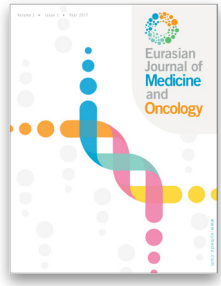
EJMO

DOI: 10.14744/ejmo.2018.74745
EJMO 2018;2(2):97-104



Research Article

Modulation of Vascular Endothelial Growth Factor and Tissue Necrosis Factor Alpha Using Ethanol Stem Bark Extract of *Boswellia Dalzielii H.* Attenuates Ethanol-induced Gastric Ulcer in Albino Rats

Anas Husainy Yusuf,[1] Hamza A Salami,[1] Maryam Babakura,[2] Umar Kyari Sandabe,[3] Nuhu Sambo,[4] Abdulwasiu Abubakar[1]

[1]Department of Human Physiology, College of Medical Sciences, University of Maiduguri, Nigeria
[2]Department of Biological Science, University of Maiduguri Faculty of Medicine, Borno State, Nigeria
[3]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, Nigeria
[4]Department of Human Physiology, Bingham University Faculty of Medicine, Karu, Nigeria

Abstract

Objectives: The modulation of vascular endothelial growth factor, antioxidants, and tissue necrosis factor alpha (TNF-α) using ethanol stem bark extract of *Boswellia dalzielii H.* was evaluated in an ethanol-induced gastric ulcer albino rat model.

Methods: Thirty albino rats of either sex (200–250 g) were starved for 48 h but were allowed drinking water with 8% sucrose to avoid dehydration. The rats were placed on wire gauze above the base of the cage to prevent coprophagy. At the end of the fasting period, the rats were equally divided and assigned to six treatment groups. Group A served as control and 5 ml/kg distilled water was orally administered to the rats without further treatment. Rats in group B were given 5 ml/kg distilled water and served as negative control. Rats in groups C, D, and E were pretreated with 100, 200, and 400 mg/kg of the ethanol stem bark extract of *B. dalzielii H*, respectively. Group F received 50 mg/kg ranitidine. After 1 h, all the rats in groups B–F were each given absolute ethanol 1 ml/200 g body weight of rat. All treatments were by intragastric lavage. One hour after the treatment with ethanol, all the rats in the experiment (groups A–F) were euthanized with an overdose of anesthetic ether and their stomachs were excised. The stomachs were cut along the greater curvature and washed in warm normal saline. Each stomach was stretched out and pinned on board.

Results: The results of the study revealed that pretreatment with ethanol stem bark extract of *B. dalzielii H.* decreased gross and histological gastric mucosal damage caused by intragastric administration of absolute ethanol in a dose-dependent manner when compared with controls. The gastric ulcer index and gastric tissue level of malondialdehyde (MDA) and TNF-α were significantly reduced ($p<0.001$), whereas the gastric tissue level of superoxide dismutase, catalase, total antioxidant capacity, and vascular endothelial growth factor (VEGF) was significantly increased ($p<0.001$) when compared with the controls.

Conclusion: The plant extract attenuated gastric mucosal damage induced by ethanol via upregulation in the expression of gastric tissue VEGF, reinforcement of the antioxidant system, and reduction in the gastric tissue level of MDA and the pro-inflammatory cytokine TNF-α.

Keywords: Antioxidants, ethanol, gastric ulcer, tissue necrosis factor alpha, vascular endothelial growth factor

Cite This Article: *Yusuf A, Salami H, Babakura M, Sandabe U, Sambo N, Abubakar A. Modulation of Vascular Endothelial Growth Factor and Tissue Necrosis Factor Alpha Using Ethanol Stem Bark Extract of Boswellia Dalzielii H. Attenuates Ethanol-induced Gastric Ulcer in Albino Rats. EJMO. 2018; 2(2): 97-104*

Address for correspondence: Anas Husainy Yusuf, MD. Maiduguri Universitesi, Tip Fakultesi, Insan Fizyolojisi Anabilim Dali, Maiduguri, Nigeria
Phone: +234 806 622 85 75 **E-mail:** anasyusuf2007@gmail.com
Submitted Date: November 14, 2017 **Accepted Date:** January 19, 2018 **Available Online Date:** February 05, 2018

The presence of bicarbonate, mucus, phospholipids, trefoil peptides, prostaglandins (PGs), and heat-shock proteins in and around the gastric lumen aid in the maintenance of gastric mucosal integrity and avert gastric mucosal damage.[1] These mucosal defenses can be breached by ethanol to induce gastric ulcer. Contact of gastric mucosal epithelium with ethanol results in the release of endothelins, decrease of nitric oxide synthase, and attenuation of nitric oxide synthesis that will lead to reduced blood supply to the gastric mucosal cells.[2, 3] Restriction of the microvascular circulation of the gastric epithelium ensures nutrient and oxygen deprivation to the affected mucosae, accumulation of arachidonic metabolites, generation of reactive oxygen species, polymorphonuclear (PMN) infiltration and adhesions, depletion of gastric mucus, plasma leakages of sodium and potassium ions, and edema.[4-6] These unfavorable gastric activities may overwhelm the antioxidant capacity of the system, which lead to oxidative stress that could result in gastric cell membrane damage, apoptosis, exfoliation, epithelial erosion, and formation of gastric ulcer.[5] Tissue necrosis factor-α (TNF-α) along with adenosine, histamine, and leukotrienes are considered as the mediators of early vascular injury in ethanol-induced gastric damage.[2, 3, 7] TNF-α causes polymorphonuclear (PMN) migration through upregulation of the expression of adhesion molecules in neutrophils and endothelial cells. The migration of PMN is an essential ingredient in the several pathways leading to gastric mucosal disruption.[8] However, vascular endothelial growth factor (VEGF), a glycoprotein that is homodimeric and exists in five different isoforms, stimulates vascular permeability, acts as a chemotactic agent, promotes endothelial cell mitogen,9 promotes angiogenesis, enhances epithelization, facilitates collagen deposition, actively provides gastric mucosal defense, and stimulates activities that favor the gastric ulcer healing cascade. VEGF activates the release of nitric oxide and prostacyclin, the products of nitric oxide synthase and cyclooxygenase, and promotes vasodilation and vascular permeability. These activities favor gastric mucosal defense and avert gastric mucosal damage.

The plant *Boswellia dalzielii* H is a tree in the Sapindales order and Burseraceae family. It is a moderate-sized flowering plant and can grow up to 13 m in height. It has an outer pale paper-like or white to greyish bark that peels into flakes or looks like rags. The tree is native to the Savannah regions of West Africa, Ghana, Niger, North and Eastern Nigeria, Ivory Coast, and Upper Volta and is also abundant in the tropical regions of Africa and Asia (Danlami, et al., 2015).[10] The plant is called frankincense tree because of its scent. It is referred to as Arrarabi or Hano in Hausa or Kaushi in Kanuri. Phytoconstituents in the stem bark of the plant include tannins, saponins, flavonoids, cardiac glycosides, sterols, and terpenes. Some other phytoconstituents are boswellic, oleanoic, triterpenoic, ursolic, and glycyrrhetic acids.[11] The stem bark of the plant is used to treat fever, rheumatism, and gastrointestinal problems. It is also used as an anticonvulsant[12] and for the treatment of asthma and various inflammatory conditions. Presence of pentacyclic triterpenic acids such as boswellic acid confers on the plant the potential of anti-inflammatory, antiarthritic, antirheumatic, antidiarrheal, hypolipemic, antiasthmatic, anticancer, antimicrobial, and analgesic activities.[13, 14]

The aim of this study was to establish the gastroprotective capacity of the ethanol stem bark extract of *B. dalzielii H* plant and evaluate its antioxidant capacity and determine its modulatory potentials on gastric tissue levels of VEGF and TNF-α after ethanol-induced gastric ulcer in albino rats.

Methods

Animals: The albino rats used in this study were obtained from the animal house of the Department of Human Physiology, College of Medical Sciences, University of Maiduguri, Nigeria. The rats were kept in the same environment, fed with standard diet, and had free access to drinking water. All experimental protocols strictly adhered to standard animal care and guidelines of the Ethics Committee on Animal Experimentation, College of Medical Sciences, University of Maiduguri.

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

Design of the Study and Induction of Gastric Ulcer in Rats Using Ethanol

Thirty albino rats of either sex (200–250 g) were starved for 48 h but were allowed drinking water with 8% sucrose to avoid dehydration. The rats were placed on wire gauze above the base of the cage to prevent coprophagy. At the end of the fasting period, the rats were equally divided and assigned to six treatment groups. Group A served as control and 5 ml/kg distilled water was orally administered to the rats without further treatment. Rats in group B were given 5 ml/kg distilled water and served as negative control. Rats in groups C, D, and E were pretreated with 100, 200, and 400 mg/kg of the ethanol stem bark extract of *B. dalzielii H*, respectively. Group F received 50 mg/kg ranitidine. After 1 h, all the rats in groups B–F were each given absolute ethanol 1 ml/200 g body weight of rat. All treatments were by intragastric lavage. One hour after the treatment with ethanol, all the rats in the experiment (groups A–F) were euthanized with an overdose of anesthetic ether and their stomachs were excised. The stomachs were cut along the greater curvature and washed in warm normal saline. Each stomach was stretched out and pinned on board.

Evaluation and Rating of Gastric Ulcer in Albino Rats

Gross changes observed on the gastric mucosa were evaluated and scored as follows: (i) grey coloration of stomach was rated as zero (0) mark, (ii) red coloration of stomach

was scored 0.5, (iii) appearance of hemorrhagic streak was scored 1, (iv) pin head size was scored 1.5, (v) ulcer up to 2 mm was scored 2, and (vi) ulcer greater than 2 mm was scored 3.[15, 16]

The percentage of ulcer protection was determined as follows:

$$\text{Ulcer protection (\%)} = \frac{(\text{ulcer index of control} - \text{ulcer index of test})}{\text{Ulcer index of control}} \times 100\%$$

Preparation of Gastric Tissue Homogenates for Antioxidants, VEGF, and TNF-α Assays

Gastric mucosal samples (500 mg) were obtained from the sacrificed rats. Each stomach was separately homogenized in 5 ml of cold (4°C) phosphate buffer saline (pH 7.4) using a homogenizer. The gastric tissue homogenates were centrifuged at 12.000×g for 10 min at 4°C. Antioxidants, VEGF, and TNF-α contents in the supernatants were determined using enzyme linked immunesorbent assay (ELISA).

Estimation of Catalase

Catalase was measured according to the method described by Aebi 17 using the Bio Vision's Catalase Colorimetric/Fluorometric Assay Kit was used according to manufacturer's specifications (Biovision Inc. 155 S Mipitas Boulevard Mipitas, CA 95035 USA). The method is based on the principle that catalase reacts with H2O2 to produce water and oxygen. The unconverted H2O2 reacts with Oxi Red TM probe to produce a product that is incubated at 25°C for 10 min and measured at 570 nm in a plate reader (colorimetric method). Catalase activity is reversely proportional to the signal strength. The kit can detect ≤1 µU of catalase activity in tissue homogenates and serum samples.

Estimation of Superoxide Dismutase (SOD) in Gastric Tissue Homogenate

Spectrophometric method was used for the assay of superoxide dismutase.[18] The Cayman comme rcial assay kit (Cayman chemical, 1180 East Elsworth Road Ann Arbor, Michigan 48108 USA) was utilized according to manufacturer's instructions. Superoxide radical generated by xanthine oxidases and hypoxanthine were detected by tetrazolium salts. One unit of SOD is defined as the enzyme required to show 50% dismutation of the superoxide radical. The activity was expressed as µmol/min/mg protein.

Estimation of Total Antioxidant Capacity (TAC) in Gastric Tissue Homogenate

The CUPRAC method was utilized in estimating total antioxidant capacity of gastric tissue homogenate.[19] The Cell Biolabs' OxiSelect™ TAC Assay Kit was used to measure the TAC of serum and gastric tissues (Cell Biolabs , Inc 7758 Arjons drive San Diego CA 92126). The protocol outlined by the manufacturer was followed. The TAC assay is based on the reduction of copper (II) to copper (I) by antioxidants such as uric acid. Upon reduction, the copper (I) ion further reacts with a coupling chromogenic reagent that produces a color with a maximum absorbance at 490 nm. The net absorbance values of antioxidants are compared with a known uric acid standard curve. Absorbance values are proportional to the sample's total reductive capacity. Results are expressed as "µM Copper Reducing Equivalents" or mM Uric Acid Equivalents.

Estimation of Malondialdehyde

Malondialdehyde was estimated from Thiobarbituric acid reactive substance.[20] Gastric tissue was homogenated in cold potassium chloride in a ratio of 1/10 in 1.15 % (w/v). Four milliliter of Thiobarbituric acid (TBA)-Trichloroacetic acid (TCA)- Hydrochloric acid (HCl)reagent (1:1:1ratio, 0.37% TBA, 0.25 N HCl and 15% TCA) was used to treat 0.2 ml of gastric tissue homogenate. The mixture was placed in boiling water for 15 min. There after, it was allowed cool before been centrifuged for 5 mins at 5000 rpm. The absorbance of clear supernatant was measured against blank at 535 nm. The values were calculated using molar extinction coefficient of chromophore (1.56x105 M-1cm-1).

Estimation of Vascular Endothelial Growth Factor (VEGF) in Rat Gastric Mucosa

The protein levels of VEGF in 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 (Ray Biotech,3607 Parkway Lane, Suite 100 Norcoss, GA 30082). VEGF level was expressed as pg/mg of tissue.

Estimation of Pro-inflammatory Cytokine TNF-α in Rat Gastric Mucosa

The RayBio® Rat TNF-α ELISA kit was used for determination of TNF-α in rat gastric mucosa according to manufacturer's instruction (Ray Biotech,3607 Parkway Lane, Suite 100 Norcoss, GA 30082). TNF-α 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 TNF-α content was expressed as pg/mg of total protein.

Statistical Analyses

Results of this study were expressed as mea±standard error of mean. One-way analysis of variance (ANOVA) followed by Dunnets' post hoc test was used to evaluate differences among the groups. All analyses were performed using JMP Statistical Discovery (software) from SAS, version 11.0. A p-value of <0.05 was considered statistically significant.

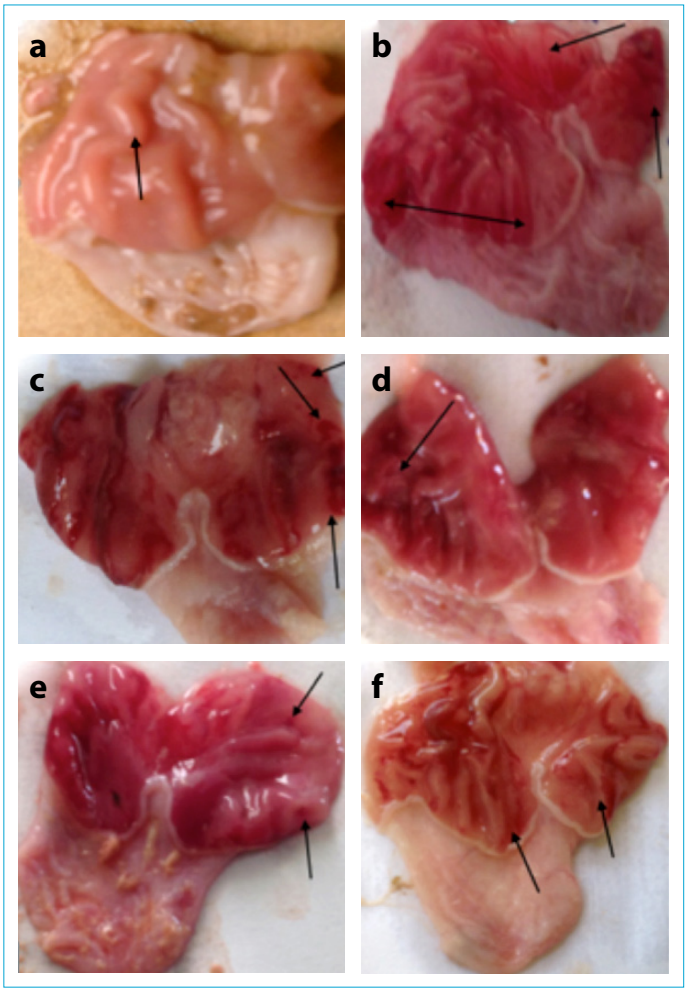


Figure 1. Gross appearance of rat stomach after treatment with absolute ethanol.

Slide A. normal stomach with no alcohol; B. alcohol alone; and C, D, and E treated with graded doses (100, 200, and 400 mg/kg) of the ethanol stem bark extract of *B. dalzielii H*, respectively, prior to oral administration of ethanol. Group F was treated with ranitidine before oral administration of ethanol.

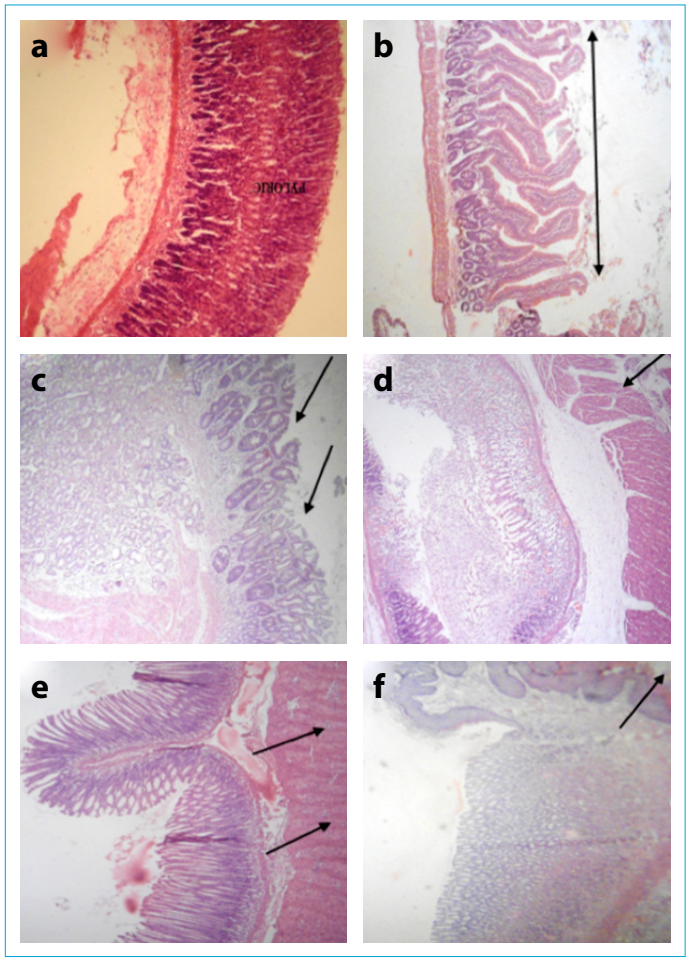


Figure 2. Photomicrographs of gastric mucosa (pylorus) in ethanol-induced gastric ulcer in albino rats.

Slide A shows the mucosa of a normal rat without treatment. Slide B indicates the gastric mucosa of rat treated with absolute alcohol. Slides C, D, and E shows the mucosa (pylorus) of rats treated with 100, 200, and 400 mg/kg, respectively, of the ethanol stem bark extract of *B. dalzielii H* prior to oral intake of absolute ethanol in each group. H&E (×400).

Results

Gross Appearance of Gastric Mucosa in Ethanol-induced Gastric Ulcer

Figure 1 shows the deleterious impact of oral administration of ethanol on gastric mucosa of rats. Slide A is a normal stomach with grey appearance of the surface lined up with rugae indicated by black arrow. Slide B is the macroscopic presentation of rat gastric mucosa treated with absolute ethanol. The surface area is reddish with erosion and has ulcer lesions all over the surface (black arrow), flattening of the entire surface, hemorrhagic band, petechial, pinpoint ulcer, and penetrating ulcer area greater than 2 mm. Pretreatment with graded doses (100, 200, and 400 mg/kg) of the ethanol stem bark extract of *B. dalzielii H* attenuated ethanol-induced damage in rat (slides C, D, and E) when compared with control (slide B). The cytoprotective activity of the plant extract was dose-dependent. The highest protection to the gastric mucosa was by 400 mg/kg of the extract, and the least protection was from 100 mg/kg of the extract. Slide 1F indicates the protective role of 50 mg/kg ranitidine on the gastric mucosa exposed to absolute ethanol. It diminished the size of gastric ulcer when compared with ethanol control.

Effect of the Ethanol Stem Bark Extract of B. Dalzielii *H* on the Gastric Histology in Ethanol-induced Gastric Ulcer

The photomicrographs in Figure 2 shows reduction in gastric ulcer sizes proportional to the doses (100, 200, and 400 mg/

Table 1. Effect of the ethanol stem bark extract of *B. dalzielii H* on ethanol-induced gastric ulcer in albino rats

Treatment	Ulcer Index (mm)	% Protection
Control (5 ml/kg)	Nil	Nil
Ethanol (absolute)	24.50	0
100 mg/kg	18.83	25.30
200 mg/kg	13.83	43.55
400 mg/kg	11.0	55.10
Ranitidine 50 mg/kg	9.66	60.57

Results are expressed as mean±SEM (n=5). One-way ANOVA along with Dunnets' post hoc test were used for statistical comparison

*p<0.05 was considered statistically significant when compared with control.

kg, respectively) of the ethanol stem bark extract of *B. dalzielii H* administered (black arrows). Slide A is the microscopic appearance of the gastric mucosa of a normal control rat that was administered with normal saline. It revealed a smooth pyloric epithelium without erosion, gastric ulcer, or glandular degeneration. Slide B depicts a deep and wide area of ulcerative lesion (black arrow) and eroded gastric mucus after oral administration of ethanol. Prior to intake of ethanol in groups C, D, and E, the rats were treated with graded doses (100, 200, and 400 mg/kg, respectively) of the ethanol stem bark extract of *B. dalzielii H*. Gastric mucosal damage by ethanol was attenuated by 100 mg/kg of the extract as shown by black arrows in slide C when compared with slide B (control). Further reduction in ulcer size and damage to the mucosal epithelium was achieved by 200 mg/kg of the extract relative to control as indicated by black arrows in slide D. Treatment with 400 mg/kg of the extract considerably averted damage to the mucosal epithelium as shown by black arrows in slide E. Slide F shows a small area of gastric ulcer in the group treated with ranitidine prior to oral administration of ethanol. A considerable reduction in the gastric ulcer size was observed when compared with the ethanol control (slide B).

Effect of Ethanol Stem Bark Extract of *B. Dalzielii* H on Gastric Tissue Antioxidants in Ethanol-induced Gastric Ulcer in Albino Rats

Single intragastric intake of absolute ethanol in rats causes a statistically significant reduction in the levels of SOD, CAT, and TAC ($p<0.05$) and increased MDA ($p<0.0001$) when compared with control as shown in Table 2. Pretreatment with oral graded doses of the ethanol stem bark extract of *B. dalzielii H* (100, 200, and 400 mg/kg) dose dependently and significantly attenuated the action of ethanol and increased the levels of the antioxidants. The extract at 100 mg/kg significantly ($p<0.001$) raised the SOD level above the ethanol control. At 200 mg/kg, it increased the SOD level by 171.4%, whereas 400 mg/kg increased the SOD level in the gastric mucosa by 210%. These increases were significant ($p<0.0001$) when compared with normal control. Intake of 200 and 400 mg/kg of the extract also increased the SOD level above normal control ($p<0.05$). Similarly, ranitidine significantly increased the SOD level when compared with normal control ($p<0.05$) and ethanol control ($p<0.0001$).

The CAT level in the gastric tissue homogenate of rats pretreated with 200 and 400 mg/kg of the extract significantly increased above the normal control ($p<0.05$). The CAT level in normal control, 200, and 400 mg/kg groups was significantly higher than that in the ethanol group ($p<0.05$). Ranitidine also significantly increased the CAT level ($p<0.05$) when compared with normal control and ethanol control as shown in Table 2.

Ethanol-induced reduction in TAC in the gastric mucosa of rats was reversed by pretreatment with the ethanol stem bark extract of *B. dalzielii H*. At 100, 200, and 400 mg/kg, the plant extract significantly elevated the TAC level ($p<0.0001$) for all the treated groups when compared to ethanol group. TAC treatment with 100 mg/kg of the extract significantly raised TAC level ($p<0.05$); treatment with 200 and 400 mg/kg of the plant extract significantly increased tissue level of TAC ($p<0.0001$) when compared to normal control. Ranitidine significantly raised the level of tissue TAC in this study ($p<0.0001$) when compared to normal and ethanol controls.

Table 2. Estimation of in vivo antioxidant capacity of the ethanol stem bark extract of *B. dalzielii H* in ethanol-induced gastric ulcer in albino rats (tissue)

Treatment	SOD (µmol/min/mg protein)	CAT (mU/mL)	TAC (mM uric acid equivalents)	MDA
Control	1.19±0.08b	1.07±0.08b	1.81±0.05c	1.60±0.2b
Ethanol Control	0.03±0.08a	0.55±0.08a	0.94±0.05d	7.21±0.2d
100 mg/kg	0.05±0.08ac	0.95±0.08	2.40±0.05ac	4.11±0.2ab
200 mg/kg	2.04±0.08ac	1.14±0.08b	2.95±0.05dc	3.82±0.2ac
400 mg/kg	2.50±0.08ac	1.59±0.08ab	3.62±0.05dc	3.30±0.2ac
Ranitidine 50 mg/kg	4.50±0.08ac	1.84±0.08ab	3.12±0.05dc	4.40±0.2ac

Results are expressed as mean±SEM (n=5). One-way ANOVA along with Dunnets' post hoc test were used for multiple statistical comparison.

a=significant when compared to control $p<0.05$, b=significant when compared to ethanol control $p < 0.05$, c = significant when compared to ethanol control $p <0.0001$, d=significant when compared to control $p<0.0001$.

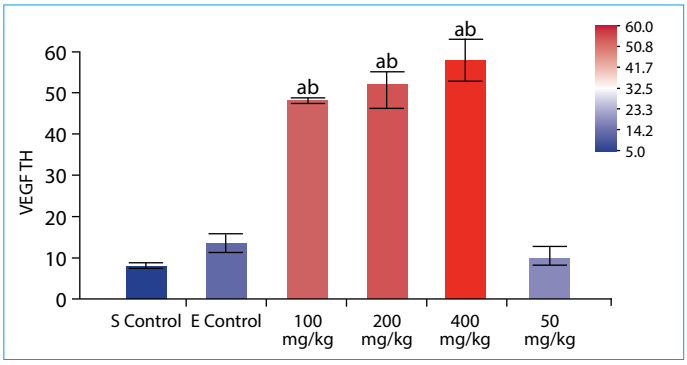


Figure 3. Effect of ethanol stem bark Extract of *B. dalzielii H* on gastric VEGF (pg/mg of tissue) in ethanol-induced gastric ulcer in albino rats

Results are expressed as mean±SEM (n=5). One-way ANOVA along with Dunnets' post hoc test were used for multiple statistical comparison.

p<0.05 is significant, a=p<0.0001 significant compared with normal control, b=significant relative to ethanol control (p<0.0001).

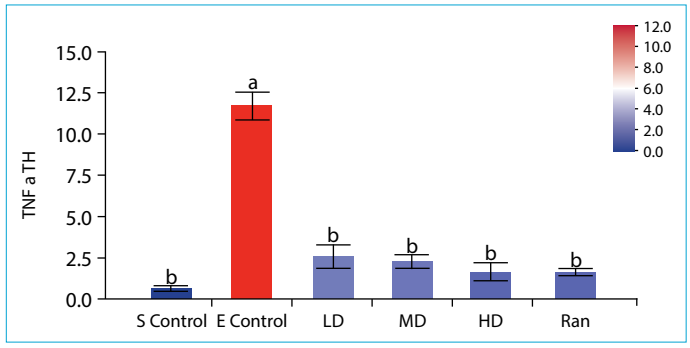


Figure 4. Effect of ethanol stem bark extract of *B. dalzielii* H on gastric TNF-α (expressed as pg/mg of total protein) in ethanol-induced gastric ulcer in albino rats

a=significant (p<0.0001) when compared to normal control, b=significant (p<0.0001) when compared to ethanol control.

Increase in gastric tissue level of MDA caused by oral intake of ethanol was significant (p<0.0001) when compared to control. Oral intake of ethanol stem bark extract of 200 and 400 mg/kg *B. dalzielii H* prior to ethanol ingestion significantly reduced (p<0.0001) the gastric tissue level of MDA in rats, whereas 100 mg/kg of the extract also significantly attenuated (p<0.05) ethanol-induced increase in gastric tissue level of MDA. However, the decrease caused by the plant extract (at 100, 200, and 400 mg/kg) was significantly higher than the normal control (p<0.05). Administration of ranitidine in the study caused a significant decrease in the MDA level (p<0.05) when compared to control. It also caused a significant reduction when compared to ethanol control (p<0.0001) as presented in Table 2.

Effects of the Ethanol Stem Bark Extract of *B. dalzielii* H on Gastric Tissue VEGF in Ethanol-induced Gastric Ulcer in Albino Rats

Intragastric administration of absolute ethanol to rats (1 ml/200 g BDW) in this study significantly increased the expression of VEGF in a dose-dependent manner when compared with normal control (p<0.0001) and also when compared with ethanol control (p<0.0001) as presented in Figure 3.

Effects of the Ethanol Stem Bark Extract of *B. dalzielii* H on Gastric Tissue TNF-α in Ethanol-induced Gastric Ulcer in Albino Rats

Oral intake of absolute ethanol significantly elevated the gastric tissue level of TNF-α when compared with normal control (p<0.0001). Treatment with graded doses of the extract significantly downregulated the expression of TNF-α when compared to normal control (p<0.0001). The increase in tissue levels of the extract-treated groups was not significant when compared with normal control (p<0.05) as shown in Figure 4.

Discussion

The anti-ulcer, antioxidants, and immunomodulation potential of ethanol stem bark extract of *B. dalzielii H* was evaluated in ethanol-induced gastric ulcer albino rat model. Oral administration of absolute ethanol to rats (1 ml/200 g BWT) in this study caused extensive erosions with severe bleeding in the glandular stomach. It also showed multiple elongated hemorrhagic streaks and gastric ulcers of different shapes and sizes in the corpus mucosa along the long axis of the stomach of the ethanol control group (Fig. 1). Histological assessment of the ethanol control group revealed severe disruption of the surface epithelium, necrotic lesion that extended deep into the mucosa, and moderate acute inflammatory cells predominantly infiltrating in the submucosa along with dilated and congested vascular channels (Fig. 2). The gastric ulcer index (Table 1) was statistically significant when compared to normal control (p<0.001). This result is consistent with the findings of previous studies[21, 7] that reported severe disruption of the surface epithelium with noticeable gastric mucosal injury grossly and histologically after oral intake of ethanol in rats. The results of this study also show decreased SOD, CAT, GSH, and TAC levels (Table 2) with elevation in the MDA levels. Expressions of VEGF and TNF-α increased when compared with normal control (Figs. 3 and 4). The result obtained is in accordance with previous studies that reported downregulation of enzymatic and non-enzymatic antioxidants. It was also reported that intragastric administration of absolute ethanol in rats increases the levels of VEGF,[22, 23] TNF α, and MDA in gastric tissue homogenates.[24, 25]

Pretreatment of rats with graded doses of the ethanol stem bark extract of *B. dalzielii H* provided cytoprotection, signifi-

cantly decreased ($p<0.0001$) gastric ulcer index, and ameliorated gastric mucosal injury by ethanol in a dose-dependent manner when compared to ethanol control. The treatment also significantly increased ($p<0.001$) the SOD, CAT, and TAC levels when compared with control. Cytoprotection is generally achieved by generation of prostaglandins or blockade of back diffusion of H+ ions.[6] The flavonoids, tannins, and saponins in the plant extract may have exhibited their antioxidants capacity by preventing ethanol-induced depletion of the antioxidants and also enhanced the levels of enzymatic and non-enzymatic antioxidants. Tannins avert gastric ulcer occurrence by causing protein precipitation and microvascular constriction at the ulcer margin that is further augmented by its astringent action, which creates an impervious protective layer on the gastric mucosal epithelium to resist chemical irritation or prevent deformation due to mechanical trauma. Although accumulated level of tannin can cause inflammation, diarrhea, vomiting, and coagulation of protein at the inner layers of the gastric mucosa.[26] Ethanol stem bark extract of *B. dalzielii H* used in this study protect the gastric mucosa against injury by ethanol. Similar results were obtained in previous studies. It was reported that water extract of the *B. dalzielii H* plant has anti-ulcer activity.[11]

The increase in the gastric tissue levels of SOD, CAT, and TAC in this study may be linked to the presence of antioxidant micronutrients such as Cu, Zn, and Mn present in the plant. The synergistic activities of the various components of the plant may avert oxidative stress and exert antioxidant prowess that mitigate cellular damage to the gastric mucosa. Zinc was reported to prevent gastric lesion in ethanol-induced gastric model through reinforcement of the antioxidant activity, stimulation of prostaglandin E_2, and upregulation of HSP70.[27] Similarly, flavonoids are well-known anti-ulcer agents.[28]

It has been established in previous studies that intragastric intake of absolute ethanol in rats decreases gastric blood flow, increases the level of IL-1β and TNFα, and generates ROS that results in lipid peroxidation expressed by elevation in MDA and 4-hydrononenal levels.[29] The downregulation of MDA, an index of PMN accumulation in the gastric mucosa, and the reduction in TNF-α, an inflammatory marker, by ethanol stem bark extract of *B. dalzielii H* after ethanol-induced gastric ulcer in this study suggest that the plant extract may have inhibited neutrophil infiltration and neutrophil-associated TNF-α in order to avert gastric damage. It was argued[30] that TNF-α causes PMN migration through upregulation of the expression of adhesion molecules in neutrophils and endothelial cells. The migration of PMN is an essential ingredient in several pathways leading to gastric mucosal disruption.

However, ethanol-induced gastric lesion is caused by decreased production of nitric oxide synthase and attenuation of nitric oxide synthesis. The increase in the formation of reactive oxygen species and inhibition of the expression of cyclooxygenases[21] lead to disturbance in gastric microvascular circulation to promote hemorrhagic necrosis and depletion of gastric mucus, a necessary factor in gastric mucosal defense. The erosion of the gastric mucus leads to sodium ion influx, potassium ion outflow, increase pepsin secretion, and elevation in gastric hydrochloric concentration and histamine, with their eventual discharge into the gastric lumen.[27, 7] The results of this study show an elevated VEGF level after oral intake of absolute ethanol in rats. It was argued that VEGF is elevated to dilute ethanol and other ulcerogenic products as a gastric protective mechanism.[22, 31, 23] VEGF prevents gastric mucosal injury by creating perivascular edema around subepithelial capillaries in the gastric mucosa to delay the absorption of toxic chemicals and minimize endothelial cell exposure to reduce or avert gastric injury.[32]

Ranitidine is a reversible, competitive blocker of histamine at H_2 receptor on the parietal cells to decrease gastric acid secretion, reduce gastric juice volume, and reduce H+ concentration.[33, 34] Based on these potentials, it probably explains why in this study, macroscopic and histologic damages in the gastric mucosa of ranitidine-treated group was reduced when compared with control.

Conclusion

The ethanol stem bark extract of *B. dalzielii H* attenuates ethanol-induced gastric mucosal damage through reinforcement of the antioxidant system, upregulation in the expression of VEGF, and downregulation of MDA and TNF-α in gastric tissue of albino rats.

Disclosures

Acknowledgments: The authors sincerely appreciate the contributions of Mr. Ibrahim Wiam of the Department of Veterinary Histology, Faculty of Veterinary Medicine, for preparing the slides and Dr. Bukar M, Consultant Pathologist, University of Maiduguri Teaching Hospital, for interpreting the slides. Thank you all.

Ethics Committee Approval: This study was approved by the Ethics Committee on Animal Experimentation, College of Medical Sciences, University of Maiduguri.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship contributions: Concept – A.H.Y.; Design – A.H.Y.; Supervision – N.S.; Materials – M.B.; Data collection &/or processing – A.A.; Literature search – H.A.S.; Writing – A.H.Y.; Critical review – U.K.S.

References

1. Tarnawski AS. Cellular and molecular mechanisms of gastrointestinal ulcer healing. Dig Dis Sci 2005;50 Suppl 1:S24–33.

2. Szabo S, Trier JS, Brown A, Schnoor J. Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. Gastroenterology 1985;88:228–36.
3. Rahgozar M, Pazokitoroudi H, Bakhtiarian A, Djahanguiri B. Diazoxide, a K(ATP) opener, accelerates restitution of ethanol or indomethacin-induced gastric ulceration in rats independent of polyamines. J Gastroenterol Hepatol 2001;16:290–6. [CrossRef]
4. Peskar BM. Neural aspects of prostaglandin involvement in gastric mucosal defense. J Physiol Pharmacol 2001;52:555–68.
5. Sahoo SK, Sahoo HB, Priyadarshini D, Soundarya G, Kumar CK, Rani KU. Antiulcer Activity of Ethanolic Extract of Salvadora indica (W.) Leaves on Albino Rats. J Clin Diagn Res 2016;10:FF07–FF10.
6. Garg S, Pal K, Sharma A, Garg K. Ethnopharmacological Evaluation of Antiulcer Activity of Caralluma attenula. Int Journal of Pharmacy and Life Sciences 2014;5:3585–91.
7. Hussein SA, El-Senosy YA, Marwa FD. Gastroprotective, antiapoptic and antiinflammatory effect of alpha- lipic acid on ethanol induced gastric mucosal lesion in rats. Am J of Biochem and Mol Bio 2017;4:48–63. [CrossRef]
8. Nishida K, Ohta Y, Ishiguro I. Role of gastric mucosal constitutive and inducible nitric oxide synthases in the development of stress-induced gastric mucosal lesions in rats. Biochem Biophys Res Commun 1997;236:275–9. [CrossRef]
9. Bao P, Kodra A, Tomic-Canic M, Golinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. J Surg Res 2009;153:347–58. [CrossRef]
10. Danlami U, Daniel GJ, David BM, Galadanchi KM. Phytochemical, Nutritional and Antimicrobial Screening of Hexane, Ethyl Acetate and Ethanolic Extracts of *Boswellia Dalzielii* Leaves and Bark. American Journal of Bioscience and Bioengineering 2015;3:76–9. [CrossRef]
11. Nwinyi FC, Binda L, Ajoku GA, Aniagu SO, Enwerem A, Orisade A, et al. Evaluation of the aqueous extract of *Boswellia dalzielii* stem bark for antimicrobial activities and gastrointestinal effects. African Journal of Biotechnology 2004;3:284–8. [CrossRef]
12. Nazifi AB, Danjuma NM, Olurishe TO, Ya'u J. Anticonvulsant activity of methanol stem bark extract of *Boswellia dalzielii* Hutch. (Burseraceae) in mice and chicks. African Journal of Pharmacology and Therapeutics 2017;6.
13. Shama A, Chhikara S, Ghodekar SN, Bhatia S, Kharya MD. Phytochemical and pharmacological investigation on Boswellic serrata. Phcog Rev 2009;3:206–2.
14. Siddiqui MZ. Boswellia serrata, a potential antiinflammatory agent: an overview. Indian J Pharm Sci 2011;73:255–61.
15. Gulia Y, Choudhary M. Peptic ulcer disease review. Pharmacologyonline 2011;3:48–70.
16. Shawon L, Palit G. An Overview of the Current Methodologies Used for Evaluation of Gastric and Duodenal Anti-ulcer Agents. Pharmacologia 2012;3:249–57. [CrossRef]
17. Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
18. Ukeda H, Maeda S, Ishii T, Sawamura M. Spectrophotometric Assay for Superoxide Dismutase Based on Tetrazolium Salt 3'-{1-[(Phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzenesulfonic Acid Hydrate Reduction by Xanthine–Xanthine Oxidase. Anal Biochem 1997;251:206–9.
19. Apak R, Güçlü K, Ozyürek M, Karademir SE, Altun M. Total antioxidant capacity assay of human serum using copper(II)-neocuproine as chromogenic oxidant: the CUPRAC method. Free Radic Res 2005;39:949–61. [CrossRef]
20. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271–8. [CrossRef]
21. Hajrezaie M, Salehen N, Karimian H, Zahedifard M, Shams K, Al Batran R, et al. Biochanin a gastroprotective effects in ethanol-induced gastric mucosal ulceration in rats. PLoS One 2015;10:e0121529. [CrossRef]
22. Gu JW, Elam J, Sartin LW, Roach R, Thomas HA. Moderate levels of ethanol induce expression of vascular endothelial growth factor and stimulate angiogenesis. Am J Physiol Regulatory Integrative Comp Physiol 2001;281:R365–72. [CrossRef]
23. Jones MK, Itani RM, Wang H, Tomikawa M, Sarfeh IJ, Szabo S, et al. Activation of VEGF and Ras genes in gastric mucosa during angiogenic response to ethanol injury. Am J Physiol 1999;276:G1345–55. [CrossRef]
24. Matsui Y, Mitsuyama K, Tomiyasu N, Toyonaga A, Sata M. Efficacy of vascular endothelial growth factor in the treatment of experimental gastric injury. Digestion 2002;66:99–105. [CrossRef]
25. Abebaw M, Mishra B, Gelayee DA. Evaluation of anti-ulcer activity of the leaf extract of Osyris quadripartita Decne. (Santalaceae) in rats. J Exp Pharmacol 2017;9:1–11. [CrossRef]
26. Sumbul S, Ahmad MA, Mohd A, Mohd A. Role of phenolic compounds in peptic ulcer: An overview. J Pharm Bioallied Sci 2011;3:361–7. [CrossRef]
27. Golbabapour S, Hajrezaie M, Hassandarvish P, Abdul Majid N, Hadi AH, Nordin N, et al. Acute toxicity and gastroprotective role of M. pruriens in ethanol-induced gastric mucosal injuries in rats. Biomed Res Int 2013;2013:974185. [CrossRef]
28. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002;13:572–84. [CrossRef]
29. Kwiecień S, Brzozowski T, Konturek PCh, Konturek SJ. The role of reactive oxygen species in action of nitric oxide-donors on stress-induced gastric mucosal lesions. J Physiol Pharmacol 2002;53:761–73.
30. Wantabe T, Higuchi K, Tominaga K, Fujiwara Y, Arakawa T. Acid regulates inflammatory response in a rat model of induction of gastric ulcer recurrence by interleukin 1β. Gut 2001;48:774–81.
31. Beregovyi S, Chervinska T, Olefir Y, Beregova T, Tolstanova G. Expression Pattern of Angiogenic Growth Factors at Early Stages of Experimental Gastric Ulcers Pathogenesis. RJPBCS 2015;6:817–22.
32. Suzuki H, Nagahashi S, Isii H. In: Yoshikawa T, editor. Oxidative Stress and Digestive Diseases. New York: Karger; 2001.
33. Dursun H, Albayrak F, Bilici M, Koc F, Alp HH, Candar T, et al. Gastroprotective and antioxidant effects of opipramol on indomethacin-induced ulcers in rats. Yakugaku Zasshi 2009;129:861–9. [CrossRef]
34. Sharkawi SM, El-Sherbeny GA, Ain-Shoka AA, El-Sayed ME. Prophylactic Role of Echinacea, Green Tea and Boswellia Extracts in Pyloric Ligation-Induced Gastric Ulcer in Rats. British Journal of Pharmacology and Toxicology 2012;3:197–204.