THE GASTROPROTECTIVE EFFECT OF TADALAFIL ON STRESS-INDUCED GASTRIC ULCER USING WISTAR RATS

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ABSTRACT

This study was carried out to investigate the gastro protective property of tadalafil in stress – induced gastric ulcer. The wistar rats of male sex (wt =180 – 252 g) were divided into 6 groups (n= 5) and pretreated with the drugs for two weeks prior to gastric ulcer induction. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. **Group 1** received control (distilled water); **Group 2 and 3** received Tadalafil (50 and 100mg/kg); **Group 4 and 5** received tadalafil (50 and 100mg/kg) + Omeprazole 1.75mg/kg) and Group **6** received positive control/ standard Omeprazole (30mg/kg). The ulceration was induced with0.5 ml of 95% ethanol and 0.25 g/kg reserpine respectively on end the 14 days pretreatment course, 1h after the last dose. The ulceration was induced by immersion and mobilization at the end of the 14 days pretreatment course, 1h after the last dose of drugs was given. All drugs were administered through the aid of orogastric cannula. 6 h later, the animals were sacrificed by cervical dislocation, dissected and the stomachs were isolated and carefully opened along the greater curvature. Mucous secretion was evaluated. Gastric tissues were obtained and fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. The Ulcer score, Ulcer index, and Preventive ratio of drugs were calculated. From the study, tadalafil 50mg/kg and 100mg/kg reduced significantly (P< 0.05) the level of ulcer index with elevation in percentage protective ratio. The histopathology

revealed normal stomach tissue wall and absence of distortion with tadalafil 50mg/kg and 100mg/kg and their various combinations with standard, omeprazole.

KEYWORDS: Gastric ulcer, Tadalafil, ulcer scores, histopathology.

INTRODUCTION

Drug repositioning is a system whereby drugs already in use is redirected and channeled for another therapeutic use. It is the system of redirecting the use of drugs already in existence for another clinical indication. Drug repurposing involves the utilization of a drug for a totally different therapeutic application. It is also known as drug rechanneling, re-profiling, or re-routing (Emdormi et al., 2020). Drug repurposing is necessitated by the fact that traditional drug discovery and development process has become quite expensive taking an average of US$1.8billion and a long duration of time with an average of (13-15) years. The long development process, high cost, drug resistance, toxicity and a very low success rate have revealed the unavoidable need for drug repurposing of old conventional drugs for a new therapeutic application (Wuerth et al., 2016).

Gastric mucosa is frequently opened to so many substances ranging from food, vital nutrients and many other deleterious agents. These substances given via the oral route can lead to destruction of gastric-mucosal integrity. Some of these substances have deleterious impact on the gastric mucosa which is the cause of some Gastric ulcers and acute mucosal damage (Chavez-Pina et al., 2010). Such substances could be ethanol/alcohol, Nicotine, ingestion of non-steroidal anti-inflammatory drugs (NSAIDs- Ibuprofen, Indomethacin), pepsin, smoking etc. The gastric membrane protective system that maintains and upholds its integrity include: epithelial cells secreting mucus, endogenous prostaglandin (Takeuchi, 2014), bicarbonates, normal gastric blood flow (Zhu and Kaunitz, 2008). Gastric lesions are the detectable effects of these aggressive factors which are linked to cellular influx, release of free radicals such as reactive oxygen species, cytokines and growth factors.

Tadalafil is PDE5 inhibitors just like sildenafil and vardenafil. It hampers with cGMP breakdown by the PDE5 (Phosphodiesterase enzyme 5), thereby leading to the buildup of cGMP which invariably bring about the dilation of smooth muscle of the blood vessels. Elevation of cGMP level enhances PDE5 actions (Cruz-Burgos et al., 2021). cGMP builds up to excite its metabolism; however, the pharmacological PDE5 inhibition hinders this negative feed-back procedure. Tadalafil is particular for PDE5 and in a lesser percentage inhibits

PDE6 (Ahmed, 2019), which functions for visual transduction in the retina. In Ajiboye and Oluwole, (2012), they proposed that Tadalafil significantly reduced indomethacin-linked gastric ulcer compare to control at high doses. They recorded significant variations in (area, depth & width) of the ulcer when Tadalafil (10 mg kg -1 BW) group were compared to the control. This study seeks to find out the effect of tadalafil on stress-induced gastric ulcer by immersion on ulcer scores, ulcer index, lipid profile, hematology parameters, biochemical parameters and stomach tissue wall (looking at the photomicrograph).

METHODS

Effect of Tadalafil on Water Immersion-Stress Model of Gastric Ulceration in Rats

This procedure was carried out according to Senay and Levine (1967) with modifications. Wistar rats of male sex (weighing 180 -220g) were divided into 5 groups (n=6). Food was removed 24 hrs and water 2hrs prior the experiment.

The wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tadalafil 50mg/kg/Omeprazole 1.75mg/kg and tadalafil 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer induction of stress with cold water bath immersion method.

They were vertically immersed individually in a compartment of cage water tank containing water and the temperature of the tank sustained between 15 – 20 °C using ice pack to generate stress ulceration**.**

Group 1 received distilled water, no drugs

Group 2 received Omeprazole 1.75mg/kg;

Group 3 and 4 received Tadalafil 50 & 100mg/kg respectively;

Groups 5 and **6** received Tadalafil 50 & 100mg/kg/ Omeprazole 1.75mg/kg.

The ulceration was induced by immersion and mobilization at the end of the 14 days pretreatment course, 1h after the last dose.

All drugs were administered through the aid of orogastric cannula.

6h later, the animals were sacrificed by cervical dislocation. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of

lesions. The Ulcer score (Lau and Ogbe, 1981), Ulcer index, and Preventive ratio of drugs were evaluated.

Macroscopic examination was carried out with a hand less and scored for the presence of lesions

Calculation of Ulcer Index

This was carried out as described by (Martin-aragon et al., 1994).

It is shown below:

M XN 100

Where: M = Mean number of ulcers per rat in the group

N= Percentage of rats with ulcer in the group.

Determination of adherent gastric mucus content

The extraction of mucus was done by the method described by Ettarh and Okwari, (1999). The rats were fasted overnight and after administration of anesthesia, their abdomens were opened and the stomachs cut and washed in saline and opened along the greater curvature and slightly stretched and supported with dissecting pins on a corkboard. The accumulated mucus was removed using a blunt scalpel into a pre-weighed beaker holding 4ml of water (M). The final weight of the beaker plus the mucus (N) minus M gives the weight of the mucus (Z) for each animal in all the groups, i.e. (N –M = Z g)

Determination of Antioxidant activity

Sample preparations

Prior to homogenization, the stomach tissues were washed with distilled water to reduce the effect of hemoglobin with free radicals and to get rid of blood attached to the mucous membrane. The tissues were sliced into piece and homogenized using Teflon homogenizer (Polytron, Heidolph RZR 1 Germany) with the right buffer and centrifuged at 18,000rpm for 15 minutes at $4\Box$ C. The supernatant was collected in a beaker for further analysis.

Determination of superoxide dismutase (SOD)

This procedure was carried out according to Sun et al. (1988). The activity of the enzyme was investigated by estimating its ability to suppress the photochemical reduction of nitro-blue tetrazolium (NBT).

In a dark chamber, I mL of the reactant (50mM phosphate buffer, 100nM EDTA and 13mM 1- methionine, pH 7.8) was blended with 30 µ l of 2 µm riboflavin. The resulting solution in

a tube was exposed to fluorescent light bulbs (15W) for 15 minutes and completely read using spectrophotometer at 560nM.

Note: In this assay, the photochemical reduction of riboflavin produces O^{2-} which breakdown the NBT to yield formazan salt that absorbs at a wavelength of 560nM.

Determination of membrane lipid per oxidation, MDA

Gastric content of lipid peroxidation was carried out following the Ohkawa et al. (1979). Determination of the rate of lipoperoxidation in the gastric mucus membrane was evaluated by assaying of malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) test. Supernatant obtained after homogenization was mixed with 400 μl of 0.6% thiobarbituric acid, and incubated at 95 -100□ C for I h and absorbance read at 532 nM. Using 1, 1,3,3-tetramethoxypropane, a standard curve was obtained. Consequently, the result was expressed as nmol of MDA/mg protein (Bradford, 1976).

Determination of Alanine aminotransferase (ALT)

ALT was assayed using (Reitman & Frankel, 1957).

Principle

a-oxoglutarate + L-Alanine L- Glutamate + Pyruvate Alanine amino transferase was determined by regulating the concentration of pyruvate hydrazone developed with 2, 4-dinitrophenylhydrazine. Reagents (R) R1: Buffer (phosphate buffer 100mmol/L, pH 7.4, L-alanine 200mmol/L, a-oxoglutarate 10mmol/L) R2: 2, 4-dinitrophenylhydrazine 2.0mmol/L

Procedure

Two test tubes which contained blank and samples were adequately labeled.

R1 (at 0. 5ml) was introduced into the both test tubes containing both the blank and samples. Distilled water of (0.1ml) was also introduced to the sample alone. The two test tubes were kept warm for (30 min) at (37°C) after which (0.5ml) of R2 was added to each of the tubes, blend thoroughly and left to settle for (20 min) at 25°C.

At this point, (5ml) of Sodium hydroxide was introduced to both tubes, mixed carefully and the sample read was determined against the blank after (5 min) at (546nm).

Determination of Aspartate Amino Transferase (AST)

The AST concentration assessment was carried out using Reitman and Frankel (1957) method.

Principle

AST was assayed by regulating the concentration of oxaloacetate hydrazone created with 2,4-dinitrophenylhydrazine. α-oxoglutarate + L-aspartate L-glutamate + oxaloacetate Reagent (R) R1 : Buffer (phosphate buffer 100mmol/L, pH 7.4, L-aspartate 100mmol/L and a-oxoglutarate 100mmol/L) R2 : 2,4-dinitrophenylhydrazine (2mmol/L)

Procedure

Two test- tubes, the blank and samples were adequately labeled. R1(0.5ml) was introduced into tubes containing the blank and samples.

Distilled water (0.1ml) was also introduced to the sample alone. The (2) test- tubes were warmed for (0.5hr) at (37°C). Then R2 (0.5ml) was put in both tubes containing blank and samples, mixed appropriately, permitted to stay for (20 min) at (25°C). At this point, Sodium hydroxide (5ml) was introduced to the two test-tubes containing the blank and samples. The tubes were properly mixed and the sample read/determined against the blank after (5 min) at 546nm.

Determination of Alkaline Phosphate (ALP)

ALP concentration examination was executed by the aid of Randox kit following Deutsche Gesellschaft fur Klinische Chemmie (GSCC) i.e. German Association of Clinical Chemistry recommendation.

Principle

P-nitrophenylphosphate is being hydrolyzed by ALP to yield phosphate and P-nitrophenol P-nitrophenylphosphate + H20 phosphate + P-nitrophenol
Reagent 1a: Buffer (Diethanolamine buffer 1mol/pH9.8, MgCl2 0.5mmol/L)
Reagent 1b: Substrate (p-nitrophenylphosphate 10mmol/L)

Procedure

0.05 ml of sample and 3.00ml reagent 1a (Diethanolamine buffer 1mol/pH9.8, MgCl2 0.5mmol/L) and reagent 1b (p-nitrophenylphosphate 10mmol/L) were pipette into a cuvette. Absorbance of the mixture was read at time (0, 1, 2, & 3 at 405nm).

Determination of Urea (Jung et al., 1975)

The reagents for urea assay were arranged following Jung et al., (1975) directives.

Jung working reagents consist of

Working reagents: (100 mg/L) o-phthal-aldehyde, (215 mg/L) N-(1-naphthyl) ethylenediamine, (2.5 mol/ L) sulfuric acid, (2.5 g/L) boric-acid, and (0.03%) Brij-35. Modified reagents: These include; (100 mg/L) o-phthal-aldehyde, (513 mg/L) primaquine bis-phosphate, (2.5 mol/ L) sulfuric-acid, (2.5 g/L) boric-acid, and (0.03% Brij-35). Standard: Double-distilled water and (5.00 mg/dL) urea

Procedure

Water (50 µL), standard of (50 µL and 5.00 mg/dL) , and (50 µL) of each sample was moved into distinct well of a clear flat-bottom 96-well plate. 200 µL of newly arranged working reagent was included and combined by shaking the plate. At room temperature, the reaction was incubated. Measurement of optical densities was done at 430nm and 505nm on the plate reader for determination utilizing the modified reagent and the standard Jung reagent inclusively.

Determination of serum creatinine concentration (Roscoe, 1953)

Reagent 1 (R1) working solution: Sodium hydroxide: 0.20 mol/l.

Reagent 2 (R2) working solution: Picric acid Standard solution of creatinine.

Procedure

Preparation of alkaline creatinine picrate was done by adding 2ml of 0.75N NaOH and 2ml of saturated picric acid to 6ml of a Standard solution consisting of 0.25-1.0mg of creatinine per 100mls. Linear color response (orange) was seen on a logarithmic scale. The absorbance was read at 500nm against a blank prepared with distilled water (in place of standard solution), NaOH and Picric acid. For the various blood samples, precipitation of the serum was done by adding 2 volumes of a serum dilution, one part of sodium tungstate 5% and one part of either 0.33N H_2SO_4. The fundus section of the treated animals' stomach was homogenized (5%) in ice cold 0.9% saline. The mitochondrial fraction was collected via centrifugation and utilized for the Analysis of the enzymatic Antioxidants such (SOD & CAT). The protein was analyzed in mucosal homogenate to reveal the activities of SOD and CAT per milligram (mg) of protein.

Hematology Assay

Blood specimens were acquired from the treated reserpine-induced, ethanol-induced and stress-induced gastric ulcers rats via cardiac puncture after sacrificing the animal using anti-

coagulant EDTA bottle. Hematological parameters and indices were determined from unclotted blood samples using standard protocols (Jain, 1986). Hematological analysis was performed for parameters such as; WBC, RBC, Platelet count, Hematocrits.

Measurement of serum lipid profile

The serum lipid profile of total cholesterol (Roeschlau et al., 1974), serum triglyceride (Buccolo and David, 1973) high density lipoprotein (Burstein et al., 1970) were measured while very low density lipoprotein(VLDL) was calculated as triglyceride/5 and low density lipoprotein (LDL) was calculated using the equation: LDL = total cholesterol − (HDL + VLDL).

3.2.12 Histopathological Analysis

The stomach tissues obtained from the animals was subjected to histopathological examination using the method of Drury (1983).

Fixation and washing

To preserve the tissues, formalin (10%) was utilized. A minute portion of the tissues (1- 2cm in diameter) were sliced using a razor blade that is sharp. Small pieces of tissues that were kept in the 10% formalin and the container mixed quietly to ensure that the reagent penetrated all the tissues and also to avoid them gumming to the surfaces. At 25°C they were incubated and allow to be properly fixed. Subsequently they were washed with running water for 24 hours to wash off to much of the fixatives.

Dehydration

It was ensured the tissues were without H2O before embedding them in paraffin. Tissues were submerged in automatic tissue processor consisting of 12 jars in other to attain the dehydration. 70, 90 and 95% absolute alcohol was introduced in the first three containers respectively. The essence of this is to get rid of the water content in the tissues. Fresh absolute alcohol was reintroduced to ascertain complete water elimination. Similar procedure was done in the other jars of the automatic tissue processor.

Clearing

At his point, Xylene solutions were utilized in the clearing of the tissue sections. This procedure was indicated in the other jars of the automatic tissue processor. Xylene solution was preferred because it is miscible with both alcohol and paraffin before penetration occurs.

The essence of carrying out clearing was to get rid of opacity from dehydrated tissues. The tissue stayed in the solution for 15 minutes before it was removed.

Infiltration with paraffin

The tissues were infiltrated with paraffin wax for 50-52°C. They were moved to a bath with molten paraffin. They were incubated for 30-60 minutes in the first bath and thereafter, transferred to a fresh dish containing paraffin in fourth jars containing automatic tissue processor for the same duration of time.

Embedding (Blocking) with Paraffin

The tissues were completely soaked with paraffin and the paraffin allowed to solidify in and out of the tissues.

Paraffin Sectioning

The soaked sections of the tissues were sliced into squares and fixed in the microtome knives for partitioning and thereafter passed through the water bath.

Mounting

Thin layer of the albumen fixative was prepared on a clean glass slides. The slides were used to obtain the required section from the other partitions in the water. The partitions on the glass slides were moisturized before staining was carried out.

Staining with Haematoxylin

Series of Jars containing alcohols of reducing strength and different staining solutions were brought and the slides passed through each of them. Microscopic Observation of Slide Slides were made ready and viewed under the photomicroscope one after the other at 400 magnification power of the microscope. Each of the slides was photographed. Various rats were obtained after carrying out Ethanol-induced and indomethacin-induced gastric acid ulcer models. They were dissected through the large curvature. The injuries were located, sliced and fixed in ALFAC solution for (24 hr) at 4 °C. Processing of the samples was carried out through embedding in paraplast. They were cut into 7u thick sections of which they were stained with periodic acid-schiff (PAS), (Vacca, 1985) and hematoxylin-eosin (Behmer et al., 1976).

Analysis of the sample was done with a Leica microscope in conjunction with Leica Qwin software (Leica England).

Methods of Data Collection

At the end of each experiment both the test animals and the controls were sacrificed under chloroform anesthesia. One milliliter (1ml) of blood was collected from each rat via cardiac puncture. Blood samples were obtained in anticoagulant bottle (EDTA) bottle for lipid, biochemical and hematological assessments. The stomach were carefully harvested and fixed with formalin for histo-pathological assessment.

Statistical Analysis

The data obtained from this research were reported as mean ± SEM. Statistical significance were also computed by the aids of One-Way Variance of Analysis followed by Turkey's Honesty Significant Difference (HSD) test at the level of significance ($p < 0.05$).

Ethical Approval

This research was approved by the UPH Research Ethical Committee. The set guidelines and regulations pertaining to experimental animal management were accurately followed.

RESULTS

Calculation of Ulcer score, Ulcer Index and Percentage Preventive Ratio

Ulcer scored for the presence of gastric lesion following rating scale of Lau and ogbe (1981) as follows: 0.0 = usual color of,   1.5 = hemorrhagic lines, 2.0 = ulcers having (>3 but =5mm2) area, 3.0= ulcers > 5mm2,

Ulcer index was calculated using severity scores and average number of ulcers per animal.

Severity was scored as: 0 - Normal stomach, 0.5 - Red coloration, 1 - Spot ulcers,

1.5 - Hemorrhagic streaks, 2 - Ulcer > 3 mm but ‹ 5 mm, 3 - Ulcers > 5 mm

Ulcer index (UI) = UN + US + UP × 10,

Where UI = ulcer index, UN = average number of ulcers per animal, US = average of severity score, UP = percentage of animals with ulcer.

$$\text{Percentage protective ratio} = 100 - \frac{[\text{UI pretreated}]}{[\text{UI control}]} \times 100$$
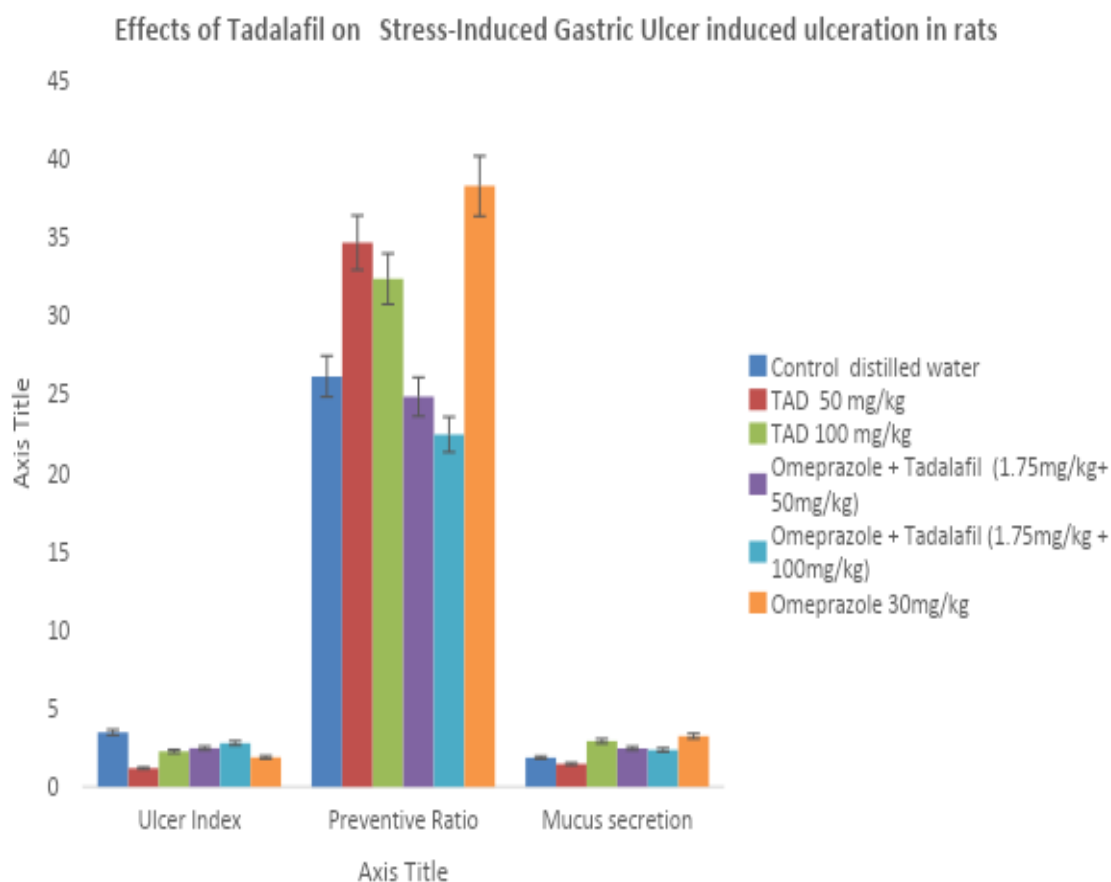
FIG 4.1: Bar chart representing ulcer index, preventive ratio and mucous secretion in stress-induced model. In this model of gastric ulceration, tadalafil 50mg/kg and tadalafil 100mg/kg + omeprazole 1.75mg/kg revealed a significant reduction in ulcer index. The percentage protective ratio is 26.10, 34.60, 24.80, 22.40 and 38.20 respectively. The mucous secretion is significantly elevated ($p<0.05$) with tadalafil 100mg/kg and omeprazole 1.75mg/kg + tadalafil 100mg/kg as compared to standard.

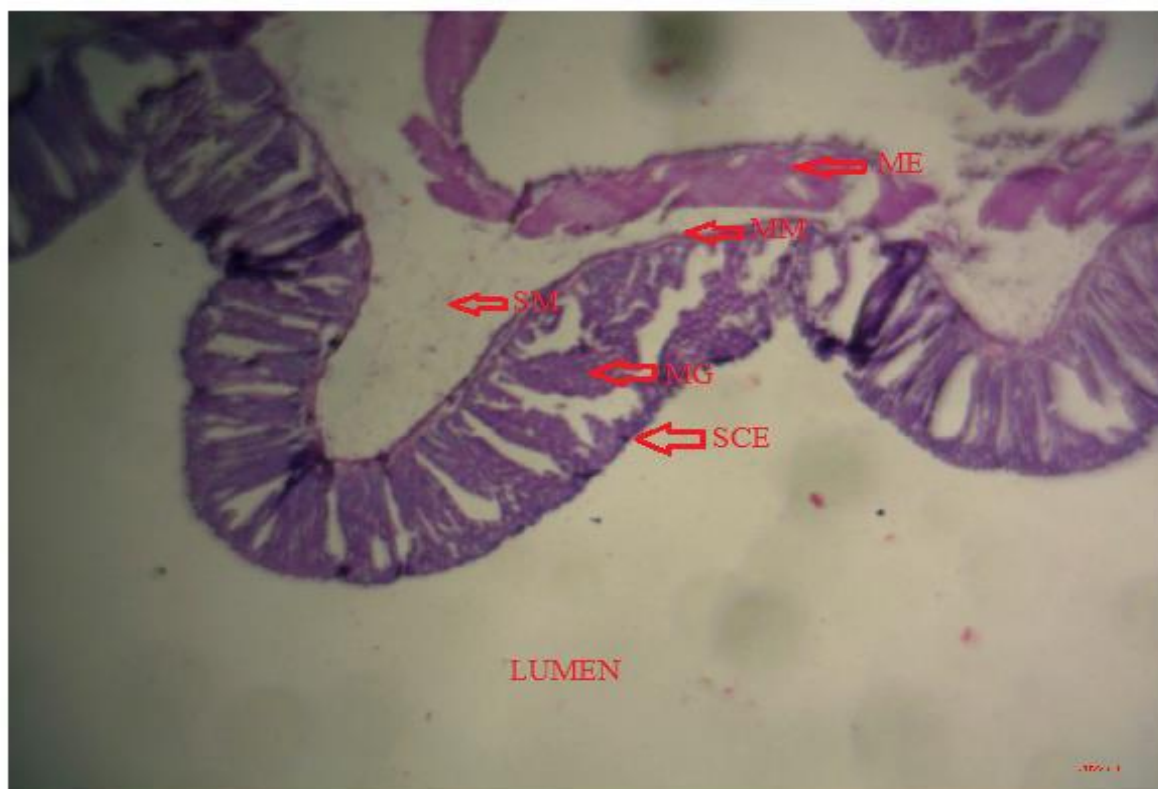**Investigation of effect of tadalafil on stress- induced ulcer**

This investigation was executed on six groups of adult male wistar rats of five rats in each group. The wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer induction with stress. The group pretreated with distilled water functioned as the negative control whereas the group pretreated with only Omeprazole served as a positive control. The wistar rats were sacrificed under diethyl ether anesthesia 4 hrs after ulcer generation. The gastric tissues obtained from each of the rats were correctly prepared for histological examination via a microscope. The representative photomicrographs achieved from the study are presented below, showing the

negative control and revealed some v-shaped histological abrasions, ulcer, indicated with arrows. Also, a figure obtained from the positive control (Omeprazole pretreated group) has normal histological. The subsequent two plates; figures 4.17 are the representative photomicrographs gotten from the wistar rats exposed to tadalafil 50mg/kg for 14 days. The two plates also depicted some distortions. More so, figures 4.18 which represent the tadalafil 100mg/kg pretreated group retained the appearance of a histologically normal gastric tissue.
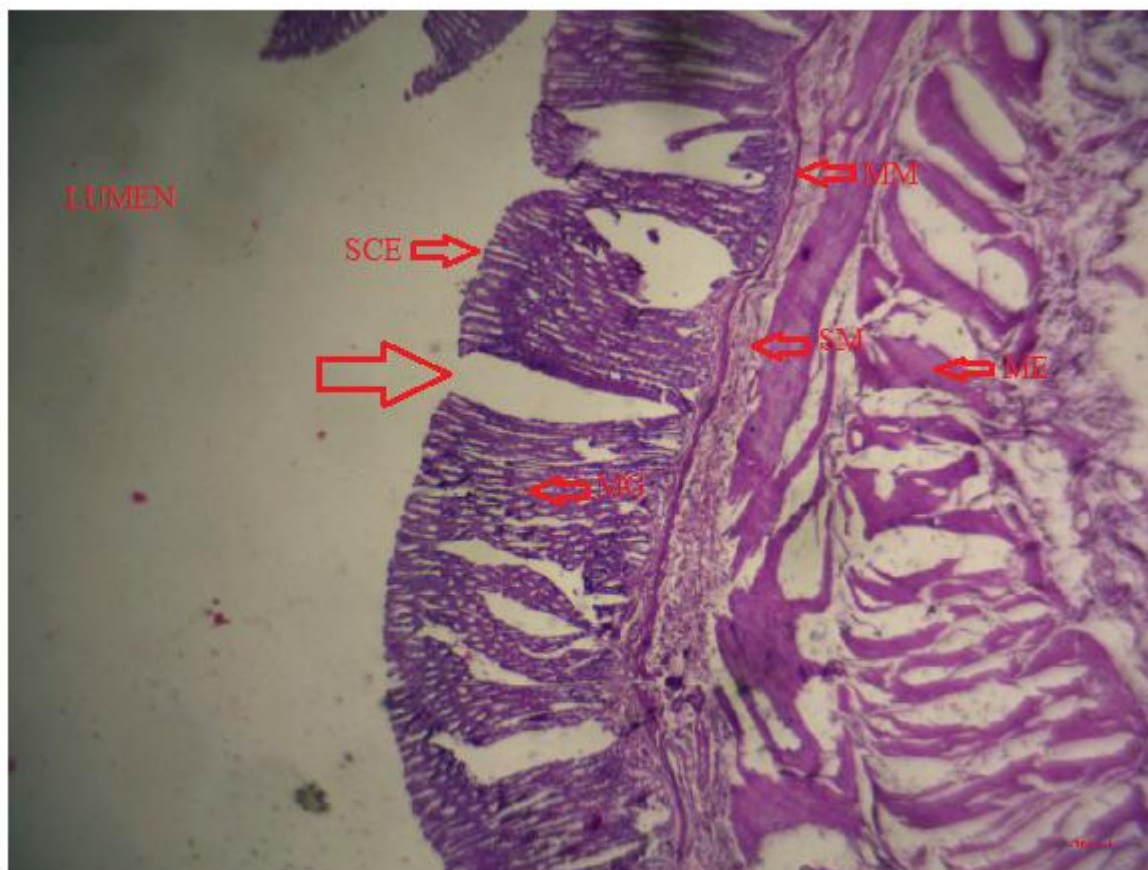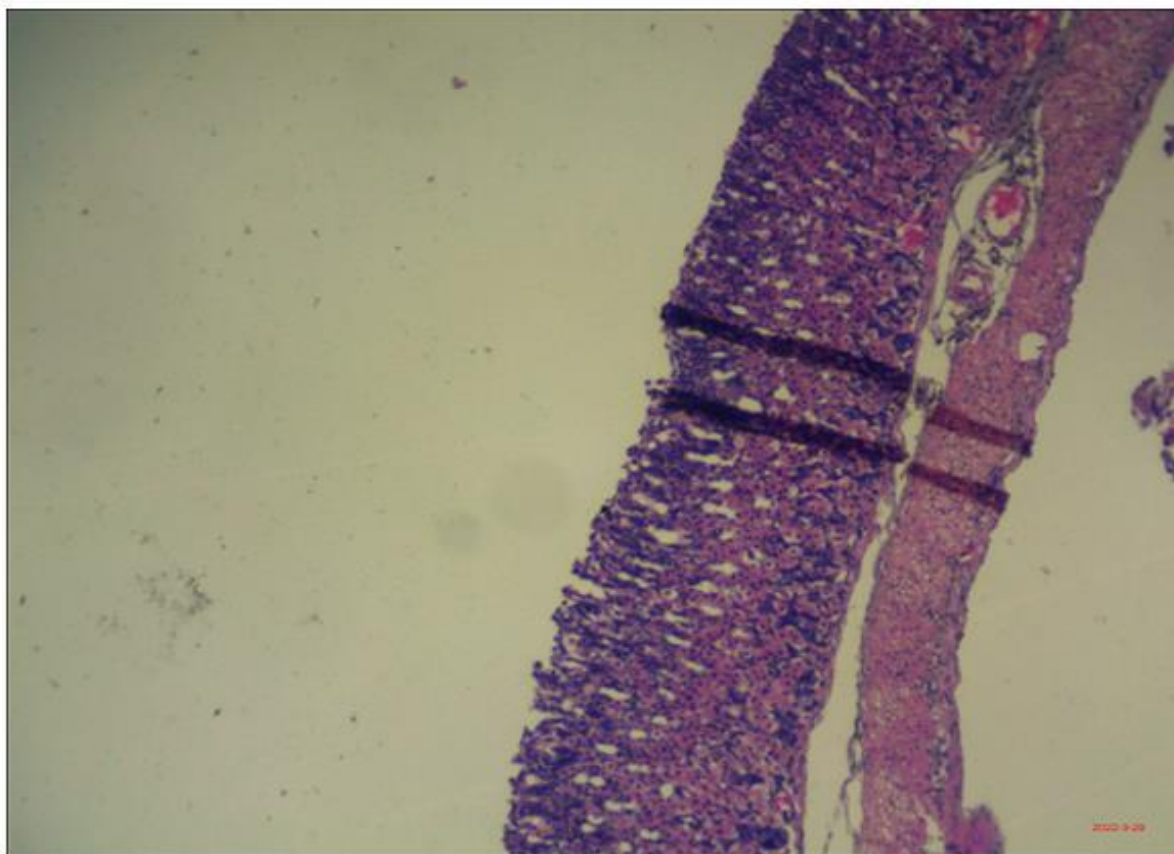

Photomicrograph of rat's stomach tissue showing the effect of stress (water immersion) on the stomach tissue through water immersion. The photomicrographs obtained from this group retained the features of histological **DISTORETED** stomach tissue which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification).
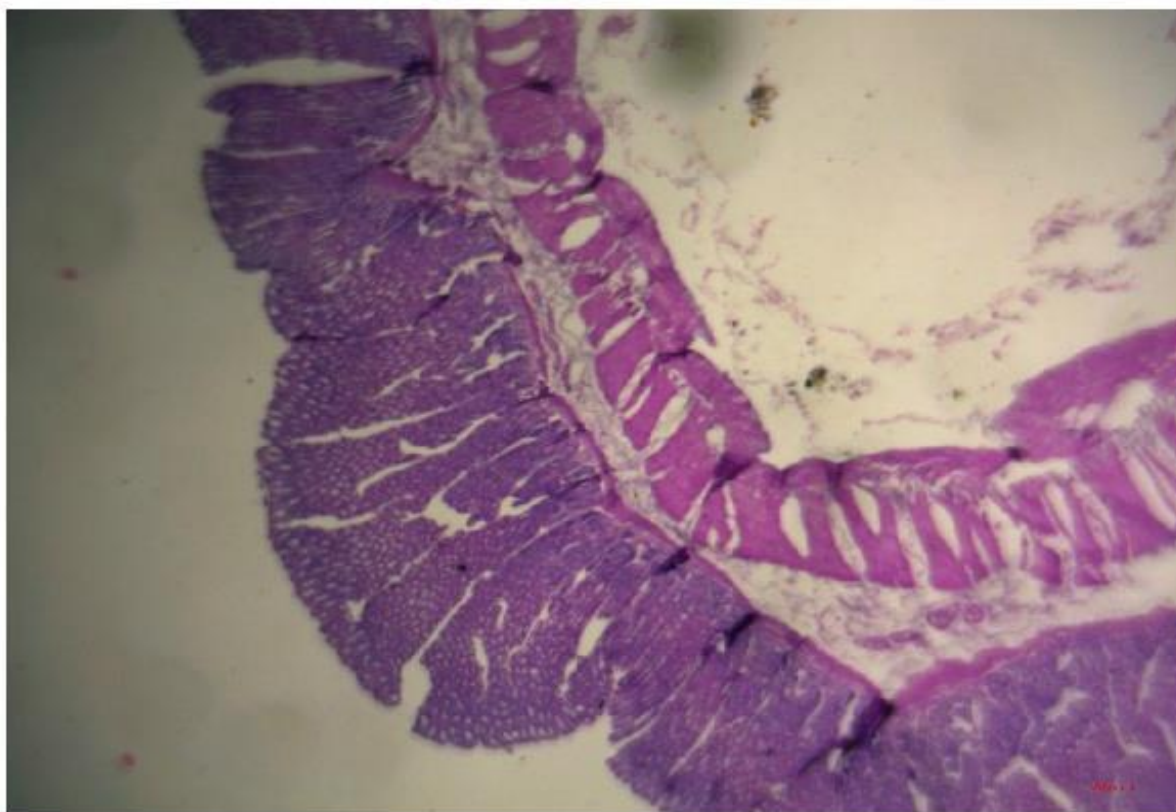
24

Photomicrograph of rat's stomach tissue showing the effect of omeprazole 1.75mg/kg on stress –induced ulcer. The photomicrographs obtained from this group has **normal** stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)

3

Photomicrograph of rat's stomach tissue showing the effect of omeprazole 1.75mg/kg on stress –induced ulcer. The photomicrographs obtained from this group has **normal** stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)
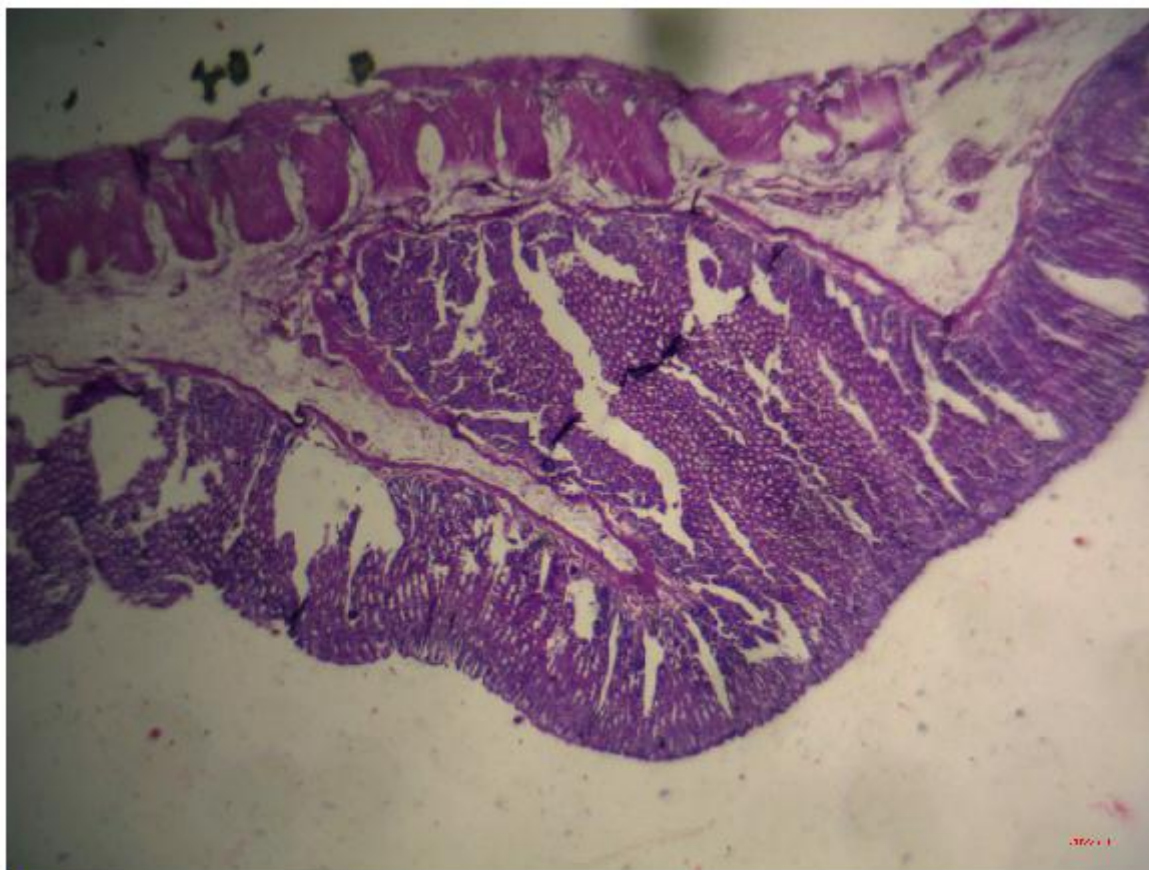
5

Photomicrograph of rat's stomach tissue showing the effect of tadalafil 50mg/kg on stress – induced ulcer. The photomicrographs obtained from this group has normal stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)

11

Photomicrograph of rat's stomach tissue showing the effect of tadalafil 100mg/kg on stress – induced ulcer. The photomicrographs obtained from this group has **normal** stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)

12

Photomicrograph of rat's stomach tissue showing the effect of tadalafil 50mg/kg / Omeprazole on Stress –induced ulcer. The photomicrographs obtained from this group has **normal** stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)

24

Photomicrograph of rat's stomach tissue showing the effect of tadalafil 100mg/kg and omeprazole on Stress –induced ulcer. The photomicrographs obtained from this group has **normal** stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)

Assessment of hematological effect of tadalafil in stress- induced ulcer

This study was performed on six groups of adult male wistar rats of 5 rats each. The Wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer induction with stress. The group pretreated with distilled water functioned as the negative control whereas the group pretreated with only Omeprazole served as a positive control. The wistar rats were sacrificed under chloroform anesthesia 4 hrs after ulcer generation. Blood samples obtained from the wistar rats were used to assess the hematological effect of tadalafil on the animals. The result obtained from this investigation is presented on table below

Hematological effect of tadalafil on Stress-induced Ulcer
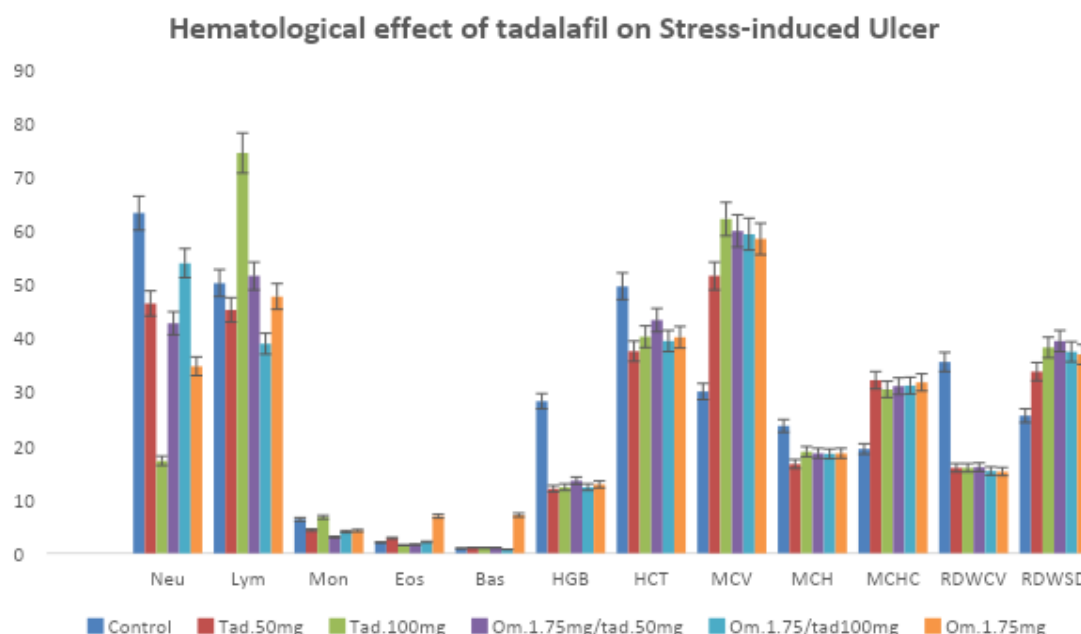
The ANOVA result revealed a significant alteration in the white blood cells counts with (P = 0.003 < 0.005). The Means comparison of hematological parameters gathered from different groups of the wistar rats equally demonstrated a significant change (P = 0.001< 0.005). From the analysis, ethanol produced the following effect on the hematology profile of the rats: The **neutrophils, and lymphocytes** has an elevated mean score values of the treated groups as compared to the control as compared to the control while **eosinophil, monocytes and basophils** which are component of WBC, has reduced mean score value compared to the standard. The **hemoglobin** level is low when compared to the control whereas the hematocrits /red cell component of the blood was highly elevated in comparison to the control.

Investigation of biochemical effect of tadalafil in stress induced ulcer

This investigation was executed on six groups of adult male wistar rats of 5 rats each. The Wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer induction with stress. The group pretreated with distilled water functioned as the negative control whereas the group pretreated with only Omeprazole served as a positive control. The wistar rats were sacrificed under chloroform anesthesia 4 hrs after ulcer generation. Blood samples obtained from the wistar rats were used to assess the biochemical effect of   tadalafil on the animals. The result obtained from this investigation is shown on below 4.45.
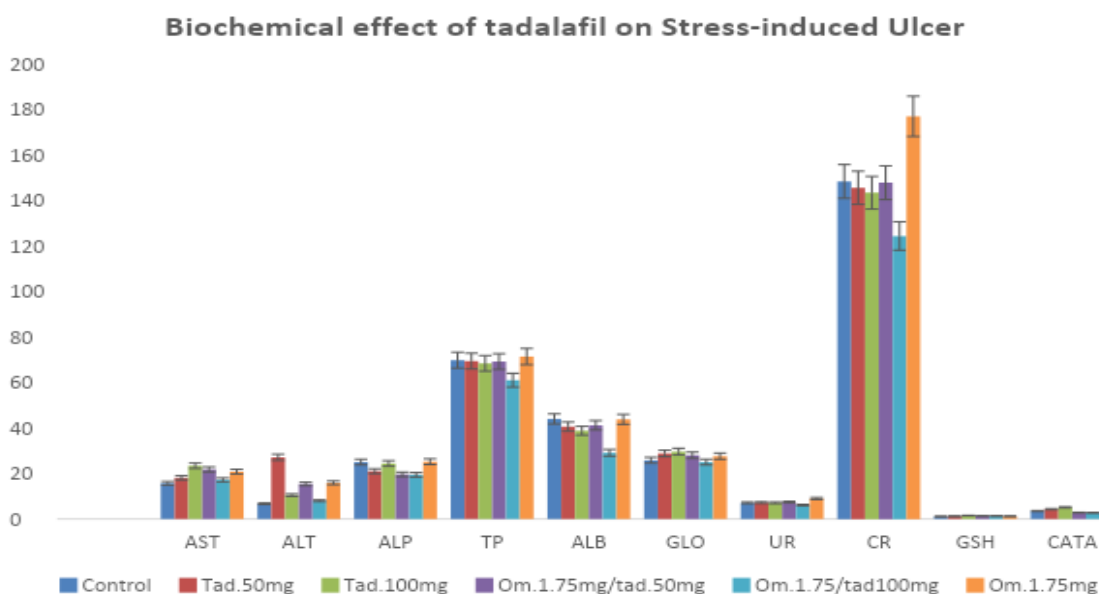
FIG 4.45: The ANOVA result showed a significant change in the means of ALT, GLO, UR and CR with P= 0.001, 0.001, 0.001, 0.001 and 0.001 < 0.05 respectively compared to the negative control. From the analysis, ethanol produced the following effect on biochemical parameters.

Aspartate transferase, AST: From the result chart, tadalafil 100mg/kg, omeprazole 1.75mg/kg +tadalafil 50mg/kg and omeprazole alone yielded a higher mean score value when compared to control. **Alanine transaminase, ALT**: The result demonstrated that tadalafil 50mg/kg produced a higher mean score value than other groups, followed by omeprazole 1.75mg/kg +tadalafil 50mg/kg and omeprazole alone. **Alanine phosphatase, ALP**: Here, tadalafil 100mg/kg and omeprazole standard drugs produced a lesser mean score value compared to control. **Total protein, TP**: From the bar chart, there was an elevated mean score value from all the groups with the standard drugs omeprazole yielding higher value. **Albumin, ALB**: The result obtained from the various group from the analysis induced an increase in the albumin mean score value with the standard drugs omeprazole showing a higher level of effect. **Globulin, GLO:** The result revealed that the various groups treated possess equipotent or similar mean score value which is comparable to control. **Urea, UR**: There was a reduced mean score value of urea level obtained by the various groups as compared to standard**. Creatinine, CR:** From the bar chart, there was an elevated mean score value from all the groups treated as compared to the standard with the highest peak. **Glutathione (GSH) and Catalase (CAT):** There was a reduced mean score value of glutathione and catalase level obtained by the various groups studied.

Examination of effect of tadalafil on lipid profile in stress induced ulcer

This investigation was performed on six groups of adult male wistar rats of 5 rats each. The wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer generation with ethanol. The group placed on only distilled water served as the negative control while the group pretreated with only omeprazole played the role of a positive control. The wistar rats were sacrificed under chloroform anesthesia 4 hours after ulcer induction. Blood samples obtained from members of various treatment groups of wistar rats were used to examine the biochemical effect of tadalafil on the rats.
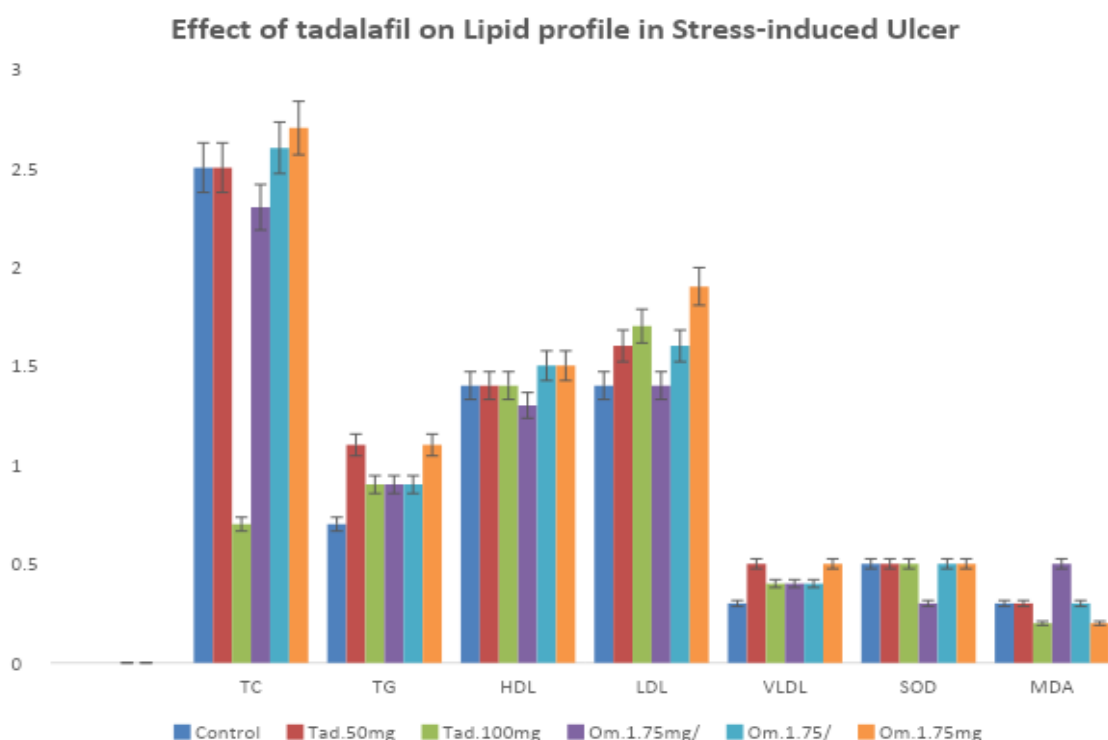

FIG4.48: The ANOVA result revealed a significant alterations in the means TG, LDL, VLDL and MDA, with P-value = 0.001, 0.001, 0.001, 0.001, < 0.05 correspondingly compared to the negative control. From the result obtained, the TC, TG, HDL, LDL and VLDL produced an extremely elevated mean score value with all the treated groups (NOTE: TG, total glyceride, TC, total cholesterol, HDL, high density lipoprotein, LDL, low density lipoprotein, VLDL, very low density lipoprotein, VLDL,)

DISCUSSION

Prior to generation of ulcers with stress model in the different treated groups of wistar rats, the animals were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for 14 days.

The mechanism behind stress gastric ulcer model involves release of histamine and elevation of gastric acid secretion and output with diminished mucus production and poor gastric blood flow (Dejban et al., 2020). This model is known to reduce the amount and content of mucus secretion and production through the diminished synthesis of its component, mucin. This makes this model important in investigating mucosal and cyto-protective properties (Kuna et al., 2019). The stress equally activates gastrointestinal motility with elevated vagal activity.

The reduction in ulcer index, elevation of preventive protective ratio and reduced mucus secretion seen from the result obtained, revealed that tadalafil has some element of protective activity on stress triggered on the mucosal wall by protecting the gastric mucosa layer and reduce gastric acid secretion.

Meanwhile, tadalafil acts by increasing the blood flow to gastrointestinal tissues following increased cGMP levels. As a phosphodiesterase inhibitor, tadalafil enhances the endogenous synthesis of NO (Ahmed, 2019) and this will invariably produce anti-inflammatory effects by enhancing cGMP production as shown in figure above. Tadalafil bring about production of more NO. NO is widely known as a vasodilator via its capacity to enhance blood flow in GIT tissues thereby reducing tissue breakdown.

The photomicrographs of the negative control (stress with cold-water immersion) revealed a v-shaped histological abrasions representing ulcers. This is a confirmation of the ulcerogenic activity of stress (Chuang et al., 2021). Also, plates obtained from the positive control (Omeprazole pretreated group) equally retained normal histological features. Photomicrographs pretreated with tadalafil 50mg/kg and 100mg/kg showed no histological disruption. The gastric mucosa integrity was maintained. This suggests that tadalafil exhibits cyto-protective effect especially at high dose (100mg/kg) (Abd Al Haleem et al., 2021).

Investigation of impact of tadalafil and omeprazole combination regimen on stress induced ulcer revealed photomicrographs from wistar rat gastric tissue pretreated with a combined

regimen of tadalafil 50mg/kg and Omeprazole 1.75mg/kg. The photomicrographs revealed the characteristics of a histologically normal wistar rat gastric tissue. Interestingly, Omeprazole / tadalafil 50mg/kg combination regimen completely protect the wistar rats against stress induced gastric ulceration.

It therefore suggests that even tadalafil at 50mg/kg has some intrinsic cyto-protecting activity which may not be observable in mono-therapy (Abd Al Haleem et al., 2021). However, tadalafil 50mg/kg synergizes with Omeprazole to produce a greater or a detectable gastro-protective effect. Also, photomicrographs gotten from the stomach tissue of wistar rats pretreated with tadalafil 100mg/kg / Omeprazole 1.75mg/kg combination treatment are equally without histological abrasions. This proposes that although tadalafil exhibits its observable cyto-protective effect at high dose (100mg/kg) when used a monotherapy, in a combination regimen with Omeprazole satisfactory gastro-protective effect can be produced even with tad 50mg/kg instead of tad 100mg/kg.

Assessment of **hematological effect of tadalafil in stress induced** ulcer exposed the result obtained from this investigation. The result revealed a significant alteration in the white blood cells counts for the group pretreated with only distilled water (negative control). The Means comparison of hematological parameters gathered from different treatment groups of the wistar rats equally demonstrated a significant change in the white blood cell count and red blood cell count. The significant elevation in the white blood cell count of the negative control appears to be a validation that stress really is an ulcerogenic substance; the increase in white blood cell count is a normal system reaction to injury or inflammation. The effect on red blood cell was insignificant, implying that each or both of the drugs (tadalafil and Omeprazole have no direct impact on erythrocyte (Wang et al., 2017).

Investigation of **biochemical effect of tadalafil in stress induced ulcer** exposed the significant changes in the means of AST, ALT, GLO, UR and CR respectively compared to the negative control. The statistics points to hepatotoxic and nephrotoxic effects of the test substances (tadalafil and Omeprazole).

However, examination of effect **of tadalafil on lipid profile in stress induced ulcer** was carried out .The result obtained from this investigation revealed significant alterations in the means TG, LDL, VLDL and MDA, correspondingly compared to the negative control. The significant elevation in the total protein may be an indication of inflammatory state following

the ulcer. But a significant increase in the plasma level of LDL caused by the tadalafil appears to command some apprehensions.

Investigation on the effect of tadalafil both single administraion50mg/kg and 100mg/kg and the various combination with standard drug omeprazole on the photomicrographs, revealed that the various combinations has protective effects on the gastric mucosa wall by producing **normal** stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the sub-mucosal later. This effect is produced by the ethanol model, reserpine model and stress-induced model. Tadalafil is a phoshodiesterase V inhibitor that act by increasing blood flow to tissues in response to increased cGMP levels. They are also Nitric Oxide (NO) donors. NO is a potent vasodilator which increases blood flow in tissues where present thus preventing tissue damage (Ajiboye and Oluwole, 2012).

SUMMARY OF FINDINGS
The study shows that Omeprazole pretreatment can offer full gastro-protection against stress linked ulcer. More so, tadalafil mono-therapy and tadalafil/Omeprazole combination pre-therapy can also provide a satisfactory gastro-protection against stress-related gastric ulcer.

More so, tadalafil100mg/kg and both tad 50mg/k and tad 100mg/kg /Omeprazole combined treatment regimens all showed full gastro-protective effects against stress-linked gastric ulcer.

The result obtained from the effect of tadalafil on hematological parameters on ethanol induced ulcer investigation documented a significant increase in the white blood cell counts. The result obtained from the investigation of the impact of tadalafil on the hematological parameters of stress induced wistar rats model of ulcer recorded a significant elevation in the white blood cells counts.

Furthermore, the result gathered from the study for determination of activity of tadalafil and tadalafil Omeprazole combined treatment also revealed a significant alteration in the white blood cells counts. Again, the result also recorded significant different in AST, ALT, GLO,

UR and CR respectively compared to the negative control in stress induced wistar rats gastric ulcer model.

5.1 Limitations

This study has triggered the urge for realization of the time/effect relationship of tadalafil only and tadalafil/Omeprazole combination pretreatment on different gastric ulcer models. It would have been interesting to know if tadalafil/Omeprazole combination pretreatment can provide the required gastro protective effect within a shorter course than either of the agents used alone. Nevertheless, this study failed to consider this in its design.

CONCLUSION

Tadalafil at the doses of 50 and 100mg/kg used alone or in combination with Omeprazole 1.75mg/kg possess satisfactory gastro-protective impact against stress-induced gastric ulcers. Nevertheless, these pre-treatment regimens (tadalafil and Omeprazole appear to be nephrotoxic and hepatotoxic as evidenced by their impacts on the liver enzymes and other biochemical parameters.

Recommendations

Tadalafil at the doses of 50 and 100mg/kg may be used alone or in combination with Omeprazole to safeguard people who are at risk against gastric ulcer.There is an important demand for agents that can protect the liver and kidney from the deleterious effect of tadalafil and Omeprazole. A further study shall reveal the time/effect relationship of tad/omeprazole combined therapy compared to either drug used alone.

Contributions to Knowledge

Tadalafil at the doses of 50 and 100mg/kg pretreatment can guard against stress induced ulcer in wistar rats. The alterations in leucocytes count following tadalafil and Omeprazole treatment may not be a direct impact of the test drugs but part of the normal gastrointestinal tract protective mechanisms against some deleterious activities of the drug at other regions. Tadalafil alone or in combination with Omeprazole seem to be nephrotoxic and hepatotoxic. Tadalafil alone or in combination with Omeprazole appear to have insignificant effect on lipid profile in wistar rats.

Conflict of Interest

There was no conflict of interest among the authors while carrying out this research.

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