TMJ
Original research **Published by** OJS
Doi: 10.4314/tmj.v33i3.494.g303

OPEN ACCESS JOURNAL

Gastric Histological Changes Induced by the Use of Tumbo Mixture

Ali K. Hamad[1]*, Afadhali D. Russa[1], Ramadhan H. Mbassa[2]

[1]Department of Anatomy, School of Medicine, Muhimbili University of Health Sciences and Allied Sciences, Dar es Salaam, Tanzania
[2]Department of Anatomy, Military Collage of Medical Sciences, Dar es Salaam, Tanzania

Corresponding author:

Dr. Ali K. Hamad

Muhimbili University of Health Sciences and Allied Sciences

P. O. Box 65001

Dar es Salaam, Tanzania

Email: habibkham@yahoo.com

TMJ
Original research
Published by OJS
Doi: 10.4314/tmj.v33i3.494.g303
OPEN ACCESS JOURNAL

Abstract

Background
In the past few years, there has been a rising interest in alternative therapies and the usage of herbal products in the treatment of peptic ulcer disease. Tumbo Mixture® is amongst medicinal plant used in Tanzania in the management of the gastric ulcers, however, to date scant information is available on effectiveness of treatment of gastric ulcers. Our study intended to investigate possible histological changes in the stomach of ulcerated rats fed with Tumbo Mixture® so as to confirm the claim by traditional healer that this blended product has anti-ulcer effects.

Broad objective
The aim of the study was to evaluate the histological changes on ethanol-induced acute gastric ulcers in rats fed with Tumbo Mixture®.

Methods
Completely randomized block design conducted at MUHAS, Tanzania. Experiment involved 20 Wister rats weighing 200 - 250g, age range of 9-12 weeks. Animals were divided equally into NCG: given only normal saline and served to compare a normal gastric mucosa with that of an ulcerated one, UCG: given only ethanol without treatment, PTG and UTG. Ethanol was administrated to UCG and UTG orally through gastric gavages (2.5mls/kg) to induce gastric ulcers. Tumbo Mixture® (45 ml/kg) was given three times daily for 14 days. At the end of treatment animals were euthanized, stomachs opened and washed, photographed, fixed with 10% neutral buffered formalin, stained with H&E and evaluated for gastric changes. Materials used: Tumbo mixture, Wistar rats, absolute ethanol, digital camera, digital microscope, formaldehyde, ethanol, xylene, potassium alum, mercuric oxide, sodium phosphate monobasic and sodium phosphate dibasic.

Results
Gastric mucosa of ethanol-induced gastric ulcers rats fed with Tumbo Mixture® showed different histological changes including; decreasing in gastric lesions, focal dilated blood vessels, mild oedema and limited inflammatory cells infiltration. Those results signified that Tumbo Mixture contributes in alternative treatment of peptic ulcers. In the pre-treated group normal mucosa was shown with new formation of uniform blood vessels.

Conclusion and recommendation
Tumbo Mixture® was effective on the treatment of an ethanol-induced acute gastric ulcers as demonstrated by cell proliferation, secretary and ant-inflammatory effect which lead to repairing of ethanol-induced gastric damages. Further research aiming at cellular mechanisms for this product is recommended.

Key words: *Therapies, Medicinal plant, Peptic ulcers, Tumbo mixture, Tanzania.*

Published by OJS
Doi: 10.4314/tmj.v33i3.494.g303

Introduction

The goal of therapy for peptic ulcer disease (PUD) is to relieve symptoms, heal craters, prevent recurrences, and prevent complications. (1) Medical therapy should include treatment with drugs, and attempt to reduce gastric acidity by mechanisms that inhibit or neutralize acid secretion, coat ulcer craters, provide a prostaglandin analog, remove environmental factors, and reduce emotional stress. (2) Antacids neutralize gastric acid and are more effective than nonsteroidal anti-inflammatory drugs in healing gastric and duodenal ulcers. (3) However, antacids have to be taken in relatively large doses and may cause side effects. (4,5) The major side effect of magnesium-containing antacids is diarrhea caused by magnesium hydroxide. (6) In the past few years, there has been a rising interest in alternative therapies and the usage of herbal products, in particular, those produced from medicinal plants. (7) Plant extracts and their crude chemical ingredients are the most significant sources of new drugs and have shown promising results in the treatment of the gastric ulcers as well. (8) Due to that, investigations of new pharmacologically active agents through the screening of different plant extracts led to the discovery of effective and safe drugs with gastroprotective activity. Especially, plants with antioxidant capability as the main mechanism are used as herbal reservoir for treatment of peptic ulcer disease. (9)

This study was intended to investigate one of the herbal products used in Tanzania known as Tumbo Mixture® which is used as an alternative therapy for peptic ulcer disease (PUD). However, up to date scant information is available on effectiveness of treatment of gastric ulcers. The product is produced by the Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Science (MUHAS), Dar es salaam, Tanzania. The product contains two main components, Glycyrrhiza glabra which promotes mucus secretion and life span of surface cells in the stomach and Plantago lanceolata that act as a bacteriostatic and bactericide. (10,11) Thereby, the aim of this work was to evaluate the histological changes on ethanol-induced gastric ulcers in rats fed with Tumbo Mixture®. In doing so, the findings may add to the overall value of the medicinal potential in management of peptic ulcers disease, also confirming the claim by traditional healer that Tumbo Mixture® has anti-ulcer activities.

Materials and Methods

A completely randomized block design (CRBD) was used for the current experiment with 20 albino rats (Wister strain) of either sex weighing between 200 to 250g with age range of 9-12

weeks were used. The study was performed in accordance with the ethical guidelines for investigations of laboratory animals in the Department of Anatomy after obtaining approval from the MUHAS Ethical Committee (IRB#: MUHAS-REC-06-2020-311). **(12)** Rats were purchased from the animal breeding unit of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The animals were acclimatized for one week before experimentation; they were maintained on synthetic pellet feed (Hill Pellet Broiler Feeds, Tanzania) with unlimited supply of clean water. Animals were housed in controlled conditions with temperature of 22°C ± 2°C and 12/12 h light-dark cycle environment; they were randomly allocated to different experimental groups and placed in cages with saw dust bottom to prevent coprophagy. Four groups of albinos (Wister) rats five per each group were divided. The first group was taken as a negative control group (NCG) which received normal saline daily and were sacrificed after 14 days while the second group was taken as ulcer control group (UCG). In UCG, rats were fasted 24 hours and then absolute ethanol was administered orally through the gastric gavages (2.5mls/kg). The rats were killed 4 hours after ethanol administration. The stomachs of group 2 (UCG) were opened along the lesser curvature, isolated, washed in normal saline and photographed (Panasonic, Lumix, DMC-FH25, China). Then, the stomachs were observed with the help of a magnifying lens, and its external and internal surface were studied with the following features being observed: hemorrhage, ulceration, perforation, size and number of ulcers was evaluated according to the severity of ulcers. The stomachs were fixed in 10% neutral buffered formalin for 24 hours. The third group was taken as a PTG pre-treated group, given Tumbo Mixture® group. Tumbo Mixture® was purchased from the Institute of Traditional Medicine (ITM) and was administered orally through the gavage to rats in the dose of 45 ml/kg. The pre-treatment schedule was three times a day and it was continued for 14 days. After completing the 14 days, rats were sacrificed, stomachs were opened and washed with normal saline, photographed and fixed for 24 hours in 10% neutral buffered formalin. The fourth group was taken as an ulcer treatment group (UTG). In this group, rats were fasted 24 hours and then absolute ethanol was administrated orally through the gastric gavages (2.5mls/kg) for the induction of the gastric ulcer. Four hours after the administration of ethanol they were given Tumbo Mixture® at a dosage of 45 ml/Kg three times a day orally using oral gavages. This treatment was continued for 14 days, at the end of the last day rats were killed, stomachs were opened along the lesser curvature, isolated and washed in normal saline. After that, the stomachs were observed with the help of a magnifying lens, and its external and internal surface were studied with the following features being observed: hemorrhage, ulceration,

perforation, size and number of ulcers was evaluated according to the severity of ulcers, photographed and preserved in 10% neutral buffered formalin for 24 hours. Thereafter, all fixed tissues were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in liquid paraffin wax. Tissues were sectioned at 5µm using the Heitz 150 rotary microtome (Cambridge model). The sections were then subjected to Erlich's Haematoxylin and Eosin (H&E) staining technique using Baker and Silverton method. Histological sections were randomly selected, examined and photographed using swift binocular microscopes with in-built lighting system (Leica® DM 750, Icc50 HD-47142065, United States), and evaluated by the investigators with the assistance of the experts from the histopathology unit at Muhimbili National Hospital (MNH), Tanzania. The same histological preparation was done for all groups.

Results

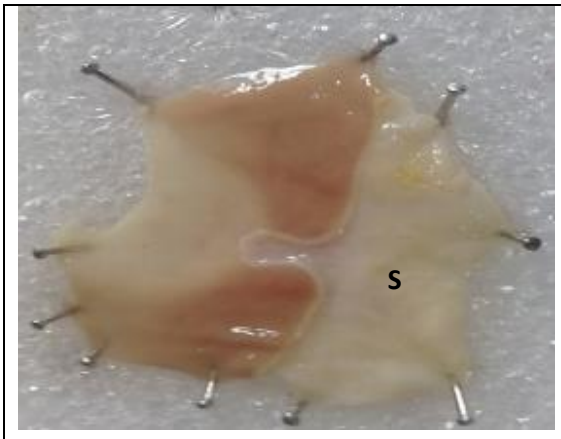
General sign and behavior of the rats

Following 14 days of the study, the behavioral patterns were observed in the first 24 hours, with special attention being given during the first 4 hours. Thereafter, observation was continued daily for a total of 14 days. The animals in all groups were normal and did not present any significant changes in behavior, fur erection, breathing, impairment in food intake and water consumption and postural abnormalities except two rats from ulcer control group (UCG) and one from ulcer treatment group (UTG) which died due to bleeding three hours after an absolute ethanol was administered orally.

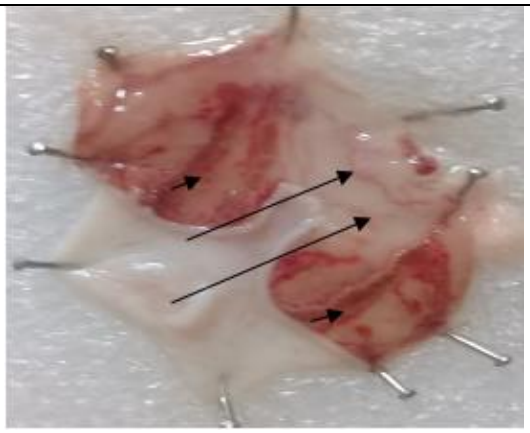
Macroscopic findings

On macroscopic examination, serosal surface of the stomach in the ulcer control group showed marked indurations, dilated blood vessels and ecchymosis sites (Figure 1b) when compared with a normal control group (Figure 1a). Severe gastric lesions such as gastric hyperemia and features of perforations were observed in the mucosa layer (Figure 1b), whereas in the pretreated group normal mucosa was shown with new formation of uniform blood vessels (Figure 1c) while partial healing gastric mucosa was revealed in the treated group (Figure 1d).

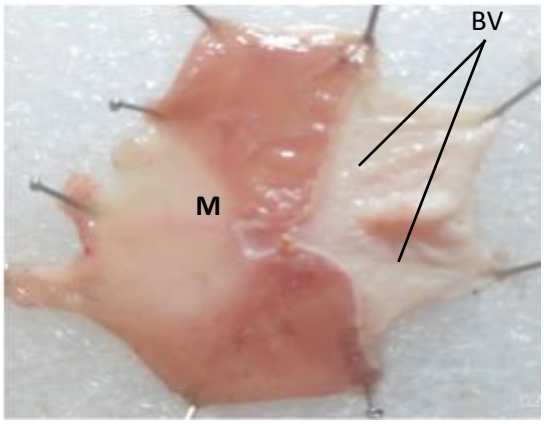
Published by OJS
Doi: 10.4314/tmj.v33i3.494.g303



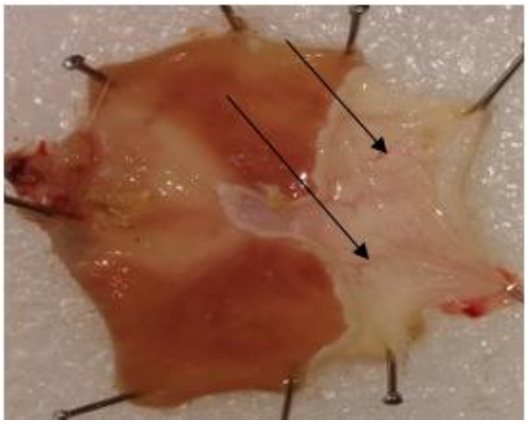
a) Photograph showing normal rat stomach



b) Photograph showing the ethanol-induced ulcers in rat stomach



c) Photograph showing the Tumbo Mixture® pretreated rat stomach



d) Photograph showing the Tumbo Mixture® treated rat stomach

Figure 1. Photographs of rat stomach: a) normal b) ethanol induced c) Tumbo Mixture® pretreated d) Tumbo Mixture® treated Shot arrows: induced ulcers; Long arrows, dilated vessels, BV: Blood vessels, S: Serosa layer. M: Mucosa layer

Histological Examination

Negative Control Group (NCG)

The epithelial of fundic region in the negative control group consists of simple columnar epithelia without goblet cells, gastric pits were oval shape and normal state occupied by gastric glands, the distribution of simple tubular gastric glands were regular, the parietal cells oval or pyramidal in shape fill with granules with the rounded nucleus located in central of cells (Figure 2A). In the pretreated group, an intact epithelium and gastric glands with the

Published by OJS
Doi: 10.4314/tmj.v33i3.494.g303

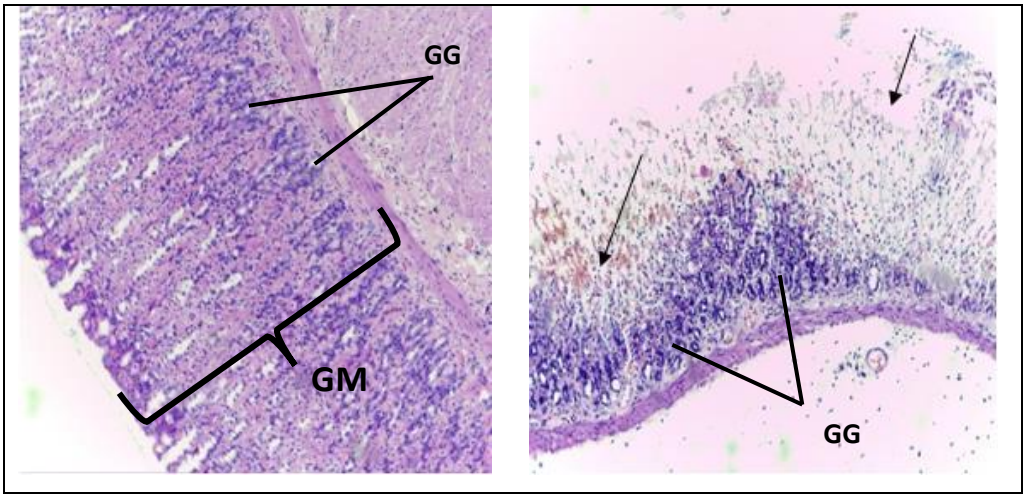
formation of new blood vessels and abundant granules in the cytoplasm of parietal and chief cells were observed (Figure 2C).

Ulcer Control Group (UCG)

Histological observation demonstrated comprehensive damage to the gastric mucosa in the ulcerated control group (UCG) of animals. Stomach of UCG rats showed loss of epithelium integrity and glandular structure with severe mucosal and submucosal damage, oedema of submucosal cell and inflammatory response as compared to the negative control group (Figure 2B) and (Figure 3A). Likewise, hyperemia was more evident in the ulcer control group which received only ethanol when compared to the ulcer treated groups which first received ethanol then treated with Tumbo® Mixture (Figure 2D).

Ulcers Treatment Group (UTG)

The gastro-mucosal changes in 14 days revealed partial healing in ulcers treated group, these findings showed decreasing in the gastric lesions, gastric glands were almost normal in appearance. Also, there were some focal dilated blood vessels, mild oedema and limited inflammatory cells infiltration (Figure 2D). The stomach in treated group was lined by a simple columnar epithelium with a lightly stained cytoplasm. The general histological appearance of the parietal cell which depicted in a triangular shape with the apical region of the cell forming the apex of the triangle, which borders the lumen of the gastric gland, decrease in number if it's compared with the ulcer control group (UCG), which had pink lightly cytoplasm and decrease in cytoplasm granules (Figure 2B).



Published by OJS
Doi: 10.4314/tmj.v33i3.494.g303

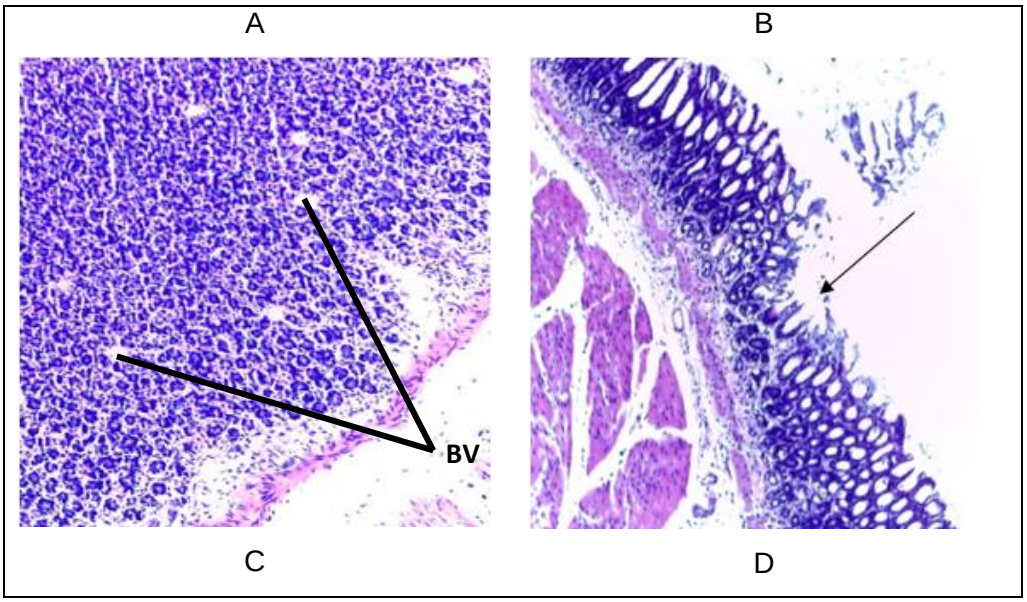
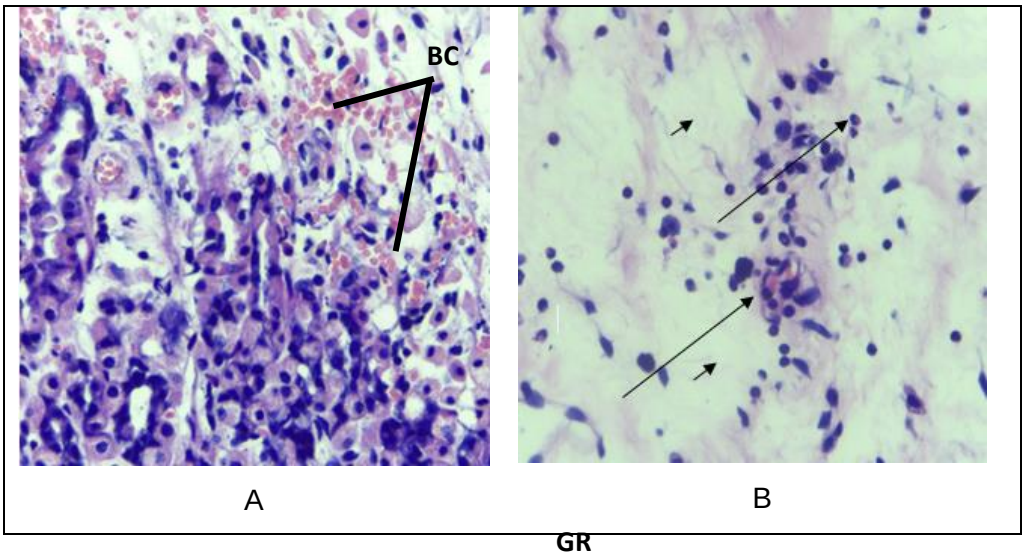


Figure 2. Histological sections of rats with: (A) normal gastric mucosa, (B) ethanol-induced gastric ulcers, (C) pretreated treated gastric mucosa, (D) treated gastric ulcers, (BV) newly formed blood vessels, (GM) gastric mucosa, (GG) gastric glands, (Arrows) showing severe ulcerative lesions (H&E ×10)



GR

Published by OJS
Doi: 10.4314/tmj.v33i3.494.g303

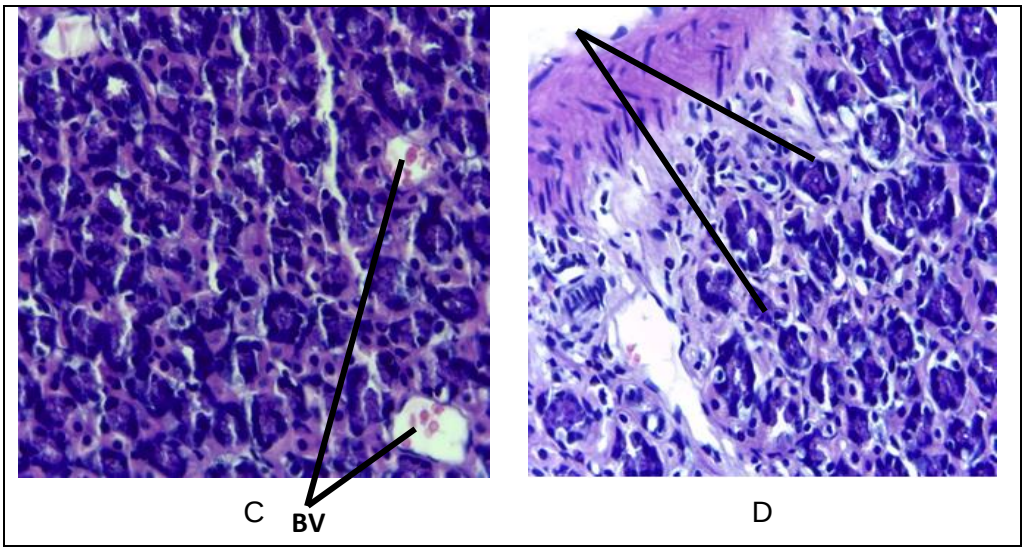


Figure 3. Histological sections of rats with (A) and (B) ethanol-induced gastric ulcers, (large arrows) and oedema in submucosa (small arrows). (C) and (D) treated gastric ulcers, (BC) extravasation of blood cells, (BV) showing angiogenesis, (GR) granules, (large arrows): inflammatory cells, (small arrows): oedema in submucosa (H&E ×40)

Discussion

Studies suggest that the ethanol-induced damage to the gastro-intestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting. (13) The current investigation found out that all ethanol-induced lesions were observed in the mucosal layer of the stomach. Ethanol induced both superficial ulcers and petechial lesions of different size within a short time. (14) In our study, the gastric ulcers were located mostly on the mucosa layer of fundic region of the stomach, the portion of the stomach secreting acid and pepsin. However, no visible ulcers found in the non-secretory part of the stomach. These findings tally with previous studies which showed very clear acetic acid-induced ulcer margins with deep defects in the fundic region of mucosal surface of the stomach in rabbits. (15) Because of the presence of visible dilated blood vessels in the treated group, it indicates that there was still healing process taking place because dilatation of the blood vessels allows cell requirements to reach the damaged site to support cells proliferation and secretory activities of the cells. (16) Additionally, a large number of inflammatory cells were found in sub-mucosa after absolute ethanol administration, however, following administration of Tumbo Mixture® the gastric mucosa displayed that the inflammatory cells were less in number with

large amount of fibroblasts, this can be compared with the study done by Rahnama (2013) which showed that the gastric glands were almost normal in appearance, there was mild oedema with limited eosinophilic infiltration and minimal lesions after treatment of Glycyrrhiza glabra. (17,18) Glycyrrhiza glabra commonly known as liquorice, has been widely used in folk medicine as an anti-inflammatory agent in the treatment of allergic reactions, antimicrobial, antiulcer, expectorant and anxiolytic activities. (19) Macroscopically, the gastric mucosa of negative control group (NCG) rats showed normal appearance because they received normal saline which has normal effect to the tissue while in the pretreated group showed two important activities, new formation of uniform blood vessels that conclude that there was a formation of new cells in the mucosa, and similarly there was increasing in granules and cytoplasm in parietal as well as chief cells which suggest that there were secretory activities taking place. The main limitation which faced our investigation was that some rats in the ulcer control group died two days following the ethanol-induced gastric ulcers.

Conclusion

Our findings suggest that Tumbo Mixture® is effective in the management of an acute gastric lesion which was demonstrated on the macroscopic as well as microscopic changes such as cell proliferation, secretory and ant inflammatory effect which led to the repairing of ethanol induced gastric damages. However, the cellular mechanisms for these actions remain to be established. Therefore, the results support the claim by traditional healer that the Tumbo Mixture® has anti-ulcer activity. Studies using human tissues with naturally occurring gratis may be needed to corroborate these findings.

Acknowledgement

We are very grateful to the personnel of the Institute of Traditional Medicine (ITM) for allowing us to use their product (Tumbo Mixture®) without any term and condition and Histopathology Department at Muhimbili National Hospital (MNH) for facilitating the laboratory part of our investigation.

Author contributions

AKH contributed to data interpretation, drafting, critically revised and editing the manuscript. ADR contributed to conception, supervision and critically review of the manuscript. RHM contributed to design, data acquisition, and data interpretation and performed all statistical analyses.

Published by OJS
Doi: 10.4314/tmj.v33i3.494.g303

References

1. Kumar A, Ashwlayan V, Verma M. **Diagnostic approach & pharmacological treatment regimen of Peptic Ulcer Disease.** Phar Pharm Res Open Acc J.2019; 1(1): 1–12.
2. Shiotani A and Graham D. **Pathogenesis and therapy of gastric and duodenal ulcer disease.** Med Clin N Am. 2002; 86: 1447–1466.
3. Robinson M, Rodriguez-Stanley S, Miner PB, Mcguire AJ, Fung K & Ciociola AA. **Effects of antacid formulation on postprandial oesophageal acidity in patients with a history of episodic heartburn**. Aliment Pharmacol Ther. 2002; 16: 435-443.
4. Malfertheiner P and Schulza C. Review Article: Peptic Ulcer: Chapter Closed? Dig Dis 2020; 38:112–116.
5. Chanda S, Baravalia Y, Kaneria M. **Protective effect of Polyalthia longifolia var. Pendula leaves on ethanol and ethanol/HCL induced ulcer in rats and its antimicrobial potency.** Asian Pac. J. Trop. Med. 2011; 4: 673–679.
6. Guerrera M, Volpe S and Mao J. **Therapeutic Uses of Magnesium.** Am Fam Physician. 2009; 80(2): 157-162.
7. Rates SM. **Plants as source of drugs.** Toxicon 2001; 39: 603–613.
8. Falcao HS, Mariath IR, Diniz MF, Batista LM, Barbosa-Filho JM. **Plants of the American continent with antiulcer activity.** Phytomedicine 2008; 15: 132–146.
9. Palle S, Kanakalatha A, Kavitha C. **Gastroprotective and antiulcer effects of Celastrus paniculatus seed oil against several gastric ulcer models in rats.** J. Diet. Suppl. 2018; 15: 373–385.
10. Heo H, Lee H, Kim C, Son K, Kim Y, Kim YC, Kang S, Park Y, Kim YH, Seo U, Seok J and Lee C. **Glycyrrhizin and Morroniside stimulate mucin secretion from cultured airway epithelial cells.** Korean J of Physiol and Pharmacol.2006; 10(6):317-321.
11. Sahakyan N, Ginovyan M, Petrosyan M and Trchounian. **Antibacterial and anti-phage activity of Plantago major L. Raw material.** Chemistry and Biology. 2019; 53(1): 59–64.
12. Ogden B, Pang W, Agui T, Lee B. **Laboratory Animal Laws, Regulations, Guidelines and Standards in China Mainland, Japan, and Korea.** ILAR Journal.2016; 57(3): 301–311.
13. Szabo S, Kusstatscher S, Sakoulas G, Sandor Z, Vincze AA, Jadus M (1995). **'Growth factors: New "endogeneous drug" for ulcer healing',** Scandinavian J. Gastroenterol. 210: 15-18.

14. Gomez OG, Rodríguez RG, Corona LQ, Borja RP, Ramírez NL, Castro ER, Gutiérrez SP, Ramos JP, Cevallos GA. **Amelioration of Ethanol-Induced Gastric Ulcers in Rats Pretreated with Phycobiliproteins of Arthrospira (Spirulina) Maxima.** Nutrients. 2018; 10(6): 763.
15. Mahmood HB, Dawood2 GA and Bargooth AF. **Histological Investigations for Cordia Myxa during the Treatment of Gastritis in Local Rabbits.** JKU.2017; 15(1): 31-39.
16. Rodrigues M, Kosaric N, Bonham C, and Gurtner G. **Wound healing: A cellular perspective.** Physiol Rev. 2019; 99: 665–706.
17. Rahnama M, Mehrabani D, Japoni S.**The healing effect of licorice (Glycyrrhiza glabra) on Helicobacter pylori infected peptic ulcers.** J Res Med Sci.2013; 18: 532–533.
18. Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A and Smolic M. **Peptic Ulcer Disease: A Brief Review of Conventional Therapy and Herbal Treatment Options**. J. Clin. Med. 2019; 8: 179.
19. Onwuchekwa C and Oluwole F. **Anti-Ulcer Effect of Risperidone in Rats.** Afr. J. Biomed. Res. 2015; 18: 225- 231.