ejpmr, 2022,9(2), 28-37

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

THE EFFECT OF D-RIBOSE-L-CYSTEINE ON INDOMETHACIN INDUCED OXIDATIVE STRESS AND GASTRIC ULCERATION IN ADULT MALE ALBINO WISTAR RATS

Rosemary N. Orjiako*[1], Edwin Nwobodo[2], Peter Okonudo[3], Chukwudi Onyeka Okonkwo[4], Uzozie Chikere Ofoego[5], Chukwunenye Chibuike Ubah[6]

[1,2,3,4]Department of Human Physiology, Nnamdi Azikiwe University,Okofia Campus, Nnewi, Anambra State, Nigeria.
[5]Department of Human Anatomy, Nnamdi Azikiwe University, Okofia Campus, Nnewi, Anambra State, Nigeria.
[6]Newday Specialist Hospital, Ikate Surulere, Lagos State, Nigeria.

***Corresponding Author: Rosemary N. Orjiako**
Department of Human Physiology, Nnamdi Azikiwe University,Okofia Campus, Nnewi, Anambra State, Nigeria.

Article Received on 06/12/2021 **Article Revised on 26/12/2021** **Article Accepted on 16/01/2022**

ABSTRACT
Gastric ulceration is the most predominant gastrointestinal syndrome ever recognized. The major etiological factors in the pathogenesis of peptic ulcer disease which is *H.Pylori* as well as increasing use of NSAIDS lead to increased production of ROS by immune and epithelial cells and by activated neutrophils respectively, resulting in oxidative stress. However, studies have shown that Riboceine is a potent anti-oxidant. The aim of the study was to investigate the effect of D-Ribose-L-Cysteine on indomethacin induced Oxidative stress and gastric ulceration in adult male albino wistar rats. 24 adult male albino wistar rats were randomly divided into four groups of six rats (n=6); normal control group (Grp. 1-received normal saline only), ulcer control group (Grp. 2- received normal saline with indomethacin), treatment group 3 (pre-treated with riboceine for three days), treatment group 4 (pre-treated with riboceine for seven days). Riboceine was administered at 2mg/kg to the animals in the treatment groups. Riboceine administration significantly reduced stomach weight (P<0.05). The increased ulcer index due to indomethacin administration was significantly ihibited following Riboceine pre-treatment for three days and seven days (P<0.05). Riboceine administration for three and seven days significantly reduced malondialdehyde level (P<0.05). Riboceine administration for seven days significantly increased catalase activity (P<0.05). Administration of Riboceine for seven days also significantly increased SOD (P<0.05). In conclusion, this study showed that riboceine can be used in the prevention of gastric ulceration due to its gastroprotective and antioxidant potentials.

KEYWORDS: Riboceine, indomethacin, oxidative stress, gastric ulceration, antiulcerogenic, antioxidant.

1. INTRODUCTION

Oxygen is vital for aerobic life processes. However, about 5 percent of then inhaled oxygen is converted to reactive oxygen species (ROS) such as superoxide anion (O_2), hydrogen peroxide (H_2O_2) and hydroxyl ion (OH), by univalent reduction of oxygen (Gupta, Nain and Sidana, 2012). Also, in the course of normal human activities- energy production, detoxification of pollutants and immunologic defense mechanisms, free radicals are produced (Senoner and Dichtl, 2019). These free radicals are unstable molecules that can extract an electron from a neighboring molecule, causing damage in the process and, when unchecked, the free radical production accelerates the pathogenesis of human disease and aging (Zawn, 2017).

The stomach is a sensitive digestive organ that is susceptible and exposed to exogenous pathogens from the diet. In response to such pathogens, the stomach induces oxidative stress (Suzuki, Nishizawa, and Hibi, 2012). Oxidative stress occur when there is an imbalance between the production of these reactive oxygen species (free radicals) and the biological system's ability to readily detoxify the reactive intermediates (Priya, Main and Jaspreet, 2012). When this imbalance occur, it ultimately results in the oxidative deterioration of proteins, lipids and nucleic acids, which in humans, is involved in the pathology of many diseases such as Atherosclerosis, Parkinson's disease, Heart Failure, Myocardial Infarction and Chronic Fatigue Syndrome (Priya *et al.,* 2012).

There is some evidence that psychological stress, in addition to physical stress such as surgical intervention as well as microbial infection including *Helicobacter pylori* (*H. pylori*), leads to oxidative stress in the stomach (Suzuki *et al.,* 2009). Physiological responses of the stomach to stressors include increased activity of the

hypothalamic-pituitary-adrenal axis as well as changes in gastrointestinal tissue (Suzuki *et al.,* 2009). According to Selye's formulation of the general adaptation syndrome, an increase in adrenocortical activity is related to an increase in the incidence of gastric ulceration (Suzuki *et al.,* 2009).

It has been proposed that neutrophil- and oxygen radical-dependent microvascular injuries are important prime events that lead to gastric mucosal injury induced by indomethacin (Naito et al, 2006). Reactive oxygen species (ROS) produced by activated neutrophils after indomethacin treatment cause gastric mucosal injury via ROS-mediated oxidation of important biomolecules such as lipid, protein, and DNA. In addition, it has been revealed that indomethacin-induced gastric mucosal injury occurs via gastric epithelial cell apoptosis. (Naito and Yoshikawa, 2006).

To counteract oxidative stress, the body produces an armory of antioxidants such as gluthathione and alpha lipoic acid to defend itself (Gupta *et al.,* 2012). It is the job of the antioxidants to neutralize the free radicals that can harm the cells, but, the internal production of antioxidants is not enough to neutralize all the free radicals (Gupta *et al.,* 2012).
One of the major paradigm shifts in the field of medicine is the refocusing of medical therapies from curative medicine to preventive medicine. In the domain of ulcer and its treatment, not much can be said to have been achieved in terms of preventing ulcer and it's attributed to the multiple causative sources (aggressive factors) which has made prevention an exceptional task (Ajiboye *et al*, 2014). Most therapies to date have focused on isolating and treating the aggressive factor(s) responsible for the ulcer while others have generally relied on careful and strict abstinence from harmful causative sources (Ajiboye *et al*, 2014).

MaxOne™ is a dietary supplement that contains Riboceine™ compound (Max International, 2019). Riboceine™ is one of the synthetic antioxidants that help cells produce glutathione on- demand (Falana, Adeleke, Orenolu, Osinubi and Oyewopo, 2017). Its active ingredient is D-Ribose- L- Cysteine (Falana *et al.,* 2017). Although Scientists have known for decades about the health benefits of glutathione, the challenge has been how to safely and effectively increase its level in cells (Max International 2019). Whole glutathione consumption cannot be effective because, it would be destroyed in the digestion process before reaching the cell. The ribose component of the riboceine thus solves these challenges by effectively protecting and delivering the fragile cysteine molecule to cells, enabling the cells to produce glutathione when they need it most (Falana *et al*., 2017).

Studies have shown that Riboceine is effective in increasing glutathione levels and effectively fighting against the effects of oxidative stress (Robert *et al.,* 1987). It has also been shown to be 300% more effective than N-Acetyl Cysteine (NAC) in raising glutathione levels in a liver cell model (Roberts *et al.*, 1987). Hence this research project is aimed at investigating the effect of oral administration of Riboceine on Indomethacin induced oxidative stress and gastric ulceration in adult male albino wistar rats.

2. MATERIALS AND METHODS

2.1 Animal Selection/Handling

A total of 24 adult male albino Wistar rats weighing between 150-180g, were used for this study. The animals were kept in the Laboratory animal house of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. They were housed in well-ventilated cages and were acclimatized for two weeks (14days) under standard environmental conditions (12 hour light/dark cycles). During the entire period of the study, animals were fed with Vital Feed Growers Marsh (manufactured by Grand Ceareals Ltd, a subsidiary of UAC Nigeria PLC, Plateau State) and had access to water (distilled water) *ad libitum*.

2.2 Ethical Approval

This was obtained from the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus Okofia, Anambra State. Rat handling and treatment will conform to the guidelines of the Nnamdi Azikiwe University Animal Research Ethics committee (NAU-AREC) for laboratory animal care and use.

2.3 Drug Procurement/ Administration

Riboceine capsules (containing 125mg riboceine/capsule) were purchased from a subsidiary of Max international, Lagos State, Nigeria. 2mg/kg b.w of the drug was administered orally, once daily via an orogastric canula. The Riboceine was dissolved in distilled water to form a solution before administration.

2.4 Acute Toxicity Studies (LD50)

Acute toxicity study followed the guidelines according to Lorke (1983). The median Lethal dose (LD50) of D-Ribose-L-Cysteine was determined in the Laboratory of the department of human physiology, Nnamdi Azikiwe University, Okofia Campus Nnewi, Anambra State. The method has two phases, phase 1 and phase 2 respectively and a total of twelve (12) rats were used.

Phase 1

Nine (9) rats were used in this phase. They were grouped into three (3), with each group having three (3) rats.
Group 1 received- 100mg/kg body weight
Group 2 received- 500mg/kg body weight
Group 3 received- 1000mg/kg body weight

Phase 2

In this phase, three rats were used. They were assigned into three groups of one animal each.
Group 1 received- 1600mg/kg body weight

Group 2 received- 2900mg/kg body weight
Group 3 received- 5000mg/kg body weight

The animals were observed for 24hrs for changes in behavior and mortality.

2.5 Determination of Sample Size
The sample size was obtained using the resource equation method (Jaykaran and Kantharia, 2013). Where E (Sample size) = Total number of animals- Total number of groups
E= 24 - 4
E= 20 which is within the acceptable limit and thus considered adequate

2.6 Experimental Design
The animals were re-weighed and randomly divided into four (4) groups with six (n=6) rats in each group. The treatment group received Riboceine for the number of treatment days as stipulated below; while the control group received normal saline before indomethacin was administered inorder to induce oxidative stress and gastric ulcers. A breakdown of the treatment protocol for each group is stated below:
Group I. (**Normal Control**) received 8ml/kg bw of normal saline.
Group II (Ulcer control) received 8ml/kg bw of normal saline + indomethacin (40mg/kg bw)
Group III- Riboceine 2mg/kg B.W for 3 days + indomethacin (40mg/kg bw)
Group IV- Riboceine 2mg/kg B.W for 7 days + indomethacin (40mg/kg bw)

2.7 Laboratory Procedure
2.7.1 Ulcer Induction
Gastric ulceration was induced in the animals according to the procedure described by Sayanti *et al.,* (2007). The animals were deprived of food for 24 hours prior to experimentation but had free access to water. The Negative Control (group I) received normal saline only, while Group II (positive control group), Group III and Group IV animals received a single oral dose of indomethacin (40mg/kg bw) at the end of their supplementation periods respectively. All animals were sacrificed 4 hours after indomethacin administration, when various degrees of ulceration have manifested (Sabiu *et al.,* 2015). They were sacrificed with ether overdose, laparotomy was done and their stomach was removed and assessed for ulcer lesions.

2.7.2 Determination of Ulcer scores
This was based on a modified method as described by Peskar et al, (2002). Ulcers were independently assessed and severity factor was scored by two observers using the following criteria: 0 = no lesions; 1 = lesions < 1 mm length; 2 = lesions 2-4 mm length; and 3 = lesions > 4 mm length.

2.7.3 Index of Ulceration
The ulcer index (UI) for each rat was calculated as the number of lesions multiplied by their respective severity factor and the mean for each group was taken (Abdallah et al., 2011, Akpamu et al., 2013).
2.7.4 Percentage Inhibition
% Inhibition = $[UI_{ulcer\ control} - UI_{ulcer\ treated}]$ X 100. Where $UI_{ulcer\ control}$ is the ulcer index for Control group and $UI_{ulcer\ control}$
$UI_{ulcer\ treated}$ = the ulcer index for the treated group (Ahammed *et al.,* 2014).

2.7.5 Histological Assessment
Histological profiling of the ulcerated gastric mucosa was done using a standard approach (Laine and Weinstein, 1988; Wei *et al.,* 2018). Small sections were taken from each stomach and fixed in 10% formalin, dehydrated to remove water, cleared by removing alcohol, impregnated and infiltrated to remove clearing agent and embedded in paraffin wax. It was trimmed at 15 microns and sectioned at 5microns using a rotary microtome and dried in a hot plate for staining, using hematoxylin and eosin (H&E) and then the gastric tissue integrity (mucosa-submucosa) was assessed for damage

2.7.6 Preparation of stomach homogenate
After ulcer scoring, the stomach tissue of each rat in each group was grounded in a mortar. The ground tissues were homogenized in cold 0.1M phosphate buffer saline (1:4(w/v), pH 7.4) and the homogenates were centrifuged at 12000rpm for ten (10) minutes at 4^0C. The resulting supernatants were frozen at -200^0C. This is to ensure the maximum release of the enzyme located in the tissue.

2.7.7 Determination of Oxidative Stress Biomarkers
The activities of Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA-a product of lipid peroxidation) level were determined using the stomach homogenate. The methods used are described below:

2.7.7.1 Determination of Superoxide Dismutase (SOD) activity
SOD was assayed by colorimetric method of Misra and Fredovich, (1972).

Principle
Adrenaline undergoes auto oxidation at pH 10.2 to form adrenochome which has an absorption maximium at 480 nm. The presence of superoxide dismutasein the reaction mixture inhibits the auto oxidation of adrenaline and the decrease in the formation of this adrenochrome is proportional to the rate of superoxide dismutase activity in the sample.

Procedure
Eighty (80 µl) of sample/blank was added into a clean test tube containing 1000 µl of carbonate buffer (pH 10.2). The resulting solution was mixed thoroughly, and allowed to equilibrate by incubating at 37 °C for 5 minutes. Thereafter, 600 µl of freshly prepared

epinephrine was added and the reaction mixture was read at 30 seconds interval for 150 seconds at 480 nm. The blank was treated the same way except that 80 ul of distilled water was used instead of plasma. The changes in absorbances of both test and blank were determined. The % inhibition of auto oxidation of epinephrine by SOD was calculated and the plasma SOD activity was expressed as U/ml. One unit of SOD activity was equivalent to the amount of SOD that can cause 50% inhibition of epinephrine.

Calculation
Actual OD reading (R) = OD150 – OD30/2
% inhibition = (Rblank – Rtest / Rblank) X 100
Enzyme Unit (U/ml) = (% inhibition/50) X dilution factor.

2.7.7.2 Determination of catalase Activity
Catalase activity will be determined by colorimetric method as described by Sinha, (1972).

Principle
Hydrogen peroxide in the presence of catalase is broken down into oxygen and water, and this reaction is inhibited by the addition of dichromate acetic acid mixture which is reduced to chromic acid when heated in the presence of the remaining hydrogen peroxide. The chromic acetate then produced is measured colorimetrically at 570 nm and the colour intensity is proportional to the unused hydrogen peroxide which is used to determine the activity of catalase in the sample.

Procedure
One milliliter (1 ml) of phosphate buffer (pH 7.0) was mixed with 0.4 ml of 0.2 M hyrdrogen peroxide and allowed for 3 minutes to equilibrate at room temperature. 0.1 ml of sample was added into the reaction mixture and was gently swirled at room temperature. 2 ml dichromate/acetic acid reagent was added after one minute and was thoroughly mixed. The solution was incubated in boiling water for 10 minutes and allowed to cool. The sample blank was prepared and treated the same way except that dichromate/acetic acid mixture was added before the addition of the sample. The color developed was read at 570 nm and the activity of catalase in U/ml which is equivalent to the amount of hydrogen peroxide (μmol) degraded per minute was calculated using 40 μmol of hydrogen peroxide as standards.

Calculation
Actual OD of test = ODblank – ODtest
Activity of catalase (U/ml) = Actual ODtest/ODstd X Std concentration.

2.7.7.3 Determination of Malondialdehyde (MDA) level
MDA level will be determined by the colorimetric method as described by Gutteridge and Wilkins, (1982).

Principle
Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 532 nm. The intensity of colour generated is directly proportional to the concentration of MDA in the sample.

Procedure
To 0.1 ml of sample in test tube was added 1 ml of 1% Thiobarbituric acid dissolved in alkaline medium (0.05 M sodium hydroxide). The mixture was mixed thoroughly, and 1 ml of glacial acetic acid was added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100 °C) for 15 minutes. It was allowed to cool and the turbidity removed by centrifugation at 3000 rpm for 10 minutes. Thereafter, the supernatant was read at 532 nm. The same volume of TBA and glacial acetic acid was added to the blank, but 0.1 ml of distilled water was added to the blank instead of plasma.

Calculation
The level of MDA in the serum is expressed as nmol/ml using the molar extinction coefficient for MDA (1.56x105 M-1cm-1).
MDA (nmol/ml) = (OD X 1000000)/ E532
Where:
E532 = Molar extinction coefficient for MDA (1.56x105 M-1cm-1)
1000000 = conversion of mMol to nMol

2.8 Statistical Analysis
Data was analyzed using Statistical Package for Social Sciences (SPSS version 23) software package. The results were expressed as Mean ± SEM and analyzed using one-way analysis of variance (ANOVA), followed by LSD post HOC test for multiple comparisons. Results were considered significant when $p<0.05$.

3. RESULTS
The results obtained from this study are represented using tables and presented as mean ± standard error of mean (SEM) with p-value ≤ 0.05 considered statistically significant and discussed as follows:

3.1 Effect of Riboceine on Stomach Weight
Table 3.1 Effects of Riboceine on stomach weight.

TREATMENT	STOMACH WEIGHT
Normal control	1.12±0.06a
Ulcer control	1.20±0.05a
Riboceine(3 days)	0.98±0.02b
Riboceine(7 days)	0.98±0.02b

Data was analyzed using ANOVA followed by LSD post-hoc test. Values are presented as mean ± SEM. Mean on the same column with different letter superscript are statistically significant at P <0.05.

The result above(Table 4.1) shows that Riboceine administration for three days significantly reduced stomach weight when compared with normal control and ulcer control(P<0.05). Administration of Riboceine for seven days also significantly reduced stomach weight when compared with normal control and ulcer control (P<0.05). When the treatment duration was compared with each other, there was no significant difference in the stomach weight (P>0.05).

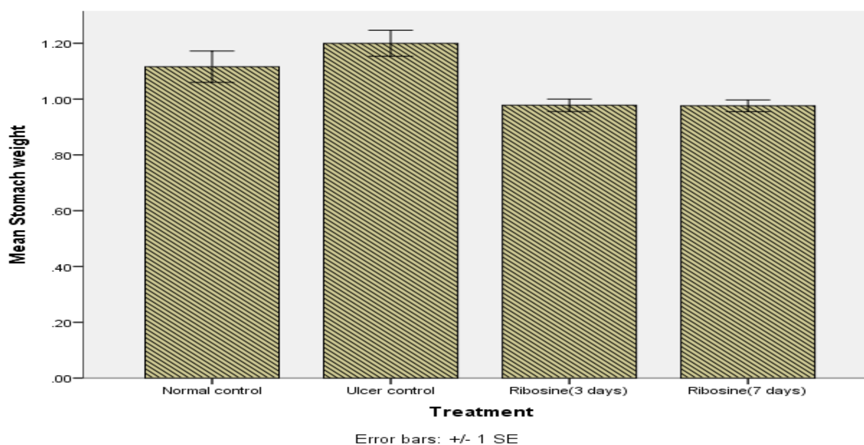


Figure 3.1 Effects of Riboceine on stomach weight.

3.2 Effect of Riboceine on Ulcer Index

Table 3.2 Effects of Riboceine on ulcer index.

TREATMENT	ULCER INDEX
Normal control	0.00±0.00a
Ulcer control	8.80±1.3b
Riboceine (3 days)	2.00±0.9a
Riboceine (7 days)	1.40±0.2a

Data was analyzed using ANOVA followed by LSD post-hoc test. Values are presented as mean ± SEM. Mean on the same column with different letter superscript are statistically significant at P <0.05.

The result above (Table 4.2) shows that there was a significant increase in ulcer index when ulcer control was compared with normal control. This increased ulcer index was significantly reduced following Riboceine pretreatment for three days and seven days (P<0.05). The duration of administration did not significantly affect ulcer index when pretreatment for seven days was compared with pretreatment for three days (P>0.05).

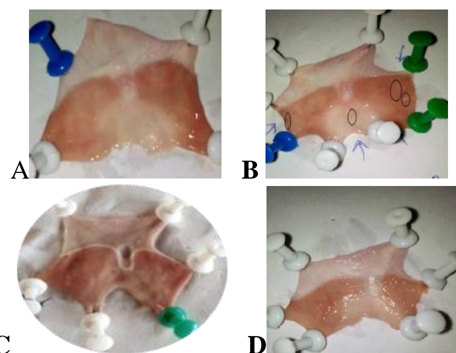


Fig 3.2 Gastroprotective effect of riboceine on indomethacin induced gastric ulcers in adult male albino wistar rats. A (Normal control), B(Ulcer control), C(Riboceine pretreatment for 3 days), D(Riboceine pretreatment for 7 days)

3.3 Effect of Riboceine on MDA, Catalase and SOD.

Table 3.3: Effects of Riboceine on MDA, Catalase and SOD.

TREATMENT	MDA	CATALASE	SOD
Normal control	1.03±0.02a	42.53±2.19a	18.13±0.24a
Ulcer control	1.22±0.06b	36.47±1.05b	18.67±1.25a
Riboceine(3 days)	1.00±0.02a	38.47±0.99ab	20.53±0.03a
Riboceine(7 days)	0.79±0.02c	42.80±1.87a	23.23±0.73b

Data was analyzed using ANOVA followed by LSD post-hoc test. Values are presented as mean ± SEM. Mean on the same column with different letter superscript are statistically significant at P <0.05.

The result above (Table 4.3) shows that ulcer (Indomethacin) significantly increased MDA when compared with normal control (P<0.05). Riboceine administration for three and seven days significantly reduced MDA when compared with ulcer control (P<0.05). Riboceine administration for three days did not show any significant difference when it was compared with normal control (P>0.05). Duration of administration significantly reduce MDA level when Riboceine administration for seven days was compared with administration for three days (P<0.05). Catalase was significantly reduced in the ulcer control when compared with the normal control (P<0.05). Riboceine

administration for three days had no significant effect on catalase when compared with ulcer control (P>0.05). Riboceine administration for seven days significantly increased catalase when compared with ulcer control (P<0.05).

Riboceine administration for three days had no significant effect on SOD when compared with ulcer control and normal control. Administration of Riboceine for seven days significantly increased SOD when compared with ulcer control and normal control (P<0.05). There was a significant difference in SOD when administration for seven days was compared with three days administration (P<0.05).

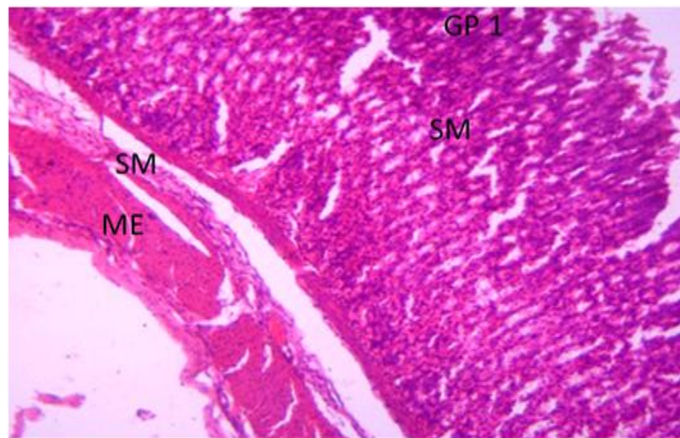
3.4 Acute Toxicity Study

Observations

Table 3.4: Acute Lethal Dose Determination.

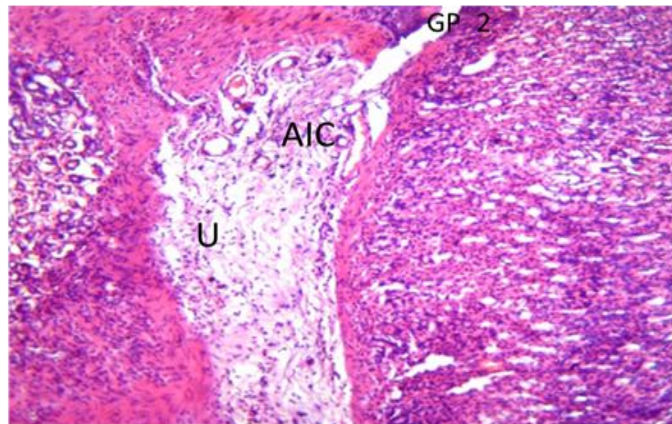
Phase	DOSE	DEATH	OBSERVATION
I	100mg/kg	0/3	Animals were calm
	500mg/kg	0/3	Animals were calm
	1000mg/kg	0/3	Animals were calm
II	1600mg/kg	0/1	Animals were calm
	2900mg/kg	0/1	Animals were calm
	5000mg/kg	0/1	Animals were calm

3.5 Histology of The Stomach Tissue



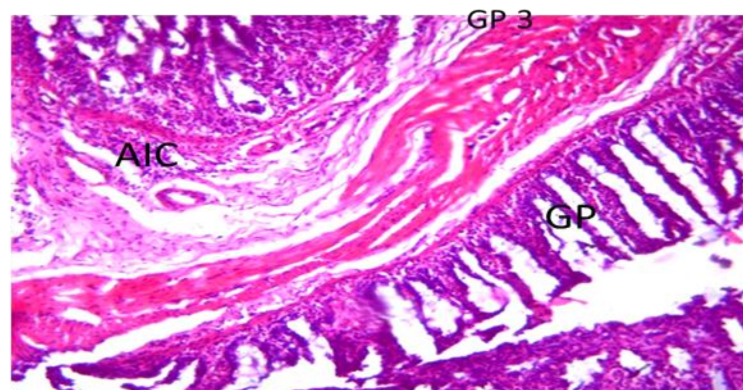
Photomicrograph of group gp 1 control section of stomach (x150)(H/E) shows normal stomach with muscularis externa(ME), ,the muscularis mucosa (MM) and submucosa (SM)

Fig. 3.3: Photomicrograph of group 1 (normal control) section of stomach (x150)(H/E) shows normal stomach with muscularis externa(ME), the muscularis mucosa (MM) and submucosa (SM).



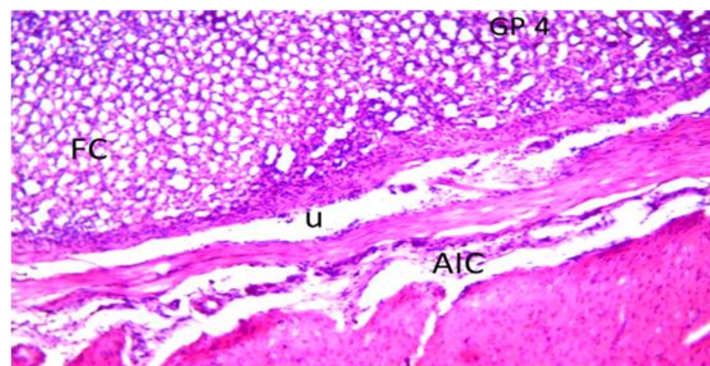
Photomicrograph of group GP 2 section of stomach administered indomethacin only (x150)(H/E) shows sever degeneration **of the lining of the stomach with severe ulceration (U) and moderate aggregate of inflammatory cell (AIC) within the ulcerated area submucosa layer .**

Fig. 3.4: Photomicrograph of group 2 (ulcer control) section of stomach administered indomethacin only.



Photomicrograph of group GP 3 section of stomach administered indomethacin and ribceine (x150)(H/E) shows mild healing with **moderate aggregate of inflammatory cell (AIC) within the submucosa layer and necrotic appearance of the gastric pit (GP)**

Fig. 3.5: Photomicrograph of group 3 section of stomach administered indomethacin and pre-treated with riboceine for 3 days.



Photomicrograph of group GP 4 section of stomach administered indomethacin and ribceine (x150)(H/E) shows mild to moderate healing with mild ulceration (U), **mild aggregate of inflammatory cell (AIC) within the submucosa layer and moderate fatty change (FC) within the** muscularis mucosa

Fig. 3.6: Photomicrograph of group 4 section of stomach administered indomethacin and pretreated with riboceine for 7 days.

4. DISCUSSION

It is a known fact that reactive oxygen species (ROS) are constantly produced in biological tissues, and in the stomach it also results in oxidative stress and gastric ulceration when its production becomes abnormally high, overwhelming the body's endogenous defense mechanisms (Priya, *et al.*, 2012). D-ribose-l-cysteine (DRLC), an analogue of cysteine has been reported to boost cellular antioxidant capacity by enhancing intracellular biosynthesis of glutathione (GSH) (Tanjina *et al.,* 2014). Since treatment with indomethacin can lead to increased production of ROS by activated neutrophils (Naito *et al*, 2006), this present study, investigated the effect of d-ribose-l-cysteine on indomethacin induced oxidative stress and gastric ulceration in wistar rats so as to ascertain the gastroprotective and antioxidant potential of DRLC.

Findings from this present study demonstrate that Riboceine administration significantly reduced stomach weight when compared with normal control group and ulcer control group (P<0.05). The reduction in stomach weight may be as a result of the fact that riboceine which promotes optimal levels of glutathione, is involved in the removal of harmful toxins and elimination of cellular inflammation (Lobo *et al.,* 2010; Max international, 2019).

In this present study, there was a significant increase in ulcer index when ulcer control group was compared with normal control group. This is also observed when the photomicrograph of group 1(normal control) is compared with the photomicrograph of group 2 (ulcer control) and it could be as a result of the inhibitory action of indomethacin on prostaglandin synthesis leading to vasoconstriction and low gastric blood volume, inhibition of bicarbonate secretion and low mucin content coupled with free radicals formation as a result of inflammation and neutrophil infilteration (Brunton *et al.,* 2011; Yoshikawa *et al.,* 1993; Amole *et al.,* 2020). These have been opined as critical biochemical events in the pathogenesis of gastric ulceration (Naito *et al.,* 2006). Decreased prostaglandin level has been attributed to impaired gastroprotection and increased gastric acid secretion which are also important events in the etiology of mucosal ulceration. This is in agreement with the findings of (Bech *et al.,* 2000) and (Muhammed *et al.*, 2012) where indomethacin was reported to have caused alterations in gastric secretions of rats.

Conversely, pre-treatment with Riboceine for three days and seven days significantly reduced the ulcer index (P<0.05). This is in agreement with the findings of (Amole *et al.,* 2020) and is observed in the photomicrographs of group 3 and 4 stomach section. It may be attributed to a combination of events including release of preformed mucus (which provides significant buffering capacity for the neutralization of stomach acid, offers protection against both endogenous aggressors and exogenous gastrotoxic agents such as indomethacin), wound retraction and re-epithelialization which are involved in ulcer-healing process after toxicological injury by indomethacin (Szabo and Hollander, 1985; Naito *et al.,* 1995). Besides the antioxidant action of riboceine which helps to protect the mucus layer and arrests ulcer progression, ulcer healing process which followed pre-treatment with riboceine may also be associated with decreased pepsin activity and elevated mucin level in the gastric mucosa (Sabiu *et al.,* 2015). This is suggestive of the enhanced mucus secretory potential of riboceine and their significant role in ulcer healing process. Also in this present study, there was no significant difference in ulcer index when pretreatment for seven days was compared with pretreatment for three days (P>0.05). This indicates that the potency of riboceine as an antiulcer agent does not depend on how long it is taken.

Cells or tissues are in a stable state if the rates of free radical formation and scavenging capacity are essentially constant and in equilibrium (Sabiu *et al.,* 2015). However, when an imbalance between them occurs, it results in oxidative stress which further deregulates cellular functions leading to different pathological conditions (Sabiu *et al.*, 2014). The results from this present study shows that ulcer (Indomethacin) significantly increased MDA when compared with normal control (P<0.05). Findings from this study also showed significant reduction in the activity of SOD in the untreated groups. There was no significant change in SOD activity in group three, pre treated with riboceine for three days. Catalase was significantly reduced in the ulcer control when compared with the normal control (P<0.05). The increased concentration of MDA as well as reduced activity of SOD and catalase in the stomach of indomethacin-ulcerated rats may have been as a result of increased lipid peroxidation and over production of free radicals resulting in mucosal damage. This is in agreement with the findings of Halici *et al.,* (2005) and Odabasoglu *et al.,* (2006), that reported the ability of free radicals to thwart antioxidant enzyme activities and initiate lipid peroxidation which is important in the mechanism of indomethacin toxicity and that, indomethacin decreases antioxidant enzyme activity in the stomach of the rats thereby inducing gastric ulceration. Odabasoglu *et al.*, (2006) also reported that Indomethacin decreased antioxidant enzyme and it is associated with overpowering of the cellular antioxidant defense systems by free radicals ravaging influence which subsequently results in stomach oxidative injury. However, findings from this present study shows that Riboceine pretreatment for three and seven days significantly reduced MDA when compared with ulcer control (P<0.05), Riboceine pretreatment for seven days significantly increased catalase when compared to ulcer control and also riboceine pretreatment for seven days significantly increased SOD when compared to ulcer and normal control groups. This is in agreement with the findings of (Akingbade *et al.,* 2020). The significantly reduced concentrations of MDA coupled with marked

increase in the activity of SOD and catalase in rats pretreated with riboceine is an obvious indication of antiperoxidative potential and thus antioxidative potential of riboceine.

CONCLUSION

Riboceine is effective in removal of toxins and elimination of cellular inflammation; this was evident in the reduction of the stomach weight of the rats in the treated groups, it can therefore be used in the prevention of gastric ulceration due to its gastro protective and antioxidant potentials.

REFERENCES

1. Abdallah, I. Z.A., Khattab, H.A.H. and Heeba, G.H. Gastroprotective effect of *Cordia Myxa* L. fruit extract against indomethacin induced gastric ulceration in rats. *Journal of Life Science,* 2011; 8(3): 433-445. Retrieved from http://www.lifesciencesite.com, 21 November, 2021.
2. Ajiboye, T., Iliasu, G., Ibitoye, O.B. and Abdulazeez, A.T. Sorghum-based alcoholic beverage, Burukutu, perturbs the redox status of the liver of male rats. *Journal of Food Science and Nutrition,* 2014; 2(5): 591-596. Doi:10.1002/fsn3.139 21 November, 2021.
3. Akingbade, B.T., Ojieh, A.E., Nwogueze, B.C., Omeru, O. and Ebuwa, E.I. Modulatory role of Riboceine and Vitamin C supplementation on altered oxidative stress status of alloxan-induced diabetic wistar rats. *Journal of Bioscience Biotechnology Research communications,* 2020; 13(4): 2130-2138. Doi: 10.21786/bbrc/13.4/75 6 December, 2021.
4. Akpamu, U., Owoyele, V.B., Ozor, M. and Osifo, U.C. Indomethacin- induced gastric ulcer: model in female wistar rats. *International Jouurnal of Basic, Applied and Innovative Research,* 2013; 2(4): 78-84. Retrieved from https://www.ajol.info on 6 December, 2021.
5. Amole, O.O., Akinyede, A.A. and Adewale, M.T. Effect of Riboceine (cellgevity) on ulcer models in rats. *Journal of Pharmacology and Toxicology,* 2020; 4(1): 28-34. Retrieved from https://www.scigreen.com/index.php /JBPT/article/view/69 on 6 December, 2021.
6. Bech, P.L., Xavier, R., Lu, N. and Nanthakumar, N.N. Mechanism of NSAID-induced gastrointestinal injury defined using mutant mice. *Journal of gastroenterology,* 2000; 119(3): 699-705. Doi: 10.1053/gast.2000.16497. 10 December, 2021.
7. Brunton, L., Chebner, B., Knollman, B., Goodman &Gilmans manual of pharmacology and therapeutics. 12th ed. New York: McGraw Hill companies, 2011; 39
8. Falana B.,Adeleke O., Orenolu M., Osinubi A. and Oyewopo A. Effect of D- ribose –L- cysteine on alluminium induced testicular damage in male Sprague- Dawley rats. *JBRA Assisted Reproduction*, 2017; 21(2): 94-100.
9. Gupta P., Nain P., and Sidana J. A review of Oxidative Stress Induced Ulcer protected by natural antioxidant. *International Research Journal of Pharmacy,* 2012; 3(5): 76-81. Retrieved from https://www.researchgate.net/publication/292380619 14 November, 2021.
10. Halici, M.B., Odagbasolu, F., Suleyman, H. and Cakir, A. Effects of water extract of Usnea longissima on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. *International Journal of Phytotherapy and Phytopharmacology,* 2005; 12(9): 656-662. Doi: 10.1016/j.phymed.2004.06.021 5 December, 2021
11. Jaykaran, C. and Kantharia, N.D. How to calculate sample size in animal studies. *Journal of Pharmacology and pharmacotherapy,* 2013; 4(4): 303-306. Doi: 10.4103/0976-500X.119726 6 December, 2021.
12. Kader, T., Porteous, C.M., Williams, M.J.A., Gieseg, S.P. and McCormick, S.P.A. Ribose-cysteine increases glutathione-based antioxidant status and reduces LDL in human lipoprotein(a)mice. *International Journal for Research and Investigation on Atherosclerosis and Related Diseases,* 2014; 37(2): 725-733. Doi: 10.1016/j.atherosclerosis.2014.10.101. 12 December, 2021.
13. Laine, L. and Weinsteine, W. M. Histology of alcoholic hemorrhagic gastritis: a prospective evaluation. *Gastroenterology,* 1988; 94(6): 1254-1262. Doi: 10.1016/0016-5085(88)90661-0 10 December, 2021.
14. Lorke, D. A new approach to practical acute toxicity testing. *Archives of Toxicology,* 1983; 54(4): 275-287. Doi: 10.1007/BF01234480 10 December, 2021.
15. Max international *Independent Studies on Riboceine.* Retrieved from http://diet234.com/wp-content /uploads/2017/04 on 9 August, 2021.
16. Muhammed, A.V.K., Thamotharan, G., Sengottuvelu, S., Haja-Sherief, S. and Sivakumar, T. Evaluation of anti-ulcer activity of Ficus pumila L. leaf extract in albino rats. *Global Journal of research on medicinal plants and indigenous medicine,* 2012; 1(8): 340-351. Retrieved from https://www.sciencedirect.com /science/article/pii/S2214750015000037. 13 December, 2021.
17. Naito Y. and Yoshikawa T. Oxidative Stress involvement and gene expression in indomethacin induced gastropathy: *Redox Report*, 2006; 11(6): 243-53. Doi: 10.1179/135100006X155021 12 June, 2021.
18. Naito, Y., Iinuma, S., Yagi, N. and Boku, Y. Prevention of indomethacin induced Gastric Mucosal injury in *Helicobacter pylori-* negative healthy volunteers: A comparison study Rebamipide vs Famotidine. *Journal of Clinical Biochemistry and Nutrition,* 2006; 43(1): 34-40. Doi: 10.3164/jcbn.2008041 12 December, 2021.

19. Naito, Y., Yoshikawa, T., Tanigawa, T., Sakurai, K., Yamasaki, K., Uchida, M. and Kondo, M. Hydroxyl radical scavenging by rebamipide and related compounds: Electron paramagnetic resonance study. *Free Radical Biology and Medicine,* 1995; 18(1): 117-123. Doi: 10.1016/0891-5849(94)00110-6 6 December, 2021.
20. Odagbasolu, F., Cakir, A., Suleyman, H., Aslan, A., Bayir, Y., Halici, M. and Kazaz, C. Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. *Journal of Ethnopharmacology,* 2006; 103(1): 59—65. Doi: 10.1016/j.jep.2005.06.043 10 December, 2021.
21. Peskar, B.M., Ehrlich, B.A. and Peskar, B.A Role of ATP- Sensitive potassium channels in prostaglandin-mediated gstroprotection in rats. *Journal of Pharmacology and Experimental Therapeutics,* 2002; 310(3): 969-974. Retrieved from https://www.sciencedirect.com/science/article/pii/S22147500017300963.
22. Priya G., Nain P. and Jaspreet S. Oxidative stress induced ulcer protected by natural antioxidant: A review. *International Research Journal of Pharmacy,* 2012; 3(5): 76-81. Retrieved from http://www.researchgate.net/2923. 13 November, 2021.
23. Sabiu, S., Garuba, T., Sunmonu, T., Ajani, E., Sulyman, A., Nurain, I. and Balogun, A. Indomethacin- induced gastric ulceration in rats: Protective roles of Spondias mombin and Ficus exasperate. *Journal of Toxicology Reports*, 2015; 2: 261-267. Doi: 10.1016/j.toxrep.2015.01.002. 12, December, 2021.
24. Sabiu, S., Wudil, A.M. and Sunmonu, T.O. Combined administration of Telfaria occidentalis and Veernonia amygdalina leaf powders ameliorates Garlic-induced hepatotoxicity in wistar rats. *Journal of Pharmacology,* 2014; 5(5): 191-198. Doi: 10.5567/pharmacologia.2014191198 4 December, 2021.
25. Suzuki H., Nishizawa T., Tsugawa H., Mogami S. and Hibi T. Role of Oxidative stress in stomach disorders. *Journal of Clinical Biochemistry and Nutrition,* 2012; 50(1): 35-39. Doi: 10.3164/jcbn.11-115SR 20 August, 2021.
26. Szabo, S. and Hollander, D. Pathways of gastrointestinal protection and repair: Mechanism of action of sucralfate. *American Journal of Medicine,* 1989; 86(6): 23-31. Doi: 10.1016/0002-9343(89)90153-8 13 December, 2021.
27. Sayanti, B., Susri C.R., Subrata, C. and Sandip, K.B. Healing properties of some Indian medicinal plants against indomethacin-induced gastric ulceration of rats. *Journal of Clinical Biochemistry and Nutrition,* 2007; 41(2): 106-114. Doi: 10.3164/jcbn.20070154 December, 2021.
28. Senoner, T. and Ditchi W. Oxidative Stress in Cardiovascular Diseases. *Nutrients,* 2019; 11(9): 2090. Doi: 10.3390/nu11092090 13 November, 2021.
29. Yoshikawa, T., Naito, K.A., Tomil, T., Kaneko, T., Iinuma, S., Ichikawa, H., Yasuda, M., Takahashi, S. and Kondo,M. Role of active oxygen, lipid peroxidation and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. *Gut,* 1993; 34(6): 732-737. Doi: 10.1136/gut.34.6.732. 10 December, 2021.
30. Zawn, V. Free *Radicals: How they affect the body. Retrieved from* www.medicalnewstoday.com on 15 October, 2021.