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Histomorphometric and Immunohistochemical Effects of Coconut Oil on Alcohol-Induced Gastric Ulcer

Busuyi Kolade AKINOLA[1], Bolanle Christiana FALEYE[2], Toluwase Solomon OLAWUYI[1], Priscilla Adebimpe AKINOLA[3]
[1]Department of Human Anatomy, Federal University of Technology, Akure. Ondo State, Nigeria.
[2]Department of Chemical Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji. Osun State, Nigeria
[3]Department of Biology, Federal University of Technology, Akure. Ondo State, Nigeria.

Corresponding Author:- Busuyi Kolade AKINOLA

Abstract:-

- ***Background***

Alcohol is a well-known damaging agent to the gastric mucosa. Excessive alcohol ingestion is no doubt a cause of human gastric ulcer. Coconut oil has been reputed to possess antioxidant and anti-inflammatory properties. It contains polyphenol and other very important compound of medical importance. The aim of this study is to evaluate the immunohistochemical effects of Coconut Oil (CO) on gastric ulcer in wistar rats.

- ***Materials/Method***

Twenty rats weighing 80-120g were divided into four groups with five rats in each group. Induction of gastric ulcer was done through oral administration after the animals were fasted for 24hrs with 0.5ml of 90% ethanol. Group A (control) received distilled water; Group B received alcohol + Coconut oil; Group C received alcohol + Omeprazole while Group D received alcohol once daily. Animal treatment was for 4 weeks after which they were sacrificed by cervical dislocation. The stomach was harvested and blood samples obtained by cardiac puncture. Samples were subjected to immunohistological and biochemical analysis.

- ***Results***

The results showed that there was gastric ulceration in group D while groups B and C showed marked mucosa repair on histological evaluation. There was increased presence of Nrf2 in group D whereas it was significantly reduced in groups B and C. There was significant increase in MDA levels in group D while it was significantly decreased in groups B and C when compared with group A ($P<0.05$). There was significant decrease in SOD levels in group D while it was significantly increased in groups B compared with the control ($P<0.05$).

- ***Conclusion***

The results from this study showed that Coconut oil has ameliorative potential on alcohol induced gastric ulcer.

Keywords:- *Gastric ulcer, Coconut Oil, Immunohistochemistry, Alcohol, Omeprazole.*

I. INTRODUCTION

Sores in the stomach lining or the duodenum are referred to as a gastric ulcer. It is one of the most frequent illnesses that individuals, particularly in underdeveloped nations, suffer from (Okafor *et al.,* 2018). When the impacts of some aggressive elements grow more evident and overwhelm the mucosa protective mechanism, gastric ulceration occurs. Alcohol, Helicobacter pylori infection, nonsteroidal anti-inflammatory drugs (NSAIDs), cigarette smoke, hereditary factors, and certain diets are among these aggressive variables (Omojola *et al.,* 2019). It has been reported that in Nigeria, at least one family member has suffered from or is currently suffering from stomach ulcers. Helicobacter pylori infection and/or the use of nonsteroidal anti-inflammatory drugs (NSAIDs) produce gastric ulcers. There is little or no effective control of pharmaceuticals by authorized government organizations in poor nations. Antisecretory medicines and cyto-protective medications are currently used to treat stomach ulcers. When used to treat ulcers, some drugs cause unwanted side effects or resistant strains of H. pylori, in addition to being expensive. This has sparked scientists' interest in producing ulcer cures based on natural plant components that don't build resistant bacteria strains and have few adverse effects; the field of "nutraceuticals" has sprung up as a result. Nutraceuticals, as described by Stephen De Felice, are foods or dietary components that give medical/health benefits, such as illness prevention and therapy (Okafor *et al.,* 2018).

Ethanol is a toxic substance linked to a variety of diseases, and it can be used to produce acute stomach ulcers in experimental animals. Ethanol-induced gastric ulcers occur when the stomach mucosa is disrupted, resulting in increased mucosal permeability and bleeding. Infiltrating white blood cells such as neutrophils causes an overproduction of reactive oxygen species (ROS) and other inflammatory mediators, leading in oxidative damage (Sineenart *et al.,* 2022).

Virgin coconut oil (VCO) is oil extracted from fresh mature coconut (Cocosnucifera) kernels using mechanical or natural methods, with or without the use of heat, and without the use of any chemical refining or bleaching agents. It has been reported that tropical countries have used

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coconut from the tree *Cocosnucifera*, Family Aracaceae (palm family) as an integral part of their diet and livelihood. The composition of Fatty acids in VCO as determined by Gas Liquid Chromatography include Saturated fats: Lauric acid (45%to52%), Myristic acid (16% to 21%), Palmitic acid (7% to 10%), Caprylic acid (5% to 10%), Capric acid (4%to 8%), Stearic acid (2% to 4%), Caproic acid (0.5% to1%) and Palmitoleic acid (in traces) and Unsaturated fats: Oleic acid (5% to 8%), Linoleic acid (1% to 3%) and Linolenic acid (up to 0.2%) (Pavan *et al.*, 2018). Coconut oil (CO) is colorless, rancid-free, and has a distinct fresh coconut scent. CO is high in polyphenols, which explains why it has anti-oxidant, anti-inflammatory, and analgesic qualities. Cocosnucifera oil has been shown to be an excellent wound healer as well as a tissue repair agent. However, very few studies have explored the effects of coconut oil on gastric mucosal injury. This study therefore will provide information on the effects of coconut oil on gastric ulcer in adult wistar rats.

II. MATERIALS AND METHODS

➢ *Materials Used for the Study*

Materials used for the study include: Ethanol, formalin, oral canula, coconut oil, gloves & syringes, universal bottles, microscope, microscope slides, dissecting set, finisher's feed, weighing scale, normal saline.

➢ *Experimental Animals Care and Maintenance*

Twenty wistar rats (males & females) weighing between 80-120g were purchase from the central animal house of the Biochemistry Department, Federal University of Technology, Akure. The animals were acclimatized for one week in a ventilated cage at optimum temperature. The animals were fed on standard animal feeds and water without restrictions. The experiment was done in accordance to the present rules and regulations that has been established for laboratory animals (NRC, 2011).

➢ *Induction of Gastric Injury*

Induction of gastric injury was done after the animals were fasted for 24hrs by oral administration of 0.5ml/90% ethanol once.

➢ *Experimental Design*

The twenty wistar rats were divided into four groups with five rats in each group:

- **Group A:** The positive control group, received feed and distilled water for 14 days.
- **Group B:** Received 7.5ml/kg body weight (bwt) of coconut oil once daily for 14 days after induction.
- **Group C:** This group received 0.6mg/kg (bwt) omeprazole orally once daily for 14days after induction.
- **Group D:** Received 90% ethanol (5ml/kg) after 24 hours of fasting at induction.

➢ *Histological Analysis*

Paraffin-embedded tissues were sectioned at a thickness of six to seven microns using a rotary microtome. The sectioned ribbons containing colon sections were then stained by hematoxylin and eosin (H&E) for histological evaluation of gastric mucosa damage (x40 and x100 magnification). In a typical tissue, nuclei are stained blue by the hematoxylin while the cytoplasm and extracellular matrix show varying degrees of pink staining from the eosin. Nucleoli stain with eosin.

➢ *Biochemical Analysis*

The remaining part of the tissue was homogenized in phosphate buffer (pH 7) formalin and centrifuged. Superoxide Dismutase (SOD) and Malondialdehyde (MDA) levels were evaluated.

➢ *Measurement of Superoxide Dismutase (SOD)*

50ul of the sample was added to 1000ul of 50mM Na_2CO_3 (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 17ul adrenaline. The cuvette contained 50ul of sample, 1000ul of buffer and 17ul of adrenaline (substrate). The increase in absorbance at 480nm was monitored every 15secs for 120seconds.

➢ *Determination of Malondialdehyde (MDA)*

150ul of the serum sample was placed in a test tube and mixed with 8.1% of Sodium Dodecyl Sulphate (SDS) of 150ul, then 250ul of acetic acid (pH 3.4) was added and also 200ul of 0.6% of Thiobarbituric acid (TBA) was added. The mixture was heated in boiling water for 60 minutes. The test tubes were cooled at room temperature and the absorbance was determined at 532nm using the spectrophotometer.

➢ *Immunohistochemical Detection of Nrf2*

Gastric sections were defrosted at room temperature following removal from −20◦C and quenched for endogenous peroxidase activity using Bloxall (Vector Laboratories Ltd) for 10 min at room temperature. Sections were gently washed with PBS and incubated with avidin D for 15 min at room temperature, excess avidin D solution gently blotted off, and the section then incubated for a further 15 min at room temperature with biotin blocking solution (Vector Laboratories Ltd) according to the manufacturer's instructions. Gastric sections were permeabilised with 1 g l−1 Triton-X 100 (Sigma) in PBS with 10% normal donkey serum and then incubated with primary antibodies goat anti-glial fibrillary acidic protein (GFAP, 1:400) and rabbit anti-Nrf2 (1:100) overnight at 4◦C. GFAP was also identified by fluorescence, and sections incubated with secondary antibodies (donkey anti-goat Cy5, 1:500) for 1 h at room temperature. To quantify Nrf2 by immunohistochemistry, gastric sections were incubated with goat anti-rabbit biotinylated secondary antibody and ABC reagent to amplify the DAB reaction product signal. Gastric sections were washed twice with distilled water and stained with DAPI (2 μg/ml) for 5 min. Quantification using our immunohistochemical technique is critically dependent on the stability of the tissue in the first few seconds after application of the DAB–H2O2 reaction mix. We found that preincubation of sections with ascorbic acid (1 mM) and DAB for 30 min, before application of the reaction mix, eliminated tissue movement.

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Microscopy of the immunohistochemically stained tissue sections was performed by a pathologist blinded to the experimental condition. Evaluation of the sections was undertaken by assessing the intensity of staining (4 grades) as follows: "0" indicates very low density of positive staining; "1" indicates moderate density of positive staining; "2" indicates a higher but submaximal density of positive staining; and "3" indicates the highest density of positive staining.

III. DATA ANALYSIS

The differences between the treatment and control groups were statistically analyzed by one-way analysis of variance (ANOVA). All statistical analyses will be carried out using the Statistical Package for Social Sciences (SPSS version 16.0 for Windows). The data were represented as mean value ± SEM (Standard error of mean). All results will be mean of 5 data samples and the statistical analysis will be carried out using students' t-test.

IV. RESULTS

- *Effects of Coconut Oil on the Histology of the Stomach of Wistar Rats*

Plate 1-2 is the photomicrograph of the histological sections of the gastric mucosa at varying magnification across groups. Group A (control) shows normal gastric morphology and histoarchitecture. There were heavy glandular damage, oedema, and infiltration of inflammatory cells and cell necrosis in group D (ethanol only), however, the administration of coconut oil and omeprazole tends to ameliorate the cytoarchitectural and histomorphological distortions of the gastric mucosa as seen in group B (ethanol + coconut oil) and C (ethanol + omeprazole) when compared to group D (ethanol only).

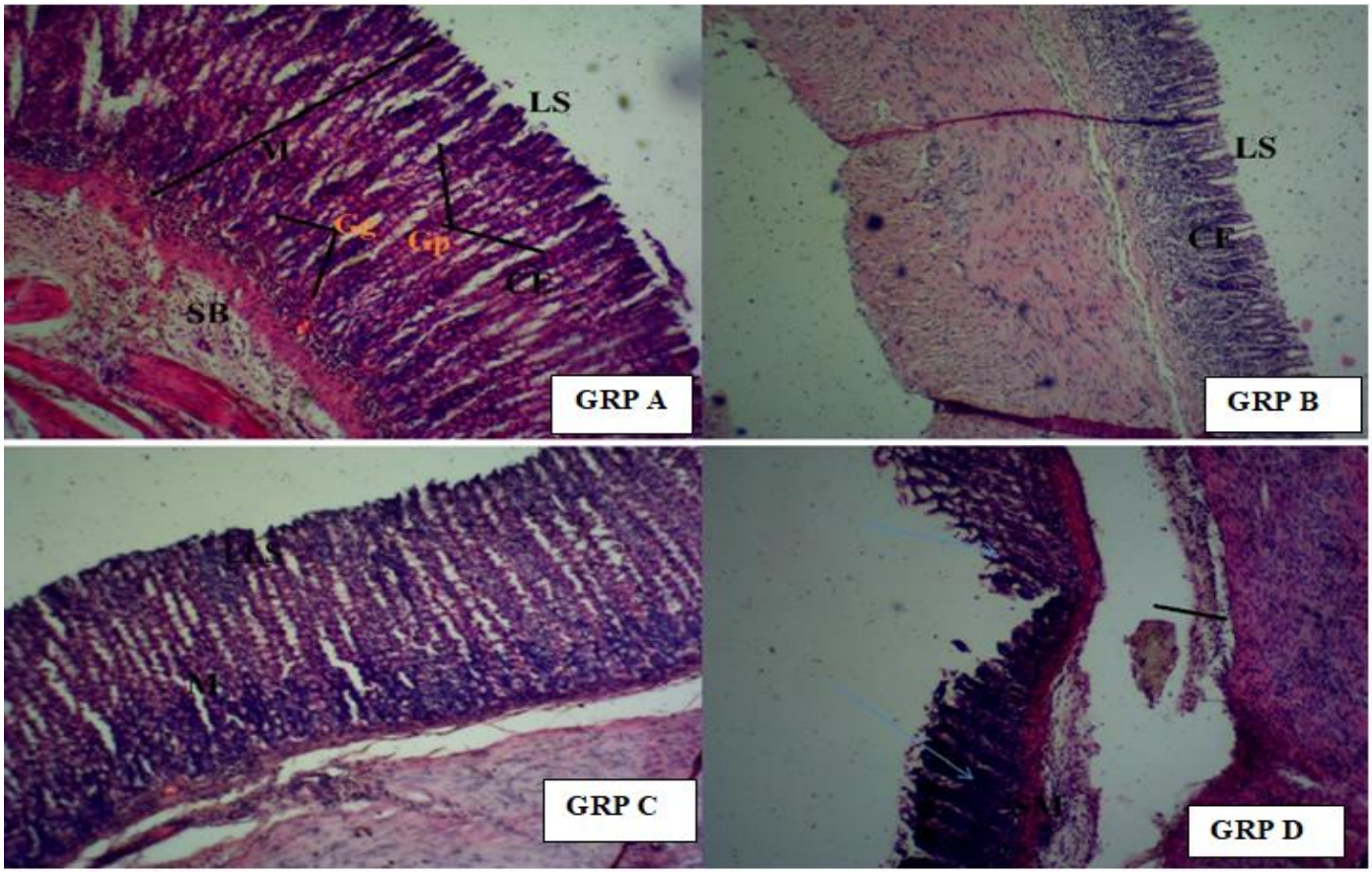

Plate 1: Photomicrograph of gastric tissue histology showing group A (control group) with normal tissue morphology; group D (ethanol only) with heavy glandular damage, oedema, and infiltration of inflammatory cells and cell necrosis; group B (ethanol + coconut oil) and group C (ethanol + omeprazole) with remarkable protection against the gastric lesion. M = mucosa; Mm = Muscularis mucosae; Gg = gastric glands; Sm = submucosa; Gp = gastric pits. Stain: H & E; Magnification X40 and X100.

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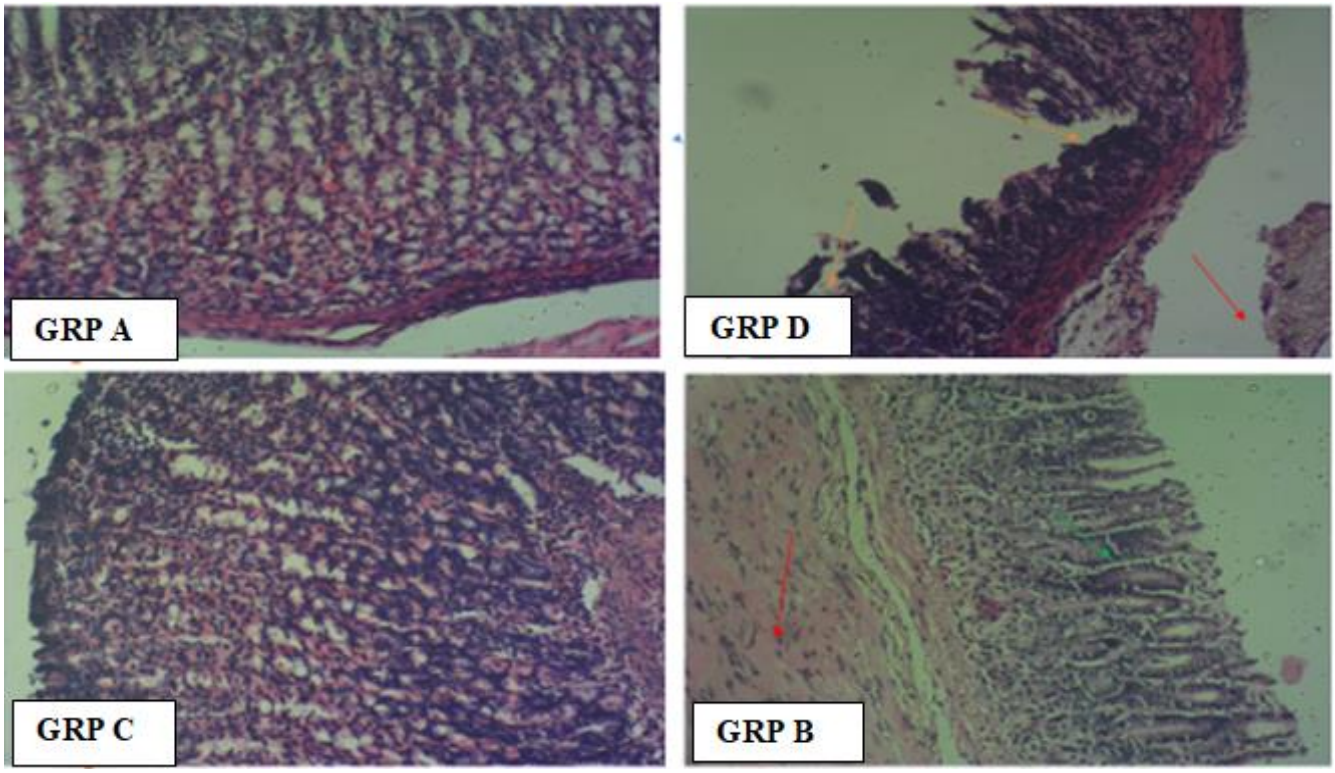

Plate 2: Photomicrograph of gastric histology showing Group A (control) with normal morphology, Group D (alcohol only) with heavy mucosal damage, oedema and cell necrosis, Group B (alcohol + coconut oil) and C (alcohol + omeprazole) with remarkable protection against the gastric lesion. Arrows shows damaged areas of the histoarchitecture of the mucosa layer and also Infiltration of neutrophils. Stain: H & E X 400.

➢ *Effect of Coconut Oil on Malondialdehyde (MDA)*

Figure 1 shows the MDA level in the stomach homogenates of wistar rats. The level of MDA was significantly high in the ulcer induced group (group D) compared to the normal control. Group B treated with coconut oil and group C treated with omeprazole were found to protect against the damage found in the ulcer induced group and led to the decreased concentrations of MDA in this groups.

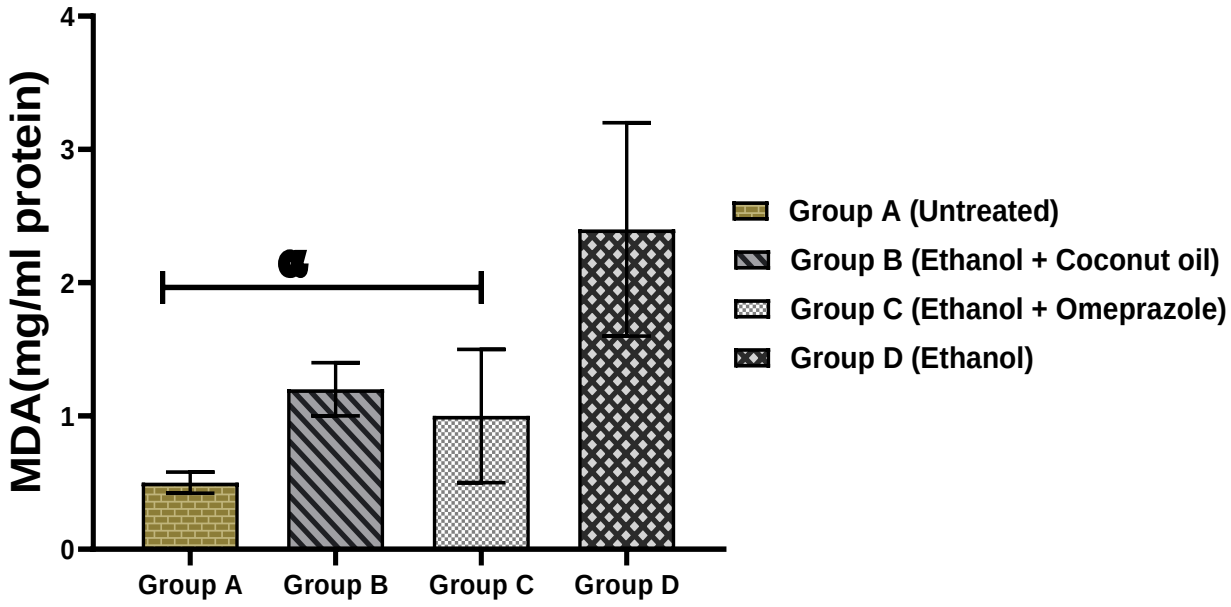

Fig 1 Effects of coconut oil on product of lipid peroxidation (Malondialdehyde, MDA). Each value represented Mean ± SEM, n = 4 readings. Values of $p < 0.05$ were considered significant. The value with superscript α = significantly different from group D. MDA– Malondialdehyde.

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➢ *Effect of Coconut Oil on Superoxide Dismutase (SOD)*

Figure 2 shows the SOD level in the stomach homogenates of wistar rats. The level of SOD was significantly lower in the ulcer induced group compared to the normal control. The level of SOD was significantly higher in the group treated with coconut oil (group B) and omeprazole (group C) compared to group D (ethanol only).

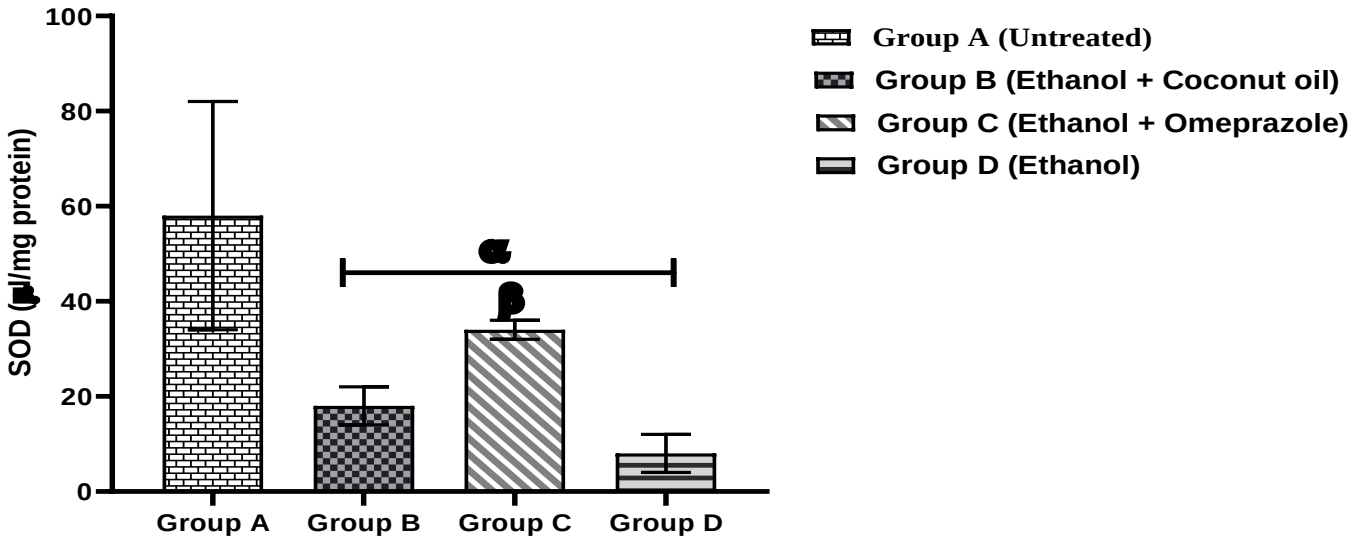

Fig 2 Effects of coconut oil on SOD activity. Each value represented Mean ± SEM, n=4 readings. Values of $p<0.05$ was considered significant. The values with superscript α = significantly different from group A, β= significantly Different from group D. SOD– Superoxide Dismutase.

➢ *Immunohistochemical Staining of Stomach Nrf2*

Plate 3-6 is the photomicrograph of the immunohistochemical localization of Nrf2 in the gastric mucosa at varying magnification across groups. The expression of Nrf2 in the gastric mucosa cells was less in group B compared to other groups as seen below.

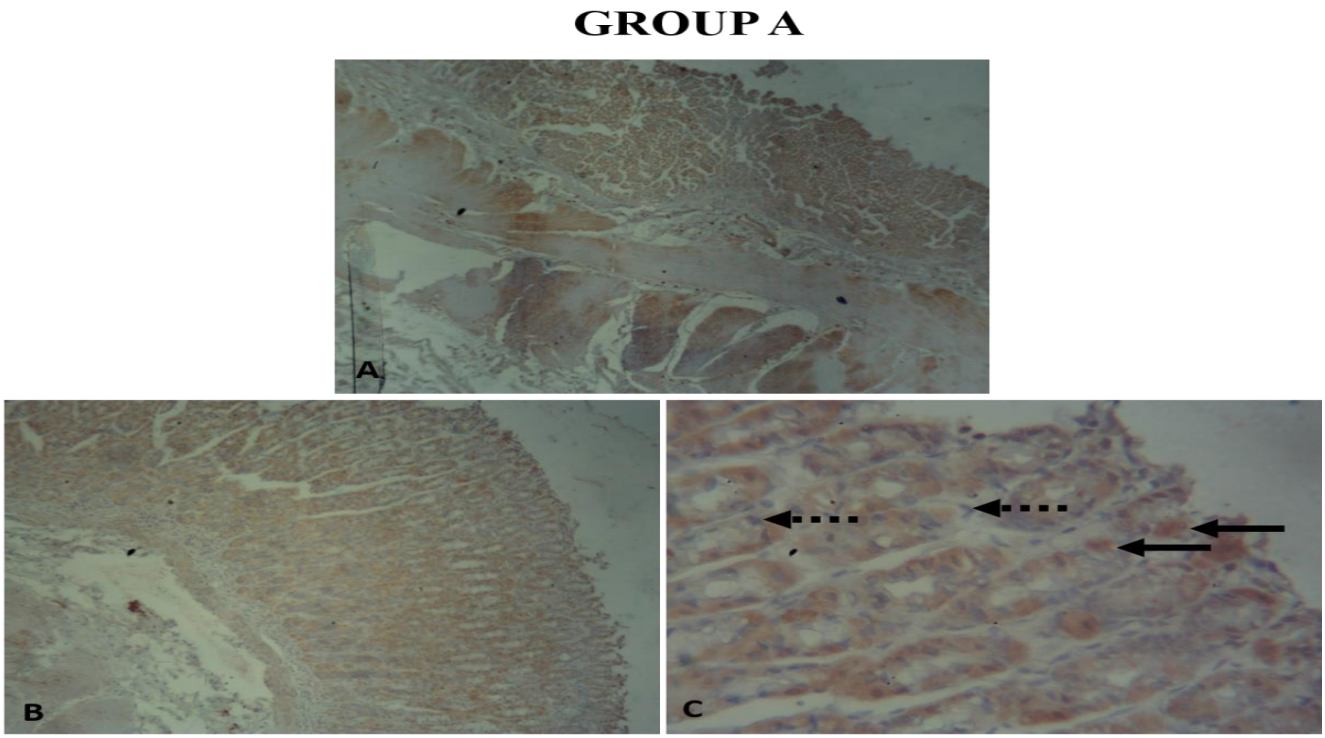

Plate 3: Photomicrographs of the mucosa layer of the stomach for control group at magnification x40 (A), x100 (B), and x400 (C). Dotted arrows denote non-Nrf2 expressing cells stained blue with heamatoxylin, while thick arrows indicate Nrf2 expressing cells stained brown with anti-Nrf2.

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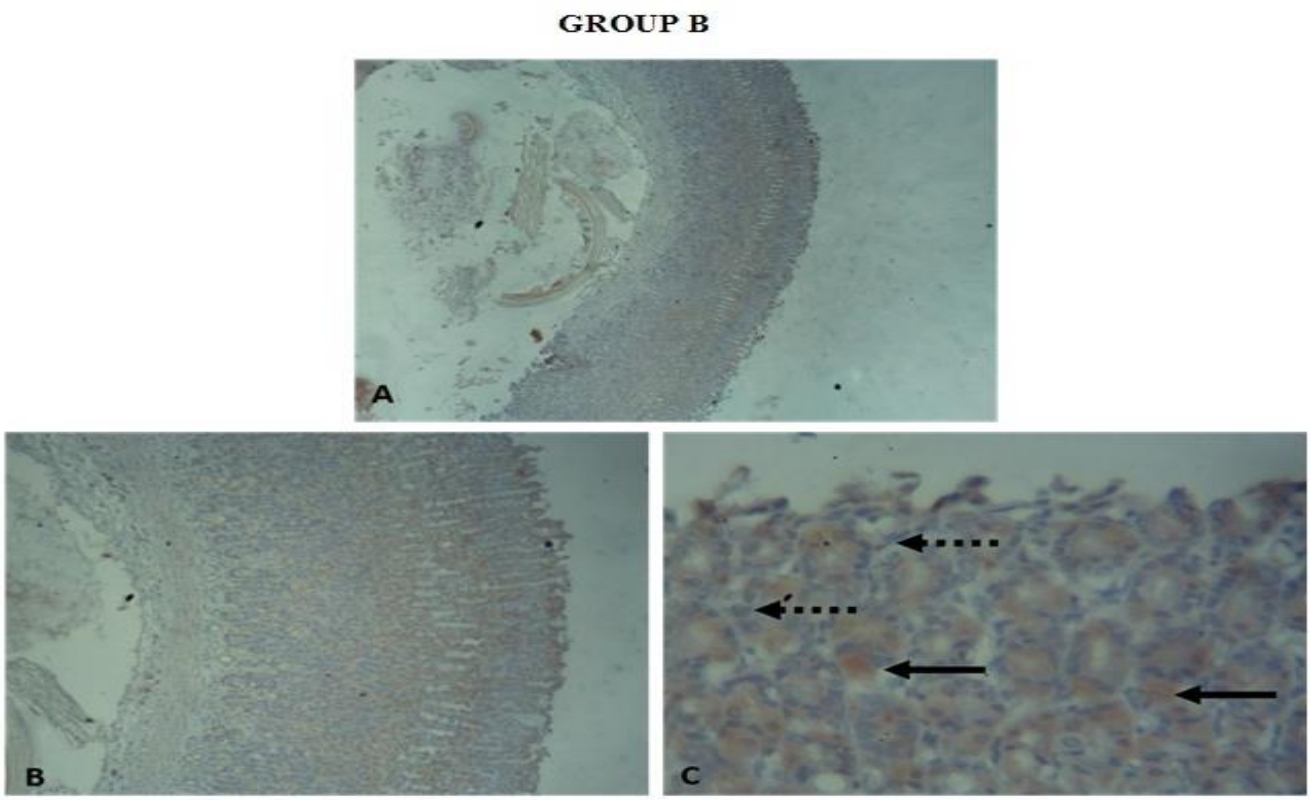

Plate 4: Photomicrographs of the mucosa layer of the stomach for group B (alcohol + coconut oil) at magnification x40 (A), x100 (B), and x400 (C). Dotted arrows denote non-Nrf2 expressing cells stained blue with heamatoxylin, while thick arrows indicate Nrf2 expressing cells stained brown with anti-Nrf2.

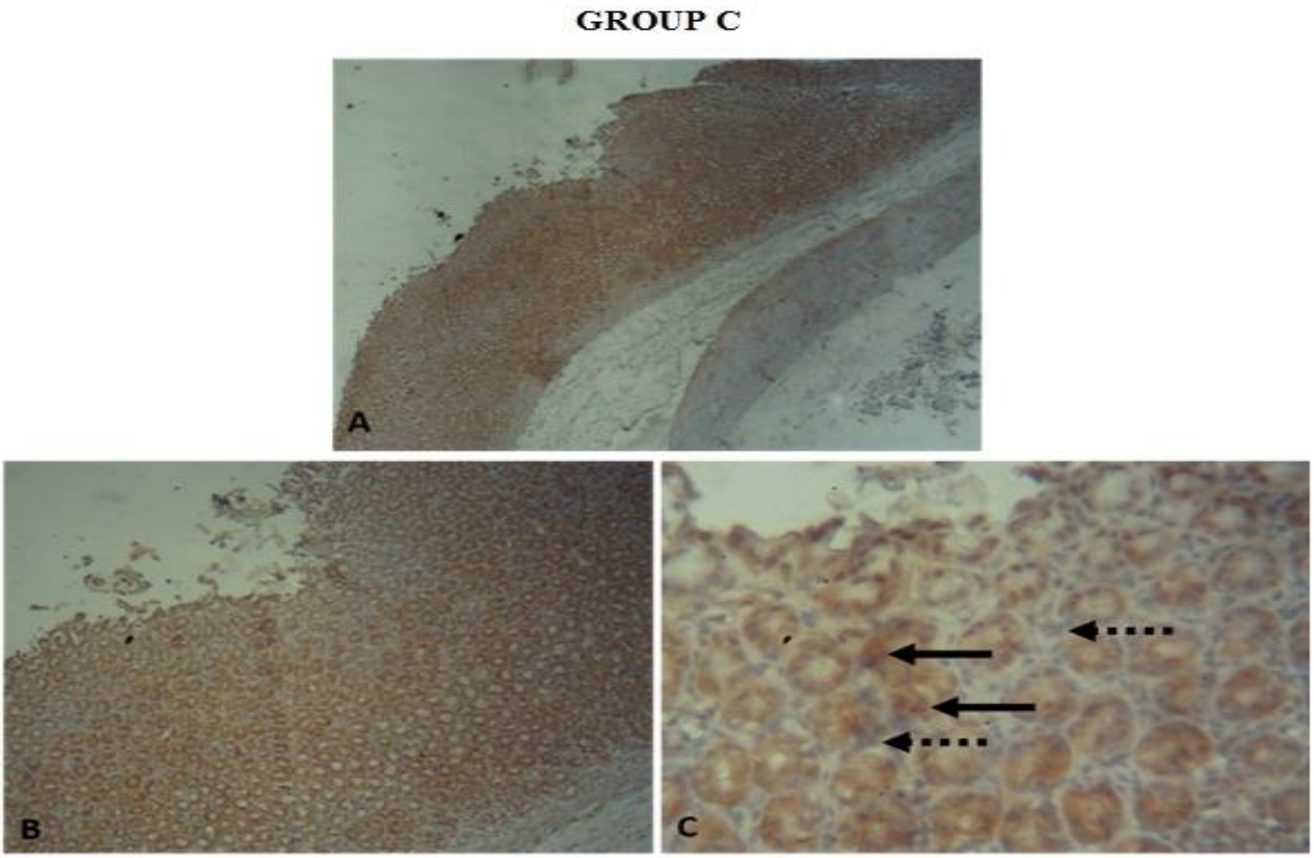

Plate 5: Photomicrographs of the mucosa layer of the stomach for group C (alcohol + omeprazole) at magnification x40 (A), x100 (B), and x400 (C). Dotted arrows denote non-Nrf2 expressing cells stained blue with heamatoxylin, while thick arrows indicate Nrf2 expressing cells stained brown with anti-Nrf2.

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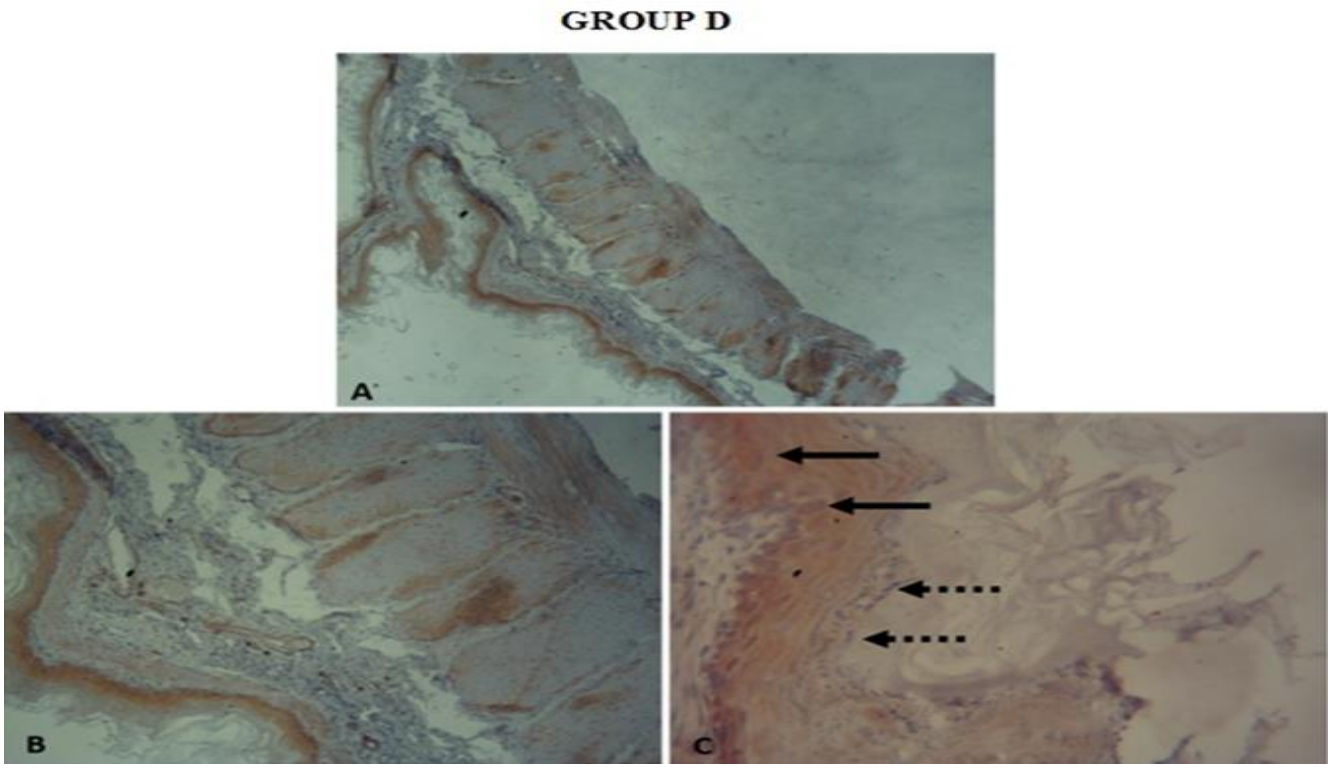

Plate 6: Photomicrographs of the mucosa layer of the stomach for group D (alcohol only) at magnification x40 (A), x100 (B), and x400 (C). Dotted arrows denote non-Nrf2 expressing cells stained blue with heamatoxylin, while thick arrows indicate Nrf2 expressing cells stained brown with anti-Nrf2.

➢ *Image J Quantification of Nrf2 Immunoreactivity Across Groups*

Figure 3 shows the percentage of Nrf2 immunoreactivity in the stomach of wistar rats across groups. There was a significant increase in Nrf2 immunoreactivity in group B (94.73 ± 2.831) and group D (98.73 ± 0.6960) compared to group A (73.23 ± 2.659). There was a significant decrease in group C (20.63 ± 4.674) compared to group A (73.23 ± 2.659), group B (94.73 ± 2.831) and group D (98.73 ± 0.6960). However, there was no significant difference between group B (94.73 ± 2.831) and group D (98.73 ± 0.6960).

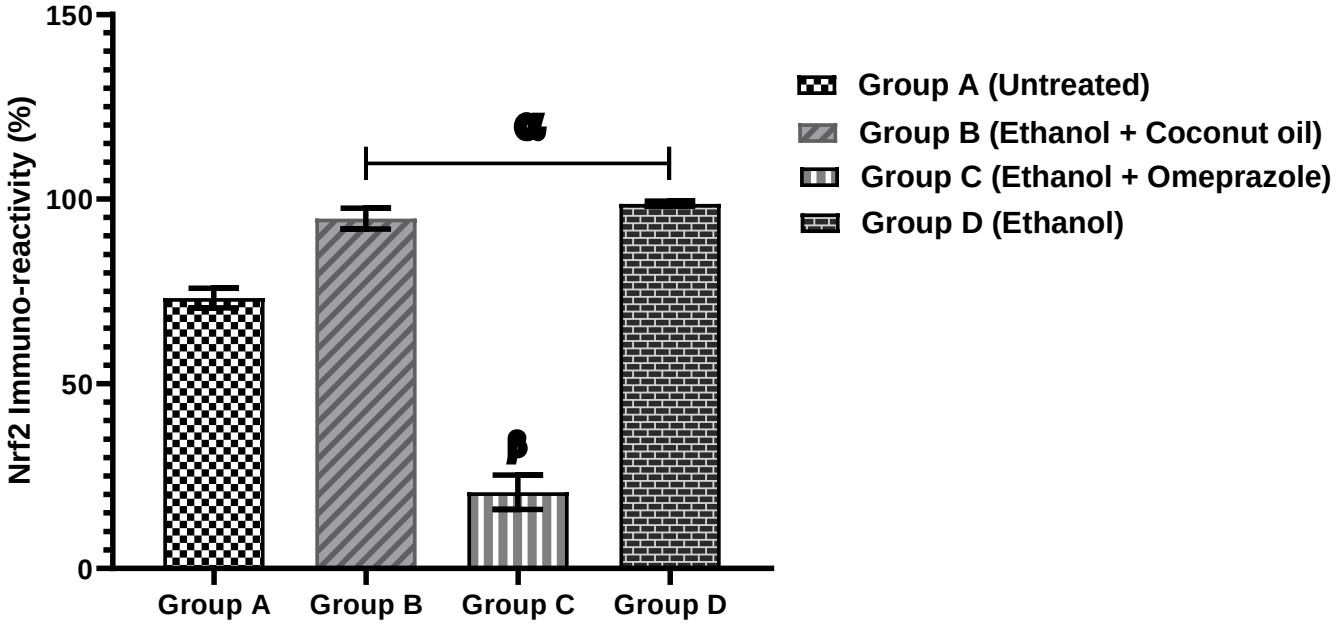

Fig 3 The percentage of Nrf2 immunoreactivity across groups. Each value represented Mean ± SEM, n = 6 readings. Value of $p < 0.05$ was considered significant. α denotes significant increase ($p<0.05$) compared to control group, β denotes significant decrease ($p<0.05$) compared to control group. One-way ANOVA followed by Turkey test.

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V. DISCUSSION

In this study, the presence of heavy glandular damage, oedema, infiltration of inflammatory cells and cell necrosis in the mucosa layer of stomach wall in the alcoholic group (Group D) confirms alcohol-induced histological damage to the stomach. This is similar to the study done by Omojola et al. (2019) whereby it was reported that ethanol induced ulceration and hemorrhagic lesions in the stomach with presence of numerous ulcers in the mucosae. The ulceration may be due to the toxic effect of ethanol on the mucosa resulting to loss of epithelium and mucus depletion and its constrictive effect on veins and arteries of the gastric mucosal, producing congestion, inflammation and tissue injury. However, the administration of coconut oil and omeprazole tends to ameliorate the cytoarchitectural and histomorphological distortions of the gastric mucosa as seen in group B (ethanol + coconut oil) and C (ethanol + omeprazole) when compared to group D (ethanol only). This is similar to the study of Okafor et al. (2018) whereby it was stated that treatments of indomethacin-induced gastric ulcer with virgin coconut oil showed some visible effect against ulceration by showing decreased trend of mucosal congestion. Saad et al. (2016) also reported that pretreatment of ethanol-injected rats with omeprazole resulted in mild histopathological changes as compared to ethanol-treated rats. Omeprazole has been used for treatment of gastric ulcer and has been used in numerous published studies to provide a gastroprotective effect (Saad *et al.*, 2016).

Oxygen derived free radicals have been said to play an important part in the pathogenesis of the injury of various tissues including the digestive system and lead to acute gastric and esophageal mucosal injury as a result ischemia, or ethanol. ROS generate lipid peroxidation which is believed to be an important cause of destruction and damage of the cellular membranes (Wi *et al.*, 2013).

The involvement of extensive lipid peroxidation in ethanol-induced gastric damage has been demonstrated by the accumulation of MDA level, as an index of lipid peroxidation. It has been demonstrated that free radical damage to the gastric mucosa can be prevented by the administration of free radical scavengers (Wi *et al.*, 2013). Ethanol injures the vascular endothelial cells of the gastric mucosa and induces microcirculatory disturbance and hypoxia, linking to the overproduction of oxygen radicals (Hidekazu *et al.*, 2012).

In the present study, the level of malondialdehye (MDA) was significantly higher in the ulcer induced group (group D) compared to the control group (group A). Group B treated with coconut oil and group C treated with omeprazole was found to protect against the damage found in the ulcer induced group which led to the decreased concentrations of MDA in these groups. Coconut oil and omeprazole was found to markedly reduce lipid peroxidation by promoting the healing of gastric mucosa lesions induced by ethanol. This is similar to the study of Jie et al. (2019) where it was reported that the level of MDA was significantly reduced by virgin coconut oil (VCO) relative to the control group. This finding indicates that VCO play a role in suppressing formation of ROS and there may be a strong correlation of its antiulcer activity with the free radical scavenging activity, similar to omeprazole.

In the present study, the level of superoxide dismutase (SOD) was significantly lower in group D (ethanol only). This is similar to the study of Dan et al., 2020 on the gastroprotective effect of gallic acid against ethanol-induced gastric ulcer in rats, whereby it was reported that ethanol exposure causes significant diminutions in GSH content, SOD and CAT activities. However, the level of superoxide dismutase (SOD) was significantly increased in group B (ethanol + coconut oil) and C (ethanol + omeprazole). This is similar to the study of Adeniyi et al. (2018) on the gastric ulcer healing effects of virgin coconut oil in experimental animals whereby a significant increase in superoxide dismutase activity was observed in animals treated with VCO. The reason for this finding may be because of the antioxidant property of coconut oil.

Nrf2 is a transcription factor that plays a vital role in antioxidant synthesis. It reduces oxidative stress by down regulation of nuclear factor κB (NFκB) to reduce inflammation, and upregulation of antioxidants. In the present study, there was a significant increase in Nrf2 immunoreactivity in the ulcer induced group (group D). This is similar to the study of Dan et al. (2020) whereby it was reported that elevated Nrf2 levels in the ulcer area in ethanol-treated group was observed. However, there was a significant decrease in Nrf2 immunoreactivity in the group treated with omeprazole (group C). Currently, there is no study directly linking omeprazole to the expression of Nrf2 level on alcohol-induced gastric ulcer. However, there is no significant difference between the group treated with coconut oil and the ulcer induced group.

VI. CONCLUSION

In conclusion, our present study revealed that VCO exerted appreciable gastroprotective effect against ethanol-induced gastric lesions in rats. As pathogenesis of ulcer disease is associated with various factors, VCO can be considered as a potential therapy to be used for treating and preventing this ailment.

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