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Effects of Aqueous Extract of *Blighia sapida* Leaves on Ethanol-Induced Gastric Ulcer in Male Wistar Rats.

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Abstract

Blighia sapida have shown promising ameliorative effect in folkloric treatment of gastric ulcer with little or no scientific basis. This study investigated the effect of Aqueous Extract of *Blighia sapida* Leaves (AEBSL) on ethanol-induced gastric ulcer in male Wistar rats. Sixty-four male Wistar rats, weighing averagely 200g, were randomly selected into eight groups; Group I (Control) received distilled water, Group II received 200 mg/kg body weight of AEBSL only, Group III received cimetidine 50 mg/kg.b.w, Group IV (ulcerated untreated) orally pretreated with 50% absolute ethanol for gastric ulceration. Groups V, VI and VII were ulcerated groups treated with AEBSL at 100, 200 and 400 mg/kg b.w and Group VIII, ulcerated group treated with cimetidine at 50 mg/kg b.w. Ulcer index, hematological and antioxidant parameters were determined using standard methods after 21 days of treatments.Results showed that pH level of gastric secretion, total protein, bicarbonate, Red Blood Cell and Hemoglobin concentrations were significantly ($p<0.05$) decreased in ulcerated untreated animals (Group IV) compared with control and extract only group. However, the effects were reversed in a concentration dependent manner in treated Groups (V, VI, VII and VIII). Also, Superoxide dismutase and Catalase activities as well as Reduced glutathione (GSH) concentration were significantly ($p<0.05$) decreased in ulcerated untreated animals, while treatment Groups V, VI, VII and VIII significantly ($p<0.05$) elevated the enzymes activities and GSH concentrations in a concentration dependent manner. Alkaline phosphatase and Pepsin activities as well as ulcer Index (UI), Free and Total acidity, White Blood Cell, Platelet, Nitric oxide and Malondialdehyde concentrations were significantly ($p<0.05$) increased in ulcerated untreated animals, while they were restored near to control level in the treated Groups (V, VI, VII and VIII) at different concentrations .Cimetidine, at 50 mg/kg b. w exhibits similar ameliorative effect comparably with AEBSL at 200 mg/kg b. w, while the extract showed antioxidant, anti-inflammatory and ameliorative effects on ethanol-induced gastric ulcer in rats.

Keywords: Ameliorative, Antioxidant, Anti-inflammatory, *Blighia sapida*, Cimetidine, Gastric ulcer.

Introduction

Gastric ulcer is a common global health problem both in terms of morbidity and mortality, occurring as a result of imbalance between aggressive and defensive factors of the gastric mucosa. The origin of gastric ulceration is usually multifactorial and some predisposing factors have been implicated. Some of these factors in ulceration includes, smoking, consumption of alcohol, diet irregularity (Gopinathan *and Nija*, 2014), duration of starvation, nature of ingested food, bile reflux, reduced mucosal resistance, gastric mucosal blood flow alteration, disruption of gastric mucosal barrier by stress, alkaline mucosal bicarbonate reduction and decreased mucus secretion, over dosage and prolonged administration of Non- Steroidal Anti-Inflammatory drugs (NSAIDs) (Suerbaum and Michetti, 2002; Berardi and Welage, 2005). Gastric ulcer can be defined as an erosion that occurred in the lining of the stomach caused by the disruption of the gastric mucosal defense systems and also, an imbalance between the rate of secretion of gastric juice and the degree of protection afforded by the gastro- stomach mucosal barrier as well as the neutralization of the gastric acid by stomach juice (Gopinathan *and Nija* , 2014). Gastric ulcer as a widespread disorder also occurred when the gastric mucosa is constantly exposed to potentially injurious agents such as bacterial products like Helicobacter pylori and drugs (Non-Steroidal Anti-Inflammatory Drugs). Pathogenesis of gastric ulcer may also be traced to increased gastric acid and pepsin secretion, prostaglandin synthesis inhibition, reduced gastric blood flow and gastric motility (Toma *et al*., 2005). Ulcer incidence varies with the type, gender and age and when it is located in the stomach it is called gastric ulcer while in the duodenum region it is called duodenal ulcer and together they are called peptic ulcer.

Persistent decrease of gastric acid production and also, stimulation of processes that causes re-enforcement of gastric mucosal production has been the major approaches to cure, prevent and ameliorates peptic ulcer (Gopinathan *and Rameela* , 2014). In any form of etiology, an increase in aggressive factors or a decrease in defensive factors will result in loss of mucosal integrity causing ulceration (Alan, 2000). Gastric ulcers are most of the time characterized by pain and this is common in older age people. Other symptoms may include nausea, vomiting and weight loss (Avinash *et al*., 2011). The most common symptoms of duodenal ulcer are waking at night with severe upper abdominal pain that may improve with eating .However, in gastric ulcer, the pain may get worsen with eating (Rao *et al.,* 2014). Other symptoms of gastric ulcer include belching, vomiting, loss of weight and poor appetite, while complications of ulceration may include bleeding, perforation, and stomach blockage with bleeding occurring in about 15% of the affected people (Milosavljevic *et al*., 2011).

Treatment of ulcer varies with degrees and different medications as younger patients having ulcer-like signs and symptoms are sometimes treated with antacids or H_2 antagonists like Cimetidine, Ranitidine and Omeprazole, before endoscopy is carried out, while People who are placed on Non-Steroidal Anti-Inflammatories (NSAIDs) drugs may also be prescribed a prostaglandin analogue (misoprostol) to help prevent ulcers (Najm, 2011). Acid reducing medication: H_2 antagonists or Proton-pump inhibitors reduces the amount of acid present in the stomach, thereby helping with healing of ulcers and when Helicobacter Pylori infection is present, the most effective treatments for elimination of the bacteria and treatment of ulcer are combinations of two antibiotics (e.g. clarithromycin, amoxicillin, tetracycline, metronidazole) and a proton-pump inhibitor (PPI), sometimes together with a bismuth compound, while the effective first-line therapy mostly use for uncomplicated ulcer cases would be the combination of amoxicillin, metronidazole and pantoprazole (PPI) (Najm, 2011). However treatment of ulcer is usually overshadowed by different side effects arising from the drugs used. For example, H_2-receptor antagonists (e.g. cimetidine) may cause headache and Itching as reported by Reilly, (1999). Management, prevention and treatment of diseases related to oxidative stress and inflammation such as ulcer has been carried out using orthodox drugs with much side effects hence, the need to source for natural products of plant origin which are believed to have little or no side effect and equally validate some of the claimed medicinal values by the local herbal practitioners.

Medicinal plants constitute an effective source of modern medicine in ulcer treatment, and have acquired greater appreciation, due to their little side effects and higher efficacy. More than 80% of the world's population depends on traditional herbal medicine as their primary healthcare (Sofowora 1982; Vijayan *et al.*, 2007), as *modern drugs employed in orthodox medicine have their origin from plants (Sofowora, 2001). According to Bubayero, in 1998 and Sofowora, (2001), 65-70% of the Nigerian populationdo patronize traditional medicine practitioners with their various forms and different methods of treatment.*

Blighia sapida (Ackee) *is a plant that belongs to the family of Sapindaceae. It is mostly found in Western Tropical Africa and was introduced into Jamaica in the late 18th century. The plant has extended to other regions of tropical America but still more widely cultivated in Jamaica than elsewhere. B. sapida in West Africa expands from Senegal to Gabon. It is also cultivated in India and tropical America. B. sapida is well distributed throughout the regions of Nigeria and also, found in drier forest of the savannah region with various applications in folk medicine (Esuoso and Odetokun, 2005). B. sapida, is noted for its highly prominent reddish fruits existing in different species, which include Blighia sapida, Blighia welwitschii and Blighia unijugata. B. sapida is a common tree usually cultivated for provision of shade from hot sun. The plant is about 25m high and 2.5m in girth, having heavy evergreen crown. It has a pale brown bark, while its leave has a stout stalk of about 5–23cm long. The leaflets, 5-15cm long by 3.5-7.5cm broad, are obovate with its lowest part almost circular and very close to the base of the leaf-stalk. The flowering of the plant begins between October-March (Esuoso and Odetokun, 2005). It has a small flower that is greenish white in colour. The fruits start appearing between the month of March to September. The fruits are obovoid and about 3.5-6cm long by 3- 5cm in diameter, bright red to yellowish in colour and often by itself splits open on the tree. The seed in the plant is covered with a glossy testa and about 2.5cm long by 2cm broad, while the aril (the edible part of the fruit) is pale yellow or cream coloured, wrinkled and about 2cm long .*

Extracts of Blighia sapida plant are commonly employed in folk medicine to treat different types of diseases, especially in developing countries, the bark pulp of the plant is used as liniment for oedema intercostal pains in Ivory Coast. The pulp is employed as eye drop in ophthalmic and conjunctivitis (Irvin, 1965). In Brazil, repeated little doses of aqueous extract of the seed of B. sapida have been administered to expel parasites. In Colombia, the leaves and bark of the plant are considered stomachic (Morton, 1987). Okogun, (1996), revealed that, the bark can be powdered and grounded with capsicum and rubbed on the body to function as a stimulant while the

dried husks ashes and seeds are used in the soap preparation, due to their richness in potash. Also, different parts of B. sapida plants are employed either alone or in combination for the treatment of various diseases like psychosis, cancer, gonorrhea, stomach ache, hernia, backache, diarrhoea and constipation (Okogun, 1996; Antwi, 2009). It is not astonishing that medicinal plants are mostly employed in the treatment, management and prevention of various ailments. However the process of isolation and elucidation of chemical structures embedded in plant extracts may not be too significant, until proper and suitable bioassays are employed to establish the biological activities exhibited by the plant extract and validate their medicinal claims, hence the basis for this study.

Materials and Method

Chemicals and Reagents.

Laboratory assay kits for determination of various biochemical indices and other chemicals such as Casein, hydrogen peroxide, Trichloroacetic acid, Ketamine hydrochloride, adrenaline were purchased from sigma Chemical Company St. Louis, MD, USA. All other chemicals and reagents for the study were of analytical grades while Cimetidine tablet , a product of bond chemical pharmaceutical industry, Awe, Oyo state, was purchased from Akol pharmacy Ogbomoso.

Plant material and authentication

Blighia sapida leaves were collected from the school farm of Ladoke Akintola University of Technology Ogbomoso, Nigeria , authenticated by plant Taxonomist at botany unit of the Department of Pure and Applied Biology of the same institution with herbarium specimen voucher number (LHO 465) deposited.

Preparation of Aqueous extract of *B. sapida* leaves

Dried powdery leaves of *Blighia Sapida* (500g) was soaked in 5000ml of distilled water, left for 3days with intermittent stirring, filtered using whatmann filter paper number 1, and the filtrate was concentrated in rotary evaporator at 35^0C and later dried using fixed drier. Percentage yield was 13.69% and part of this were used to prepare the aqueous extract at different concentrations.

Experimental animals and design.

Sixty-four Male Wistar rats weighing between 180-200g, were obtained from the animal house of the Department of Animal Production and Health, Faculty of Agriculture, Ladoke Akintola University of Technology Ogbomoso, Nigeria. They were housed in a cage under standard laboratory condition. The rats were acclimatized to laboratory condition for 14days before the commencement of the experiment. All procedures involving handling of laboratory animal were followed in accordance with the international standard on animal ethics and regulations as made available in our laboratories.

The rats were randomly divided into eight groups of eight rats per group and treated for twenty one days as follows:

GROUP I (Negative Control): Rats received standard feed and distilled water only.

GROUP II (200mg/kg.bw. extract only): Rats received 200mg/kg body weight (bw) of extract only.

GROUP III (50mg/kg. b.w. Cimetidine only): Rats received 50mg/kg body weight (bw) of cimetidine.

GROUP IV (Ulcerated untreated): Ulcer was induced with oral pretreatment of 50% ethanol (10ml/kg. bw.) once.

GROUP V (Ulcerated treated with 100mg/kg. b.w. extract): After ulcer induction, animals were treated with 100mg/kg. bw. Extract.

GROUP VI (Ulcerated treated with 200mg/kg. bw. extract): After ulcer induction, animals were treated with 200mg/kg. bw. Extract.

GROUP VII (Ulcerated treated with 400mg/kg. bw. extract): After ulcer induction, animals were treated with 400mg/kg. bw. Extract .

GROUP VIII (Ulcerated treated with 50mg/kg. bw. Cimetidine): After ulcer induction, animals were treated with 50mg/kg. bw. Cimetidine.

Induction of Ulcer

Throughout the experimental period, the animals were given standard rat pellets and distilled water *ad libitum*, however the groups to be induced with ulcer (IV, V, VI, VII and VIII) were deprived of food for 24 hours with access to drinking water only before ulcer induction as this is to ensure total emptiness of gastric contents for effective ulceration. Gastric ulcer in albino Wistar rats was induced by orogastric intubation of 70% absolute ethanol (10ml/kg. bw.) (Gopinathan *and Rameela* , 2014). After an hour of ethanol induction, an animal in each group were sacrificed, and the stomach was opened to assessed for ulceration. After twenty one days of treatment based on the various groups treatment , animals were fasted for 24 hours before the sacrifice.

Induction of gastric juice secretion

During the fasting period, the animals were placed on a raised wire mesh to avoid Coprophagy (Basso *et al.*, 1983).The animals were anaesthetized using Ketamine Hydrochloride (50mg/kg I.P) (Mabrouk *et al.*, 2009). The abdomen was opened without causing any damage to its blood supply, an incision of 1cm long was made in the abdomen just below the sternum and the stomach was exposed. A thread was passed around the pyloric sphincter and applied a tight knot using the thread, the stomach was injected with 3ml of distilled water , abdominal wall was sutured using chromic catgut and nylon filament . All the animals recovered after 1 hour and were left for 4hrs after which they were anaesthetized using chloroform and latter sacrificed. The blood samples were collected using cardiac puncture for hematological study, the stomach was removed, opened at the greater curvature, gastric juice was collected in a graduated centrifuge tube and stomach were harvested, washed carefully in 0.9% Normal saline. Both the fundic and glandular portion of the stomach were homogenized in ice using homogenizer for Biochemical analysis.

Preparation of 70% ethanol from absolute ethanol (99.9%)

Stock solution of 70% ethanol was prepared from absolute (99.9%) Ethanol by measuring out 70ml absolute ethanol with measuring cylinder and making it up to 100ml with distilled water.

Preparation of serum for hematological and biochemical analysis.

The blood was collected via cardiac puncture using a 5ml needle and syringe. 1ml of whole blood from each animal was collected for haematological analysis while the rest was transferred into plain sample bottles, centrifuged at 4000rpm for 5 minutes to collect the serum which was stored in a freezer for further biochemical analysis.

Collection of gastric juice

The gastric juice from the stomach was collected carefully in centrifuge tube and centrifuged at 1000 rpm for 10 min, decanted into another tube for the determination of free and total acidity as well as proteolytic activity of Pepsin.

Histological study

Stomach Specimens were collected from rats of the various groups at the end of the experimental period, fixed in 10% Buffered formosaline (pH 7.0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 microns thickness sections prepared and stained with heamatoxylin and eosin for examining both the fundic and glandular parts of the stomach (Bancroft and Gamble, 2008).

Determination of pH, Free and Total acidity of gastric secretion

The pH level of the gastric secretion was measured using pH meter while the free and total acidity of the gastric secretion were determined by the procedure of Janardhanan *et al., (2012).*

Determination of ulcer score, Ulcer index and Percentage Amelioration.

The stomach of the animals was removed, opened along the greater curvature, using dissecting lens, the ulcer was scored based on the number and severity of the ulcers and these were calculated according to Nwafor *et al.,* 2000, while the percentage amelioration was determined according to the method described by Dekanski *et al.,* 1975.

Determination of haematological indices and electrolytes

Determination of Red blood cells (RBCs), count and Haemoglobin (Hb), were carried out using SYSMEX KX-21N hematology analyzer machine while serum Bicarbonate (HCO_3^-) was determined using Ion Selective Electrode Machine.

Determination of proteolytic activity of Pepsin enzyme

This was determined by the method of Hawk *et al.,* (1960) ,in which 0.2ml of centrifuged gastric juice was added to 3ml of casein (3% for each rat test and blank), as 10ml of 6% Trichloroacetic acid was added to blank to stop enzyme activity. Both blank and test tubes were incubated in water bath at temperature 37^oC for 30 minutes. Proteolytic activity was determined spectrophotometrically by optical density measured at 280nm wavelength.

Determination of protein concentration in gastric tissue.

The protein concentration of the harvested gastric tissue was determined by the method that was developed by Bradford, (1976), and reported by Dewi and colleagues (2012).

Determination of lipid peroxidation and antioxidant indices

The homogenized gastric tissues (stomach) was centrifuged at 4000 revolution per minutes for 10 minutes and the supernatant was collected for biochemical analysis. Malondialdehyde (MDA) concentration was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation as described by Rice – Evans *et al.,* (1986). Reduced glutathione (GSH) concentration was determined by the method described by Anderson ,(1985).The antioxidant enzyme Superoxide dismutase (SOD) activity was determined by the methods of Mistra and Fridovich (1972) and that of Catalase activity was determined by the method developed by Sinha, (1972).

Determination of inflammatory indices

Nitric oxide (NO) concentration was determined according to the method described by Arias-Negrete et al.,(2004). White blood cell count (WBC) and Platelet concentration were determined by SYSMEX KX-21N hematology analyzer.

Statistical analysis.

All data were presented as Mean± Standard error of mean (Mean ± SEM). Statistical analysis was performed using Graph pad prism 5. One way analysis of Variance (ANOVA) was used for the comparison of relative expression levels for different groups followed by Turkey Post Hoc test.

Results

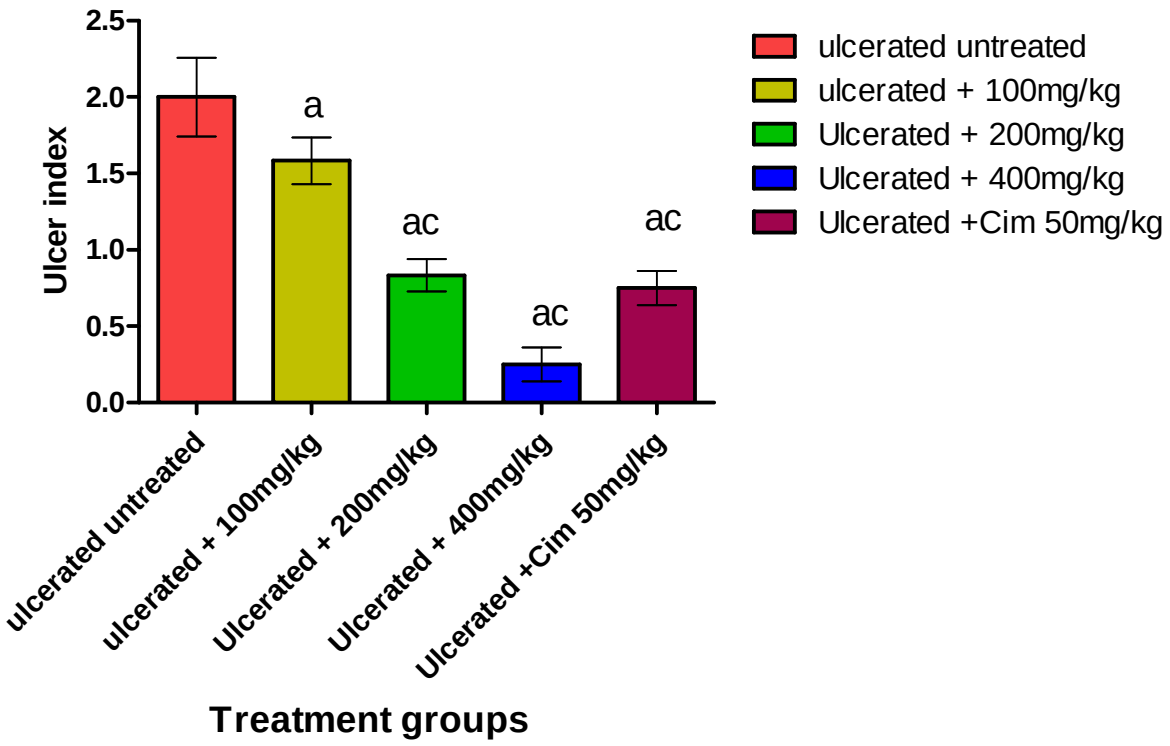

Fig. 1: Ulcer index in the stomach (gastric Tissue) of Various treatment groups

Level of significance was taken at (P< 0.05) for 6 rats per group

a Represents Significant decrease at p< 0.05 compared with control

c Represents significant decrease compared with 100mg/kg body weight of extract.

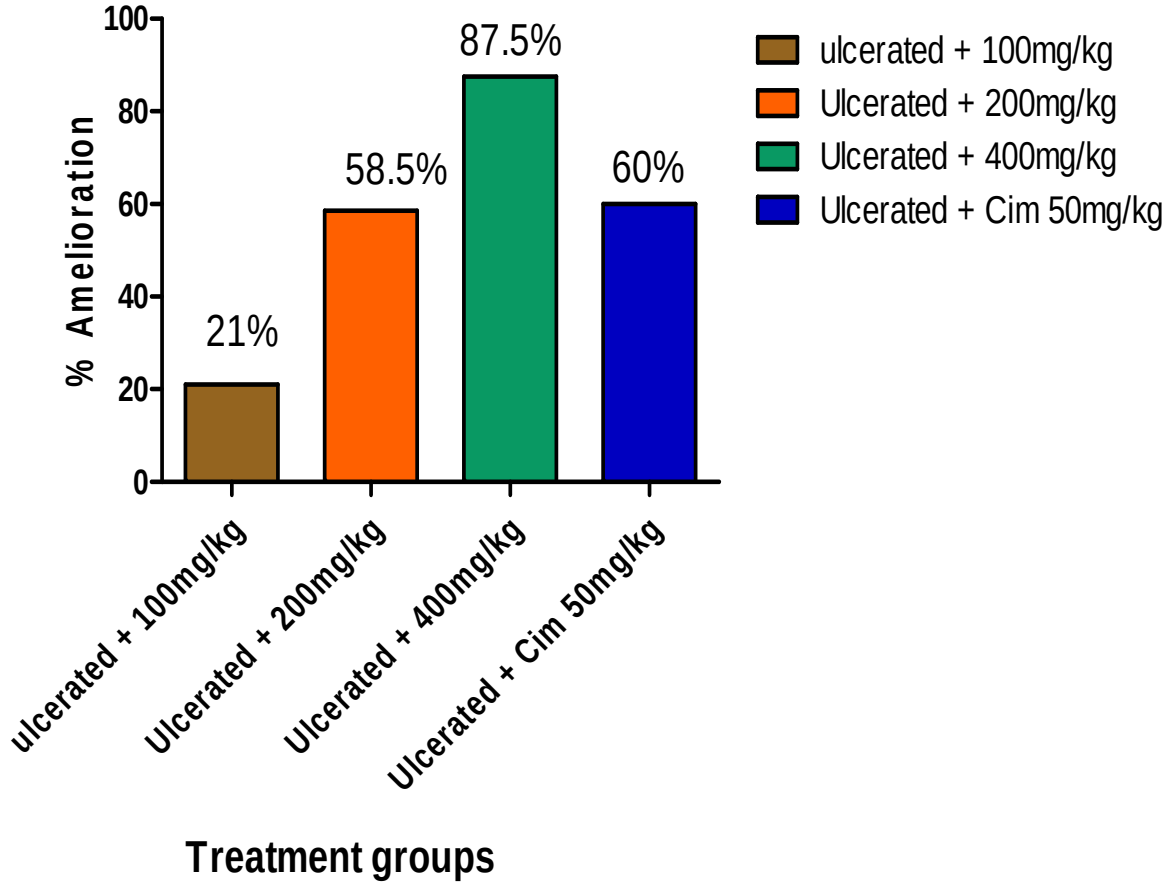

Fig. 2: Percentage (%) amelioration by different concentrations of aqueous extract of *B.sapida* leaves

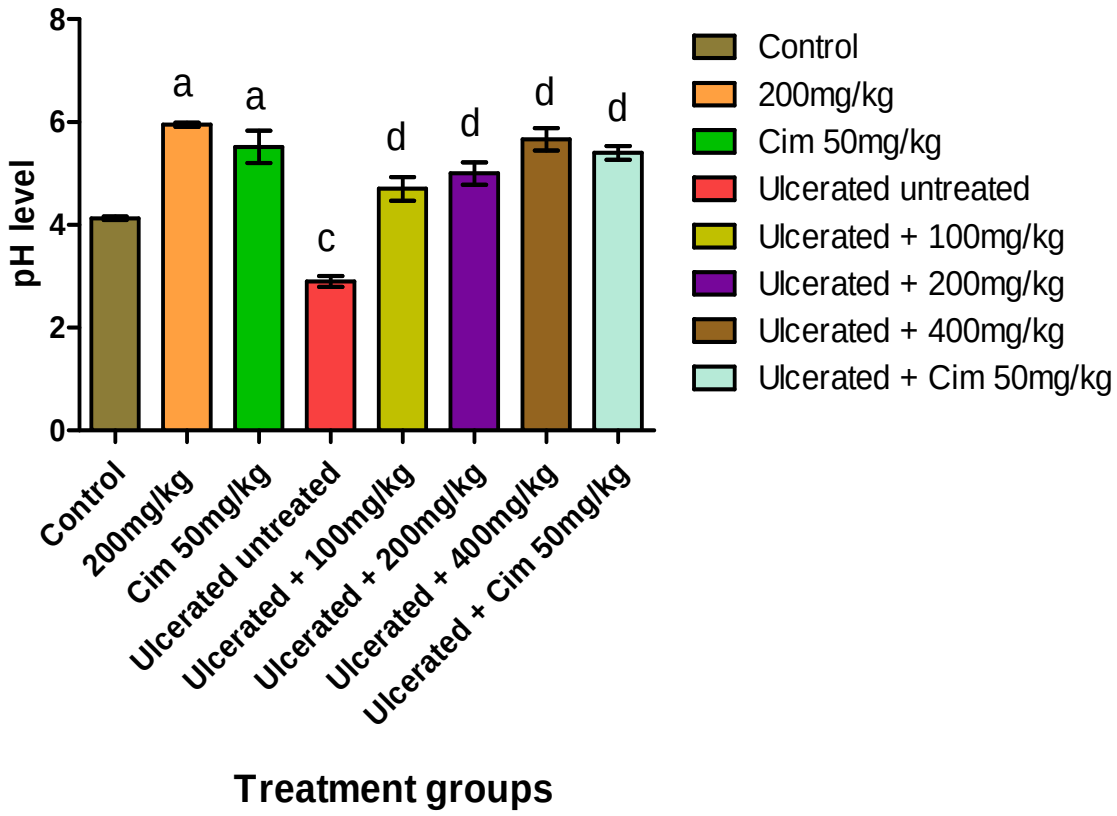

Fig. 3: pH level of gastric juice in various treatment groups.

Level of Significance was taken at (P <0.05) for six rats per group.

a Represents Significant increase at (p< 0.05), compared with control

c Represents Significant decrease at (p < 0.05), compared with control and extract only groups.

d Represents Significant increase compared with Ulcerated untreated group

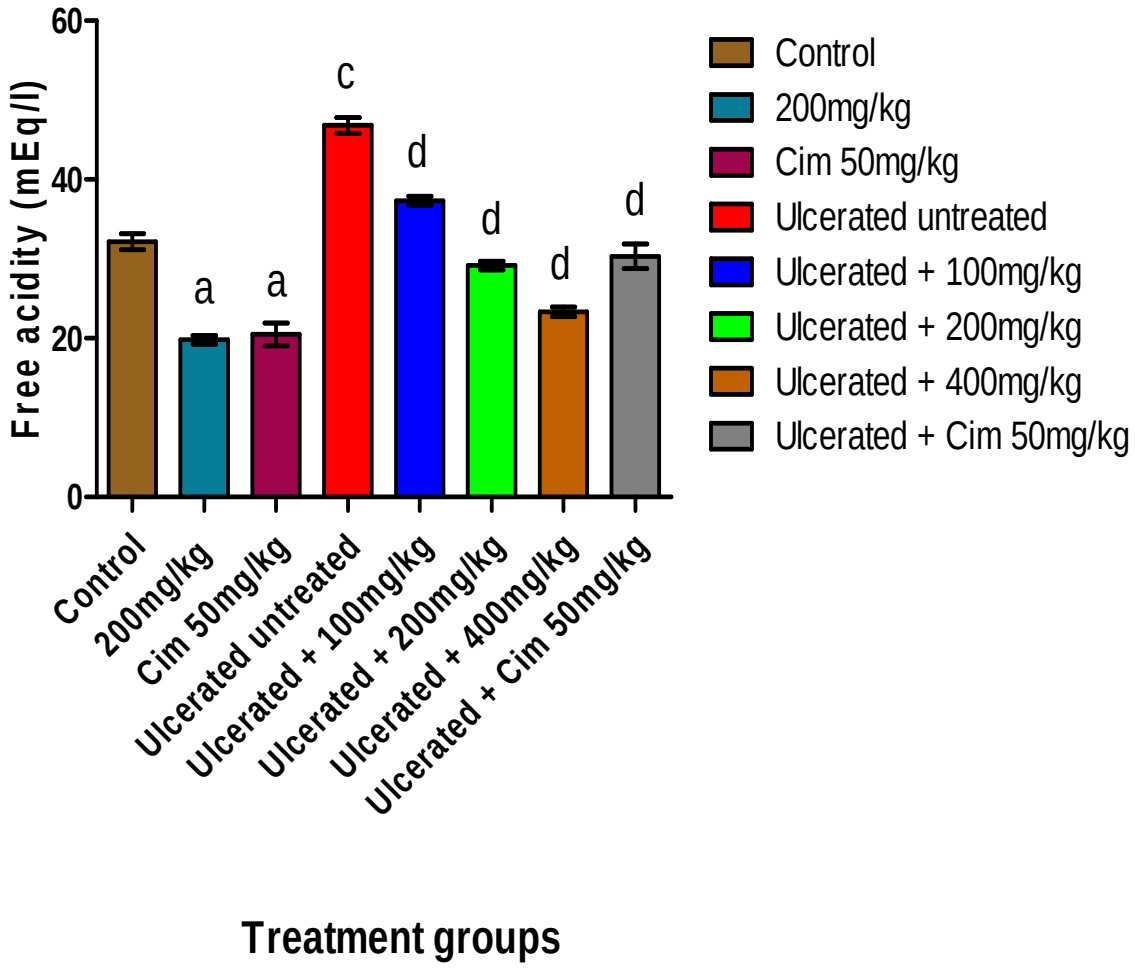

Fig. 4: Free acidity concentration in gastric juice of various treatment groups.

Level of significance was taken at P<0.05 for six rats per group

a Represents significant(P<0.05) decrease compared with control

c Represents significant increase compared with control and extract only groups

d Represents significant decrease compared with ulcerated untreated group

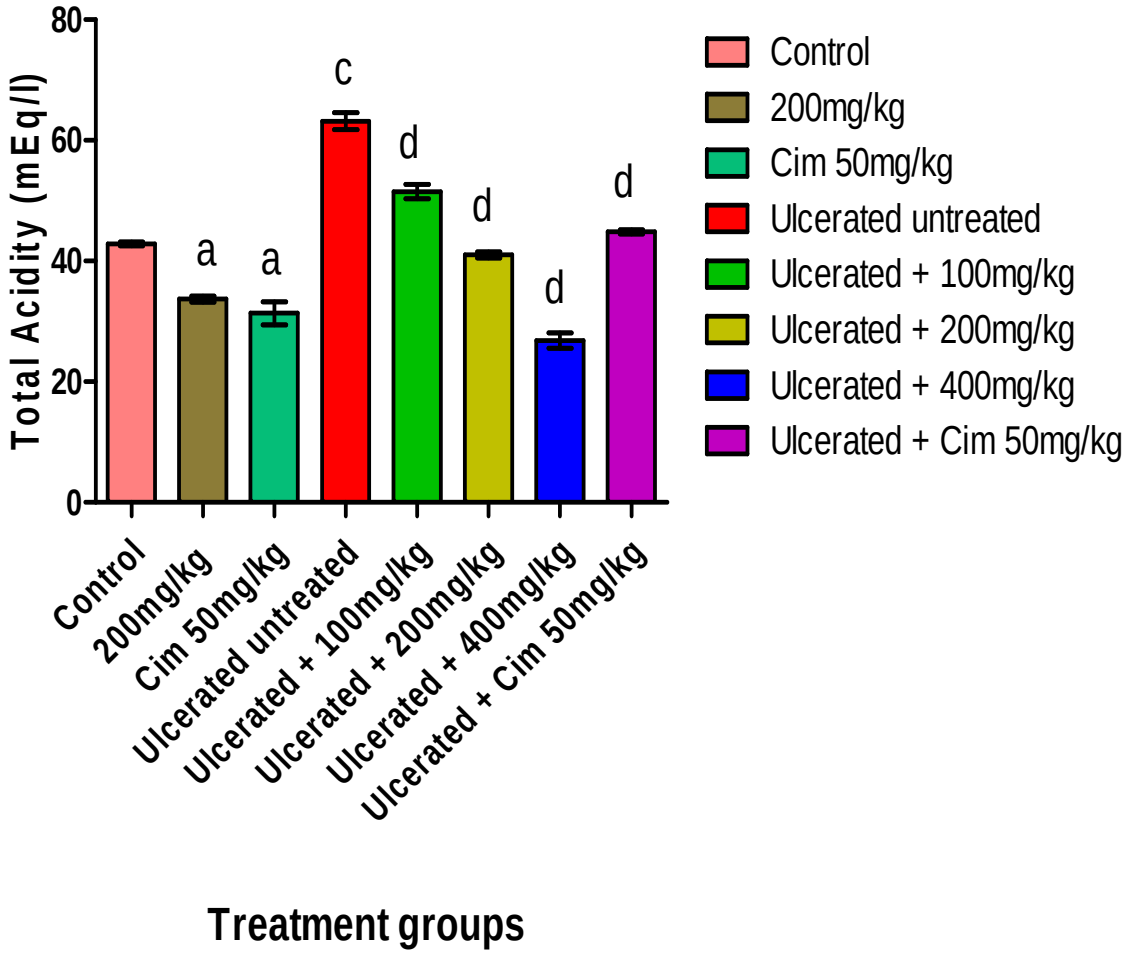

Fig. 5: Total acid output in gastric juice of various treatment groups.

Level of significance was taken at P< 0.05 for six rats per group

a Represents significant (P<0.05) decrease compared with control

c. Represents significant (P<0.05) increase compared with control and extract only groups

d Represents significant decrease compared with Ulcerated untreated group.

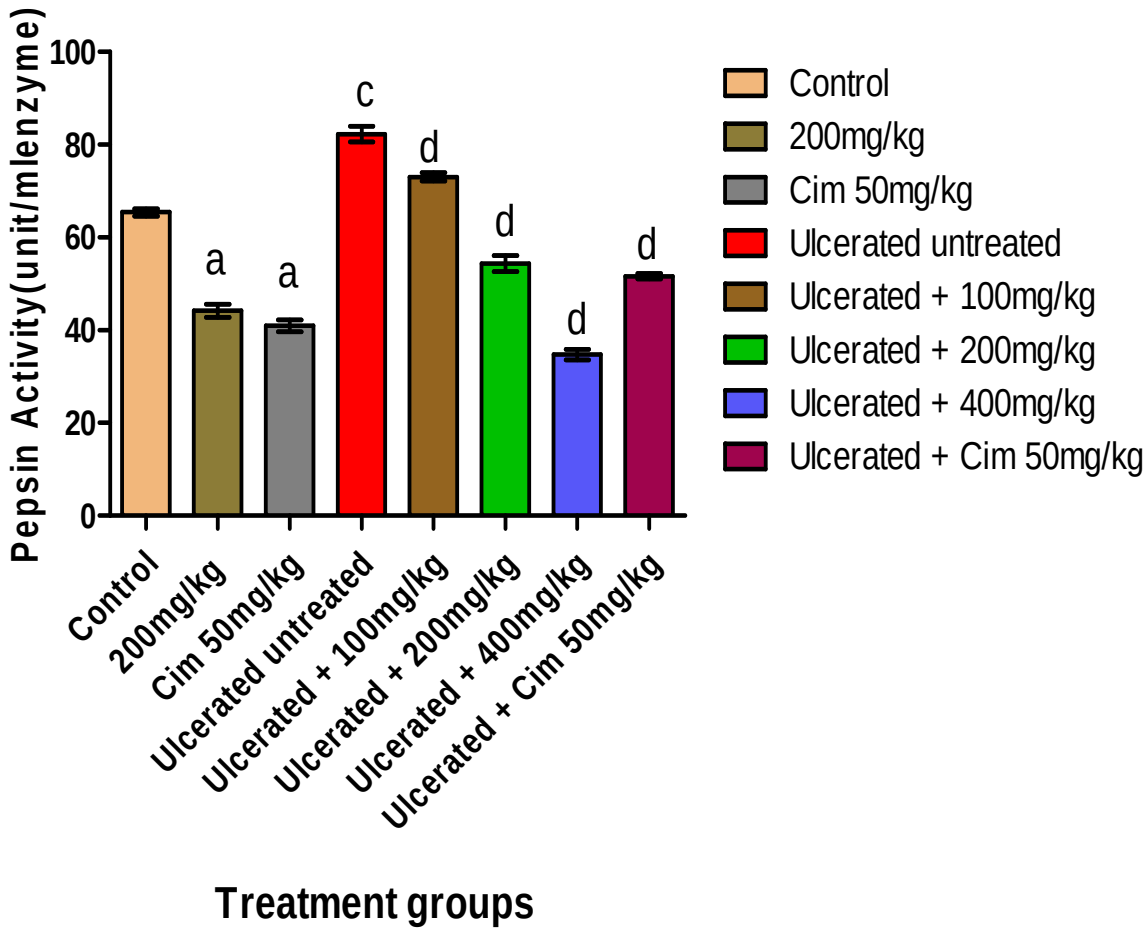

Fig. 6: Pepsin Activity in gastric juice of various treatment groups

Level of Significance was taken at P< 0.05 for six rats per group

a Represents Significant decrease compared with control animals

c Represents significant increase compared with control and extract only groups.

d Represents significant decrease compared with ulcerated untreated group.

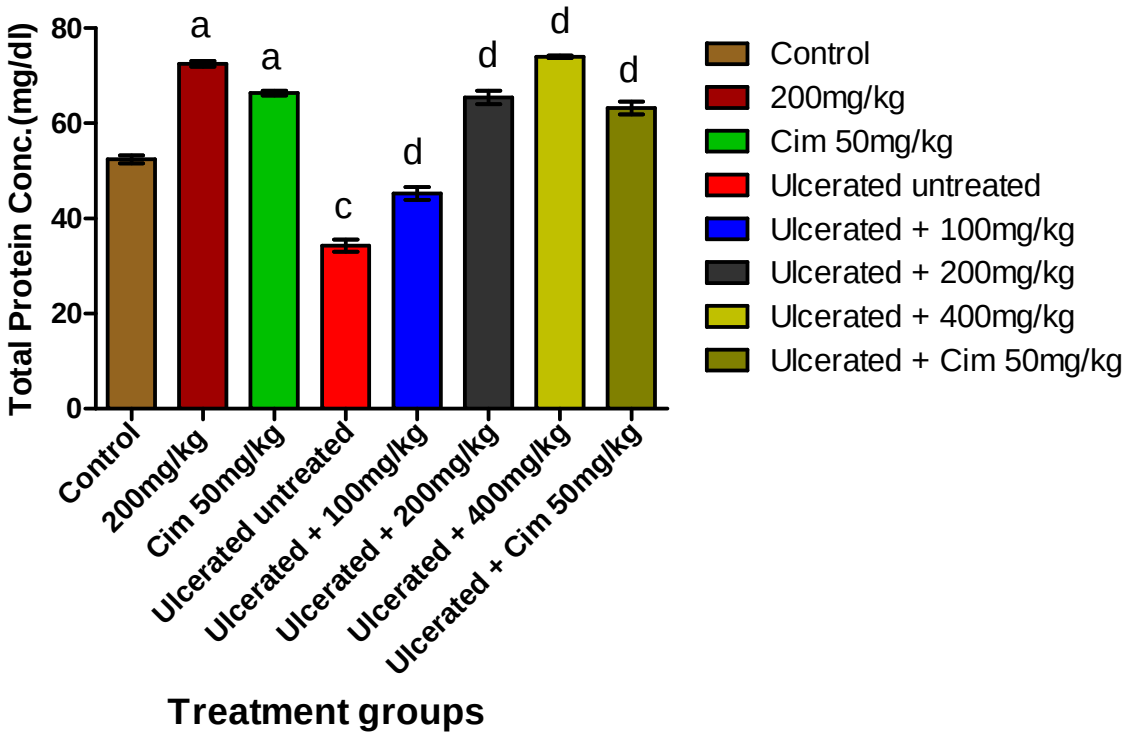

Fig. 7: Total Protein concentration in gastric tissues of various treatment groups

Level of significance was taken at P< 0.05 for six rats per group.

a Represents Significant increase compared with control animals.

c Represents Significant decrease compared with control and extract only groups

d Represent Significant increase compared with ulcerated untreated group

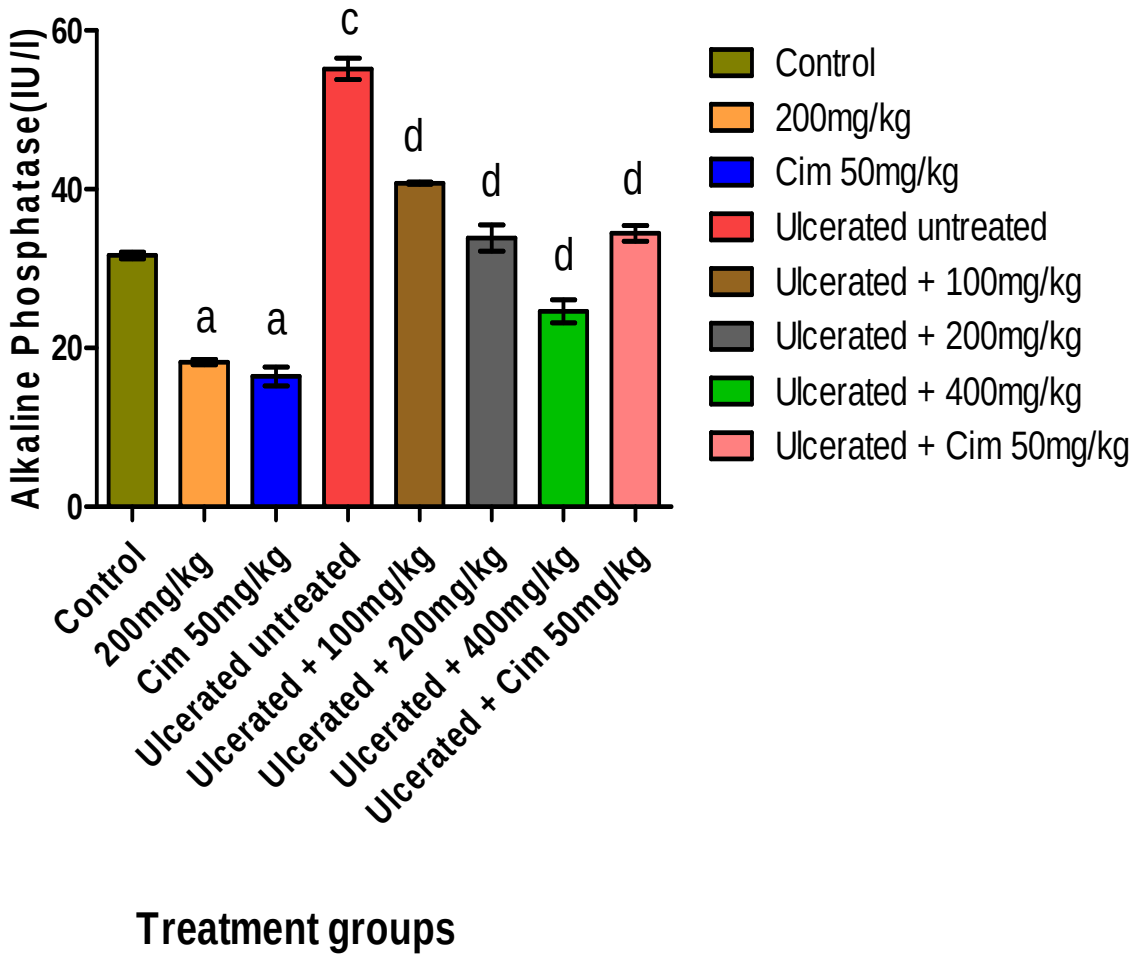

Fig. 8: Alkaline phosphatase (ALP) activity in gastric tissue of various treatment groups

Level of Significance was taken at P<0.05 for six rats per group

a Represents Significant(P<0.05) decrease compared with control

c Represents Significant Increase compared with control and extract only groups.

d Represents Significant decrease compared with ulcerated untreated group

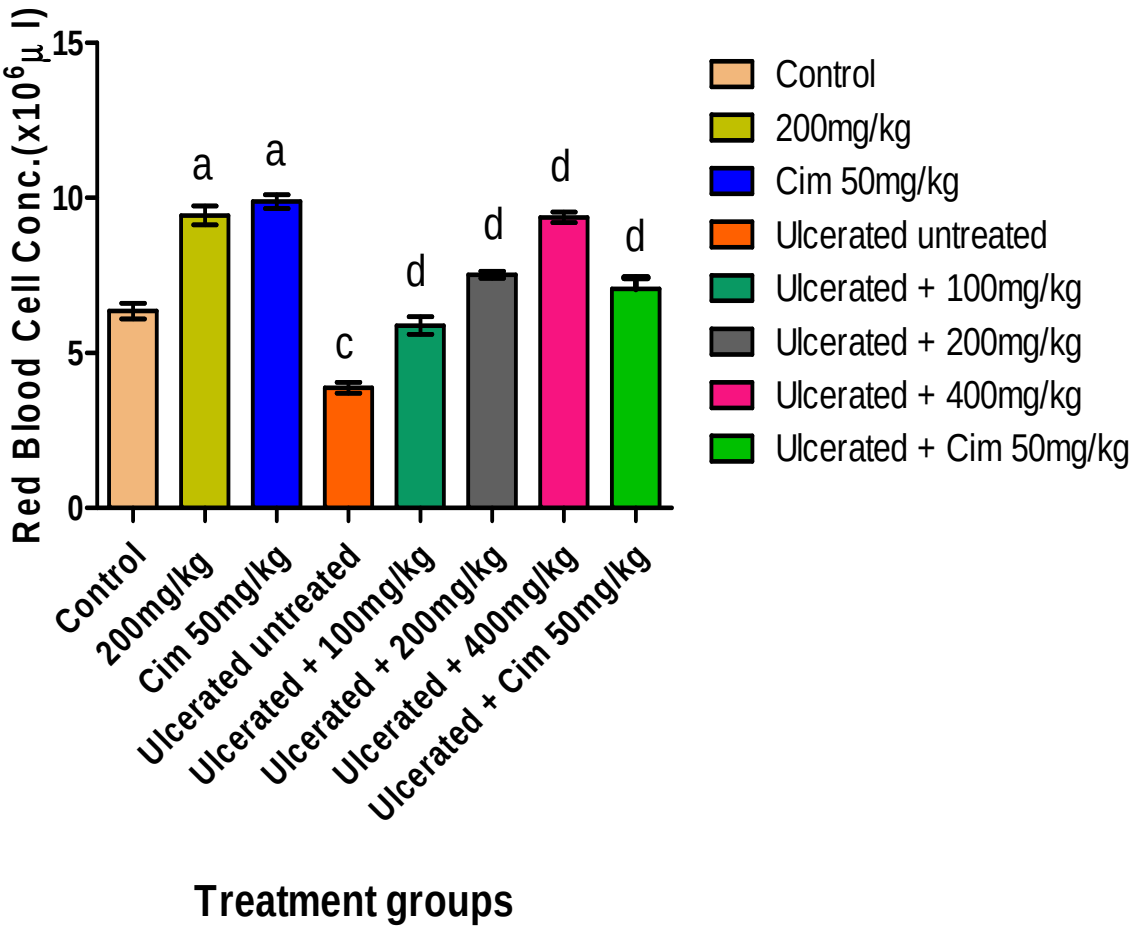

Fig. 9: Red Blood Cell count in blood of various treatment groups

Level of significance was taken at P< 0.05 for six rats per group

a Represents significant (P<0.05) increase compared with control

c. Represents significant decrease compared with control animals and extract only groups

d Represents significant increase compared with ulcerated untreated animals

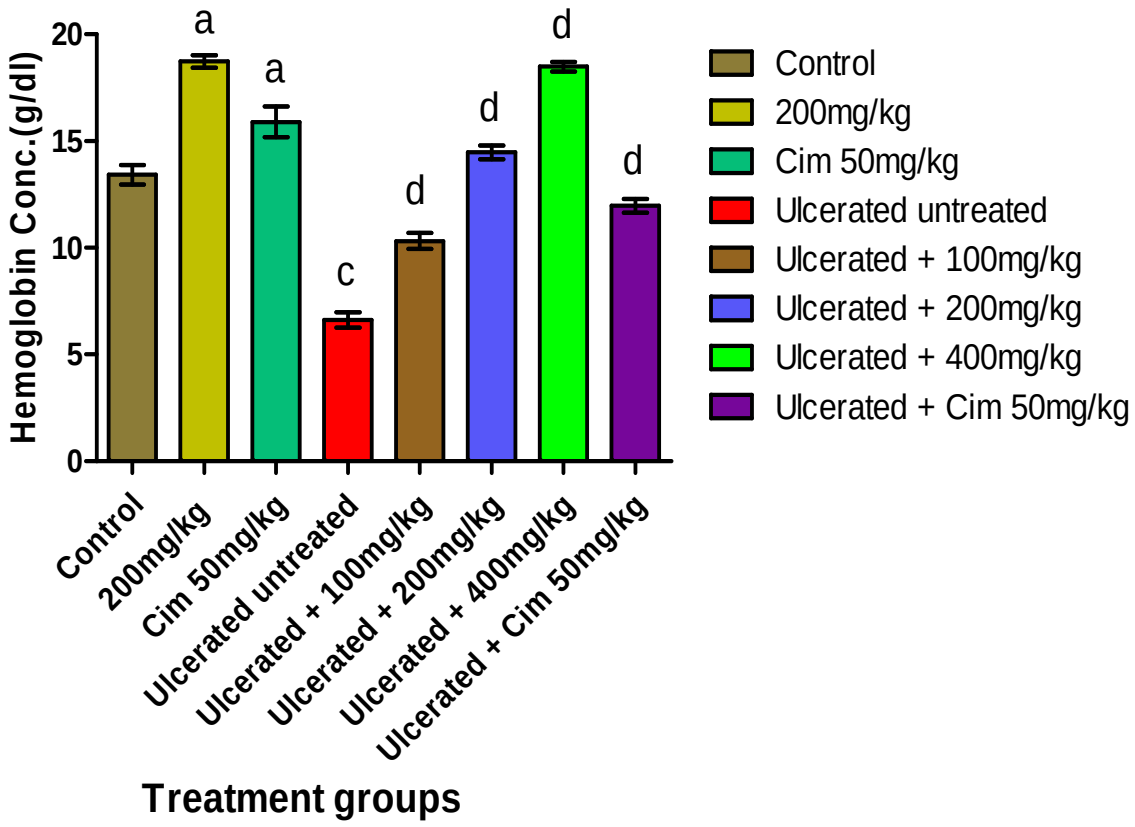

Fig . 10: Hemoglobin (Hb) concentration in blood of various treatment groups

Level of Significance was taken at P< 0.05 for six rats per group

a Represents significant increase compared with control.

c Represents significant decrease compared with control and extract only groups

d Represents significant increase compared with ulcerated untreated group.

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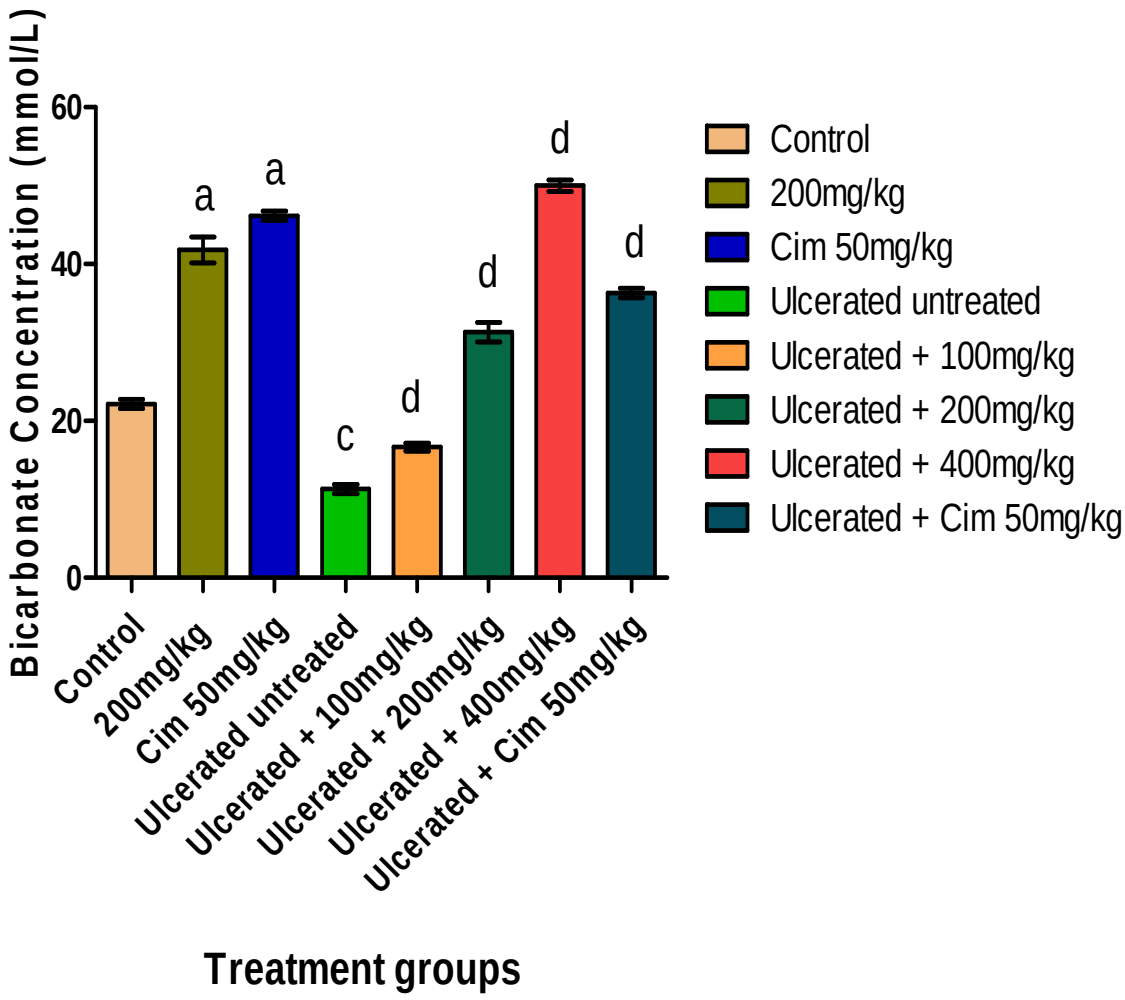

Fig. 11: Bicarbonate (HCO_3^-) concentration in serum of various treatment groups.

Level of significance was taken at $P < 0.05$ for six rats per group.

a Represents Significant increase compared with control animals.

c Represents Significant decrease compared with control and extract only groups

d Represent Significant increase compared with ulcerated untreated group

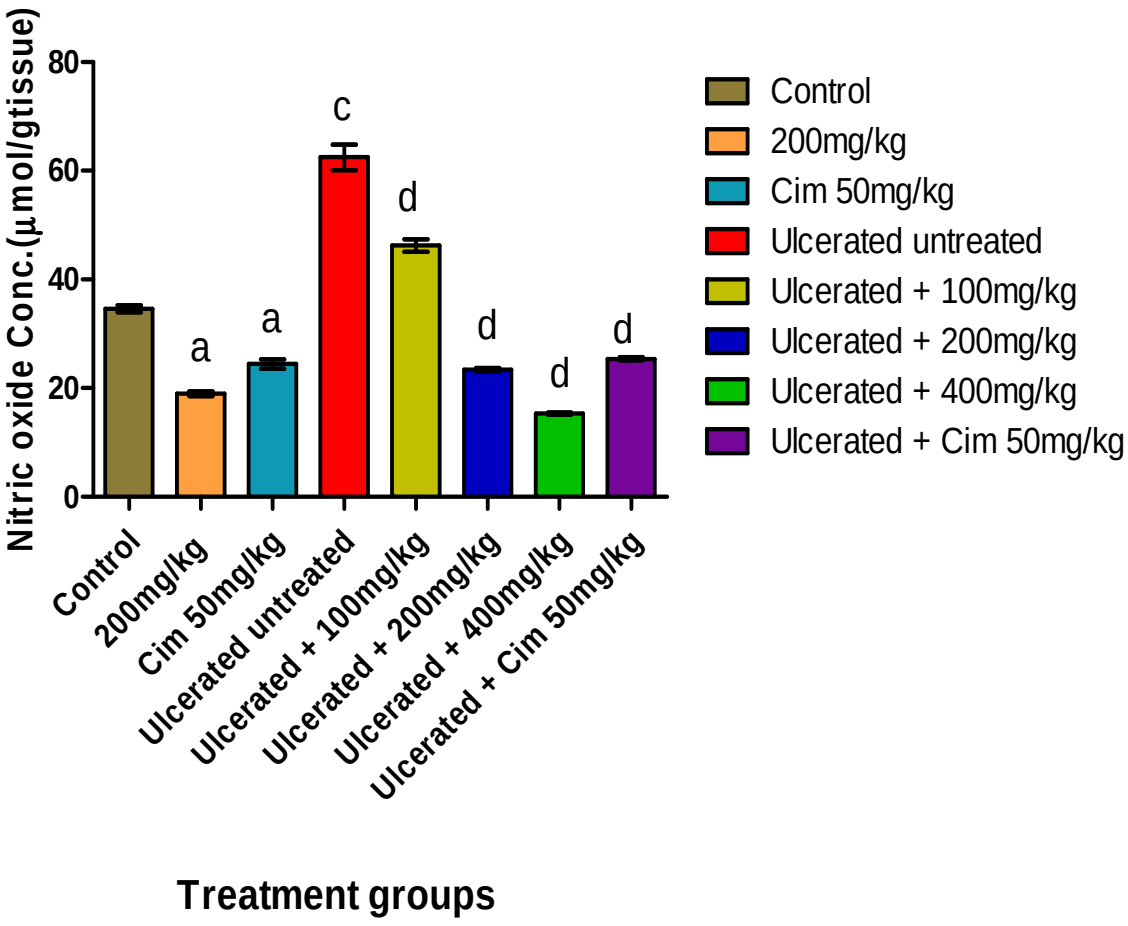

Fig. 12: Nitric Oxide (NO) Concentration in gastric tissue of various treatment groups

Level of Significance was taken at P<0.05 for six rats per group

a Represents Significant(P<0.05) decrease compared with control

c Represents Significant Increase compared with control and extract only groups.

d Represents Significant decrease compared with ulcerated untreated Group

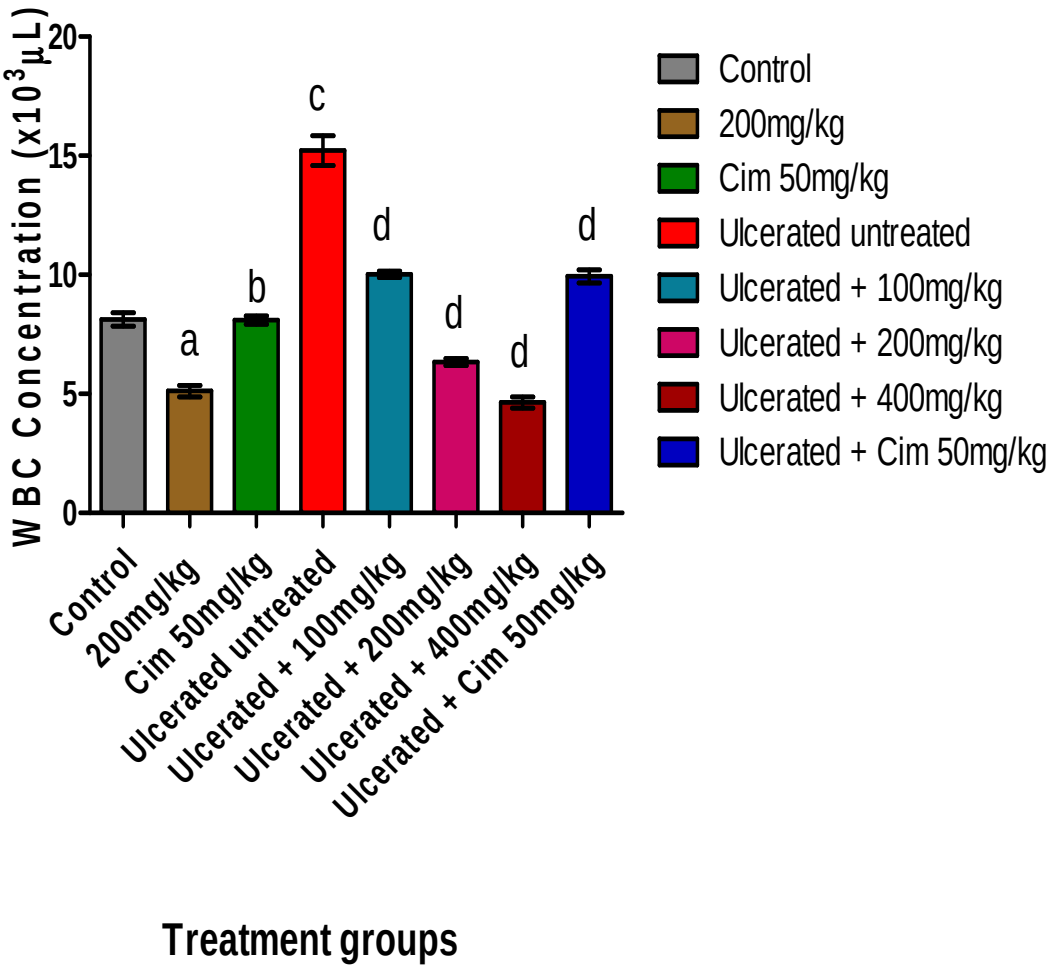

Fig. 13: White Blood Cell (WBC) count in blood of various treatment groups

Level of Significance was taken at P<0.05 for six rats per group

a Represents Significant(P<0.05) decrease compared with control

b Represents no significant decrease compared with control.

c Represents Significant Increase compared with control and extract only groups.

d Represents Significant decrease compared with ulcerated untreated Group

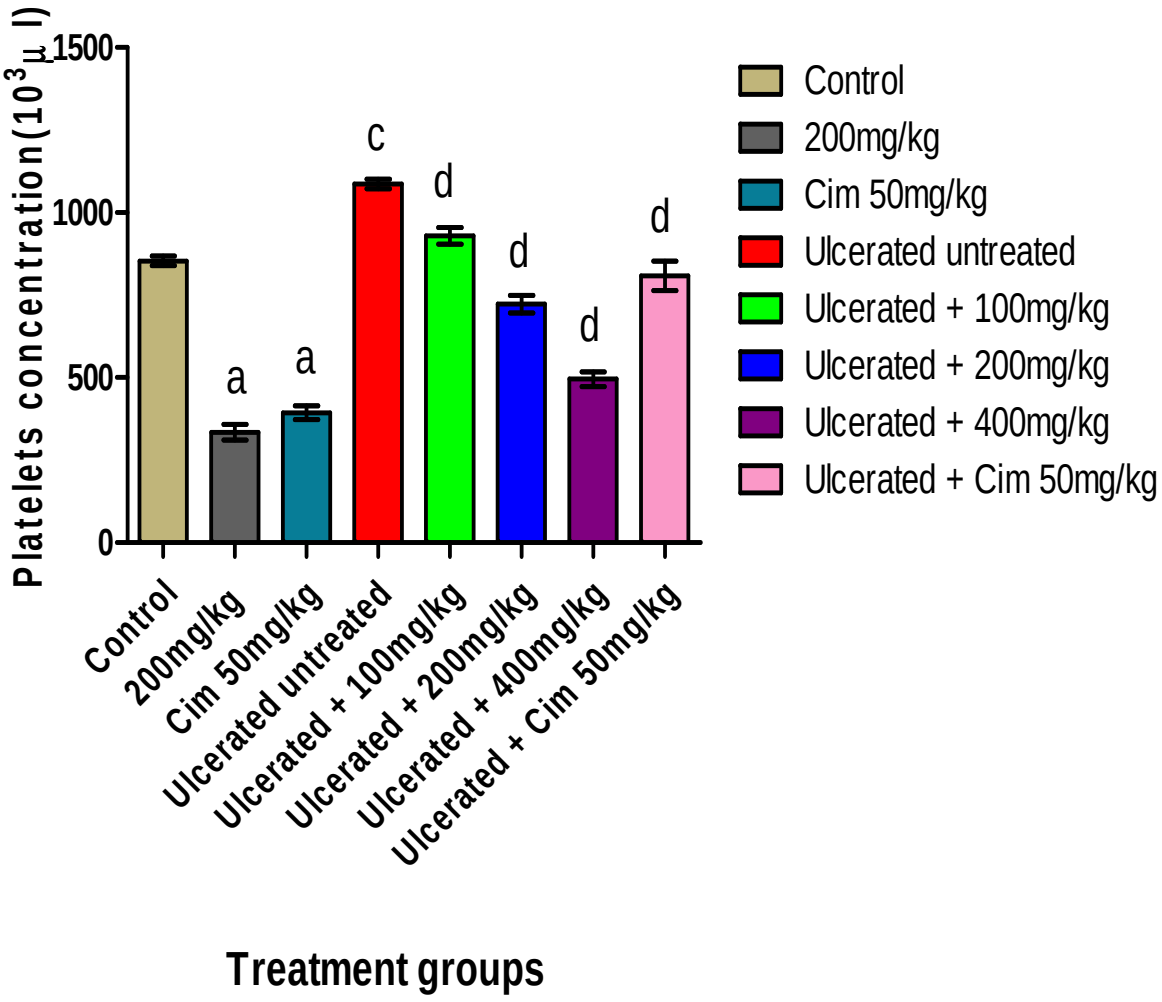

Fig. 14: Platelet count in blood of various treatment groups

Level of Significance was taken at P<0.05 for six rats per group

a Represents Significant(P<0.05) decrease compared with control

c Represents Significant Increase compared with control and extract only groups.

d Represents Significant decrease compared with Ulcerated untreated Group

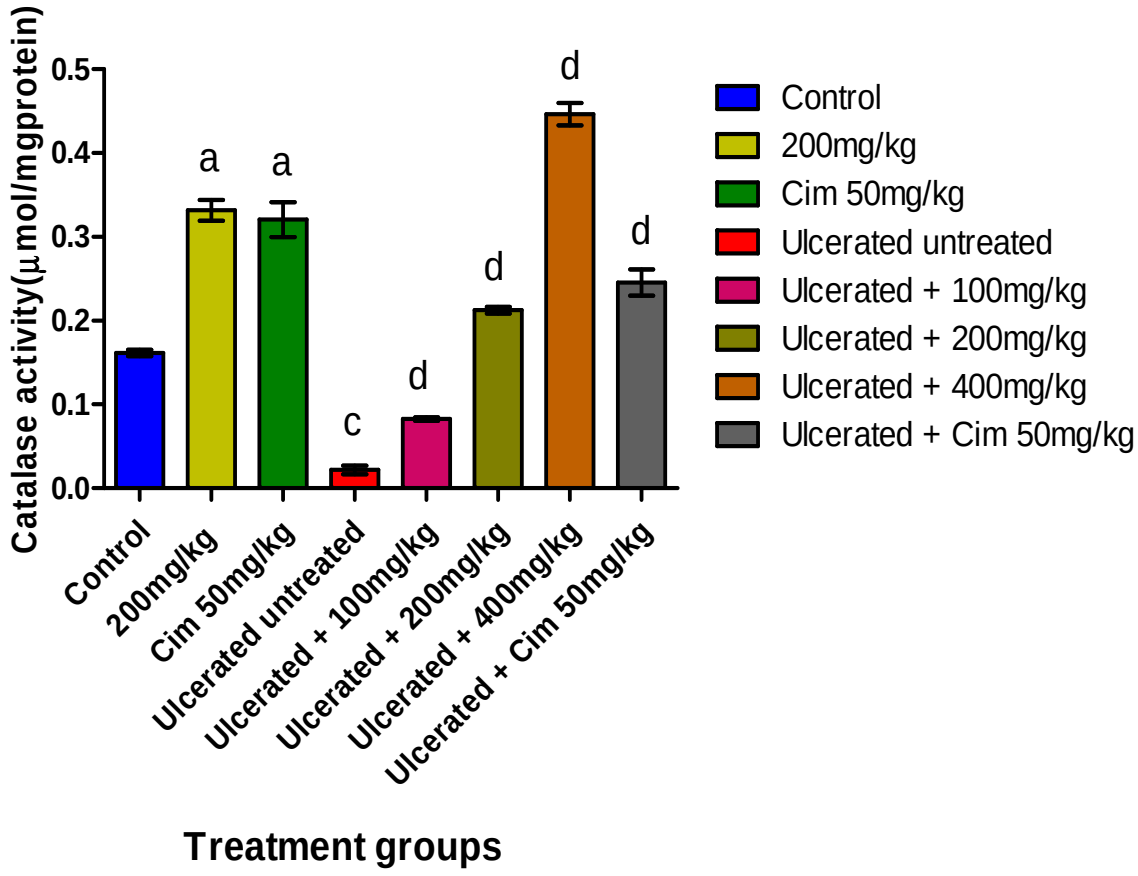

Fig. 15: Catalase activity in gastric tissue of various treatment groups

Level of significance was taken at P< 0.05 for six rats per group.

a Represents Significant (P<0.05) increase compared with control animals.

c Represents Significant decrease compared with control and extract only groups

d Represents Significant increase compared with ulcerated untreated group

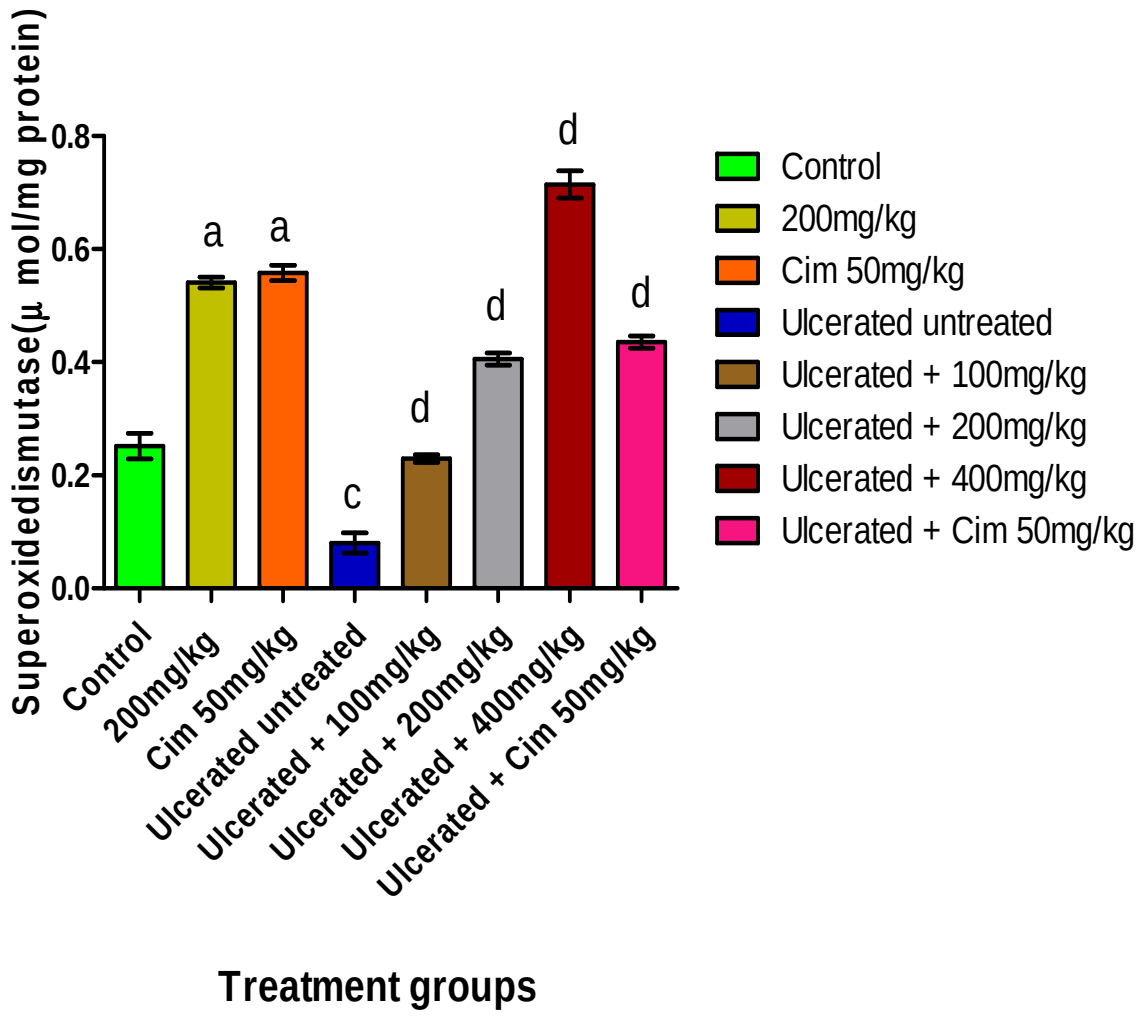

Fig.16 : Superoxide Dismutase (SOD) activity in gastric tissues of various treatment groups

Level of significance was taken at P< 0.05 for six rats per group.

a Represents Significant increase compared with control animals.

c Represents Significant decrease compared with control and extract only groups

d Represents Significant increase compared with ulcerated untreated group

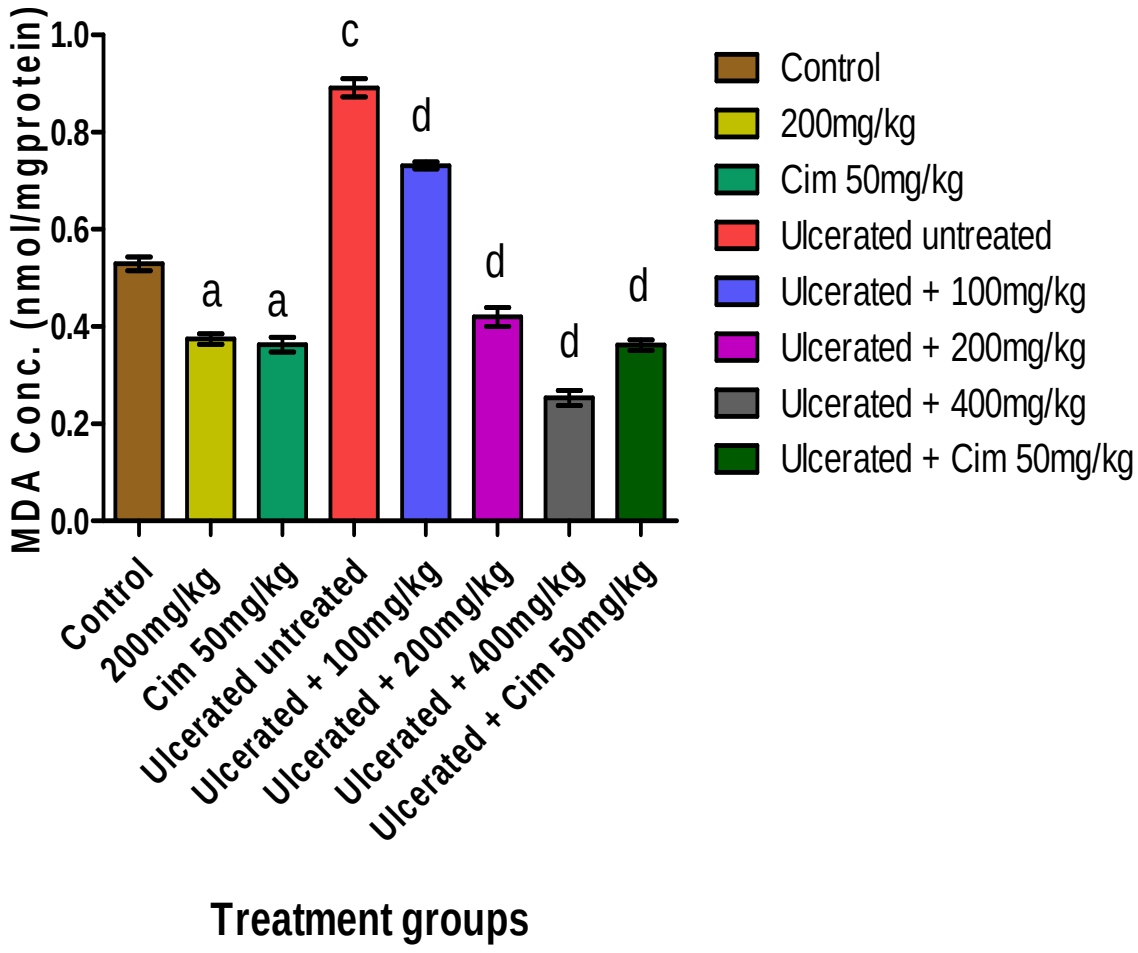

Fig. 17: Malondialdehyde (MDA) Concentration in gastric tissue of various treatment groups.

Level of Significance was taken at P<0.05 for six rats per group

a Represents Significant(P<0.05) decrease compared with control

c Represents Significant Increase compared with control and extract only groups.

d Represents Significant decrease compared with ulcerated untreated Group

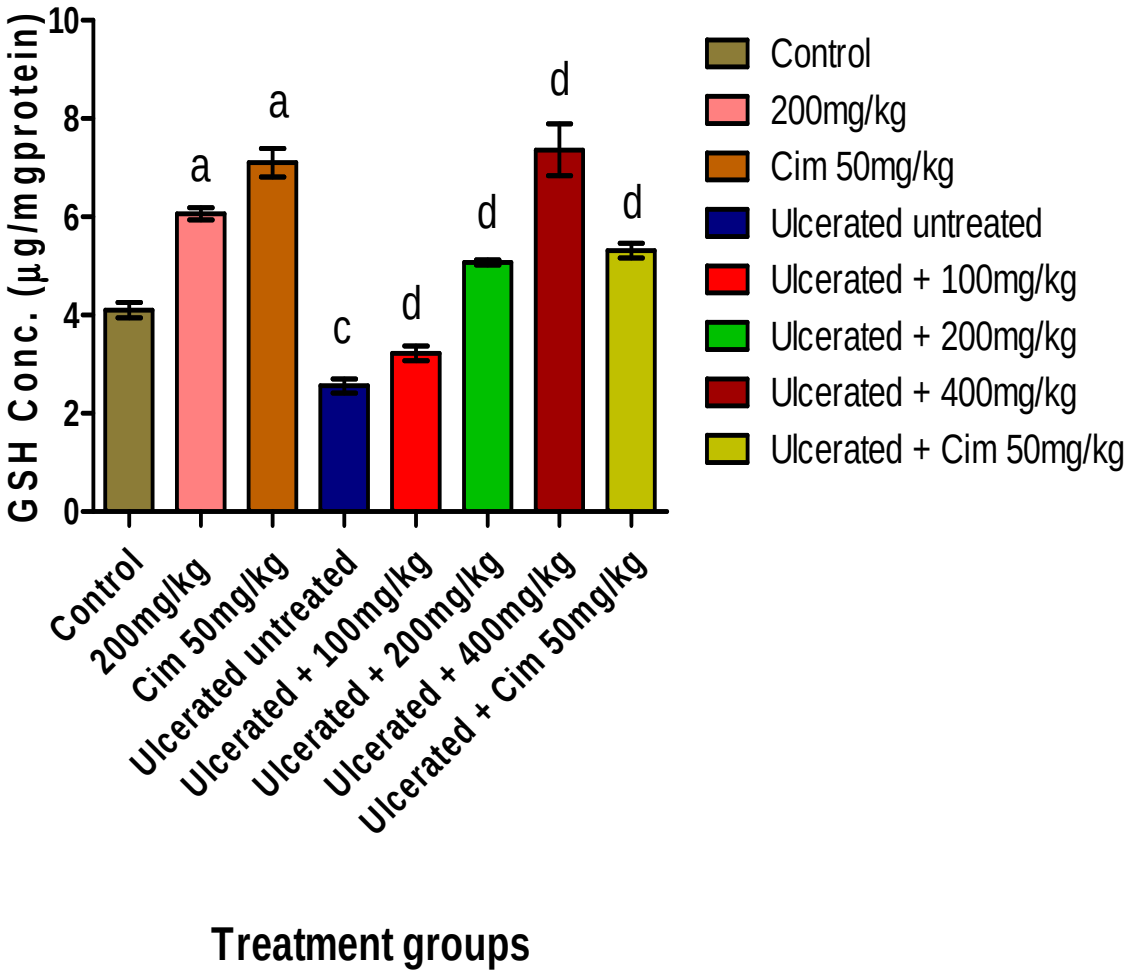

Fig. 18: Reduced Glutathione (GSH) concentration in gastric tissues of various treatment groups.

Level of significance was taken at P< 0.05 for six rats per group.

a Represents Significant increase compared with control animals.

c Represents Significant decrease compared with control and extract only groups

d Represent Significant increase compared with ulcerated untreated group

Photomicrograph showing the histology of the stomach (gastric tissue) for normal stomach, ulcerated untreated, treated with extract at different concentrations and separately with the standard drug.

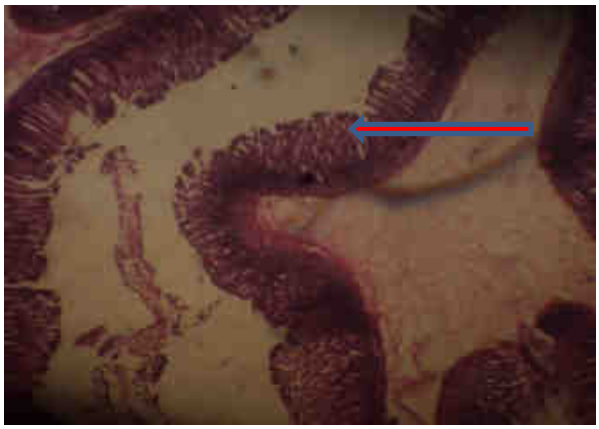

Plate.1: Normal stomach

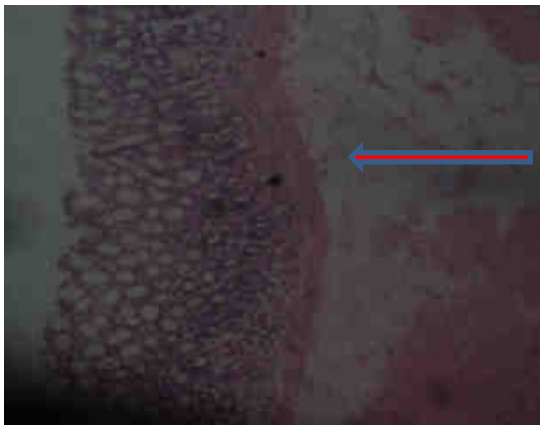

Plate.2: Ulcerated untreated stomach

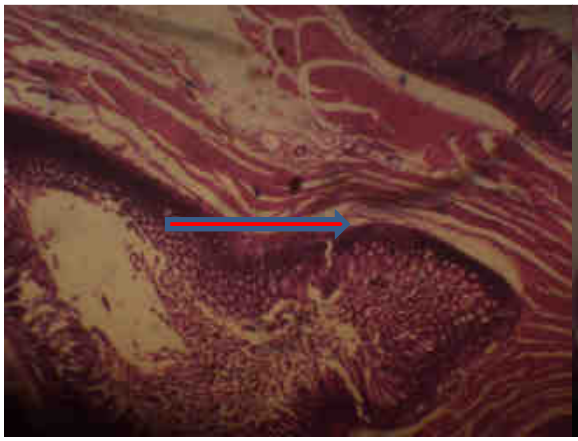

Plate .3: Ulcerated + 100mg/kg bw extract

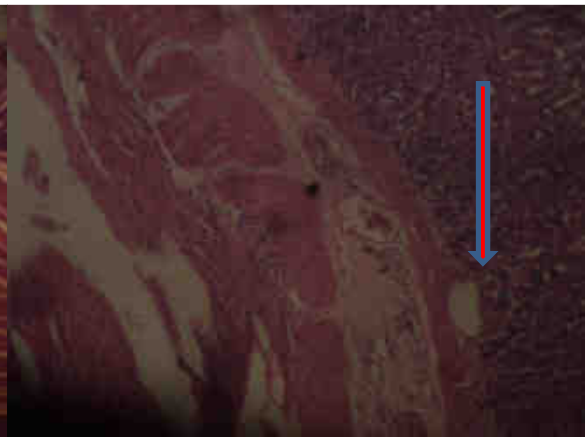

Plate .4: Ulcerated +200mg/kg bw extract

Plate .5: Ulcerated + 400mg/kg bw extract

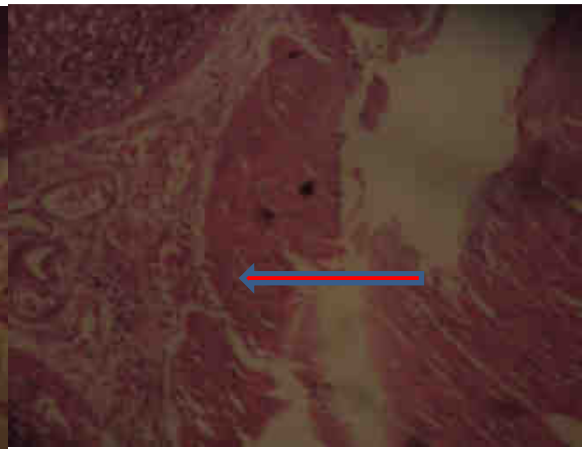

Plate .6: Ulcerated + Cim 50mg/kg bw.

Discussion

The gastrointestinal (GI) tract is a key source of ROS. Despite the protective barrier offered by its epithelial layer, pathogens and some ingested materials can cause inflammation by activating the epithelium and macrophages, to generate inflammatory cytokines and some other mediators that contribute further to oxidative stress. Various (GI) pathological conditions like gastro-duodenal ulcers, gastro-intestinal malignancies, inflammatory bowel disease (IBD) arise from oxidative stress. Oxidative and nitrosative stress have been etiologically implicated in a wide variety of disease processes which includes: aging, hypertension, atherosclerosis, diabetic neuropathies, and renal diseases. Oxidative stress also contributes to various GI diseases including gastro-duodenal ulcers (Peng *et al.*, 2008), inflammatory bowel disease (Grisham, 1994), and GI malignancies such as gastric cancer and colorectal cancer (Inokuma *et al.*, 2009: Kekec, 2009).In this study, some ulcer indices, biochemical and antioxidant indices were evaluated in rats to assess the possible effects of aqueous extract of Blighia sapida leaves in ethanol-induced gastric ulcer.

Ulcer index refers to mean ulcer score per group of animals (Nwafor *et al.*, 2000).In figure 1, Ulcer Index Value (UIV) significantly increased ($P<0.05$) in ulcerated untreated animals (Group IV) compared with other treatment groups (V, VI, VII and VIII). However administration of both plant extract and cimetidine significantly ($P<0.05$) decreased the ulcer index. The maximum inhibition of ulcer index was observed in animals treated with plant extract at 400 mg/kg body weight than those treated with standard drug (Cimetidine). Similar effect was observed at 200mg/kg body weight extract and cimetidine at 50mg/kg body weight. Also, in figure 2, the percentage amelioration was highest in group treated with 400mg/kg body weight of extract and lowest at 100mg/kg body weight of extract with similar effects observed at 200mg/kg body weight extract and cimetidine at 50mg/kg body weight, suggestive of possible cytoprotective potential of the plant extract.

Ethanol administration leads to increased production of Hydrochloric acid (HCl) from the parietal cells of the stomach, thereby making the pH of gastric secretion more acidic. Excessive acid production in gastric mucosa caused decreased pH level which has been implicated in gastric ulcer (Ian and Williams, 2005). In figure 3, the pH of gastric secretion was significantly ($P<0.05$) increased in extract and cimetidine only treated groups (Group II and III) compared with controls , the drug and the extract or its metabolites might have reduces the concentration of acid secreted in gastric juice significantly ($P<0.05$) in the ulcerated rats. The extract at 200mg/kg body weight and cimetidine at 50mg/kg body weight however elicit similar effect on the pH of the gastric secretion. Similarly in figure 4 and 5, a significant ($P<0.05$) decrease in Free and Total acidity was observed in extract and cimetidine only groups (Group II and III), compared with control animals while free and total acidity concentration was significantly increased ($P<0.05$) in ulcerated untreated rats (Group IV),

compared to control. After the treatment, it was significantly (P<0.05) decreased in the groups treated with plant extract and Cimetidine. The decrease was more pronounced in extract treated at 400 mg/kg body weight than those of cimetidine, while extract at 200mg/kg body weight and cimetidine at 50mg/kg had similar effects. This behavior is suggestive of antacid or cytoprotective effect of *Blighia sapida* extract or possibly by antisecretory effect which may be responsible for its possible ameliorative potential.

Pepsin enzyme is an endopeptidase that is responsible for initiating the process of protein digestion into smaller peptides and polypeptides in the stomach. Pepsin splits the long chain amino acid in the region of peptide bonds having aromatic amino acids. However enhanced activity of Pepsin enzymes have been implicated in gastric ulcer (Ian and William, 2005). In figure 6, a significant (P<0.05) decreased in pepsin activity was observed in extract and cimetidine only treated groups (Group II and III) compared with control animals. Proteolytic activity of Pepsin enzyme in gastric secretion significantly (P<0.05) increased in ulcerated untreated animals (Group IV) compared with control groups. Upon treatment, administration of both plant extract and cimetidine significantly (P<0.05) decreased its activity in a concentration dependent manner with similar effects observed at 200mg/kg body weight of extract and cimetidine . Ethanol induced gastric ulcer is usually associated with elevated activity of Pepsin enzyme, as ethanol stimulates increase production of Hydrochloric acid (HCl) from the parietal cells of the stomach. Enhanced HCl causes increase in Pepsin activity by facilitating the conversion of Pepsinogen (inactive form of Pepsin enzyme) to the active Pepsin enzyme (Ian and Williams, 2005). In figure 7, gastric tissue total protein was significantly (P<0.05) increased in extract and cimetidine only treated groups (Group II and III) while a significant (P<0.05) decrease was observed in ulcerated untreated group (Group IV). However after treatment, total protein concentration increased significantly (P< 0.05) in a dose dependent manner with similar effect observed at 200mg/kg bodyweight of extract and cimetidine at 50mg/kg body weight. The decrease in protein concentration in ulcerated untreated animals (Group IV) may be ascribed to tissue damage in ulceration and subsequent leakage of these constituents into the gastric juice as reported by Gopinathan and Nija, (2014). The phytochemicals or bio actives constituents present in the plant extract or its metabolites may be responsible for healing of the stomach ulcer wounds and thus prevents protein degradation.

Alkaline phosphatase (ALP) is an enzyme that functions at alkaline pH of 10, it works by removing 5' phosphate from DNA and RNA and also from protein and nucleotides creating an alkaline pH. Increased activity of Alkaline phosphatase has been reported in gastric ulceration (Holtz and Kantrowitz, 1999). In figure 8, a significant (P<0.05) decrease in Alkaline phosphatase activity was observed in extract and cimetidine only groups (Group II and III) compared with control animals. Alkaline phosphatase (ALP) activity was however significantly (P<0.05) increased in ulcerated untreated animals when compared with other treatment groups. Treatment with extract and cimetidine significantly (P<0.05) decrease the activity of this enzyme in a concentration dependent manner with similar effects shown by extract at 200mg/kg bodyweight and the standard drug.

In figure 9 and 10, significant (P<0.05) increases was observed in Red Blood Cell (RBC) and Haemoglobin counts in group II and III compared with controls . Ulcerated untreated (Group IV) animals showed significant (P<0.05) decrease in Red Blood Cell and Hemoglobin concentration. In ulceration, there is

usually a profound haemorrhage due to lesions in gastric mucosa , and this was further supported by significant reduction in the level of Hemoglobin and Red Blood cells in ulcerated untreated animals in this study. However with the administration of various treatments, increases in red blood cell and haemoglobin concentrations were observed with the extract which favourably compared with the standard drug at 200mg/kg body weight.

Bicarbonates are integral indices of ulceration, in figure 11, serum Bicarbonate was significantly ($P<0.05$) increased in extract and cimetidine only administered rats (Group II and III) compared with controls. However a significant ($P<0.05$) decrease was observed in ulcerated untreated group (Group IV) compared with other treatment groups. Administration of the extract increases bicarbonates in a concentration dependent manner in the ulcerated rats treated with the extract suggestive of the possible ability of the extract or its metabolites to stimulate mucus and Bicarbonate to counteract the degenerative effects of reactive oxygen species (ROS) present in the gastrointestinal lumen as a result of a possible buffering action. (Bhoomannavar *et al.*, 2011; Borrelli and Izzo, 2000).

Nitric oxide (NO), functions as a neurotransmitter and vasodilator and also has important role in inflammatory processes, being a mediator of macrophage function. Nitric oxide, generated by the enzyme nitric oxide synthase, reacts rapidly with oxygen free radicals to form peroxynitrite. When Peroxynitrite is protonated, it decays rapidly to OH and NO_2 which are highly potent biological oxidants (Zang and Li, 2006). Result obtained in figure 12, showed significant ($P<0.05$) decreases in nitric oxide level in extract and cimetidine only treated groups (Group II and III) compared with control animals. Nitric oxide (NO) concentration was significantly ($P<0.05$) increased in ulcerated untreated animals (Group IV), while treatment with extract at different concentration and cimetidine reversed this trend significantly.

Elevated level of white blood cell have been implicated in ulceration and inflammation (Gopinathan and Rameela, 2014). In figure 13, the concentration of white blood cell (WBC) was significantly ($P<0.05$) increased in ulcerated untreated animals (IV) compared with other treatment group while a significant ($P<0.05$) decrease in white blood cell concentration was observed in extract only group (Group II).The ulcerated and treated animals with extract only and cimetidine only showed decreases in WBC with similar events observed in platelets concentrations when compared with controls (figure14). Elevated concentration of platelets is usually associated with gastro-duodenal ulceration, interestingly, the extract showed ameliorative effects competitively with the standard drug from the results obtained.

Enzymatic antioxidants such as Superoxide dismutase (SOD) and Catalase (CAT) are known to exhibit the first line of cellular defense against oxidative damage in tissues, they dispose superoxide ($O_2^{\cdot}$) and H_2O_2 before their interaction to form more harmful hydroxyl radical ($OH^{\cdot}$) (Lil *et al.*, 1988, Megala and Geetha, 2010). In figure 15 and 16, stomach SOD and Catalase activities were significantly ($P<0.05$) increased in extract and cimetidine only groups (Group II and III) compared with controls. Ulcerated untreated animals (Group IV) showed significant ($P<0.05$) decreases in SOD and Catalase activities. The lowered activities of SOD and Catalase occasioned by the ethanol administration were brought to near the control level in treated Groups V, VI, VII and VIII. The increase in SOD and Catalase activities elicited by the extract were in a concentration dependent manner while similar effects were observed with extract at 200mg/kg body weight

with Cimetidine. These results may imply possible antioxidant property of the extract which may elicit pathway that enhances increase in superoxide dismutase and catalase activities thus boosting the antioxidant status of the animals. Oral administration of 70% absolute ethanol induces ulcer in rats resulting in excessive free radicals production which includes hydroxyl ethyl radical, superoxide radical (O_2-), hydroxyl radical (OH^-), peroxyl radical and hydrogen peroxide (Schlorff *et al.*, 1999). These radicals were formed from the ethanol-mediated process and have the ability to react quickly with lipids turning them to lipid peroxides (LPO) (Rukkumani *et al.*, 2004). Result obtained in figure 17, showed a significant ($P<0.05$) decrease in Malondialdehyde (MDA) concentration in extract and cimetidine only administered groups (Group II and III) compared to control animals.

The concentration of MDA was significantly ($P<0.05$) increased in ulcerated untreated animals compared to control group and other treated groups, while treatment with extract and cimetidine significantly ($P<0.05$) decreased MDA concentration in a concentration dependent manner with highest effect at 400mg/kg body weight . Similar effect was also observed at 200mg/kg body weight of extract and cimetidine at 50mg/kg body weight.

Reduced glutathione (GSH) is a non-enzymatic antioxidant biological molecule present in body tissues. It aids the removal of reactive non radical compounds like hydrogen peroxide (H_2O_2), superoxide anions and alkoxy radicals from tissues, maintenance of membrane protein thiols, act as substrate for Glutathione peroxidase (GPx) and Glutathione -s-transferase (GST) (Townsend *et al.*, 2003). In figure 18, the extract and cimetidine only showed increases in stomach GSH concentration while ethanol occasioned significant ($P<0.05$) decreases in GSH in the ulcerated untreated animals (group IV) and these effects were reversed by treatment with the extract at various concentrations.

Interestingly, the histological examination of the stomach showed intact gastric gland and epithelium in normal stomach (Plate 1),while in the ulcerated untreated stomach the section showed destruction of gastric glands and epithelium, edema in lamina propria, as well as mononuclear cells infiltration (Plate 2).Treatment of ulcerated rats at different concentrations (100,200 and 400 mg/kg body weight) of the extract however showed gradual improvement in the structural morphology and integrity of the stomach near to control Plate(3-5) with normal histological morphology which favorably compete with the similar effect shown by the standard drug (Plate 6).

Conclusion: Aqueous extract of *Blighia sapida* leaves exhibited anti -ulcer, anti-inflammatory and ameliorative effects on ethanol-induced gastric ulcer in rats probably by the antioxidant effects of the extract, its bioactive constituents or metabolites which may be exploited in the development of drugs in combating ulcer and this study also validated the folkloric use of the plant in the treatment or management of ulcer while the efficacy of the extract was also comparable with Cimetidine .

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