The Protective Effect of Banana and Yoghurt on Gastric Ulceration in Rats

التأثير الوقائي للموز والزبادي على تقرح المعدة في الفئران

د.عبور محمد محمد عبد الرحمن، د.الشيماء عبد الله حافظ

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الملخص العربي

أجريت هذه الدراسة لتقدير القيمة الغذائية للموز الأخضر والزبادي وتأثيرهما الفعال في الحد من الإصابة بقرحة المعدة المحدثه بالإندوميتاسين ومضاعفاتها. وأوضحت النتائج أن الموز الأخضر يحتوي على كميات غنية من المغذيات ومضادات الأكسدة الطبيعية ونشاطها ، وكذلك احتوت الزبادي على كمية كبيرة من قيمة غذائية مرتفعة. أجريت التجربة البيولوجية على إحداث قرحة المعدة في فئران التجارب باستخدام جرعة فموية واحدة من الإندوميتاسين 50 ملجم / كجم من وزن الجسم بعد إطعام مجموعات مختلفة من الفئران لمدة أربعة عشر يومًا على النظام الغذائي الأساسي كمجوعة ضابطة سالب . تم تناول مجموعات مختلفة من الإندوميتاسين تم تغذيتها على النظام الغذائي الأساسي كمجموعة ضابطة موجب بينما تم تغذية المجموعات الثالثة على النظام الغذائي الأساسي وتم استبداله بشكل منفصل بـ 25٪ زبادي و 25٪ موز أخضر ووزن متساوى 12.0٪ لكل منها من الزبادي والموز الأخضر.

أشارت النتائج إلى أن تناول الزبادي والموز الأخضر ومزيجهما يؤدي إلى ضعف معنوي في قرحة المعدة كما يتضح من انخفاض معنوي في مؤشر قرحة المعدة وحجم عصير المعدة مع زيادة معنوية في النسبة الوقائية ونشاط البيبسين. أدت المعالجة المسبقة للزبادي والموز الأخضر ومزيجهما إلى تحسين مستوى مصل TNF-α و NO بشكل ملحوظ. أظهرت النتائج أن زيادة مستوى MDA المعدي في السيطرة الإيجابية بمقدار 402.17 ميكروجرام / جم / بروتين مقارنة بالمجموعة الضابطة كانت 133.81 ميكروجرام / جم / بروتين.

مجموعات الفئران التي تم تغذيتها على الزبادي والموز الأخضر والخليط منهما والتي تناولت الإندوميتاسين عن طريق الفم انخفض مستوى MDA تدريجيًا بمقدار

305.57 و 283.45 و 168.256 ميكروغرام / جم / بروتين ، على التوالي. اما عن مستويات مضادات الأكسدة الأنزيمية وغير الأنزيمية في المعدة مثل SOD ، و GSH مع ما يصاحب ذلك من انخفاض في مستوى MDA مقارنة مع تلك الموجودة في المجموعة الضابطة الإيجابية. أظهر تركيز سوبروكسيد ديسموتاز SOD في المجموعات المتلقية للزبادي والموز وخلطاتهما زيادة معنوية تدريجيًا بمقدار 15.810 و 18.803 و 21.793 وحدة / ملجم / بروتين عن المجموعة الضابطة الموجبة والتي سجلت (12.606 ميكروجرام / مجم / بروتين) اما مجموعة الكنترول السالب فسجلت 26.77 ش / مجم / بروتين. تمت زيادة SOD الفائق أكسيد ديسموتاز بشكل كبير عند الجرعات. تم دعم هذه النتائج بشكل كبير من خلال النتائج المرضية النسيجية التي كشفت عن تأثير علاج اللبن والموز الأخضر ومزيجهما على الصدمة النزفية التي يسببها الإندوميتاسين.

من النتائج السابقة يمكن التوصية بأن الخلطات من الزبادي والموز الأخضر أظهرت تأثير عالي في قدرة حمايه المعدة من حدوث القرحة المعدية التي يسببها الإندوميتاسين ويمكن زياده تأثيرها من خلال تثبيط الإجهاد التأكسدي والتهاب المعدة المحدثة.

الكلمات المفتاحية : الموز ، الزبادي ، المعدة ، إنزيم الكبد ، الالتهابات

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ABSTRACT

This study was carried out to evaluate the nutrition value for green banana and yoghurt and there can be effective in reducing the indomethacin-incidence of gastric ulcer and its complications.The biological experimental on gastric ulcers were induced in rats using one oral dose of indomethacin 50mg/kg body weight (5 ml/kg) after feeding different rats groups for fourteen days on basal diet as healthy control negative. Different groups were taken indomethacin was fed on basal diet as control positive and the third groups fed on basal diet and it's substituted separately with 25% yoghurt, 25% green banana and equal weight 12.0% for each from yoghurt and green banana.The results showed that increasing the gastric MDA level in the control positive by 402.17µM/g/ protein. The normal group was 133.81 µM/g/ protein .The different rat groups fed on yoghurt, green banana and its blends and taken orally Indomethacin the MDA was decreased gradually by 305.57, 283.45 and168.256 µM/g/ protein, , yoghurt, green banana and their blend pre-treatment significantly increased the gastric levels of enzymatic and non-enzymatic antioxidants namely SOD, and GSH with a concomitant reduction in MDA level compared with those in the positive control group. The superoxide dismutase SOD in the groups receiving yoghurt, banana and their blends showed a significant increase gradually by 15.810, 18.803 and 21.793u/mg/ protein. The positive group control was found to be significantly lower (12.606 u/mg/ protein) than the negative control group was26.77 u/mg/ protein. The superoxide dismutase SOD was significantly increased.

Key words: Banana, yoghurt, gastric, liver enzyme, inflammatory

INTRODUCTION

The gastrointestinal (GI) mucosa is considered the first defensive barrier against xenobiotics, but it is injured by toxic substances. Serious ulceration of the GI mucosa may result in GI bleeding or even perforation (**Zhang *et al.*, 2012**). Gastric ulceration is the most predominant GI syndrome ever recognized and is responsible for approximately 15 deaths out of every 15,000 complications per year worldwide (**Azhari *et al.*, 2018**).

The current use of ulcer drugs is limited due to its side effects and potentiality of relapse. Therefore, studies showed ascertain the antiulcer potentials of banana and the extracts of banana (*Musa paradisiaca*). The results revealed that banana's extract contains phytochemical like phenols, flavonoids and etc. The banana's extracts prevented the induced ulcer and significant rise ($p < 0.05$) in gastric juice pH (3.79 ± 0.24) noticed in the banana's treated group. However, the decrease in gastric juice volume and increased gastric wall mucus by banana was not statistically significant ($p > 0.05$). Findings from this study show that banana was able to prevent IND+PYL induced ulcer by strengthening the gastric mucosa and decreasing the gastric juice acidity (**Mahadeva Rao *et al.*, 2016).**

Gastric ulcers occur when there is an imbalance between the aggressive and defensive factors on the luminal surface of the epithelial cells. A large number of medicinal plants and their secondary metabolites have been reported, with potential activity against gastric ulcer and ulcerative colitis. The green fruit of the *Musa spp* ABB, variety Burro CEMSA, is effective as a gastroprotective agent in a model of induced acute ulcers by absolute alcohol and indomethacin (**Boffill Cárdenas *et al.*, 2016).**

Banana (*Musa sapientum* var. *paradisiaca*) was reported to possess ulcer protective and healing activity through its predominant action on mucosal defensive factors (**Goel *et al.*, 1986).** Methanolic extract of banana has shown antioxidant activity in gastric mucosal homogenates, where it reverses the increase in lipid peroxidation (LPO) and SOD induced by stress in normal rats (**Goel *et al.*, 2001).** Natural flavonoids, leucocyanidin isolated from banana have been reported to have anti-ulcer

activity (**Lewis** et al**., 1999).** Further, derivatives of leucocyanidin isolated from focus bengalensis have been reported to possess anti-diabetic and antioxidant activity (**Daniel *et al.*, 1998).**

Worldwide, yoghurt is considered one of the most popular fermented dairy products due to not only for its nutritional value but also for its health benefits (**Weerathilake *et al.*, 2014).** Buffalo milk is much preferred by consumers for its rich nutrition and is drunk or transformed into valuable products such as cheese, curd, yogurt, and ice cream. Buffalo milk contains about twice as much butterfat as cow milk and higher amounts of total solids and casein, making it highly suitable for processing various types of yogurt and resulting in creamy textures and rich flavor profiles. Although its many healthy and nutritious impacts are well-established, milk and its products are generally not regarded as a rich source for particular bioactive ingredients such as polyphenols and antioxidants (**Achi and Asamudo, 2019).** Thus, the formulation of novel dairy products using medicinal herbs, or their extracts has gotten more attention to meet the demand of health-conscious consumers (**Jamshidi-Kia *et al.*, 2018).** In this context, several new fermented dairy products enhanced with plant-derived foods (fruit, vegetables, or even their by-products) have been created and assessed (**Iriondo-DeHond *et al.*, 2018).**

The objective of this work was to evaluate in a model of chronic ulcer the antiulcer effect and the possible mechanisms by which the banana, yoghurt and both together act as an antiulcer.

MATERIALS AND METHODS

Materials

The green banana purchased from local market and it was sliced vacuum dried at 50^{o}C for 72 hours, ground and sieved. The sample was stored in polythene container for analysis.

Indomethacin was purchased from (Sigma-Aldrich. Com) dissolved in saline and given orally to rats at a dosage of 50mg/kg body weight according to **Hajrezaie *et al.*, (2012).**

Folin–Ciocalteau reagents, Gallic acid, Quercetin, DPPH· (2, 2-diphenyl-1- picrylhydrazyl), BHT: Butyl Hydroxy toluene, and, *p*-Nitrophenyl sulfite, were purchased from Sigma Chemical Co.(St. Louis, MO, USA).

Methods

Determination of nutritional content

The green banana was analyzed for proximate composition (moisture, ash, organic matter, crude protein, lipids, carbohydrate and crude fiber) according to **AOAC (2010).**Total carbohydrates were determined by difference according to **Pearson (1976).** In addition, minerals content as calcium iron, manganese, green banana was determined according to the method of the **AOAC (2010),** using Atomic Absorption Spectrophotometer (Perkin Elmer, Model 3300, Germany).

Preparation of banana extract

Dried powder of green banana (10 grams) was dispensed in 100ml of distilled water, ethanol, ethyl acetate and chloroform, overnight at room temperature using shaker. The mixture was filtered through whatman No 1 filter paper and the extraction step was repeated twice. The filtrate was then concentrated to dryness at 40 °C in a rotary evaporator. The crude extracts were stored in a refrigerator until further analysis.

Total phenolic content

The total phenolic (TP) banana extracts were spectrophotometrically determined by Folin Ciocalteu reagent assay using gallic acid as standard according to **Qawasmeh *et al.* (2012).** The absorbance was determined at 750 nm using spectrophotometer (Unicum UV 300). The total phenolic content in the samples was expressed as mg gallic acid equivalents (GAE)/g dry weight sample. All samples were analyzed in triplicates.

Total flavonoids content

Total flavonoids (TF) of green banana extracts were spectrophotometrically determined by the aluminum chloride method using quercetin as a standard according to **Eghdami and Sadeghi (2010).** The absorbance was measured against blank at 510 nm by using spectrophotometer (Unicum UV 300). Total flavonoids in sample were expressed as mg quercetin equivalents (QE)/ g dry weight. All samples were analyzed in triplicates.

Antioxidant activity

DPPH· Free radical scavenging assay

Determination of DPPH · free radical scavenging activity was measure din green banana according to **Ravichandran *et al*,. (2012**). The mixture was shaken vigorously and allowed to stand at room temperature. Butyl Hydroxy toluene (BHT, Sigma) was used as positive control while the negative control is contained the entire reaction reagent except the extracts. Then the absorbance was measured at 515 nm against blank.

The capacity to scavenge the DPPH · radical was calculated using the following equation:

DPPH · scavenging effect (Inhibition %) = [(Ac – As / Ac) × 100]

Where: Ac is the absorbance of the control reaction.

As is the absorbance in the presence of the plant extracts

Determination of nutritional value of yoghurt

Total solids content, total nitrogen and ash content were determined in yoghurt according to **AOAC (2010).** Fat content was determined by Gerber methods and the solids not fat were obtained by subtracting fat from total solids according to **AOAC (2012).** Moreover, Protein content was estimated according to **Bradley *et al*,. (1992).** Titratable acidity in terms of % lactic acid was measured. PH of the sample was measured at 17 to 20°C using a pH meter (Corning pH/ion analyzer 350, Corning, NY). Water soluble nitrogen (WSN) of yoghurt was estimated according to **Ling (1963).**

Biological experimental

For the determination of the gastro protective mechanisms, the study was carried out in conventional (30 rats) male rats with a weight comprised between 200 and 250 g, were purchased from National Organization for Drug and Control Research, Giza, Egypt. The animals were kept under ambient conditions of 22 ± 2 ^{o}C of temperature, 40-70% of humidity and light-dark cycles of 12 x 12 hours. They were fed commercial barley and the drinking watered.

Five experimental groups of 6 rats each were used for groups, the group 1 as control negative and group 2 as control positive was fed on a basal diet. The group 3 that was given the green banana at 25% substituted from the basal diet, the group 4

was given 25% yoghurt substituted from basal diet and the group 5 was given 12.5 green banana and 12.5 yoghurts for fourteen days. At the end of the administration period (fourteen days), the group 2 as positive and different groups 3, 4 and 5 taken Indomethacin orally to rats at a dosage of 50mg/kg body weight (5 ml/kg body weight) to be inducing the ulcer were considered.

The animals were sacrificed seven hours after the induction of the ulcers; the stomachs, which were opened by the greater care, were extracted, washed with saline solution, spread on a sheet of filter paper, and the damaged area was quickly measured in mm2. The gastric mucosa was taken from all animals for the determination of the enzymatic activities, determination of lipid peroxidation and antioxidant enzyme

The lipid peroxidation was determined calorimetrically as malondialdehyde (MDA) by **Yoshioka *et al.*, (1979).** Moreover, the activity of the antioxidant enzymes, plasma superoxide dismutase (SOD) was measured according to the method of **Sairam *et al.*, (2003)**, Non-enzyme Glutathione (GSH) measured by **Habig *et al.*, (1974).** Free radicals as nitric oxide (NO) were estimated according to **Miranda *et al.*, (2001).**

Determination of TNF-α. and Pepsin in stomach tissues

Stomach tissues homogenates were used to prepare nuclear extracts to determine TNF-α, using EpiQuik™ nuclear extraction kit (OP-0002, EpiGentek, NY, USA) according to **Millena *et al*,. (2004).***p*-Nitrophenyl sulfite was found to be a suitable synthetic substrate for rapid spectrophotometric analysis of pepsin in gastric secretion. When this substrate was incubated with enzyme, the maximal rate of change of absorbance at 320 nm was found to be directly proportional to enzyme concentration (**Robinson and White, (1970).**

Histopathological Investigation.

Gastric microscopic damage was scored on a 0–14 range according to the criteria previously described (**Hamdan *et al.*,2020**). Briefly, the stomach tissue was examined for epithelial cell loss (score: 0–3), edema in the upper mucosa (score: 0–4), hemorrhagic damage (score: 0–4), and the presence of

inflammatory cells (score: 0–3). Summation of the four histopathological scores gave the total microscopic score.

Statistical Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by an LSD test for multiple comparisons using Statistical Product and Service Solutions (SPSS) software (version 17). All data were presented as mean ± SE. $P < 0.05$ was allowed to forecast statistically significant.

RESULTS AND DISCUSSION

Nutrition content of green banana

Chemical composition and minerals content were determined in green banana, and the results are reported in table (1). The results indicated that the moisture content was 80.28 %; this means the high moisture content in the foodstuff is responsible for microbial spoilage, deterioration, and short shelf life **(Anhwange *et al.*, 2009).** Protein is an essential component of the diet for the survival of a human being to supply adequate amounts of essential amino acids. The crude protein total lipid ash content and crude fibers were 5.90, 1.80, 8.50 and 21.70%, respectively. The green banana mixed pulp and peel flour presented higher ash, total fiber, and total phenolic compound contents than traditional wheat flour **(Castelo-Branco *et al*., 2017).**

The mineral content of the macroelement, calcium, the major element found in banana was 119.2 mg/100g. It was followed by potassium, sodium, manganese, and magnesium. The mineral content of the microelement was 75.1, 62.1, 43.58 and 31.29 mg/100g, respectively in Table (1) and the microelement as iron was the lowest (1.52mg/100g). The green banana is rich in dietary fibre, essential minerals such as potassium **(Alkarkhi *et al.*, 2010 and Choo and Aziz., 2010).**

Table (1): Proximate analysis of green banana

Chemical analysis	g/100g dry weight	Minerals content	mg/100g dry weight
Moisture	80.28±2.07	Potassium	75.1±2.13
Total protein	5.90±0.14	Calcium	119.2±03.28
Ash content	8.50±0.21	Sodium	62.10±2.37
Total lipids	1.80±0.01	Iron	1.52±0.01
Crude fiber	21.70±0.95	Manganese	43.58±1.08
Total carbohydrates	62.10±2.17	Magnesium	31.29±0.94

Values are mean and SD (n = 3)

Total phenolic and total flavonoids of banana extracts

Table (2) showed that the determination of total phenolic and flavonoids in different extracts (aqueous, ethanol, ethyl acetate and chloroform). From the results, it could be noticed that the total phenolic and flavonoids in green banana at ethanol extract were the highest by 33.31 mg/100 GAE and 25.24 mg/100QE. This results confirmed by **Lapornik *et al.*, (2005)** observed that the ethanolic extract contains the most phenolic and flavonoid contents because it can release the cell wall-bound polyphenol from the cells and neutralize the activity of polyphenol oxidase (PPO) which degrades the polyphenol in plants. This study confirmed by **Turkmen *et al.*, (2006)** reported that the solvent with high polarity has a high content of polyphenol and antioxidant activity.

Moreover, the ethyl acetate extract from green banana contained total phenolic and flavonoids. The total phenolic and flavonoids were 22.98 mg/100 GAE and 18.45 mg/100 QE followed by chloroform extract was 21.52 mg/100 GAE and 17.33 mg/100 QE, respectively. Meanwhile, the aqueous extract from green banana was the lowest than other extracts.

Phenolic compounds are secondary metabolites produced in plants through the phenylpropanoid pathway and encompass a wide range of chemical classes, including phenolic acids, flavonoids, stilbenes and lignans **(Manach *et al.*, 2004).** They are basically involved in plant defense mechanisms and are also known to exert numerous health-promoting effects. They act as antioxidants and modulators of enzyme expression and thereby contribute to the

alleviation of a wide range of chronic diseases such as cancer, diabetes, skin damages, allergies, atherosclerosis, and viral infections **(Huang and Shen, 2012).** Furthermore, phenolic compounds are exploited in food protection against alterations by microorganisms or by lipid oxidations **(Maqsood *et al.*, 2013).**

Table (2): Total phenolic acids and flavonoids compounds in green banana

Banana Extracts	Total phenolic acids mg/100 GAE	Total flavonoids compounds mg/100 QE
Aqueous	9.89±0.14^{c}	8.75±0.24^{c}
Ethanol	33.31±1.20^{a}	25.24±1.35^{a}
Ethyl acetate	22.98±1.04^{b}	18.45±1.08^{b}
Chloroform	21.52±0.95^{b}	17.33±1.12^{b}

Values are mean and SD (n = 3); where: Mean values in the same with the letter are significantly different at $p<0.05$ levels.

DPPH scavenging activity

From the results in table (3), the ethanol, ethyl acetate and chloroform extracts, however, showed greater DPPH scavenging activity than the aqueous extracts. However, at a concentration of 50 µg/ml, the results were obtained for a green banana with ethanol, ethyl acetate and chloroform extracts by 43.2, 41.36 and 40.51%, respectively, followed by aqueous extract (36.87ug/ml). Moreover, the IC50 values were 45.19, 52.48, 60.17 and 90.28 µg/ml, respectively, compared to BHT which was 20.59µg/ml. It is better to mention that the lower IC50 value represents more potent free radical inhibitory activity. Thus, the present results indicated that ethanol, ethyl acetate and chloroform extracts have powerful antioxidant activity as compared to aqueous extract. The strong antioxidative properties of green banana extracts could be attributed to the presence of different antioxidant components **(Mrvcic *et al.*, 2012).** Furthermore, **Sultana *et al.*, (2007)** reported that the higher activity of DPPH · radical scavenging activity may be attributed

to the presence of higher levels of total phenolic and flavonoids as they play a key role as proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants

Table (3): DPPH· scavenging activity of banana extracts at different concentrations

Banana Extracts	Scavenging activity %				
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml
Aqueous	25.13 ±0.14 ^{c}	27.64 ±0.35 ^{c}	30.40 ±0.34^{d}	33.44 ±1.26 ^{c}	36.78 ±0.81 ^{c}
Ethanol	28.36 ±0.21^{b}	31.51 ±0.28 ^{b}	35.01 ±0.29 ^{b}	38.89 ±1.39 ^{b}	43.21 ±0.75 ^{b}
Ethyl acetate	27.10 ±0.23 ^{b}	30.11 ±0.34 ^{b}	33.45 ±0.31 ^{c}	37.16 ±0.67 ^{b}	41.36 ±0.43^{b}
Chloroform	26.59 ±0.27^{c}	29.54 ±0.26^{b}	32.82 ±0.28 ^{c}	36.46 ±0.91^{b}	40.51 ±0.90^{b}
BHT as standard	39.60 ±0.29^{a}	43.99 ±0.41 ^{a}	48.87 ±0.52 ^{a}	54.29 ±0.59 ^{a}	60.32 ±1.28 ^{a}

Values are mean and SD (n = 3); where: Mean values in the same with the letter ±are significantly different at $p<0.05$ levels

Nutritional value of yoghurt

Nutrition content of yoghurt was determined, and the results are tabulated in table (4). From the results, it could be observed that the total solids and solids not fat were 21.11 and 11.79%, respectively, to give pH by 4.9. These results confirmed with **Ozer *et al.*, (1998)** showed that total solids content had no adverse effect on the starter activity of coagulation time. Increasing milk total solids from 16 to 23% had a significant effect on decreasing the rate of pH during fermentation. Also, the incubation time for the milk at 4.6 pH was shorter than the time of retentate **(Tamime *et al.*, 1989).** The increase in milk fat content influences the growth and activity of starter cultures in samples with 2 levels of total solid (12 and 23%) **(Mahdian *et al.*, 2007).** The chemical composition of the milk base, especially the total solids has a

major effect on the acceptability of concentrated yoghurt. Concentrated yoghurt containing < 20% total solid was assessed as "thin and tasteless" and that with > 25% total solid it became gummy and bitter **(Robinson, 1977).**

Fat content and titratable acidity were found by 3.4 and 1/20% in yoghurt and these results confirmed with **Penna *et al*., (2007**) and **Najafi *et al*., (2008)** they found that the yoghurt should contain at least 3.25% of milk fat and 8.25% of milk solids not fat (MSNF) with a titratable acidity of not less than 0.9 %. Yoghurt has a smooth texture and a mildly sour and pleasant flavor.

Total protein:

Total protein, total nitrogen and water-soluble nitrogen were lower in yoghurt to give 3.97, 0.60 and 0.12%, respectively; this resulted in decreasing acidity content of the yoghurt to give pH 4.9. **Gawade (2017**) reported that acidity ranged from 0.61 to 0.66 per cent in yoghurt. Moreover**, Sengupta *et al*., (2014)** observed similar results with respect to the protein content of yoghurt and recorded 3.04% protein content in yogurt

Table (4): Nutrition content of yoghurt

Nutritional value	g/100g Yoghurt	Nutritional value	g/100g Yoghurt
Total solids %	21.11± 0.14	Titratable activity %	1.20±0.001
Solids not fat %	11.79±0.18	Total nitrogen %	0.60±0.001
Fat content %	3.45±0.05	Acidity %	0.86±0.001
Total protein %	3.97±0.04	Water soluble nitrogen%	0.12±0.001
Ash content %	0.52±0.001	pH value	4.9±0.02

Values are mean and SD (n = 3).

Measurement of weight:

Weight was determined in rats and the results are reported in table (5) From the results it could be noticed that the weight was significantly increased being 151.3 ,163.5 and 175 g in rats as control positive in 0 time ,after 7 days and after 14 days , respectively, This trend was observed in the negative control group and the other treated groups. After 14 day, the weights of

rats in all treated groups with yogurt, green banana and mixed between them recorded non-significant difference in weight gain, as compared to the control (-ve and +ve) groups

Table (5): Weight of rats as grams

Parameters / Groups	Weight / g		
	0 time	After 7 day	After 14 day
Control (-)	147.166 [a] ± 7.467	159.500 [a] ±9.159	171.166 [a] ± 8.704
Control (+)	151.333 [a] ± 11.93	163.500 [a] ± 9.731	175.000 [a] ± 8.579
Yogurt	148.333 [a] ± 9.973	160.333 [a] ± 9.563	170.833 [a] ± 9.703
Green banana	151.166 [a] ± 8.084	160.166 [a] ± 9.108	167.833 [a] ± 9.786
Yogurt & green banana	152.333 [a] ± 13.017	163.000 [a] ± 13.296	171.833 [a] ± 12.703

Values are mean and SD (n = 3) where: Mean values in the same with the letter ±are significantly different at $p<0.05$ levels

Measurement of antioxidant and enzymatic activities

The lipid peroxidation as Malondialdehyde (MDA), and the activity of the antioxidant enzymes Glutathione Reduced (GSH) and Superoxide dismutase activity (SOD) were determined in the different rats groups were taken orally Indomethacin to induce the gastric ulcer and the results are reported in table (6). Effect of yoghurt, green banana, and their blends on Malondialdehyde (MDA) level in the gastric tissue, the results showed that increasing the gastric MDA level in the control positive group was to 402.17µM/g/ protein. The normal group was 133.81 µM/g/ protein. The different rat groups fed yoghurt, green banana and its blends and taken orally indomethacin revealed the MDA was decrease gradually to 305.57, 283.45 and 168.256 µM/g/ protein, respectively. These results showed that the yoghurt and green

banana kept the gastric tissues healthy. It may be due to the banana contained the natural antioxidant and the yoghurt consists of high nutritional content. Several studies have shown that scavenging free radicals by antioxidant compounds prevent ethanol-induced gastric ulcer **(Alvarez-Suarez *et al.*, 2011).**

The superoxide dismutase SOD in the groups receiving yoghurt, banana and their blends showed a significant increase gradually to 15.810, 18.803 and 21.793u/mg/ protein. The positive control group was found to be significantly lower (12.606 u/mg/ protein) As compared to, the negative control group 26.77 u/mg/ protein. The superoxide dismutase SOD was significantly increased at the doses of 250 and 500 mg / kg BW and that all the doses of the suspensions of the Musa pulp were able to increase the Prostaglandins E2 (PGE 2) content of the gastric mucosa, the doses of 125 and 250 mg / Kg BW significantly and the 500 mg / Kg BW very high significantly than the negative control **(Cárdena *et al.*, 2019).**

Yoshino *et al.*, (1999) confirmed the results in table (6) for glutathione (GSH) in control positive thus, they found that the decrease of the nuclear glutathione (GSH) content in the control positive rats group isolated from rat liver nuclei, could lead to oxidative DNA damage, which in turn may be responsible for their mutagenicity and carcinogenicity. Moreover, the control negative group was the highest (104.40 m.mg/ protein). In addition, the different groups increased gradually from (GSH). The protein of green banana was 67.616 m.mg/ protein followed by green banana at 78.106 m.mg/protein. The protein of the yoghurt and banana was 92.816 m.mg/ protein for GSH.Free radicals as nitric oxide (NO) was determined in the different rats' groups and the results in the same table indicated that the control positive was the lowest (1.283 µM/g/ protein) and the control negative rats group was the highest (3,406 µM/g/ protein). As well as, the different rats' group was gradually increased to give the best group for that fed on yoghurt and green banana. The (NO) was 2.99 µM/g/ protein. These results occurred since to be that the banana is rich in sources of polyphenols and flavonoids. In addition, yoghurt has a high nutritional value and can be effective in reducing the incidence of gastric ulcer and its complications

Various reports showed that nitric oxide plays a protective role in gastric ulcer, and treatment with NO donors can accelerate the healing of gastric ulcer **(El-Abhar, 2010**).It was indicated that the protective effects of nitric oxide in gastric ulcer are related to the gastric mucosal blood flow, mucus secretion, and inhibition of inflammation **(Elliott *et al.*, 1995).** More studies demonstrated that ethanol administration decreased the gastric level of nitric oxide **(Abdulla *et al*., 2010).**

In addition, the green banana is rich in antioxidants including flavonoids and vitamins A and C, and also in resistant starch, which can present positive health effects regarding the glycemic index, cholesterol lowering and fermentation capability in the human colon. Therefore, the regular consumption of green banana could provide beneficial health effects due to its high content of these nutrient and non-nutrient compounds **(FAO, 2016).**

Diets that are rich in fruits and vegetables are associated with decreased risk of cancer and heart diseases. The protective effect of fruits and vegetables has been attributed to their antioxidants, including polyphenol, flavonoids and tannin which have biological and pharmacological properties **(Imam and Alter, 2011).**

Table (6): Antioxidant and enzymatic activities

Parameters / Groups	MDA µM/g/ protein	NO	GSH m.mg/ protein	SOD u/mg/ protein
Control (-)	133.810 ± 6.098 [e]	3.406 ± 0.369 [a]	104.400 ± 3.454 [a]	26.770 ± 1.660 [a]
Control (+)	402.170 ± 3.706 [a]	1.283 ± 0.261 [e]	50.473 ± 1.406 [e]	12.606 ± 0.520 [e]

yoghurt	305.570 ± 8.396 [b]	1.973 ± 0.321 [d]	67.616 ± 2.281 [d]	15.810 ± 0.491 [d]
Green banana	283.450 ± 4.852 [c]	2.536 ± 1.365 [c]	78.106 ± 1.939 [c]	18.803 ± 0.837 [c]
Yoghurt &green banana	168.506 ± 2.962 [d]	2.990 ± 0.010 [b]	92.816 ± 1.401 [b]	21.793 ± 0.478 [b]

Values are mean and SD (n = 6); where: Mean values in the same with the letter ±are significantly different at $p<0.05$ levels

Serum tumor necrosis factor-alpha (TNF- alpha) and pepsin

TNF- α is a major pro-inflammatory cytokine released from migrated macrophages during inflammation (**Rozza *et al.*, 2014**). It stimulates neutrophil infiltration in gastric inflamed areas (**Aziz *et al.*, 2019**) and suppresses the gastric microcirculation around ulcerated mucosa and delays gastric ulcer healing **(Hasgul *et al.*, 2014).**

As shown in table (7) TNF-α level was significantly increased in the positive control group to 301.916 pg/ml and decreased in pepsin activity to 7.93mg/ml, respectively. On the other hand, TNF-α was significantly reduced to 251.5,211.37 and 184.28, respectively in the groups pretreated with yoghurt, green banana and their blends which

were taken orally Indomethacin to induce the gastric ulcer. This result is consistent with previous reports of **Li *et al.*, (2013)** and **El-Hussieny *et al.*, (2017)** who reported an increase in gastric tissue proinflammatory cytokines due to ethanol administration. On the other hand, a dose-dependent reduction in TNF- α level was observed in the ulcerated groups pretreated with *C. ignea*, and this may be attributed to its anti-inflammatory effect.

It is worth mentioning that the improvement in pepsin activity was more obvious in groups treated with blends from yoghurt and green banana than in the animals treated with yoghurt and or banana. This is in agreement with **Puurunen (1982**) who clarified that high concentrations of ethanol can reduce peptic activity due to its ability to inhibit pepsinogen activation to pepsin. On the other hand, *C. Ignea* pretreatment improved pepsin activity in gastric secretion in a dose-dependent manner, indicating that *C. ignea* extract has the ability to regulate the ethanol effect on peptic activity.Other flavonoids that appear to exert anti-ulcer activity are monomeric leucocyanidin, a natural flavonoid and the major component present in unripe banana (*Musa sapientum* L. var. paradisiaca) and its synthetic analogues hydroxy ethylated leucocyanidin and tetrallylleucocyanidin showed protective effects against aspirin-induced gastric erosions in a prophylactic animal model, as shown by the absence of mucosal damage and a significant reduction in the ulcer index, when added to the diet at 5 mg and 15 mg per day **(*Lewis et al.*, 1999).**These compounds may be responsible for the displayed anti-ulcer properties and they suggested that the mechanism by which the active agent present in plantain banana and its synthetic analogues protects the mucosa is mediated, at least in part, by an increase in mucus thickness **(Lewis and Shaw, 2001).**

Table (7): Serum tumor necrosis factor-alpha (TNF- alpha) and pepsin

Parameters / Groups	TNF- alpha (pg/ml)	pepsin (mg/ml)
Control (-)	160.856 ± 0.686 ^{e}	15.280± 0.807 ^{a}
Control (+)	301.916 ± 2.338 ^{a}	7.930± 0.247 ^{e}
Yoghurt	251.503± 1.397 ^{b}	10.403± 0.507 ^{d}
Green banana	211.373± 2.511 ^{c}	
Yoghurt &green banana	184.283± 3.723 ^{d}	13.353± 0.517 ^{b}

Values are mean and SD (n = 6); where: Mean values in the same with the letter ±are significantly different at $p<0.05$ levels.

Histopathological Investigation.

Histological examination of stomach in the control group revealed normal structure of the glandular gastric mucosa and submucosa (**Photo. 1-2**).

In contrast to control group, several histopathological alterations were detected in model group. The glandular epithelium showed severe ulceration that accompanied by excessive multifocal hemorrhagic zones that showed brown pigmentation due to hemosiderin liberation. Some examined sections showed intense inflammatory cells infiltration in the mucosa and submucosal layer (**Photo. 3-7**).

The examination of BA (banana group) revealed few changes which characterized by mild epithelial sloughing with edema and cystic dilation of gastric acini in the mucosa. Few sections showed expansion of the submucosal layer with abundant edema associated with congested blood vessels and inflammatory cells infiltration (**Photo. 8-10**). Similar results were obtained in group Y (yoghurt group) that showed apparently normal mucosa in most examined sections except for few sections that showed degeneration and necrosis in the gastric acini (**Photo.11-15**).

Administration of BA + Y (banana and yoghurt group) resulted in the highest protection among the treated groups. Examination of gastric mucosa showed apparently normal histological structure in most sections (**Photo. 16-18**).

Histopathological lesion score

The statistical evaluation of all histopathological alterations showed a significant increase in model group compared to control or treated groups. Concerning the hemorrhage and inflammation, absence of significant difference was detected in all treated groups. Meanwhile, a significant decrease in epithelial loss and edema was recorded in group BA + Y compared to group BA (**Photo. 19-23**) **Hamdan *et al*., (2020)**

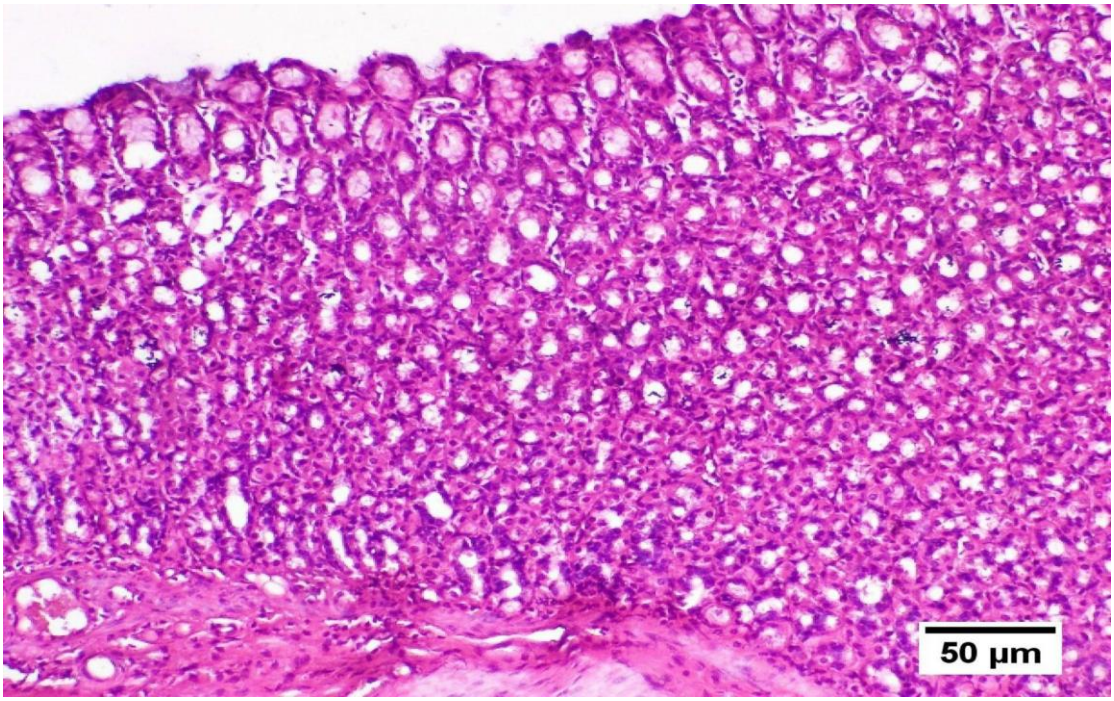

Figure 1: stomach from control group showing normal histological structure of stomach mucosa (**H&E**).

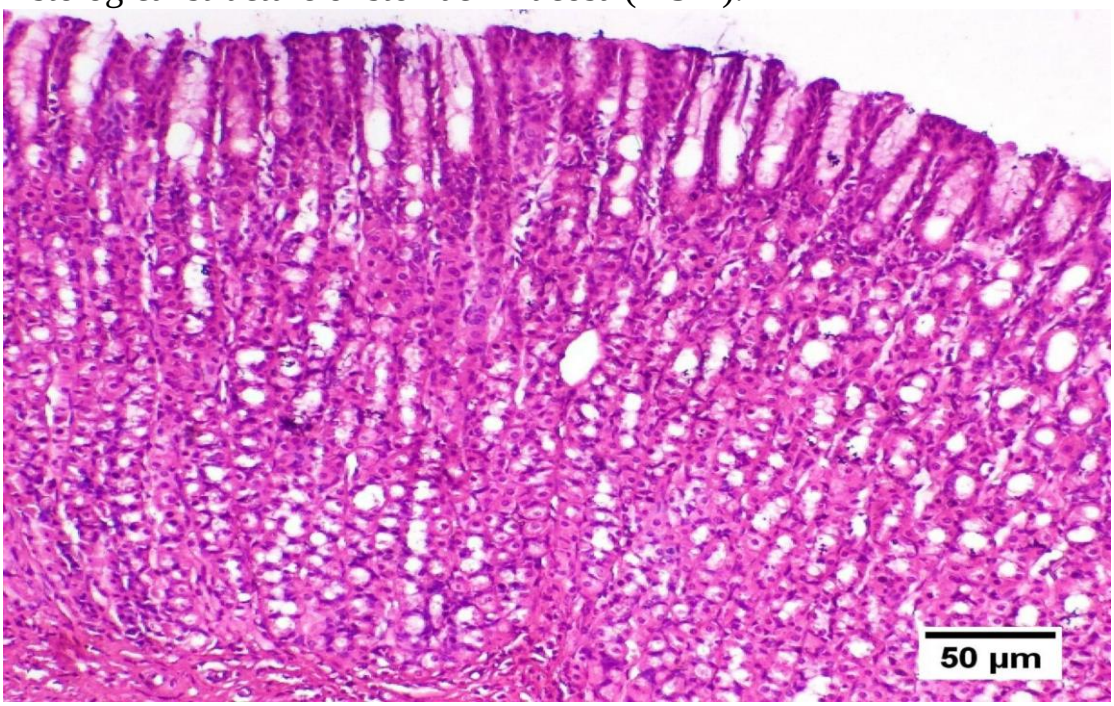

Figure 2: stomach from control group showing normal gastric mucosa (**H&E**).

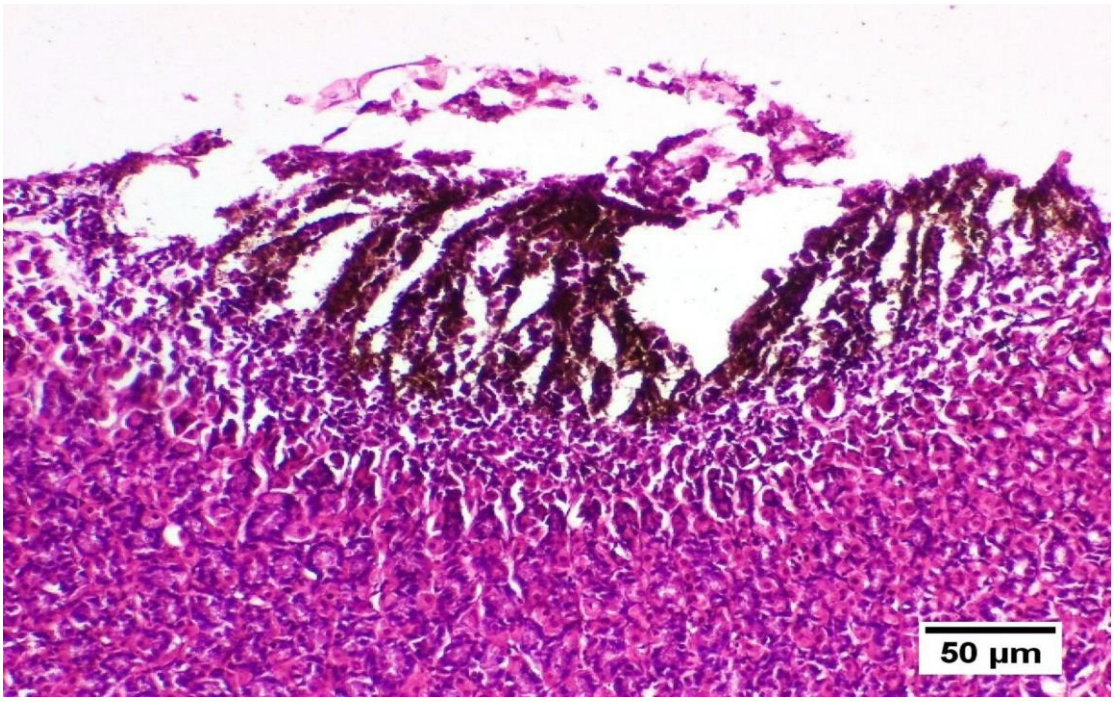

Figure 3: stomach from model group showing severe necrosis and existing of hemosiderin pigment in the gastric mucosa (**H&E**).

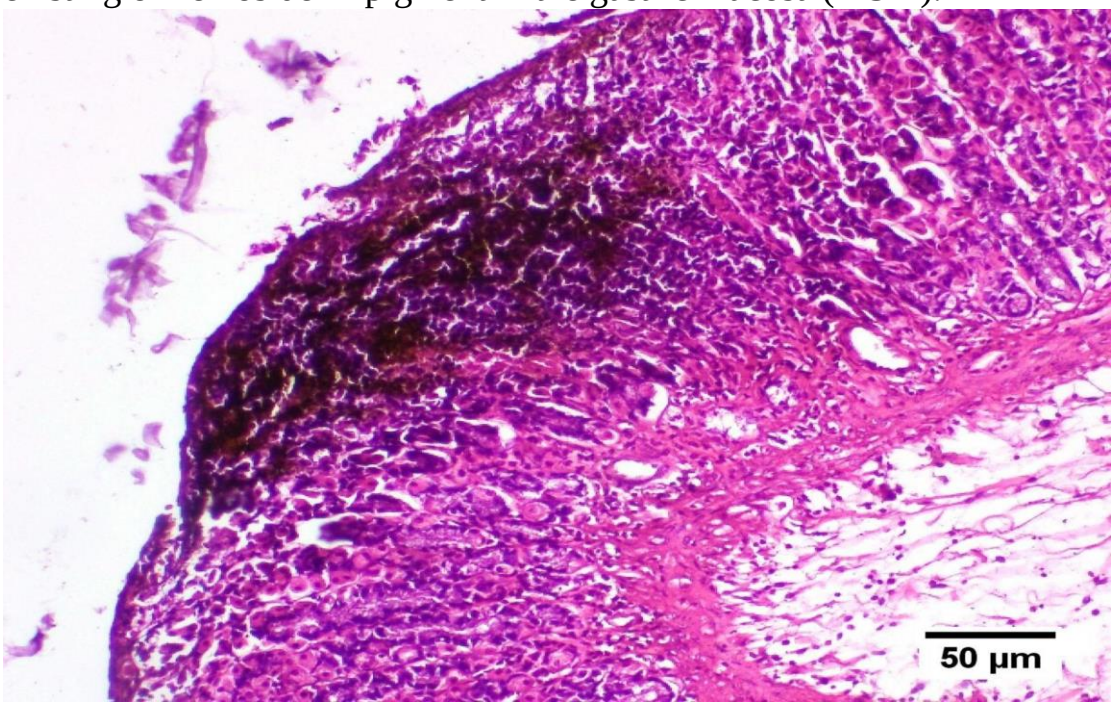

Figure 4: stomach from model group hemorrhages with hemosiderin deposition (**H&E**).

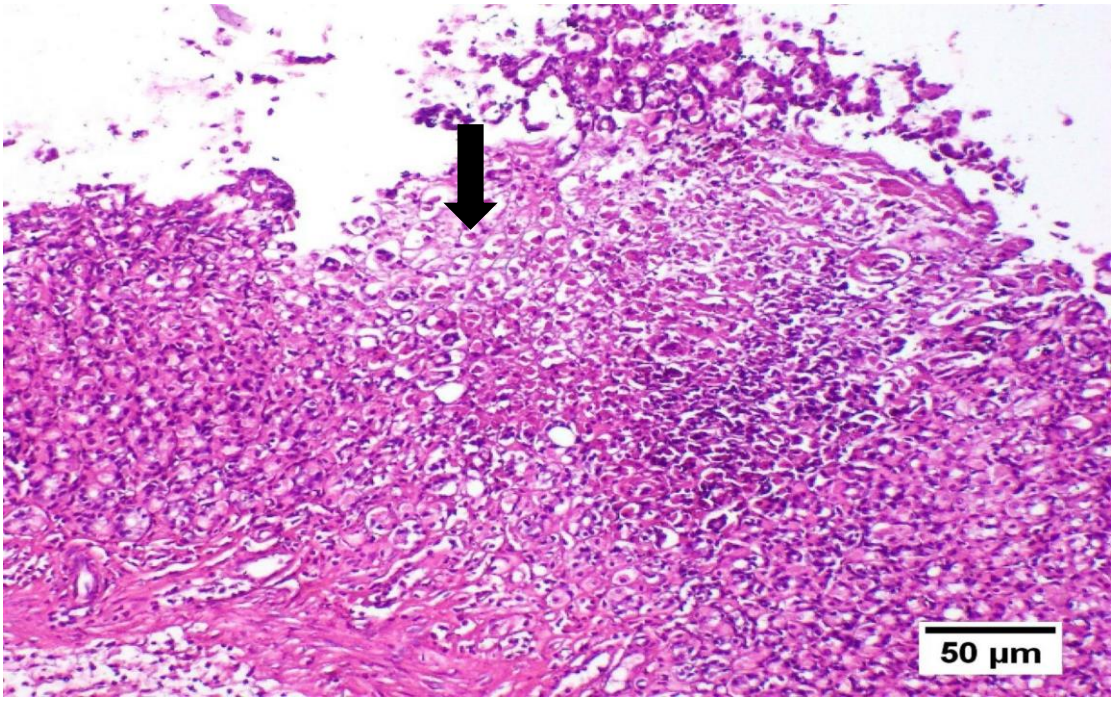

Figure 5: stomach from model group showing sloughing and necrosis of the mucosal layer (arrow) (**H&E**).

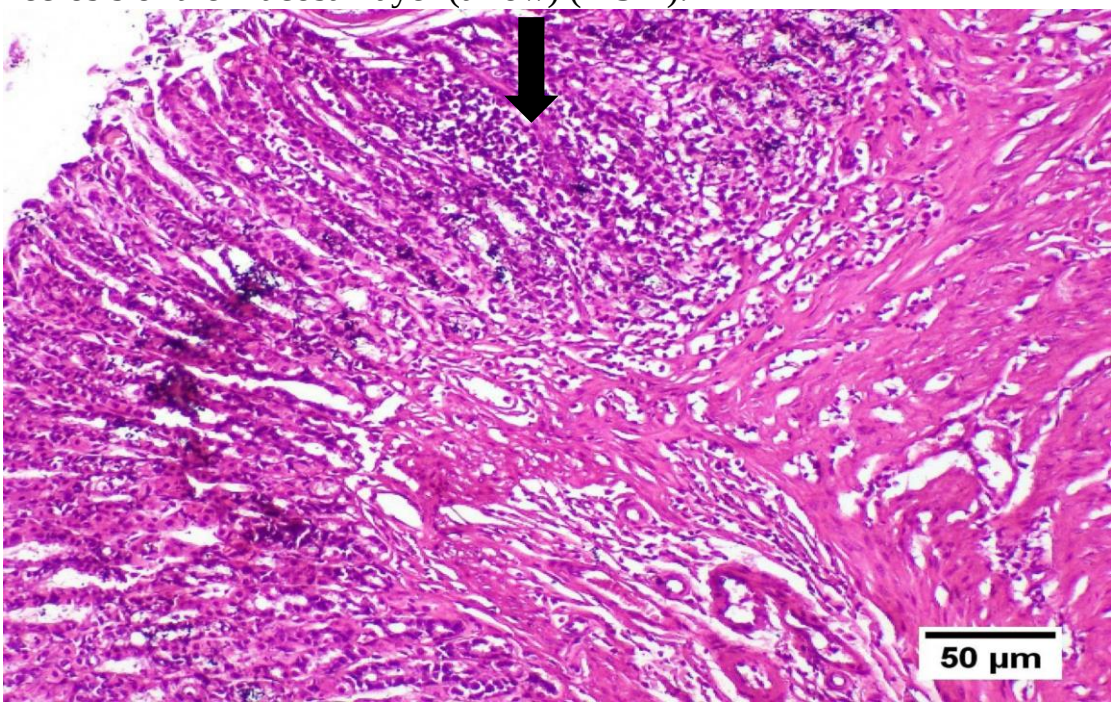

Figure 6: stomach from model group showing inflamed mucosa (arrow) (**H&E**).

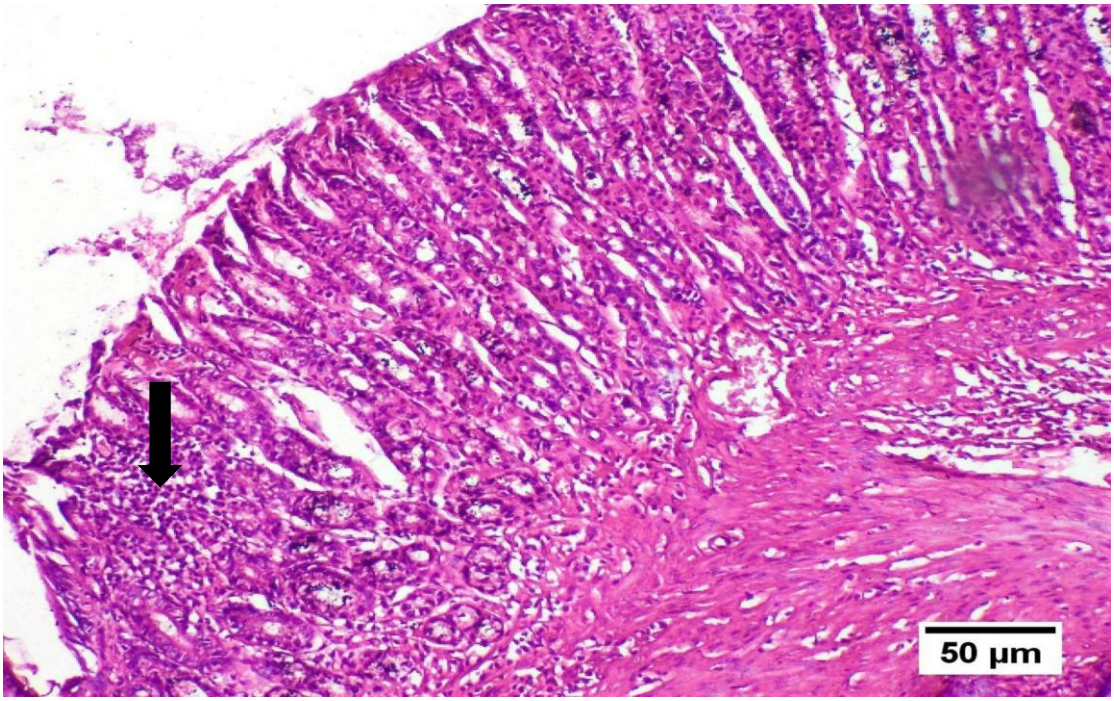

Figure 7: stomach from model group showing focal mononuclear inflammatory cells infiltration (arrow) (**H&E**).

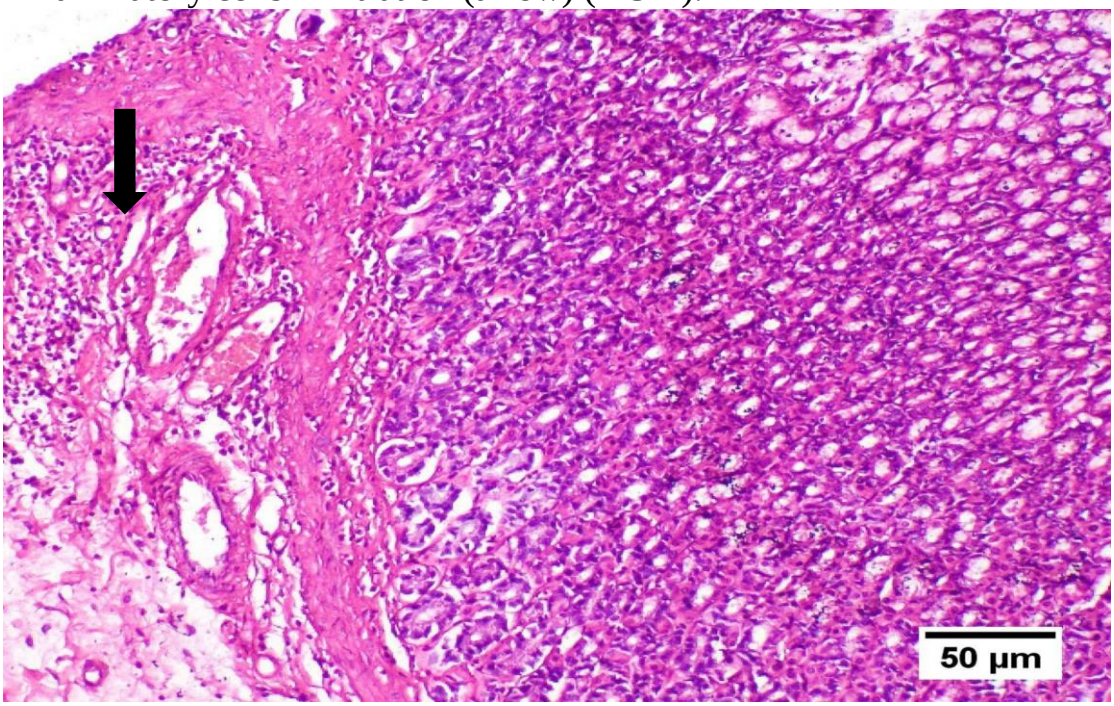

Figure 8: stomach from group BA showing submucosal expansion by inflammatory edema (arrow) (**H&E**).

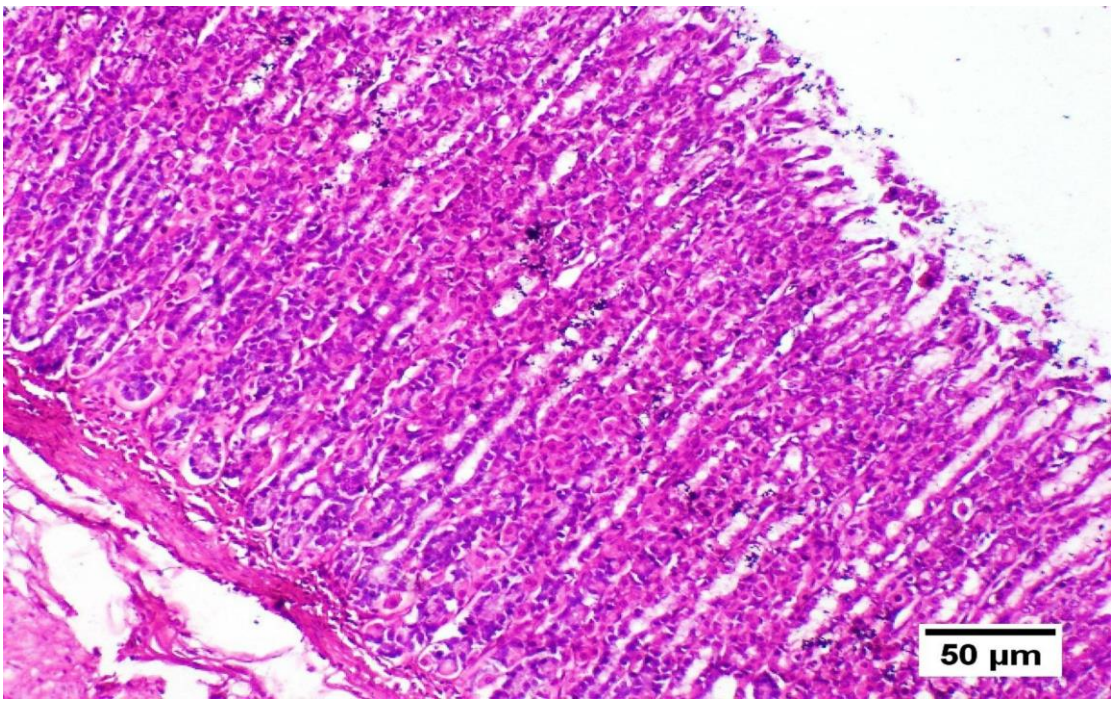

Figure 9: stomach from group BA showing mild epithelial loss (**H&E**).

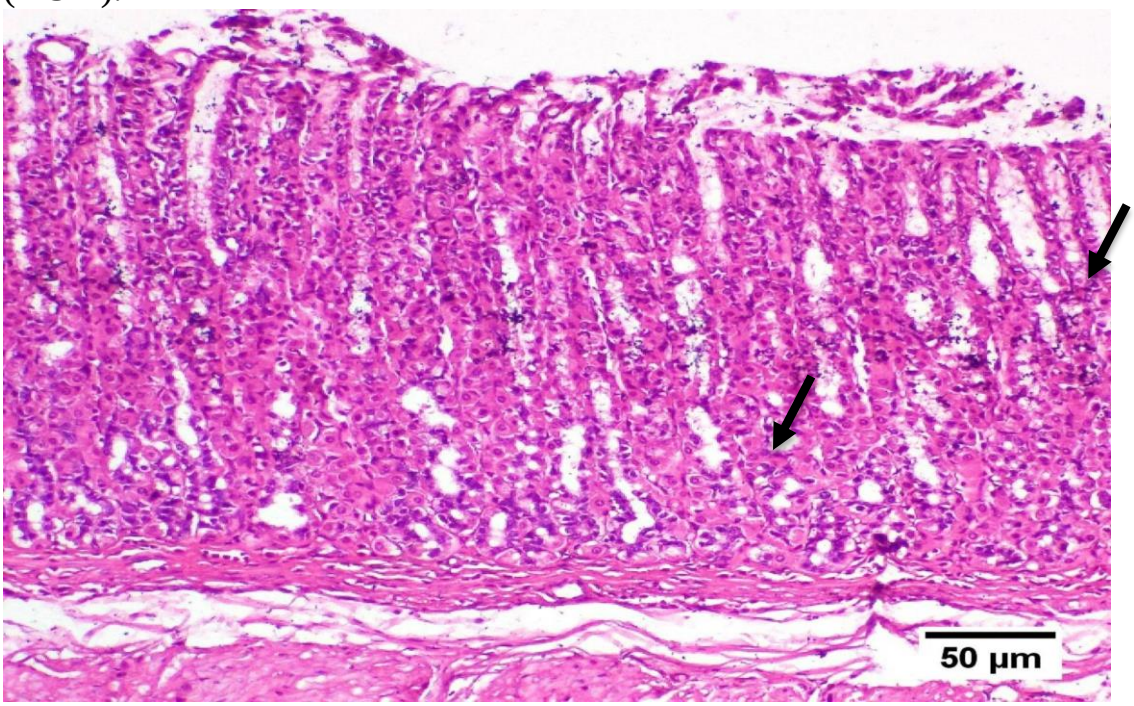

Figure 10: stomach from group BA showing cystic dilation of gastric acini (arrows) (**H&E**).

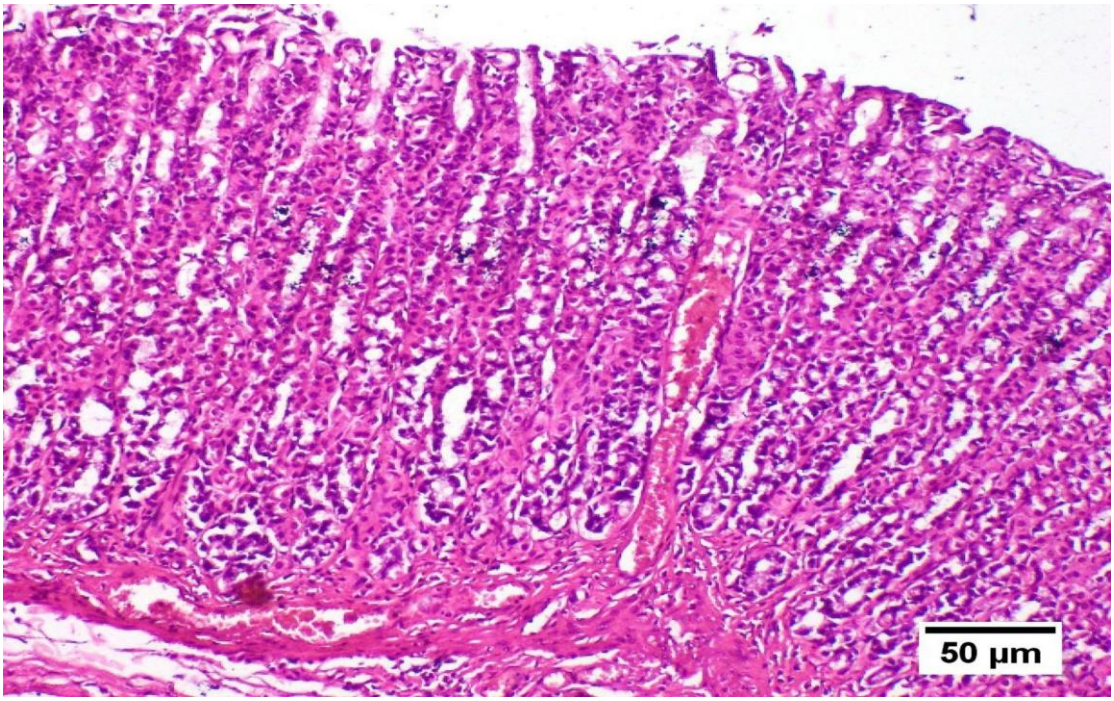

Figure 11: stomach from group Y showing congested blood vessels (**H&E**).

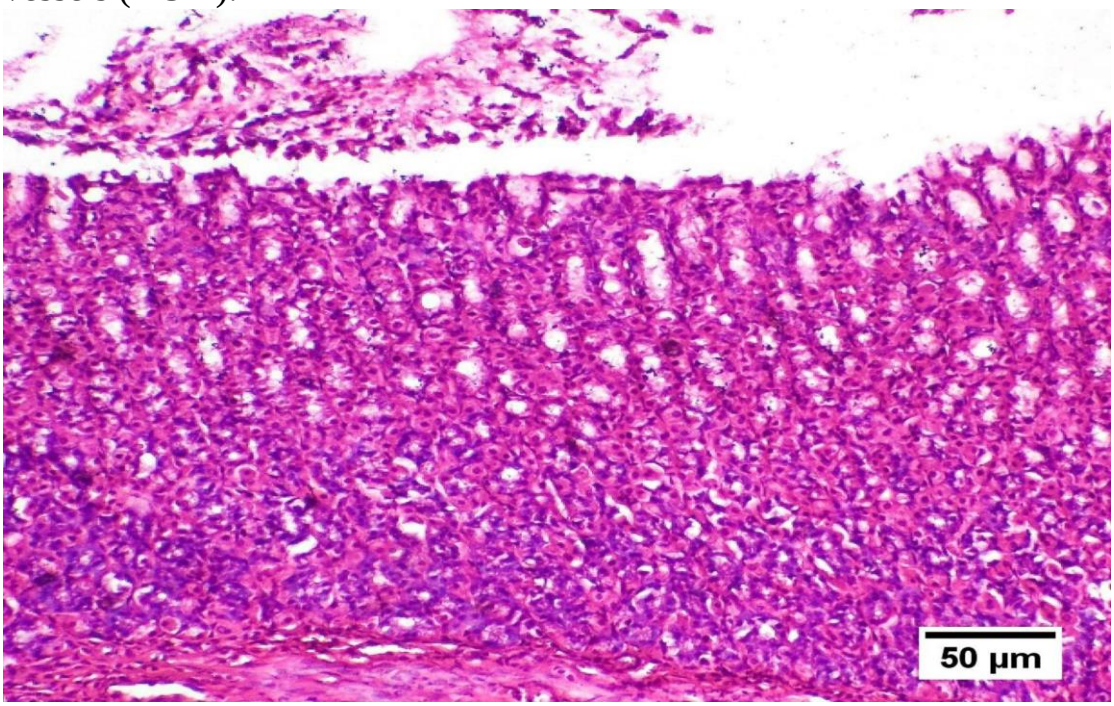

Figure 12: stomach from group Y showing apparently normal mucosa (**H&E**).

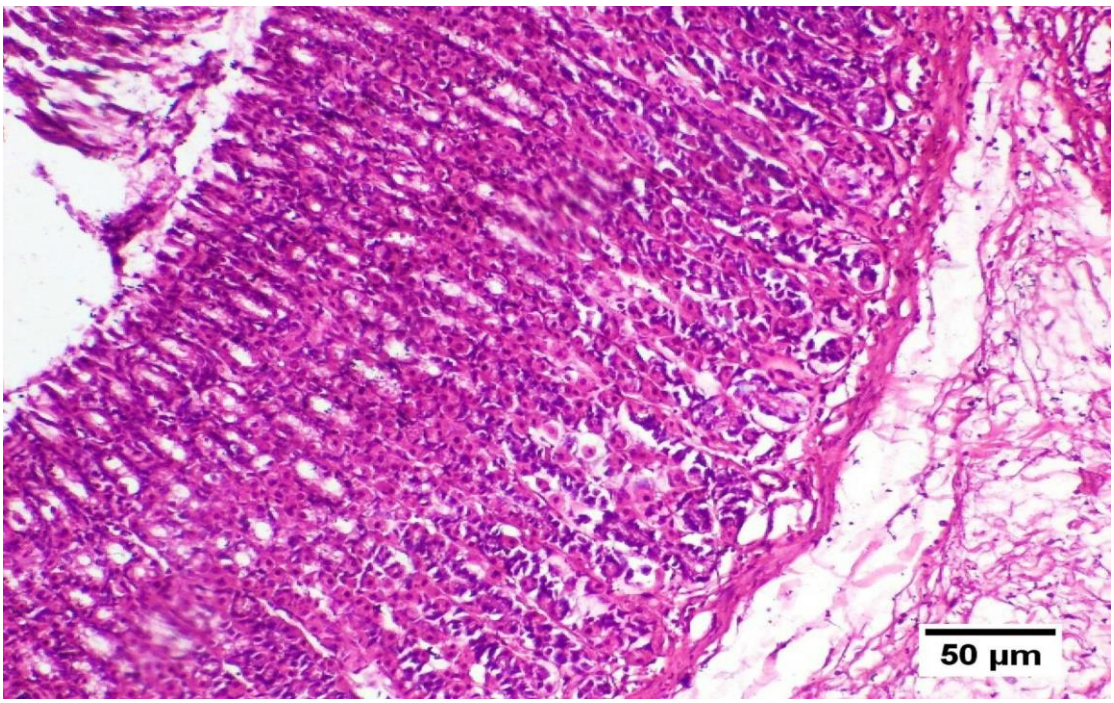

Figure 13: stomach from group Y showing apparently normal mucosa with abundant edema in the submucosal layer (**H&E**).

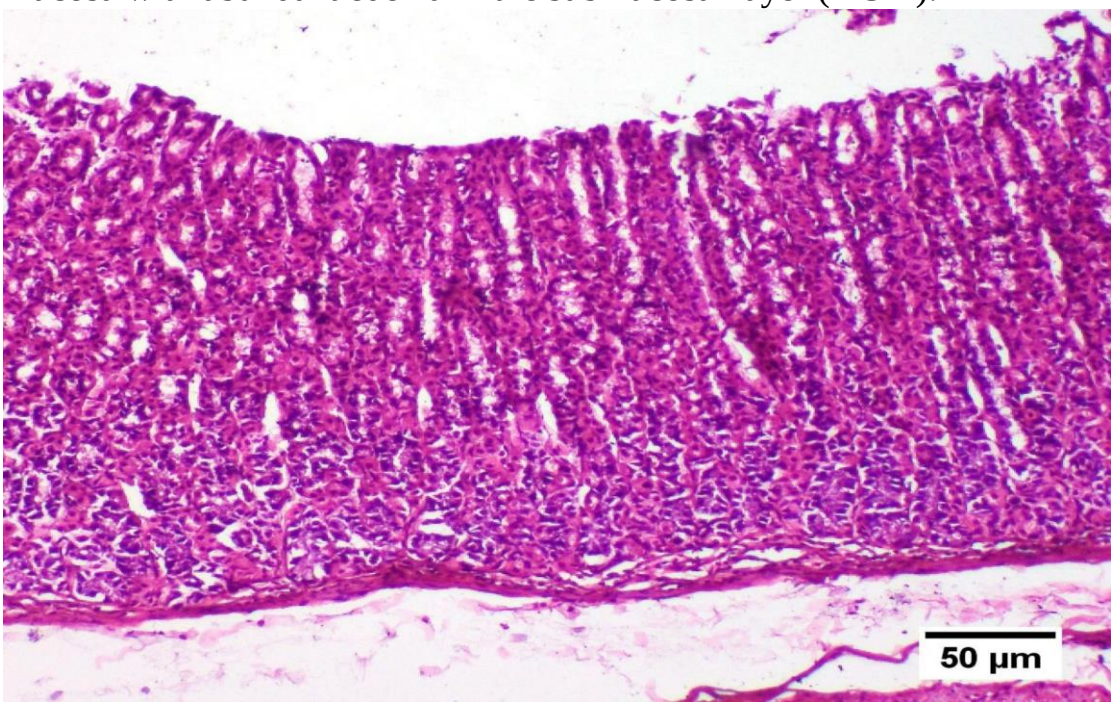

Figure 14: stomach from group Y showing apparently normal glandular mucosa (**H&E**).

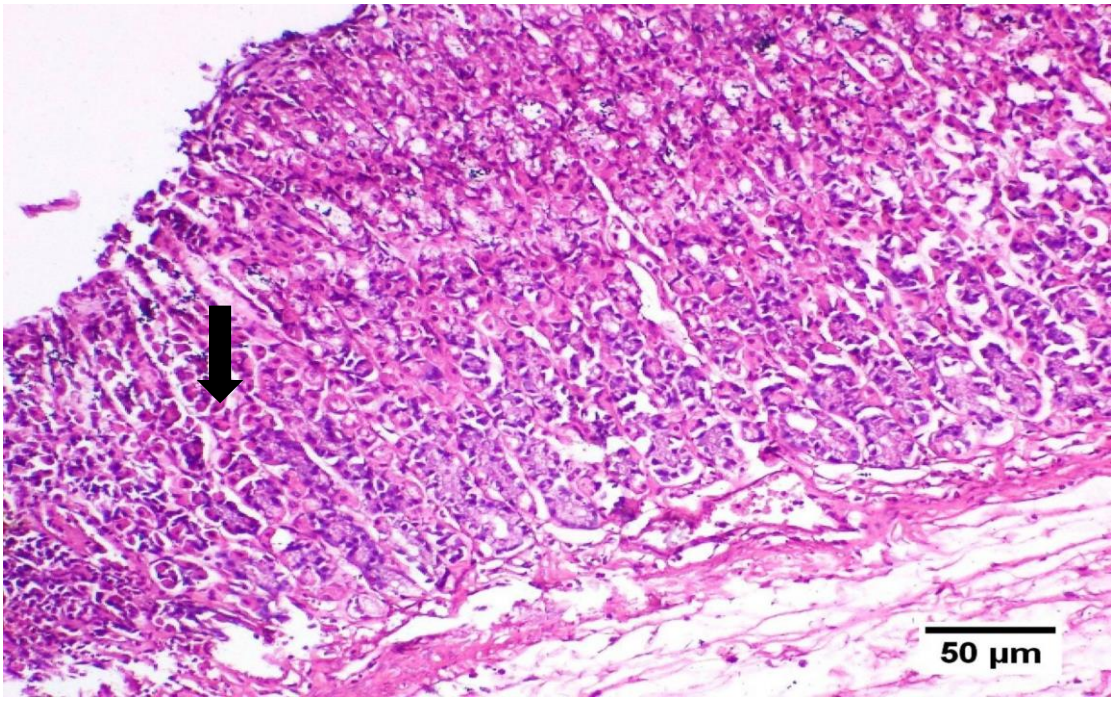

Figure 15: stomach from group Y showing few necrotic cells in the mucosa (arrow) (**H&E**).

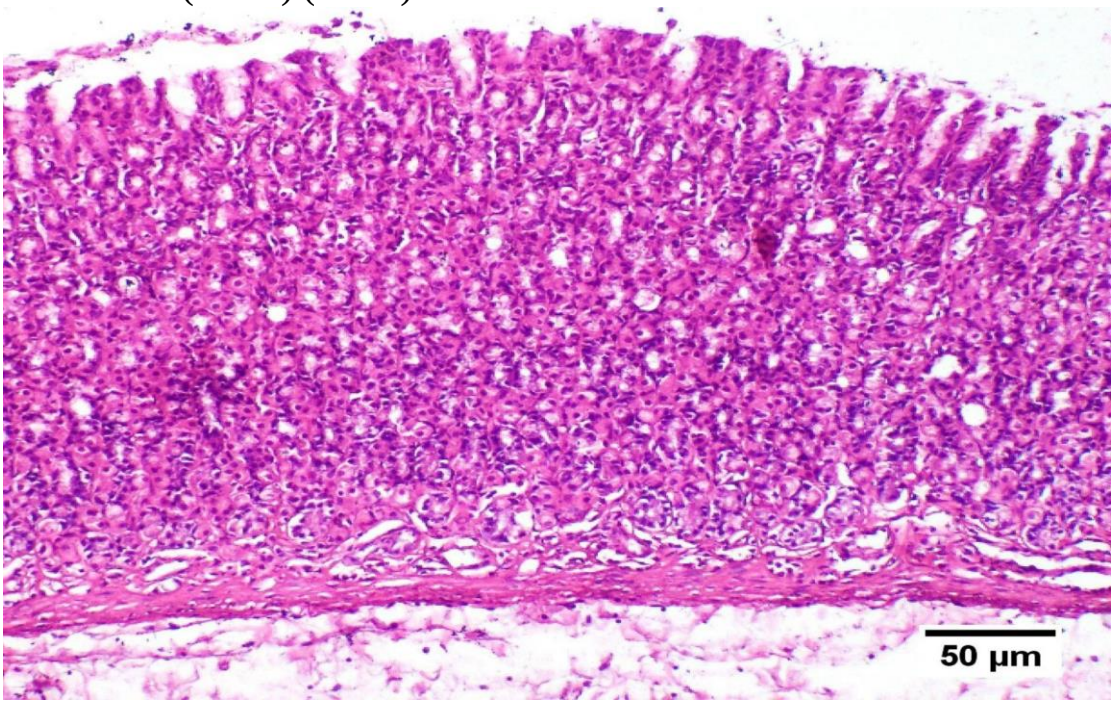

Figure 16: stomach from group BA + Y showing apparently normal epithelial covering glandular acini (**H&E**).

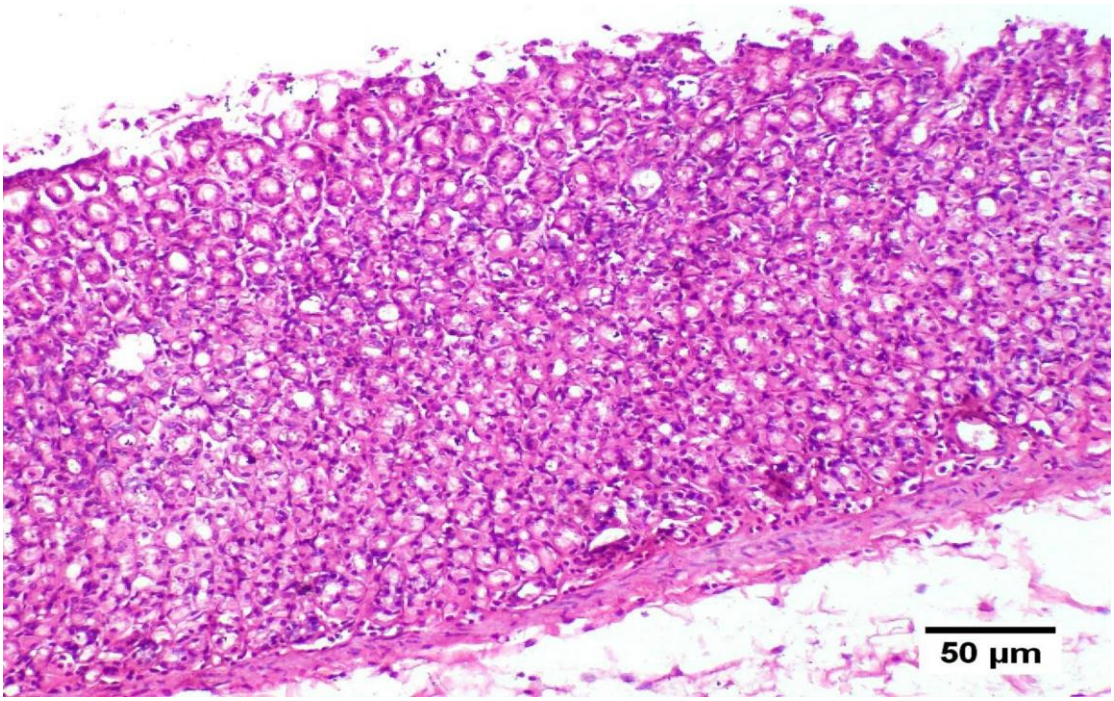

Figure 17: stomach from group BA + Y showing apparently normal mucosa (**H&E**).

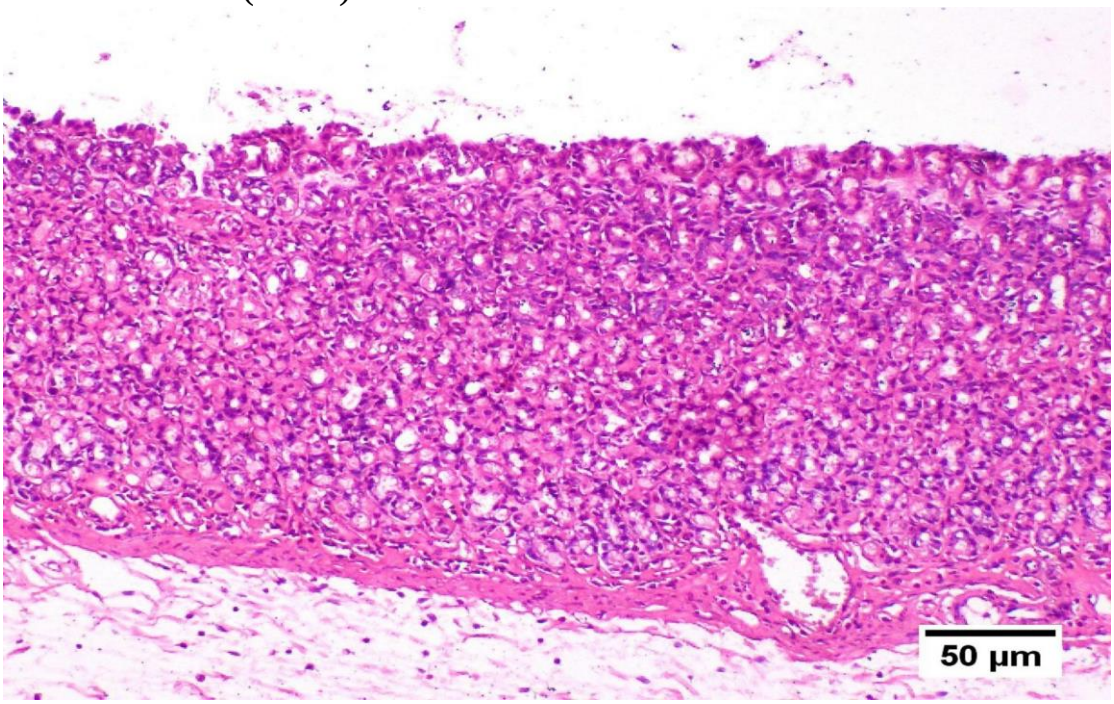

Figure 18: stomach from group BA + Y showing mild to moderate edema in the submucosal layer with apparently normal mucosa (**H&E**).

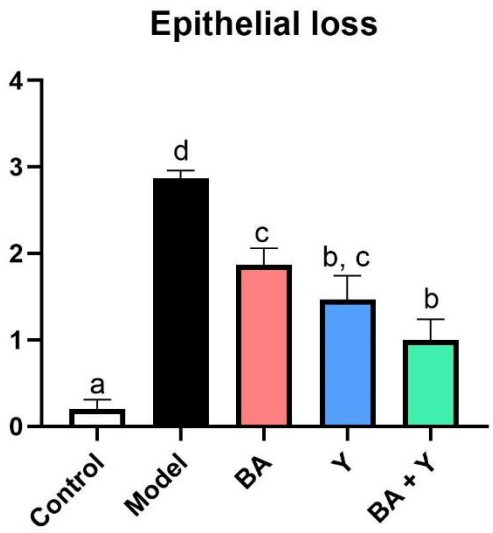

Figure 19: epithelial loss score. Data are expressed as the mean ± SE. a, b, c and d above the error bar indicate a significant difference.

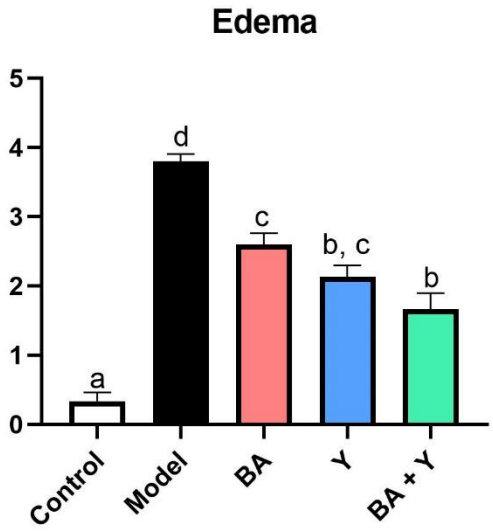

Figure 20: edema score. Data are expressed as the mean ± SE. a, b, c and d above the error bar indicate a significant difference.

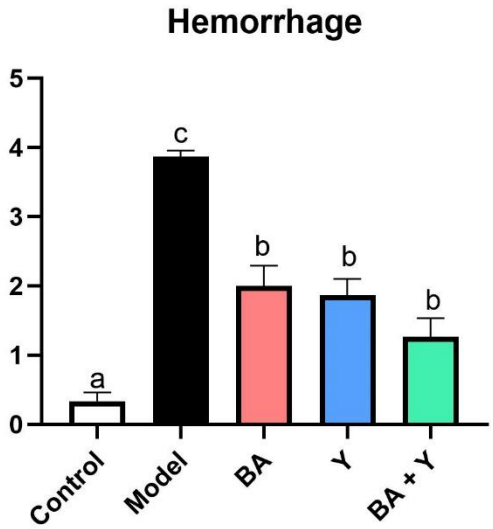

Figure 21: hemorrhage score. Data are expressed as the mean ± SE. a, b and c above the error bar indicate a significant difference.

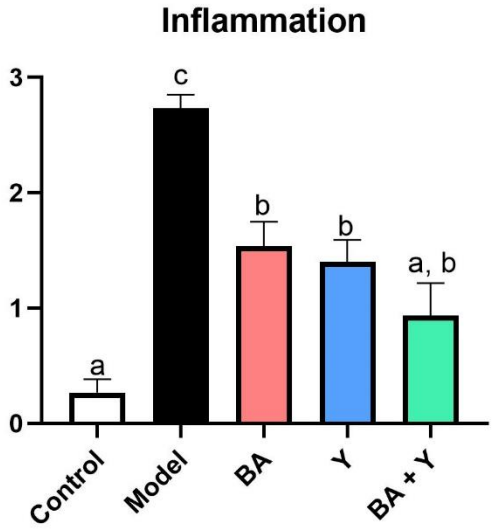

Figure 22: inflammation score. Data are expressed as the mean ± SE. a, b, and c above the error bar indicate a significant difference.

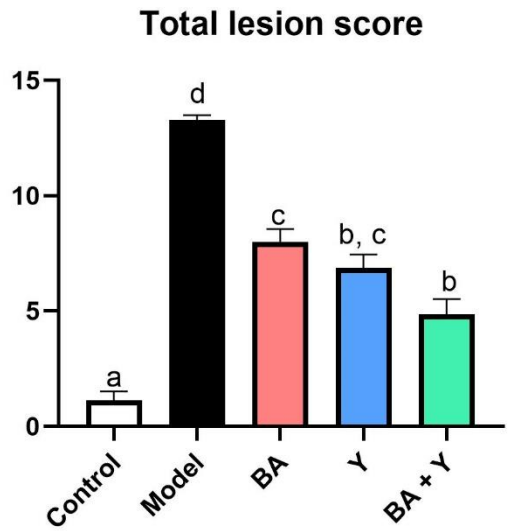

Figure 23: total lesion score. Data are expressed as the mean ± SE. a, b, c and d above the error bar indicate a significant difference

CONCLUSION

From the obvious results, it could be concluded that the study demonstrated that the indomethacin-induced gastric ulcer was caused by its antioxidant and anti-inflammatory effects. Meanwhile, using green banana, yoghurt, and their blends as a tool to the enhancement of gastric against indomethacin-induced ulceration gastric in rats could thus be possibly attributed to the presence of wealthy phytoconstituents as total polyphenols, flavonoids and nutrition content. Therefore, blends from green banana and yoghurt could be used as a promising anti-ulcer agent in the treatment of gastric ulcers

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