Wistar Albino rats.

2.0 MATERIALS AND METHODS

2.1 Chemicals and Reagents.

All chemicals used in this study were of ANALAR grade and obtained from BDH England except otherwise indicated.

2.2. Animals

Eighty healthy inbred male Wistar albino rats (200-220g) were obtained from the Department of Human Physiology, Faculty of Basic Medical Science, College of Medical Sciences, and University of Maiduguri, Nigeria. The rats were accustomed for seven days in the same environment, fed with standard diet (Growers mash, Livestock feed, Nigeria), and had free access to drinking water before being used for the experiments. All experimental protocols strictly adhered to the standard animal care and guidelines of the Ethics Committee on Animal Experimentation, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Maiduguri.

2.2.1. Experimental Design and induction of chronic gastric ulcer in rat

Eighty inbred male albino rats (200-220g) were kept for seven days to acclimatized with the study area in the animal house. The Wistar rats were fasted for 18 hours before being anesthetized with ketamine (intraperitoneal, 100 mg/kg). Laparotomy via a mid-line cut of the alba linea was done on the anesthetized rats to expose the stomach. A modified method [30]. was used to induce gastric ulcer that is chronic. A total of 0.1 ml of concentrated (100%) acetic acid was placed on the frontal end of the exposed serosa by means of the nozzle of 1 ml syringe. The surface of the serosa in contact with acetic was wiped off after forty seconds with cotton wool soaked in normal saline. The incised abdominal muscle was sutured with chromium absorbable sutures and cotton thread was used for interrupted sutures of the outer abdominal layer. Rats were kept in separate cages and provided with feed and drinking water after 12 hours. Three days after the induction of chronic gastric ulcer, the rats were arbitrarily allotted to five treatment groups of 15 rats

per group (2-6) while the remaining five rats constituted the control (group 1).

Group 1 served as a sham control with no gastric ulcer and the rats were given vehicle 5 ml/kg. Rats in group 2 had gastric ulcer and were treated with vehicle 5 ml/kg. Rats in group 3 had gastric ulcer and were given ranitidine (50mg/kg,). Rats in groups 4,5 and 6 had gastric ulcer and received 100, 200 and 400 mg/kg of the ethanolic stem bark extract of *Boswellia dalzielli* respectively. All treatments were administered through gastric intubation on a daily basis.

Five rats from each group (2-6) were sacrificed with an over dose of anesthetic ether on day 3, 7 and 9. Rats in group 1 were also sacrificed on day 9. The stomach from each of the rats was removed, cut along greater curvature. The gastric ulcer was measured to determine gastric ulcer index and percentage of healing.

Healing ratio (%) = Ulcer index of control group – Ulcer index of drug treated group ×100
Ulcer index of control group [31]

A section of the stomach was processed for histologic changes and the remaining part was prepared as homogenate.

2.2.2 Histological Evaluation of Gastric Mucosa

Gastric tissues taken from rats in all treatment and control groups were fixed in 10% formal saline, then embedded in paraffin wax, and finally stained with hematoxylin and eosin (H&E). The slides for histopathologic evaluation were prepared in Histopathology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri. The stained slides were read and interpreted by a Consultant Pathologist in the Department of Histopathology, University of Maiduguri Teaching Hospital.

2.2.3 Preparation of gastric tissue homogenate

Segment of the gastric tissue (500mg) taken from each rat stomach was separately homogenized in 5ml of cold (4^0C) phosphate buffer saline (pH 7.4) using Homogenizer (Ultra -Turrax®). Homogenates were centrifuged at 12,000 x g for 10 minutes at 4^0C. The gastric homogenates were used for the assays of TNF-α and VEGF. Blood samples were also collected from all the rats by cardiac puncture and emptied into plain sample containers. Serum was prepared for TNFα and VEGF assays.

2.2.4 Estimation of Pro-Inflammatory Cytokine TNFα in serum/gastric tissue homogenate of rat

TNFα in serum and gastric homogenates were determined using RayBio® Rat ELISA kit in accordance with manufacturer's instructions (Ray Biotech, 3607 Parkway Lane, Suite 100 Norcoss, GA 30082). TNFα present in a sample is bound to the wells by the immobilized antibody. The final product was incubated at room temperature. The absorbance was read at 450 nm. The TNFα content was expressed as pg/mg of total protein. The assay procedure for TNFα includes: 100 µl standard or sample was added to each well and incubated for 2.5 hours at room temperature .100 µl prepared biotin antibody was also applied to each well and Incubated for one hour at room temperature. A 100 µl prepared Streptavidin solution was further added and incubated for yet another 45 minutes at room temperature. 100 µl TMB One-Step Substrate Reagent was added to the mixture in each well. The final product was incubated for 30 minutes at room temperature before 50 µl Stop Solution was added to each well. The absorbance was read at 450 nm immediately. TNFα level was expressed as picogram per milligram protein.

2.2.5 Estimation of Vascular endothelial growth factor in serum/gastric tissue homogenate of rat

The protein levels of VEGF in serum and homogenized gastric tissues from both the ulcerated and non-ulcerated mucosa were measured using RayBio® Rat VEGF-A ELISA Kit according to the manufacturer's protocol. VEGF level was expressed as pg/mg of tissue. The Assay Procedure involved adding 100 µl standard or sample to each well and Incubated for 2.5 hours at room temperature. 100 µl prepared biotin antibody was added to each well, incubated for one hour at room temperature. 100 µl prepared Streptavidin solution was added and incubated for 45 minutes at room temperature. 100 µl TMB One-Step Substrate Reagent was further added to each well and incubated for 30 minutes at room temperature. In the end, 50 µl Stop Solution was applied to each well containing the mixture. Reading was taken at 450 nm immediately.
(http://www.raybiotech.com/files/manual/ELISA/ELR-VEGF

3.0 STATISTICAL ANALYSIS

All results are presented as the mean ± standard error of mean (SEM). The results were analyzed using .0ne-way analysis of variance (ANOVA) followed by Dunnetts' post-hoc test. All analyses were performed using JMP Statistical Discovery (software) from SAS, version 11.0.0. A *p* value of 0.05 was considered statistically significant.

4.0 RESULTS

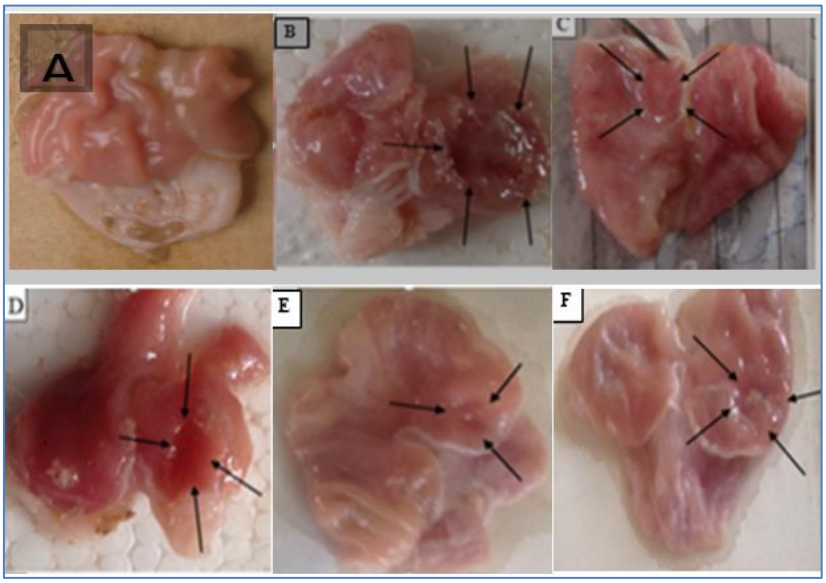

Plate-1: The gross appearance of acetic acid induced gastric ulcer in Wister albino rats after treatment with the ethanolic stem bark extract of *Boswellia dalzielli* H.

(A). Normal stomach in the control group without gastric ulcer. (B) Gastric ulcer induced by Acetic acid in the negative control group (no treatment) indicated by black arrows (after 3 days). (C). Gastric ulcer healing effect after 3 days of treatment with 400 mg/kg of the ethanolic stem bark extract of *Boswellia dalzielli* H. (D). Normal healing after nine days without treatment (E) and (F) Healing effect after nine days of treatment with the ethanolic stem bark extract of *Boswellia dalzielli* H. 400 mg/kg and ranitidine (50 mg/kg) respectively.

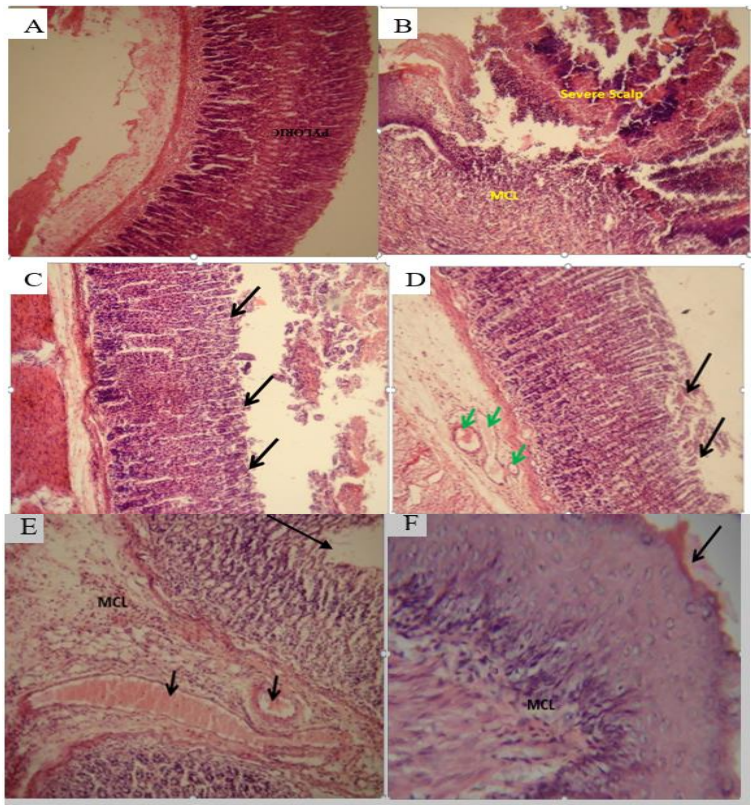

Plate-2: Photomicrographs of the effects of an ethanolic stem bark extract of *Boswellia dalzielli* H on Acetic Acid induced gastric ulcer in Wister Albino rats.

Plate a shows normal gastric epithelium without damage. Plate B shows severe scalp and area of inflammatory cell infiltration after serosal application of acetic acid. Plates C, D, E and F are photomicrographs of rat stomach treated with extract (100. 200, 400 mg/kg) and ranitidine (50 mg/kg) respectively for nine days. It shows varying degrees of damage to mucosal epithelium, mild eruption of the mucosa coat (arrow) and inflammatory cell infiltration (MCL) H&E x400.

The effect of Ethanolic stems bark Extract of *Boswellia dalzielii* on Gastric Ulcer Index in Acetic Acid Induced Gastric Ulcer

A single application of acetic acid on the serosa of each rat in all the groups produced gastric ulcer after three days with a mean gastric ulcer index of 12.96 + 0.43 in the acetic acid control group. The groups that were treated daily with 100, 200 and 400mg/kg of the plant extract respectively had accelerated wound healing and reduced the ulcer index of 11.52 + 0.43, 9.80 + 0.43 and 7.42 respectively. These reductions were significant ($P < 0.05$) when compared to Acetic acid control in day 3. Single daily treatment with the extract decreased the ulcer index significantly at day 6 (P 0.05) and 9 ($P < .0001$) when compared to control. Ranitidine treatment decreased the ulcer index at day 3, 6 and 9 significantly ($P <.0001$) when compared to Acetic acid control as shown in Figure1.

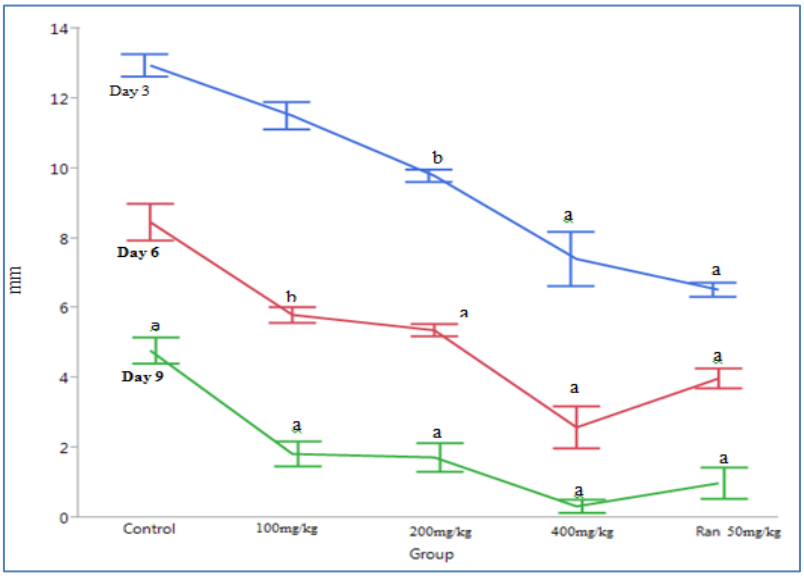

Fig-1: Effect of graded doses of ethanolic stem bark extract of B*oswellia dalzielii* h. on gastric ulcer index during the healing of acetic acid induced gastric ulcer in albino rats.

a= statistically significant when compared to control ($p<0.0001$)

b=statistically significant when compared to control ($p<0.05$)

The effect of an ethanolic stem bark extract of B*oswellia dalzielii* on gastric area healed after acetic acid induced gastric ulcer

Table 1 shows the percentage of the healing area after induction of gastric ulcer by acetic acid. The percentage of healing increased in dose and time dependent manner. The gastric ulcer healed with increased doses on day 3, 6 and 9. The reduction in gastric ulcer size due to the different doses of plant extract and ranitidine respectively, were significant ($p<0.05$). It was also observed that the extract at high dose (400mg/kg) had a higher healing potential than ranitidine (50mg/kg).

Table-1: Healed gastric ulcer area after treatment with ethanolic stem bark extract of *Boswellia dalzielii H*. in Acetic Acid induced gastric ulcer in Wistar Albino rats.

Treatment	Size of gastric ulcer area (Healed)(%)		
	Day 3	Day 6	Day 9
Control	00	34.64	62.96
100 mg/kg	11.11	55.09	85.80
200 mg/kg	24.38	58.48	86.57
400 mg/kg	42.74	79.93	97.37
Ranitidine mg/kg	49.52	69.13	92.28

Results were compared with ulcer control at day 3.

Effect of ethanolic stem bark extract of *Boswellia dalzielii H.* on serum and gastric tissue level of VEGF in Acetic Acid induced gastric ulcer in Wistar Albino rat.

Acetic acid induced gastric ulcer up-regulated the expression of VEGF in serum and gastric tissue homogenates. Treatments with ethanolic stem bark extract of *Boswellia dalzielii* H. (100, 200 and 400mg/kg respectively) increased the expression of VEGF in serum and gastric tissue homogenates as shown in table 2 and Figure 2. The increase was significant when compared to control.

Table-2: Effect of Ethanolic stem barks Extract of *Boswellia dalzielii* H. on Gastric Tissue Level of VEGF in Acetic Acid Induced Gastric Ulcer in Rat.

Treatment	Vascular Endothelial Growth Factor in Gastric Tissue		
	Day 3	Day 6	Day 9
Control	8.76±1.02^{c}	9.45±0.1.26^{d}	10.00±2.07^{d}
AA control	17.72±1.17^{b}	22.00±1.26^{a}	31.58±2.39^{a}
100 mg/kg	24.68±1.02^{ac}	23.94±1.26^{a}	51.34±2.39^{ad}
200 mg/kg	25.98±1.17^{ac}	32.29±1.46^{ac}	66.36±2.39^{ad}
400 mg/kg	32.17±1.17^{ac}	40.49 ±1.46^{ad}	184.00±2.39^{ad}
Ranitidine 50 mg/kg	23.17±1.17^{ac}	22.66±1.46^{a}	39.43±2.39^{a}

a p<.0001 when compared to sham control
b-p<0.05 when compared to sham control
c- p<.0001 when compared to acetic acid control
d- p<0.05 when compared to acetic acid control

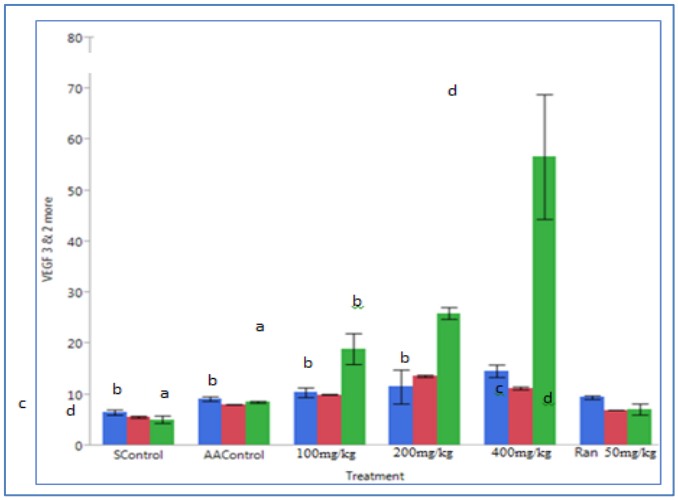

Fig-2: Effects of Ethanolic stem bark Extract of *Boswellia dalzielii* on Serum Levels of VEGF during Healing of Acetic Acid Induced Gastric Ulcer.

P<0.05 is considered significant
a= significant when compared to sham control P<0.0001
b= significant when compared to sham control P<0.05
c= significant when compared to acetic acid control P<0.0001
d= significant when compared to acetic control P<0.05

Table-3: Effect Ethanolic stem bark Extract of *Boswellia dalzielli* H. on Gastric Tissue Level of TNFα in Acetic Acid Induced Gastric Ulcer in Wistar Albino Rat.

Treatment	**Day 3**	**Day 6**	**Day 9**
Control	**3.72±1.82b**	**3.97±0.84b**	**3.19±0.51b**
AA control	27.00±2.10**	12.53±0.97**	8.22**
100 mg/kg	22.23±1.82**	6.60±0.84a	5.99±0.51*a
200 mg/kg	19.89±2.10*	6.26±0.97a	5.00±0.58a
400 mg/kg	16.27±2.10*a	5.70±0.97a	3.78±0.58a
Ranitidine 50 mg/kg	8.41±2.10b	3.16±0.97	2.31±0.58b

*=is considered statistically significant at P<0.05
**= significant when compared to normal control at p<.0001
a= significant when compared to AA control (p<0.05)
b = significant when compared to AA control (p<.0001)

Effect Ethanolic stem bark Extract of *Boswellia dalzielii* H. on Serum TNFα in Acetic Acid Induced Gastric Ulcer in Albino Rat.

Single serosal application of acetic acid for 45 seconds caused a remarkable rise in the serum level of tissue necrosis factor alpha after three days in all the treatment groups as shown in Figure 4.25. The increase was significant (P<0.0001) when compared to sham control.

Treatment with graded doses of ethanolic stem bark extract of *Boswellia dalzielii* H. (100,200 and 400 mg/kg, respectively) for three days after induction of gastric ulcer with acetic acid have attenuated the acetic acid induced increase in the serum TNF α level in this study. The down regulation of TNFα was significant and dose dependent when compared to acetic acid control.

Six days after gastric ulcer induced by acetic acid, the serum level of TNF α reduced in all the groups. The decline was not significant when compared to acetic acid control (*P*<0.05). On this day, the serum level of TNF α was significantly higher than the sham control (*P*<0.05).

The serum level of TNF α was down regulated nine days after ulcer induction in all the groups. Treatment with the graded doses of the extract has considerably resulted in a significant decrease in the serum level of TNF α when compared to acetic acid control (200 mg/kg- *P*<0.0001, 400 mg/kg- *P*<0.05).

Treatment with ranitidine for 3, 6 and 9 days after gastric ulcer induction in rat significantly reduced the serum level of TNF α in this study when compared to acetic acid control. The decrease was significant at day 3 (P<0.0001) and at day 6 (*P*<0.05).

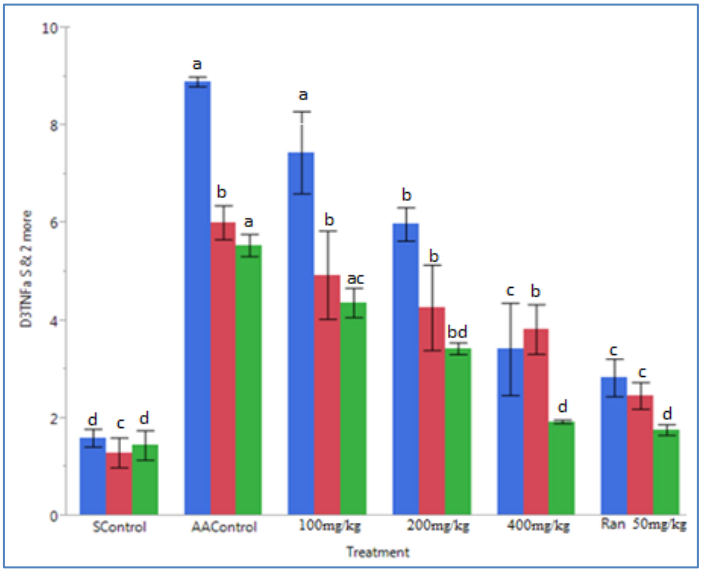

Fig-3: Serum levels of TNF α at day 3, 6 and 9 after intragastric administration of graded doses of ethanolic stem bark extract of *Boswellia dalzielii* in Wister albino rats.

P<0.05 is considered significant
a= significant when compared to sham control P<0.0001
b= significant when compared to sham control P<0.05
c= significant when compared to acetic acid control P<0.0001
d= significant when compared to acetic control P<0.05
Sc= Sham control, AAC= Acetic acid control. Day 3(blue), day 6 (red) and day 9 (green)

5. DISCUSSION

Disruption of gastric mucosal integrity is heralded by mast cell mediators like proinflammatory cytokines (TNFα, IL-Iß, IL-6), platelet-activating factor (PAF), histamine, cyclooxygenase, prostaglandins, Endothelin-I, reactive oxygen species, nitrogen oxygen species and products of phagocytic cells for gastric mucosal damage and repairs[32, 33].

Results obtained in this study presented deep and round gastric ulcers that are supported by histologic sections which revealed extensive erosion of surface epithelium, gastric mucus erosion, neutrophil infiltration and damage to the submucosa. These morphologic and histologic changes are typical presentation of acetic acid induced gastric ulcer and are in agreement with several studies [34, 35].

The ethanolic stem bark extract of *Boswellia dalzielli* remarkably attenuated macroscopic and histologic gastric mucosal damage caused by acetic acid in this study. Phytoconstituents have antioxidant, anti-inflammatory activities, gastro-therapeutic properties [36-38]. The flavonoids in the ethanolic stem bark extract of *Boswellia dalzielli* 38may be responsible for

the accelerated healing observed in this study. In this study, the serum level of VEGF increased after gastric ulcer induction with acetic acid. Similar results were obtained in previous studies [39]. Treatment of rats in the study with graded doses of an ethanolic stem bark extract of *Boswellia dalzielii* after induction of gastric ulcer with acetic acid has caused a significant increase in gastric tissue homogenates and serum levels of VEGF. The VEGF is implicated in the promotion of gastric ulcer healing in acetic acid induced ulcer. Generally, VEGF regulates the biosynthesis of nitric oxide and prostacyclin to promote vasodilation and vascular permeability. Thus, stimulates the release of von Willebrand factor to mediate platelet adhesion and aggregation to avert thrombus formation, enhance proliferation of smooth muscle cells, aid in the migration of endothelial cells [40]. The presence of flavonoids, tannins, anthocyanins and zinc in the stem bark extract of *Boswellia dalzielli* [41] may be responsible for the accelerated gastric ulcer healing via up-regulation of VEGF and down regulation of the inflammatory cytokines TNFα. Since, the ethanolic plant extract has increased the expression of VEGF. Gastric ulcer healing is largely dependent on angiogenesis for nutrient and oxygen supply. The rise in VEGF level in serum is consistent with the need to increase oxygen uptake through angiogenesis in order to heal the gastric ulcer. Although, some studies have the opinion that flavonoids inhibit VEGF/bFGF induced angiogenesis by blocking matrix degradation proteases [42] and flavonoids also inhibit hypoxia induced VEGF expression and downstream phosphorylation of phosphoinositide 3-kinase (P13K), P-38 mitogen activated protein kinases (P38MARK) [43,44]. These VEGF anti-angiogenic pathways are largely related to VEGF2 and pathologic conditions to delay or inhibit tumor growth. In other several studies, flavonoids are implicated in matrix restoration, stimulate the release of TNF-α and increase vascular penetrability, vascular growth of the embryo and VEGF receptor phosphorylation [45, 46]. This supports the idea that flavonoids may be a factor that promotes VEGF upregulation and accelerated gastric ulcer healing observed in the study. Reports indicated that wound stimulated by anthocyanin cause fibroblast and keratinocyte to produce VEGF. In addition, they weaken the union of inflammatory monocytes to endothelial cells. Tannins from medicinal plant showed evidence of angiogenesis by increasing VEGF-A expression during inflammatory phase [47]. Zinc is necessary at the core of the ion matrix metalloproteinases (MMPs), a protease that is essential for elimination of injured extracellular matrix, destruction of the capillary basement membrane to promote formation of blood vessels, contraction and reconstruction of the extracellular matrix [48]. Zinc content of the ethanolic plant extract may have contributed to the zinc pool required for the activities of the proteinases to aid in angiogenesis and accelerated gastric ulcer healing in this study.

TNF-α is an important regulator of the immune system with opposing pleiotropic function. It activates neutrophil, reduces prostaglandin production, inhibit angiogenesis and nitric oxide synthase activity, thereby interfering with nitric oxide release, blood flow, intravascular coagulation and oxygen supply leading to gastric ulcer formation and delayed gastric ulcer healing. [49]

This study revealed that the ethanolic stem bark extract of *Boswellia dalzielli* significantly decreased the expression of TNFα, a potent immunomodulator and MDA in a dose dependent fashion. It was observed that polyphenols promote gastric ulcer healing by inhibiting leucocyte mobilization through undermining the expression of VCAM-1 and E- selectin and downregulate the population of leucocyte in the damaged gastric mucosa [50]. It is likely that tannin in the ethanolic plant extract may have contributed in the healing of the acetic acid induced gastric ulcer through inhibition of TNF α and leukotrienes B4 release with eventual down regulation of leucocyte migration and upsurge in antioxidant enzyme release.

CONCLUSION

Based on the outcome of this investigation, it can be concluded the ethanolic stem bark extracts of Boswellia dalzielii (Hutch) down regulate the expression of TNFα and up-regulate VEGF to stimulate the healing and cure of acetic acid induced chronic gastric ulcer in Wistar albino rats.

Authors Contributions

Anas Husainy Yusuf designed the study and performs the methodologies. Ibrahim Lai and Lawan Adamu did the literature search and statiscal analysis.

Conflict of Interest

There is no noticeable conflict of interest in the study.

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The efforts of Mr. Ibrahim Wiam of Veterinary Histology is highly appreciated for preparing the slides used in this study. Dr. Bukar Mohammed, a consultant Pathologist with the University of Maiduguri Teaching Hospital, interpreted the slides used in this study. His contribution is highly appreciated.

REFERENCES

1. Sonnenberg, A., Sengupta, A., & Bauerfeind, P. (1988). Epidemiology of peptic ulcer disease. In *Advances in Peptic Ulcer Pathogenesis* (pp. 1-31). Springer, Dordrecht.

2. Tian, L., Qiu, H., Sun, S., Tsang, H., Chan, K. P., & Leung, W. K. (2017). Association between emergency admission for peptic ulcer bleeding and air pollution: a case-crossover analysis in Hong Kong's elderly population. *The Lancet Planetary Health*, *1*(2), e74-e81.
3. Malfertheinera, P., & Schulza, C. (2020). Peptic ulcer: Chapter Closed? Dig Dis 2020; 38:112–116
4. Malik, T.F., Gnanapandithan, K., Singh, K. (2020). Peptic ulcer disease. [Updated 2020 Jun 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-.Available from: https://www.ncbi.nlm.nih.gov/books/NBK534792
5. Prabhu, V., & Shivani, A. (2014). An overview of history, pathogenesis and treatment of perforated peptic ulcer disease with evaluation of prognostic scoring in adults. *Annals of medical and health sciences research*, *4*(1), 22-29.
6. Gulia, Y., & Choudhary, M. (2011). Peptic ulcer disease: a review. *Pharmacologyonline*, *3*, 48-70.
7. Li, L. F., Chan, R. L. Y., Lu, L., Shen, J., Zhang, L., Wu, W. K. K., ... & Cho, C. H. (2014). Cigarette smoking and gastrointestinal diseases: the causal relationship and underlying molecular mechanisms. *International journal of molecular medicine*, *34*(2), 372-380.
8. Kuna, L., Jakab, J., Smolic, R., Raguz-Lucic, N., Vcev, A., & Smolic, M. (2019). Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options. *Journal of clinical medicine*, *8*(2), 179.
9. McColl, K. E., Gillen, D., & El-Omar, E. (2000). The role of gastrin in ulcer pathogenesis. *Best Practice & Research Clinical Gastroenterology*, *14*(1), 13-26.
10. Gaton, J., & Velasco, A. (1993). The role of the neurotransmitters acetylcholine and noradrenaline in the pathogenesis of stress ulcers. *Comparative biochemistry and physiology. C, Comparative pharmacology and toxicology*, *106*(1), 125-129.
11. Srinath, R., Banik, A. (2013). Pathophysiological mechanisms and preclinical models of peptic ulcer. *International Journal of Pharmaceutics and Chemical Science*, *2*(2) 771 779.
12. Wallace, J.I., & Granger, D.N. (1996). The cellular and molecular basis of gastric mucosal defence. *The FASER Journal*. 10:731-740
13. Gray, J. L., Bunnett, N.W., Orloff, S.L., Mulvihill, S.J. and Debas, H.T. (1994). A role for calcitonin gene –related peptide in protection against gastric ulceration. *Ann Surg*. 1994 Jan; 219(1): 58–64.
14. Atuma, C. (2000). Gastrointestinal mucosal protective mechanism: Modulatory ffects of *Helicobacter pylori* on the gastric mucus gel barrier and mucosal blood flow *in vivo*. Dissertation for the Degree of Doctor of Philosophy in Physiology, Uppsala University.
15. Tarnawski, A. S. (2005). Cellular and molecular mechanisms of gastrointestinal ulcer healing. *Digestive diseases and sciences*, *50*(1), S24-S33.
16. Tache, Y. (2012). Brainstem neuropeptides and vagal protection of the gastric mucosal against injury: role of prostaglandins, nitric oxide and calcitonin-gene related peptide in capsaicin afferents. *Current medicinal chemistry*, *19*(1), 35-42.
17. Tarnawski, A., Ahluwalia, A., & K Jones, M. (2013). Gastric cytoprotection beyond prostaglandins: cellular and molecular mechanisms of gastroprotective and ulcer healing actions of antacids. *Current pharmaceutical design*, *19*(1), 126-132.
18. Aziz, R. S., Siddiqua, A., Shahzad, M., Shabbir, A., & Naseem, N. (2019). Oxyresveratrol ameliorates ethanol-induced gastric ulcer via downregulation of IL-6, TNF-α, NF-κB, and COX-2 levels, and upregulation of TFF-2 levels. *Biomedicine & Pharmacotherapy*, *110*, 554-560.
19. Mahmoodi, M., Alizadeh, A. M., Sohanaki, H., Rezaei, N., Amini-Najafi, F., Khosravi, A. R., ... & Khori, V. (2012). Impact of fumonisin B1 on the production of inflammatory cytokines by gastric and colon cell lines. *Iranian Journal of Allergy, Asthma and Immunology*, 165-173.
20. Chauhan, I., Agrawal, S., & Goel, R. K. (2018). Status of inflammatory markers and growth factor in gastric ulcer protective effects of Punica granatum L. peel extract in rat. *National Journal of Physiology, Pharmacy and Pharmacology*, *8*(1), 12-17.
21. Thalmaier, U., Lehn, N., Pfeffer, K., Stolte, M., Vieth, M., & Schneider-Brachert, W. (2002). Role of tumor necrosis factor alpha in Helicobacter pylori gastritis in tumor necrosis factor receptor 1-deficient mice. *Infection and immunity*, *70*(6), 3149-3155.
22. Arakawa, T., Watanabe, T., Tanigawa, T., Tominaga, K., Fujiwara, Y., & Morimoto, K. I. (2012). Quality of ulcer healing in gastrointestinal tract: its pathophysiology and clinical relevance. *World Journal of Gastroenterology: WJG*, *18*(35), 4811.
23. Takahashi, M., Maeda, S., Ogura, K., Terano, A., & Omata, M. (1998). The possible role of vascular endothelial growth factor (VEGF) in gastric ulcer healing: effect of sofalcone on VEGF release in vitro. *Journal of clinical gastroenterology*, *27*, S178-S182.
24. Szabo, S., Trier, J. S., Brown, A., & Schnoor, J. (1985). Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. *Gastroenterology*, *88*(1), 228-236.
25. Danlami, U., Daniel, G. J., David, B. M., & Galadanchi, K. M. (2015). Phytochemical, nutritional and antimicrobial screening of hexane, ethyl acetate and ethanolic extracts of Boswellia dalzielii leaves and bark. *American Journal of Bioscience and Bioengineering*, *3*(5), 76.
26. Nwinyi, F. C., Binda, L., Ajoku, G. A., Aniagu, S. O., Enwerem, N. M., Orisandipe, A., ... & Gamaniel, K. S. (2004). Evaluation of the aqueous extract of Boswellia dalzielii stem bark for antimicrobial activities and gastrointestinal effects. *african Journal of Biotechnology*, *3*(5), 284-288.
27. Nazifi, A. B., Danjuma, N. M., Olurishe, T. O., & Ya'u, J. (2017). Anticonvulsant activity of methanol stem bark extract of Boswellia dalzielii Hutch.(Burseraceae) in mice and chicks. *African Journal of Pharmacology and Therapeutics*, *6*(2).
28. Sharma, A., Chhikara, S., Ghodekar, S., Bhatia, S., Kharya, M., Gajbhiye, V., ... & Mahadik, K. (2009).

Phytochemical and pharmacological investigations on Boswellia serrata. *Pharmacognosy Reviews*, *3*(5), 206.
29. Siddiqui, M.Z. (2011). *Boswellic serrata*, a potential anti-inflammatory agent: an overview. *Indian Journal of Pharmaceutical Sciences*, *73*(3)255-61
30. Okabe, S. (1972). Chronicity of acetic acid ulcer in the rat. *Digestive Diseases*,*17*(7); 169-173
31. Dharmani, P., De Simone, C., & Chadee, K. (2013). The probiotic mixture VSL# 3 accelerates gastric ulcer healing by stimulating vascular endothelial growth factor. *PLoS One*, *8*(3), e58671.
32. Wallace, J. L., & Chin, B. C. (1997). Inflammatory mediators in gastrointestinal defense and injury. *Proceedings of the Society for Experimental Biology and Medicine*, *214*(3), 192-203.
33. Martin, G. R., & Wallace, J. L. (2006). Gastrointestinal inflammation: a central component of mucosal defense and repair. *Experimental Biology and Medicine*, *231*(2), 130-137.
34. Okabe, S., & Amagase, K. (2005). An Overview of Acetic Acid Ulcer Models—The History and State of the Art of Peptic Ulcer Research—. *Biological and Pharmaceutical Bulletin*, *28*(8), 1321-1341.
35. Nakao, K. I., Ro, A., & Kibayashi, K. (2014). Evaluation of the morphological changes of gastric mucosa induced by a low concentration of acetic acid using a rat model. *Journal of forensic and legal medicine*, *22*, 99-106.
36. de Lira Mota, K. S., Dias, G. E. N., Pinto, M. E. F., Luiz-Ferreira, Â., Monteiro Souza-Brito, A. R., Hiruma-Lima, C. A., ... & Batista, L. M. (2009). Flavonoids with gastroprotective activity. *Molecules*, *14*(3), 979-1012.
37. Sen, S., Chakraborty, R., De, B., & Mazumder, J. (2009). Plants and phytochemicals for peptic ulcer: An overview. *Pharmacognosy Reviews*, *3*(6), 270.
38. Sharifi-Rad, M., Fokou, P. V. T., Sharopov, F., Martorell, M., Ademiluyi, A. O., Rajkovic, J., ... & Sharifi-Rad, J. (2018). Antiulcer agents: From plant extracts to phytochemicals in healing promotion. *Molecules*, *23*(7), 1751.
39. Almasaudi, S. B., Abbas, A. T., Al-Hindi, R. R., El-Shitany, N. A., Abdel-Dayem, U. A., Ali, S. S., ... & Harakeh, S. M. (2017). Manuka honey exerts antioxidant and anti-inflammatory activities that promote healing of acetic acid-induced gastric ulcer in rats. *Evidence-Based Complementary and Alternative Medicine*, *2017*.
40. Ma, L., & Wallace, J. L. (2000). Endothelial nitric oxide synthase modulates gastric ulcer healing in rats. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *279*(2), G341-G346.
41. Abdulhamid, A., & Sani, I. (2019). Phytochemical Screening and In-Vitro Antibacterial Potential of Boswellia dalzielii (Hutch.) Stem Bark Extracts.
42. Kim, M. (2003). Flavonoids inhibit VEGF/bFGE induced angiogenesis *in vitro* by inhibiting the matrix-defrading proteases. *J of Cellular Biochem*, *89*(3),529-536
43. Schindler, R., & Mentlein, R. (2006). Flavonoids and vitamin E reduce the release of the angiogenic peptide vascular endothelial growth factor from human tumor cells. *The Journal of nutrition*, *136*(6), 1477-1482.
44. Mirossay, L., Varinská, L., & Mojžiš, J. (2018). Antiangiogenic effect of flavonoids and chalcones: An update. *International journal of molecular sciences*, *19*(1), 27.
45. Lee, Y. K., Bone, N. D., Strege, A. K., Shanafelt, T. D., Jelinek, D. F., & Kay, N. E. (2004). VEGF receptor phosphorylation status and apoptosis is modulated by a green tea component, epigallocatechin-3-gallate (EGCG), in B-cell chronic lymphocytic leukemia. *Blood*, *104*(3), 788-794.
46. Hu, W. H., Wang, H. Y., Xia, Y. T., Dai, D. K., Xiong, Q. P., Dong, T. T. X., ... & Tsim, K. W. K. (2020). Kaempferol, a Major Flavonoid in Ginkgo Folium, Potentiates Angiogenic Functions in Cultured Endothelial Cells by Binding to Vascular Endothelial Growth Factor. *Frontiers in pharmacology*, *11*, 526.
47. Gadgoli, C. H. (2016). Research in Phyto-Constituents for Treatment of Wounds. *Worldwide Wound Healing: Innovation in Natural and Conventional Methods*, 25.
48. Aziz, R. S., Siddiqua, A., Shahzad, M., Shabbir, A., & Naseem, N. (2019). Oxyresveratrol ameliorates ethanol-induced gastric ulcer via downregulation of IL-6, TNF-α, NF-κB, and COX-2 levels, and upregulation of TFF-2 levels. *Biomedicine & Pharmacotherapy*, *110*, 554-560.
49. Kaviani, A., Ohta, M., Itani, R., Sander, F., Tarnawski, A. S., & Sarfeh, I. J. (1997). Tumor necrosis factor-alpha regulates inducible nitric oxide synthase gene expression in the portal hypertensive gastric mucosa of the rat. *Journal of Gastrointestinal Surgery*, *1*(4), 371-376.
50. De Lacerda Neto L.J., Ramos, A.G.B., Sales, V.S., Gongalvas de Olivera. S.D., Dos Santos, T.L., Larisa, R.D.O. (2017). Gastroprotective and ulcer healing effects of hydroethanolic extracts of leaves of *Caryocar cariacum:* Mechanism involved in the gastroprotective activity. Chemico – *Biological Interactions*, 261; 56-62.