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Morphometric Effects of ß, Ɛ-Carotene- 3, 3'-diol on Indomethacin- induced Gastric Ulcer in Adult Male Wistar Rats

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ABSTRACT

Stomach ulcer is a disease that causes the erosion of the epithelial lining of the gastric mucosa. This study aims to determine the ameliorative effects of ß, Ɛ- carotene- 3, 3'-diol following Indomethacin-induced gastric ulcer in Wistar rats. Thirty adult male Wistar rats (7 weeks old, 160 – 165 g) were used for this study. The rats were randomized into six groups, A - F of five rats each. A –control, B – treated with 20 mg/kg body weight (bw) of indomethacin, C – treated with 20 mg/kg omeprazole, D – treated with 20 mg/kg ß, Ɛ- Carotene- 3, E – treated with 40 mg/kg ß, Ɛ- Carotene- 3, 3'-dioland F – treated with 80 mg/kg b.w of ß, Ɛ- Carotene- 3, 3'-diol respectively, 12 hourly for 14 days following Indomethacin-induced gastric ulcers. The rats were fasted for 24 hours for gastric emptying after the last administration and later sacrificed using chloroform as anesthesia. The rat's stomachs were harvested for both histological and biochemical assays. The Indomethacin-only treated rats showed an eroded epithelial lining while the treated groups showed a regeneration of the mucosa layer. A significant increase in ulcerated area, ulcer index, and a significant decrease in superoxide dismutase and catalase activity with a significant increase in tumor necrosis factor-alpha concentration compared to the control and treated groups at $p < 0.05$. ß, Ɛ- Carotene- 3, 3'-diol, showed a better curative ratio and remediation at 80 mg/kg when compared with the omeprazole-treated group.

Keywords: gastric ulcer, indomethacin, omeprazole, ß, Ɛ- carotene- 3, 3'-diol

INTRODUCTION

Peptic ulcer is a major health concern as its complications lead to morbidity and mortality[1]. It is a break or degradation in the inner lining of the stomach or duodenum[2]. Gastric ulcer is a depletion or break in the lining of the mucosa or submucosa of the stomach, which leads to cell loss, inflammation, and irritation[3]. An imbalance between protective factors such as prostaglandin synthesis, mucus secretion, and aggressive factors such as secretion of hydrochloric acid results in gastric ulcers. Several factors such as Helicobacter pylori infection, stress, prolonged intake of non-steroidal anti-inflammatory drugs, and alcohol consumption have been reported to be responsible for peptic ulcer development [4]. Some of the symptoms of gastric ulcer are nausea, vomiting, heartburn, and weight loss [5]. Omeprazole, an example of a proton pump inhibitor, helps to prevent gastric ulcers and is also used in treating ulcers with a combination of antibiotics and drugs[6]. Proton pump inhibitor drugs are associated with side effects such as diarrhea, nausea, vomiting, and anemia[6]. As a result of this, researchers have in recent times shifted their focus to plant nutrients as an alternative therapy to gastric ulcer treatment, one of such plant nutrients is ß, Ɛ-Carotene-3, 3'-diol. ß, Ɛ-Carotene-3, 3'-diol is a natural source of antioxidants and is more efficacious when compared with other forms of carotenoid. Carotenoid has two colorations either orange or red. Its coloration changes at two concentrations; it appears orange–red due to its blue color absorption at a high concentration and appears yellow at a low concentration. It is a potent, active form of carotenoid and a natural source of antioxidants, mostly consumed by people because of its availability in fruits and vegetables and also used as a food supplement with prophylactic effects against eye-related diseases such as cataracts and has prophylactic effects against health-related diseases[7,8]. ß, Ɛ-Carotene-3, 3' diol is different from other carotenoids in that it has hydroxyl groups at both ends and conjugated double bonds; this difference is what gives it its anti-inflammatory and anti-oxidant properties [9].

MATERIALS AND METHODS

ß, Ɛ-Carotene-3, 3'-diol was obtained from Ambeed Inc, United States of America, a red solid in

appearance with an average weight of 25 g, molecular weight of 568.87 g/mol, and a molecular formula of $C_{40}H_{56}O_2$ with a purity of 80.25%; Indomethacin, a product of Ningbo DHY Pharmaceutical company and Omeprazole were obtained from the Pharmaceutical store of Obafemi Awolowo University, Ile-Ife, Osun state. ELISA kit assay (Tumor necrosis factor-alpha) was obtained from Sigma Aldrich, United States of America. Thirty adult male Wistar rats (*Rattus norvegicus*), with a weight range of 160-165g used for this study were procured from the disease-free stock of Obafemi Awolowo University's Animal Holding College of Health Sciences, Ile-Ife, Nigeria. The rats were housed in plastic cages with wire mesh tops at the Animal House of the Department of Anatomy and Cell Biology, Obafemi Awolowo University at normal temperature and relative humidity. The present study was reviewed and approved by the Health Research Ethics Committee (HREC) and an approval number IPH/OAU/2032 was issued before the commencement of the study. The rats were acclimatized for two weeks before the commencement of the experiment. The experimental rats were randomly grouped into six groups, (A, B, C, D, E, and F) of five rats each. The experimental rats were fasted for 24 hours prior to ulcer induction, 20 mg/kg b.w of indomethacin was used for ulcer induction, groups B to F were administered with 20 mg/kg Indomethacin, Indomethacin was dissolved in distilled water while ß, Ɛ-Carotene-3, 3'-diol, being a lipophilic compound was dissolved in 20% Tween 80. Groups C to F were treated 24 hours after ulcer induction, the treatment was done at 12-hour interval. Administration of all substances and interventions were given via the oral route using an oral cannula.

Group A - Control (1 ml/kg distilled water)

Group B – 20 mg/kg Indomethacin only at a single dose + 1 ml/kg 20% Tween 80

Group C – 20 mg/kg Indomethacin + 20 mg/kg Omeprazole,

Group D – 20 mg/kg Indomethacin + 20 mg/kg ß, Ɛ-Carotene-3, 3'-diol

Group E – 20 mg/kg Indomethacin + 40 mg/kg ß, Ɛ-Carotene-3, 3'-diol

Group F – 20 mg/kg Indomethacin + 80 mg/kg ß, Ɛ-Carotene-3, 3'-diol

At the end of the experiment, the experimental rats were sacrificed in a laboratory outside the animal house to prevent other experimental rats from inhaling chloroform. The stomachs were harvested by making a midline incision at the anterior abdominal wall. Macroscopic examination of the stomach was done at a fixed distance using a high-resolution camera of (16M). A centimeter rule calibrated in 1 mm was photographed and imported into image J for calibration in order to enhance precise and accurate measurement. A software package, Image J was used for histomorphometric analyses of the captured images with the aid of a free hand tool in the software. The harvested stomach was used for both histological and biochemical studies.

Ulcer Index (UI) [10]:

$$UI = \frac{10}{b}$$

$$b = \frac{\text{Total mucosa surface}}{\text{Total ulcerated area}}$$

$$CR\ (\%) = \frac{(o-p)}{o} \times 100$$

o = U.I (positive control group)

p = U.I (treated group)

Data analysis

Data obtained were expressed as mean ± S.E.M. Data was analyzed using SPSS version 20. The presence of significant differences among means of the groups was determined using a one-way analysis of variance (ANOVA) with the LSD and Duncan post hoc test. Values were considered significant at $p < 0.05$.

RESULTS

Body weight change

A non-significant difference in body weight was observed across the groups from day 0 to day 10. However, there was a significant decrease in body weight of the rats administered with indomethacin only and a dose-dependent increase in body weight of the rats treated with ß, Ɛ-carotene-3, 3'-diol at $p < 0.05$.

Table 1: Effects of ß, Ɛ-carotene- 3, 3'-diol and omeprazole on body weight of Wistar rats following indomethacin-induced gastric ulcer

GROUPS	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14
A n =5	162.81 ± 7.21[a]	158.5 ± 6.33[a]	168.8 ± 7.04[a]	176.4 ± 7.14[a]	183.8 ± 7.54[a]	192.1 ± 7.85[a]	203.1 ± 7.47[b]	197.4 ± 7.16[b]
B n=6	160.3 ± 14.2[a]	155.5 ± 12.6[a]	161.3 ±11.37[a]	163.1 ± 9.29[a]	169.6 ± 9.62[a]	173.4 ±10.69[a]	178.4 ± 9.92[a]	171.9 ±9.12[a]
C n= 6	162.8 ± 6.83[a]	164.3 ± 5.86[a]	170.8 ± 6.17[a]	173.8 ± 5.87[a]	181.1 ± 6.34[a]	187.7 ±6.92[a]	193.1 ±8.48[ab]	186.2 ± 8.31[ab]
D n= 7	161.4 ± 8.59[a]	153.4 ± 8.99[a]	164.1 ± 9.09[a]	166.6 ± 7.91[a]	176.1 ± 7.86[a]	188.7 ± 7.29[a]	194.6 ± 4.66[ab]	187.6 ± 4.64[ab]
E n =6	162.2 ± 8.19[a]	159.8 ± 10.18[a]	170.1 ± 8.84[a]	176.4 ± 8.66[a]	185.6 ± 7.76[a]	196.4 ± 7.21[a]	205.3 ± 7.59[b]	198.3 ± 7.41[b]
F n =7	163.1 ± 8.20[a]	156 ± 8.99[a]	167.3 ± 8.87[a]	176.3 ± 7.98[a]	185.2 ± 8.28[a]	196.3 ± 8.74[a]	210 ± 7.41[b]	201.8 ± 7.93[b]

Data are presented as Mean ± SEM in each group as shown in Table 1. Groups: A = Control; B = treated with 20mg/kg b.w Indomethacin; C = treated with 20mg/kg b.w of Omeprazole; D = treated with 20mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, E = treated with 40mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, F = treated with 80mg/kg b.w ß, Ɛ-Carotene- 3, 3 diol

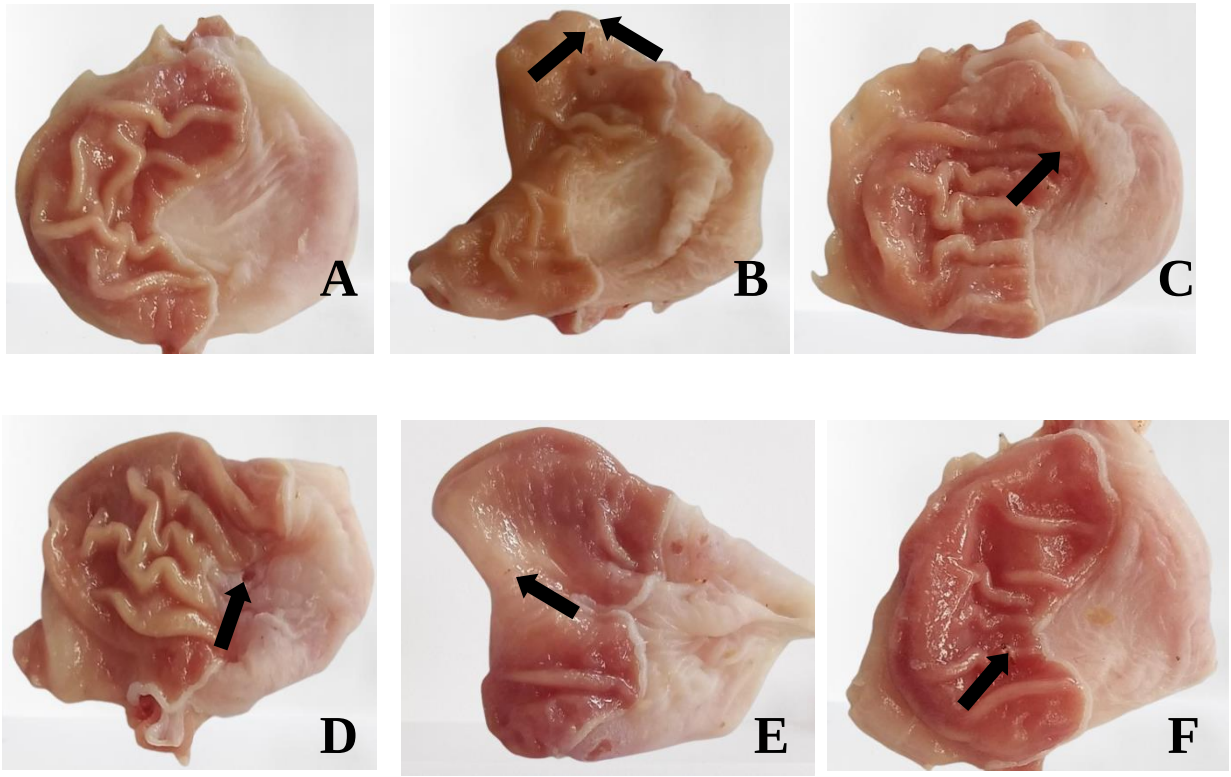

Figure 1: Macroscopic images of the stomach treated with omeprazole and ß, Ɛ-Carotene-3, 3'-diol after indomethacin - induced gastric mucosa ulceration. A = Control; B = treated with 20 mg/kg b.w indomethacin; C = treated with 20 mg/kg b.w of omeprazole; D = treated with 20 mg/kg b.wß, Ɛ-Carotene- 3, 3'-diol, E = treated with 40 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, F = treated with 80 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol. The black arrows connote ulcer

Effects of ß, Ɛ-Carotene- 3, 3'-diol on ulcerated area, ulcer index, and curative ratio

Indomethacin only treated group showed a significant increase in ulcerated area while the treated groups had a significant decrease in ulcerated area, the 40mg/kg ß, Ɛ-Carotene- 3, 3'-diol treated group showed no significant difference when compared to omeprazole treated groups which means at 40 mg/kg ß, Ɛ-Carotene- 3, 3'-diol, the healing of gastric ulcer is likened to the standard anti-ulcer drug. A significant increase in ulcer index in Group B in comparison to other groups shows the extent to which ulcers occurred at the mucosa area relative to the total surface area while the treated groups showed a decrease in ulcer index and this shows the process of healing of the mucosa injury. The % curative ratio shows the ameliorative effects of omeprazole and ß, Ɛ-Carotene- 3, 3'-diol relative to the non-treated group, B.

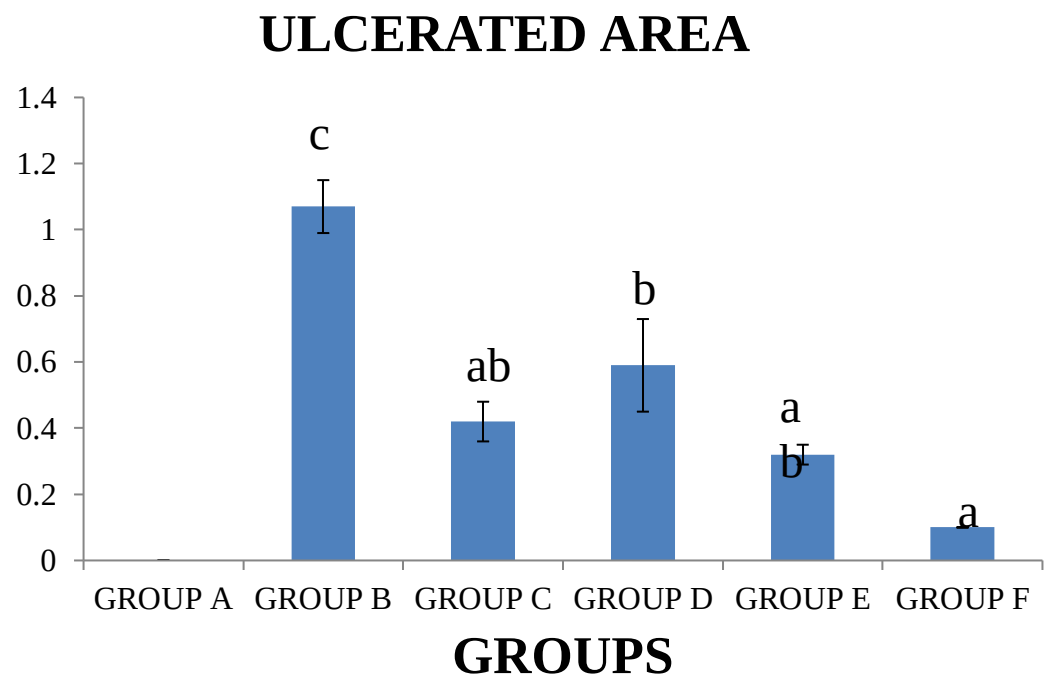

Figure 2: Shows the effects of ß, Ɛ-Carotene- 3, 3'-diol and omeprazole on ulcerated area following indomethacin-induced gastric ulcer. n = 7, mean ± SEM, ANOVA, $p < 0.05$. A = Control; B = treated with 20 mg/kg b.w Indomethacin; C = treated with 20 mg/kg b.w of Omeprazole; D = treated with 20 mg/kg b.wß, Ɛ-Carotene- 3, 3'-diol, E = treated with 40 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, F = treated with 80 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol.

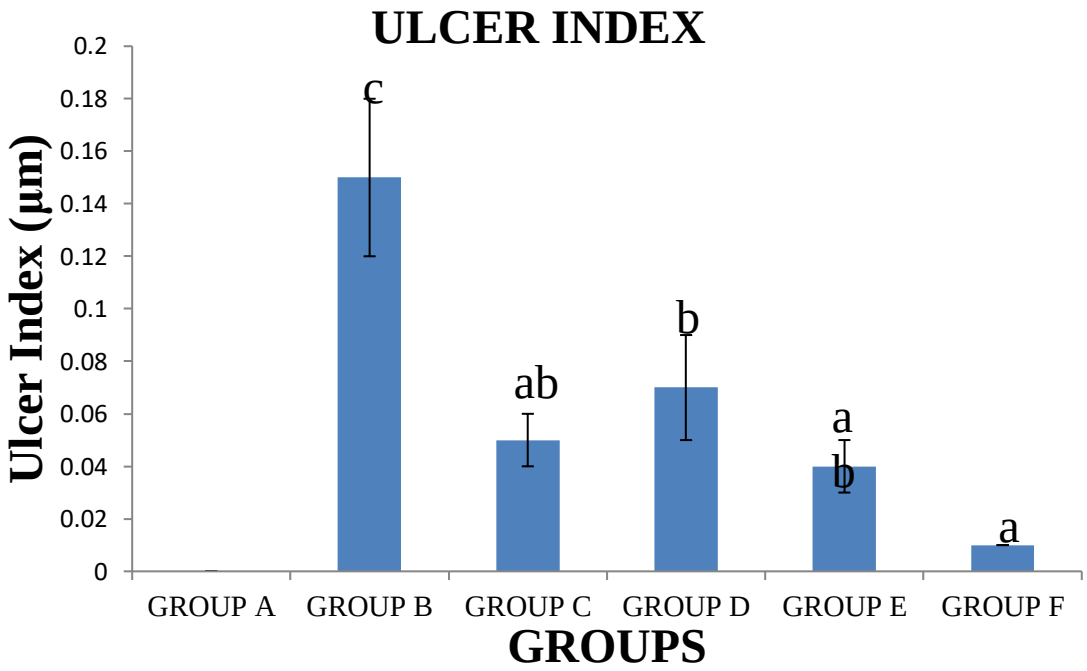

Figure 3: Shows the effects of ß, Ɛ-Carotene- 3, 3'-diol and Omeprazole on ulcer index following indomethacin-induced gastric ulcer. n = 7, mean ± SEM, ANOVA, $p < 0.05$. A = Control; B = treated with 20 mg/kg b.w indomethacin; C = treated with 20 mg/kg b.w of omeprazole; D = treated with 20 mg/kg b.wß, Ɛ-Carotene- 3, 3'-diol, E = treated with 40 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, F = treated with 80 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol.

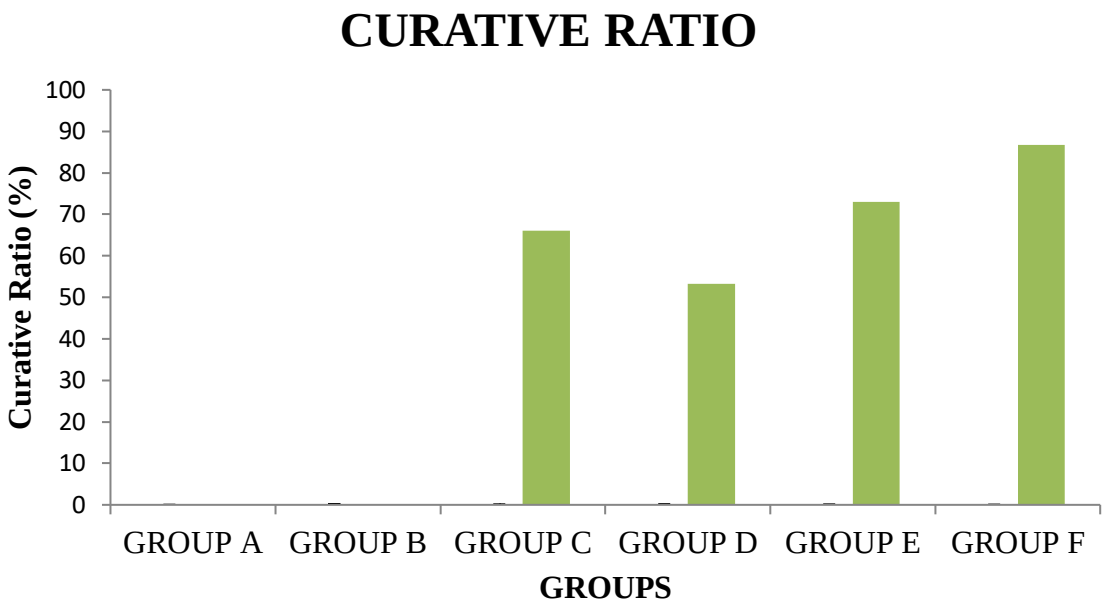

Figure 4: Shows the effects of ß, Ɛ-carotene- 3, 3'-diol and omeprazole on curative ratio following indomethacin-induced gastric ulcer. n = 7 mean ± SEM, ANOVA $p < 0.05$. A = Control; B = treated with 20 mg/kg b.w indomethacin; C = treated with 20 mg/kg b.w of omeprazole; D = treated with 20 mg/kg b.w. ß, Ɛ-Carotene- 3, 3'-diol, E = treated with 40 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol, F = treated with 80 mg/kg b.w ß, Ɛ-Carotene- 3, 3'-diol.

Histological observation

The control group (A) showed a normal histoarchitectural outline of the mucosa layer of the stomach, the mucosa layer of the non-treated group (B) showed a discontinuation of the mucosa layer while the treated groups (C, D, E, and F) showed a regeneration of the mucosa layer (Figure 5).

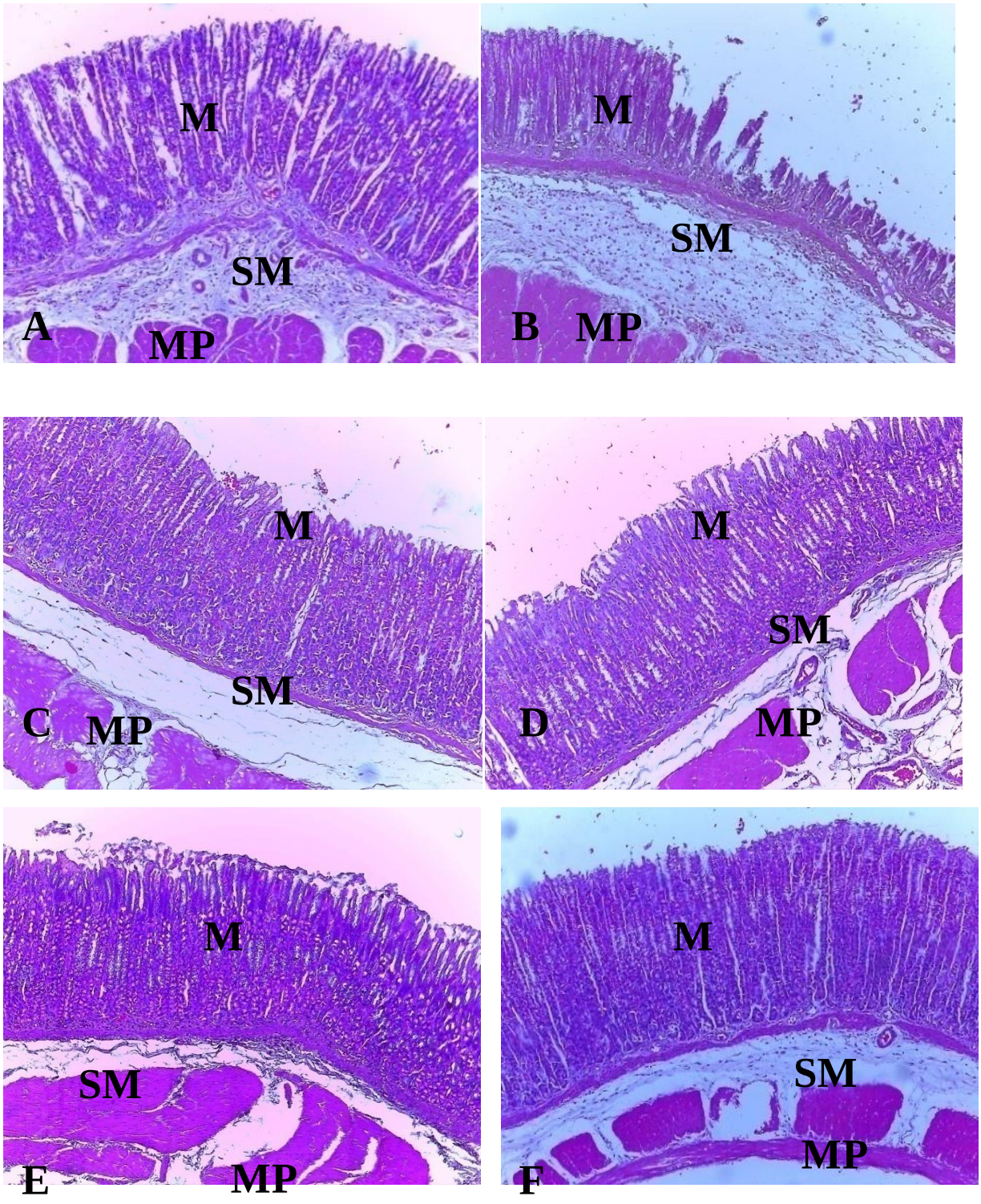

Figure 5: Representative of light micrographs of stomach subjected to indomethacin- induced mucosa ulceration and treated with omeprazole and ß, Ɛ-carotene-3, 3'-diol. H&E x100: MP- Muscularis propria, M- Mucosa, SM- Submucosa.

A (Control), B (Indomethacin only), C (indomethacin + 20 mg/kg omeprazole, D = 20 mg/kg indomethacin + 20 mg/kg ß, Ɛ-Carotene-3, 3'-diol, E = 20 mg/kg indomethacin + 40 mg/kg ß, Ɛ-Carotene-3, 3'-diol, F = 20 mg/kg indomethacin + 80 mg/kg ß, Ɛ-Carotene-3,3'-diol.

Biochemical observation

Tumor necrosis factor-alpha is an inflammatory marker and an insignificant decrease in its concentration was observed across the treated groups, the antioxidant bio-marker, Superoxide dismutase assay revealed a decrease in the non-treated groups and a dose-dependent increase in the treated groups and for catalase activity the treated groups showed the same level of catalase activity. Values are given as Mean ± SEM.

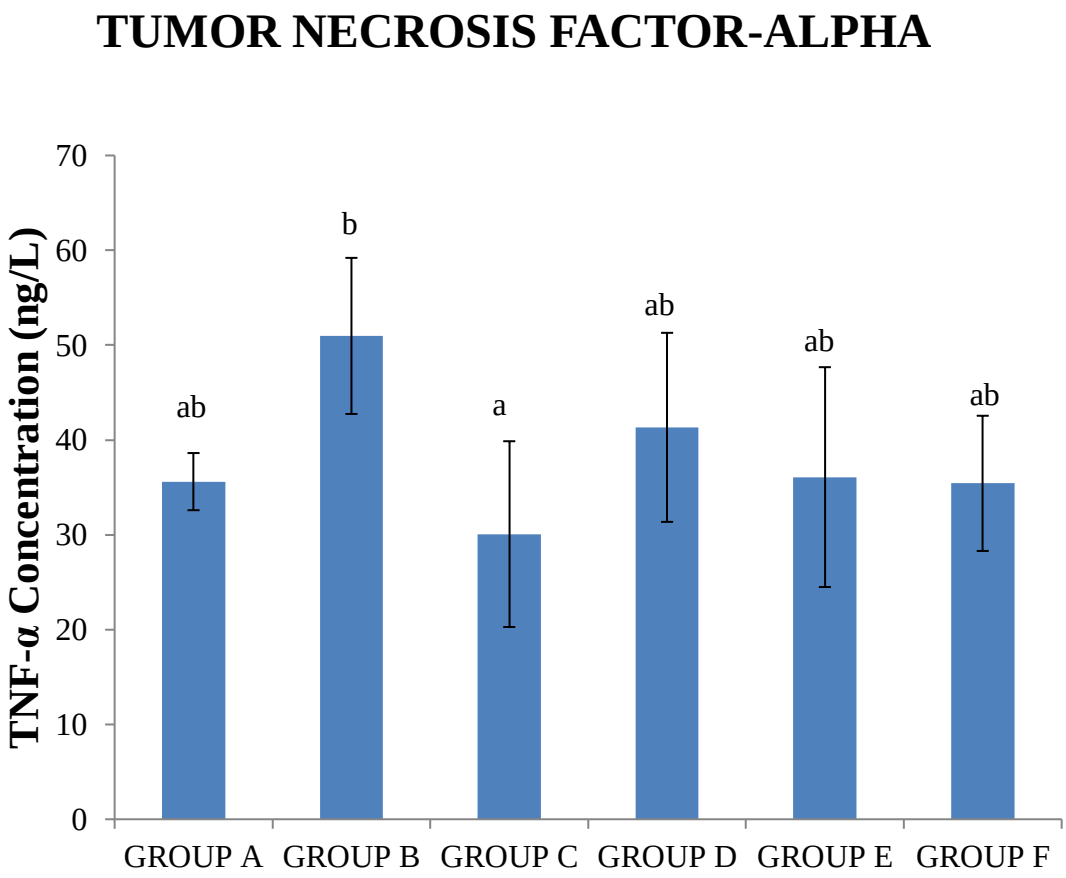

Figure 6: Tumor necrosis factor- alpha concentration (TNF-α) of rats treated with omeprazole and ß,Ɛ-carotene-3,3'-diol following indomethacin - induced Gastric Ulcers. n= 7, mean± SEM, ANOVA, p< 0.05. A: Control, B: indomethacin only, C: (indomethacin + 20 mg/kg omeprazole, D: 20 mg/kg indomethacin + 20mg/kg ß,Ɛ-carotene-3,3'-diol, E:20 mg/kg indomethacin + 40 mg/kg ß,Ɛ-carotene-3,3'-diol, F: 20 mg/kg indomethacin + 80 mg/kg ß,Ɛ-Carotene-3,3'-diol

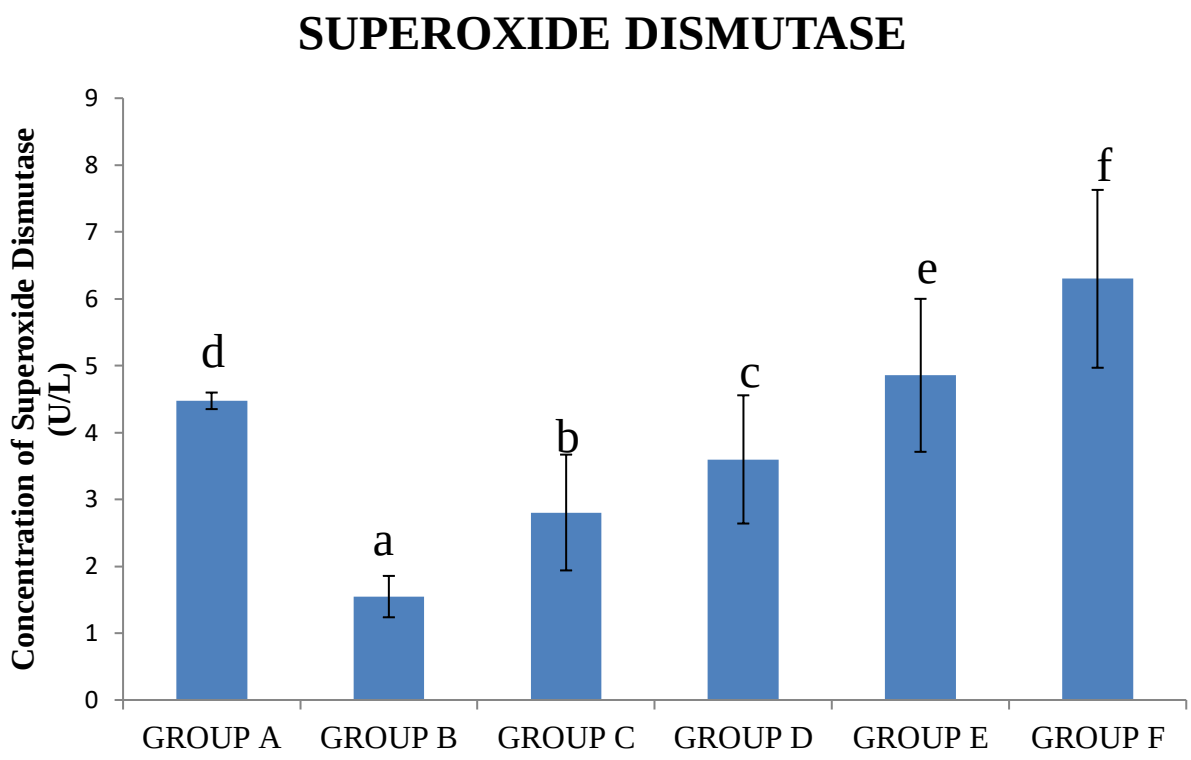

Figure 7: Concentration of superoxide dismutase (SOD) in the gastric mucosa of indomethacin treated rats. n = 7. Mean ± SEM, ANOVA, p value is significant across the groups at <0.05. A: Control, B: indomethacin only, C: indomethacin + 20 mg/kg omeprazole, D: 20mg/kg indomethacin + 20 mg/kg ß,Ɛ-carotene-3,3'-diol, E: 20 mg/kg indomethacin + 40mg/kg ß,Ɛ-Carotene-3,3'-diol, F: 20 mg/kg indomethacin + 80 mg/kg ß,Ɛ-Carotene-3,3'-diol

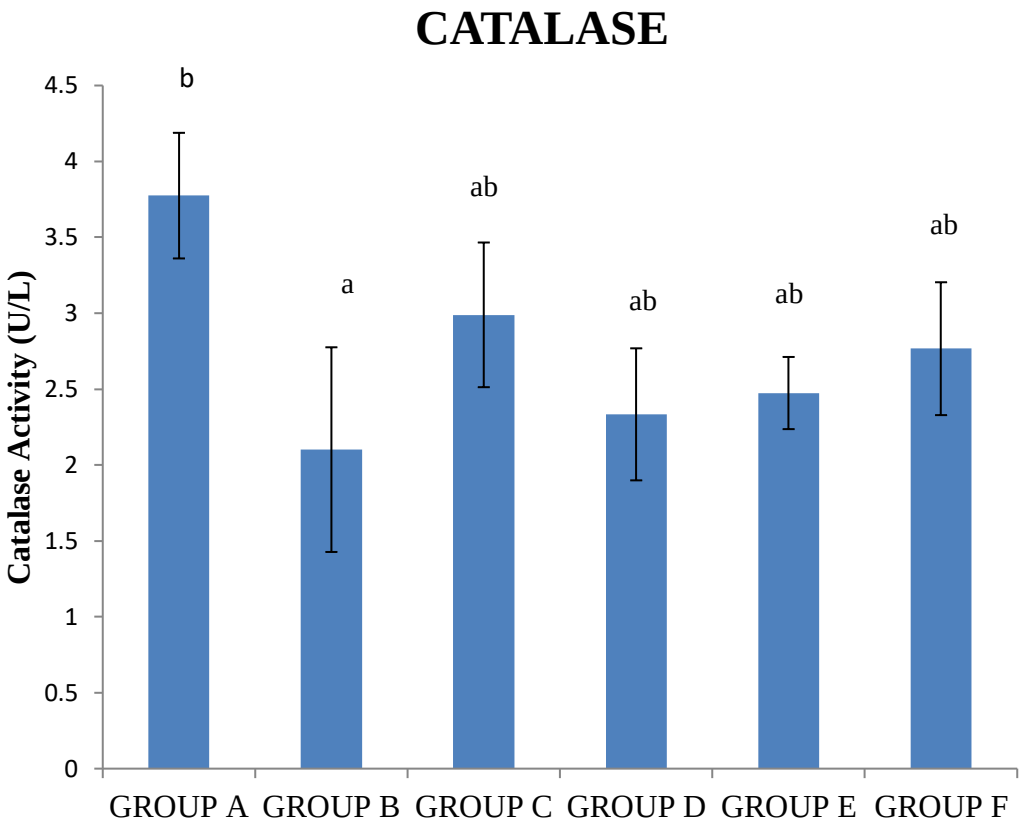

Figure 8: **C**oncentration of catalase in the gastric mucosa of indomethacin treated rats. n = 7. mean ± SEM, ANOVA, p value is significant across the groups at p <0.05. A: Control, B: indomethacin only, C: indomethacin + 20 mg/kg omeprazole, D: 20 mg/kg indomethacin + 20 mg/kg ß,Ɛ-carotene-3,3'-diol, E: 20 mg/kg indomethacin + 40 mg/kg ß,Ɛ-carotene-3,3'-diol, F: 20 mg/kg Indomethacin + 80 mg/kg ß,Ɛ-carotene-3,3'-diol

DISCUSSION

It has been reported in previous studies that gastric ulcers cause a decrease in body weight, in this study, a decrease in body weight was observed which corroborates previous findings that reduction in body weight may be due to reduction in food intake as a result of mucosa cells injury[11], however a significant difference in body weight was observed at day 12 and day 14, the reason for this is not well understood and calls for further investigation. Rats treated with ß, Ɛ-Carotene, 3, 3'diol showed a dose dependent increase in body weight and this might be as a result of a boost in the digestive functions and the recovery process within the gastric mucosa [12]. Inflammation has been reported to be implicated in gastric ulcer; several ulcerations that were observed in the indomethacin treated group might be due to a significant increase in TNF-α concentration which initiates acute inflammatory response accompanied by neutrophil infiltration into the gastric mucosa[13], while the anti-inflammatory properties of ß, Ɛ-Carotene, 3, 3'diol might be responsible for a reduction in ulcerations in the treated groups [14].

Previous findings showed that Indomethacin caused a decrease in antioxidant enzymes, a decrease in superoxide dismutase activity and catalase activity in the group treated with Indomethacin only was reported in this study and this corroborates previous studies[12], an increase in superoxide dismutase and catalase activity in ß, Ɛ-Carotene, 3, 3'diol treated groups might be due to an increase in endogenous anti-oxidant level as a result of this intervention [15]. An infiltration of Neutrophils or increase in acid secretion might be responsible for the discontinuation in epithelial lining of the gastric mucosa [16, 17]. The anti-inflammatory properties of ß, Ɛ-Carotene, 3, 3'diol might have helped to reduce the acid concentration and infiltration of inflammatory cells at the injury site and reduce lipid peroxidation which is crucial in the development of stomach ulcer [18, 19]. The deposition of collagen fibers at the site of injury can help to hasten wound healing, the dose dependent increase in curative ratio in groups treated with ß, Ɛ-Carotene, 3, 3'diol may be due to an increase in collagen fibers deposit, this is because wound healing involves collagen synthesis and tissue compaction which corroborates previous findings [20, 21].

Conclusion

The findings of this study have provided information on the use of ß, Ɛ-Carotene, 3, 3'diol in the management of gastric ulcer. At the highest dose of ß, Ɛ-Carotene, 3, 3'diol, a better remediation and ethno-medicinal value was observed and therefore can be used as an alternative therapy for gastric ulcer treatment.

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REFERENCES

1. Sung JJ, Tsoi KK, Ma TK, Yung MY, Lau JY, Chiu PW. Causes of mortality in patients with peptic ulcer bleeding: a prospective cohort study of 10,428 cases. American Journal of Gastroenterology 2010;105(1):84-9.
2. Kern TS, Berkowitz BA, Feldman EL. National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) meeting summary: Advances toward measuring diabetic retinopathy and neuropathy: from the bench to the clinic and back again. Journal of Diabetes and its Complications. 2009;23(3):219-23.
3. Dixon MF. IV. Helicobacter pylori and peptic ulceration: Histopathological aspects. Journal of Gastroenterology and Hepatology 1991;6(2):125-30.
4. Nieto Y. Therapeutic protocol of peptic ulcer. Med. 2012;11:179-82.
5. Kamada T, Satoh K, Itoh T, Ito M, Iwamoto J, Okimoto T, et al. Evidence-based clinical practice guidelines for peptic ulcer disease 2020. Journal of Gastroenterology. 2021; 56:303-22.
6. Yang JC, Lu CW, Lin CJ. Treatment of Helicobacter pylori infection: current status and future concepts. World Journal of Gastroenterology 2014;20(18):52-83.
7. Thurnham DI, Nolan JM, Howard AN, Beatty S. Macular response to supplementation with differing xanthophyll formulations in subjects with and without age-related macular degeneration. Graefe's Archive for Clinical and Experimental Ophthalmology. 2015;253:1231-1243.
8. Liu XH, Yu RB, Liu R, Hao ZX, Han CC., Zhu ZH, et al. Association between lutein and zeaxanthin status and the risk of cataract: a meta-analysis. Nutrients 2014;6(1): 452–465.
9. Zhang H, Yang W, Li Y, Hu L, Dai Y, Chen J, et al. Astaxanthin ameliorates cerulein-induced acute pancreatitis in mice. International Immunopharmacology 2018; 56:18-28.
10. Ganguly AK. A method for quantitative assessment of experimentally produced ulcers in the stomach of albino rats. Experientia 1969;25(11):1224-1224.
11. Katherine AS, Niamh MM, Steve RB. Hypothalamic regulation of appetite. Expert Review of Endocrinology and Metabolism. 2008;3(5):577-592.
12. Odabasoglu F, Cakir A, Suleyman H, Aslan A, Bayir Y, Halici M, et al. Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. Journal of Ethnopharmacology 2006;103(1):59-65.
13. Hussein SA, Karousa MM, Amin A, Awadalla MA. Evaluation of gastroprotective effect and anti-inflammatory role of resveratrol against gastric mucosal alterations in experimental model of gastritis in rats. Digestive System Diseases. 2016:6(3):1559-1570.
14. Erdman Jr JW, Smith JW, Kuchan MJ, Mohn ES, Johnson EJ, Rubakhin SS, et al. Lutein and brain function. Foods. 2015;4(4):547-564.
15. Johnson EJ. Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. Nutrition Reviews. 2014;72(9):605-612.
16. Suleyman H, Halici Z, Cadirci E, Hacimuftuoglu A, Bilen H. Indirect role of beta2-adrenergic receptors in the mechanism of anti-inflammatory action of NSAIDS. J Physiol Pharmacol. 2008;59(4):661-672.
17. Kim M, Yang J, Kwon YS, Kim MJ. Antioxidant and anticancer effects of fermented Rhus verniciflua stem bark extracts in HCT-116 cells. Science Asia. 2015;41(322.10):2306.
18. Buscemi S, Corleo D, Di Pace F, Petroni ML, Satriano A, Marchesini G. The effect of lutein on eye and extra-eye health. Nutrients. 2018;10(9):13-21.
19. He RR, Tsoi B, Lan F, Yao N, Yao XS, Kurihara H. Antioxidant properties of lutein contribute to the protection against lipopolysaccharide-induced uveitis in mice. Chinese Medicine. 2011;6(1):1-8.
20. Alese MO, Adewole OS, Ijomone OM, Ofusori DA, Alese OO. Mucus secretion and collagen fibres integrity are compromised in aspirin induced gastric lesion; protective role of Musa paradisiaca. Italian Journal of Anatomy and Embryology. 2018;123(2):136-148.
21. Ehrlich HP, Hunt TK. Collagen organization critical role in wound contraction. Advances in Wound Care. 2012;1(1):3-9.