JOURNAL OF BASIC PHARMACOLOGY AND TOXICOLOGY
Original Research Article

Effects of riboceine (Cellgevity) on ulcer models in rats

Amole OO[1*], Akinyede AA[2], Adewale MT[2]

[1]Department of Pharmacology, Therapeutics and Toxicology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria

[2]University of Lagos, College of Medicine Idi-Araba, Lagos, Nigeria

Abstract

Ulcer has been associated with acid secretion in the gastric mucosa. Recent studies have shown that prostaglandins inhibit gastric secretion, stimulates bicarbonate secretion and increases gastric blood volume, and important in the pathophysiology of peptic ulcer disease and possibly in its prevention and treatment. Studies have shown that glutathione promotes biosynthesis of leukotrienes and prostaglandins. Riboceine is a glutathione precursor. The study was conducted to determine the effect of riboceine on ulcer models in rats. Riboceine was administered at 7 mg/kg, 14 mg/kg, and 28 mg/kg for seven days orally. Misoprostol (50 µg/kg) was used a positive control orally. The prophylactic activity was investigated using ethanol, diclofenac, and pylorus-ligation-induced gastric ulcer models in rats. In the ethanol-induced gastric ulcer model, there was a significant reduction ($p < 0.05$) in the ulcer index by riboceine 28 mg/kg which produced 60% protection and lowest number of lesions. In diclofenac-induced gastric ulcer model, there was no significant reduction in the ulcer index by riboceine at all doses. Riboceine 14 mg/kg produced 72.72 % protection and lowest number of lesion. In pylorus ligation-induced gastric ulcer model, there was a significant reduction ($p < 0.01$, $p < 0.01$, $p < 0.01$) in the ulcer index by riboceine (7 mg/kg, 14 mg/kg, 28 mg/kg) respectively. Riboceine 7 mg/kg produced 87.36 % protection and lowest number of lesions which was lesser than that of misoprostol (50 mg/kg), which produced 96.84 % protection. Riboceine also showed no significant difference in the volume of gastric secretion and no significant differences in the effect of pH when compared with control.Overall, this study showed that riboceine is effective as a preventive measure in ulcer. A possible mechanism of action is by mucosal protection.

Keywords: Riboceine, Cellgevity, ulcer, mucosal protection, prostaglandins

1 Introduction

Peptic ulcer is a sore that develops in the part of the gastrointestinal tract which is exposed to gastric acid and pepsin i.e., the stomach and duodenum. The etiology of peptic ulcer is not clearly known. It results probably due to imbalance between the aggressive (acid, pepsin, bile) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins nitric oxide, high mucosal blood flow, innate resistance of the mucosal cells) factors. Also, long term use of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ibuprofen and naproxen can cause peptic ulcer [1]. Complications may include bleeding, perforation and blockage of the stomach [2].

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants [3]. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses such as glutathione [4].

Antioxidants are molecules present in cells that prevent these reactions by donating an electron to the free radicals without becoming destabilized themselves. One of the biological systems (antioxidants) that detoxifies reactive oxygen species or that repairs the resulting damages caused by free radicals is glutathione [5]. Bioavailability of orally consumed glutathione is poor because the molecules, a tripeptide, is the substrate of proteases (peptidases) of the alimentary canal and due to the absence of a specific carrier of glutathione at the levels of cell membrane [6]. Riboceine is one of the synthetic compounds that was produced to help cells manufacture glutathione. The active ingredient of riboceine is D-ribose-L-cysteine. Whole glutathione consumption cannot be effective, because it would be destroyed in the digestion process before reaching the cells. The ribose component of the riboceine solves the challenges by effectively protecting and delivering the fragile cysteine molecule, enabling the cells to produce glutathione which the need the most. Riboceine, once ingested, will be absorbed, enters the blood stream and delivers cysteine and ribose to cells, supporting glutathione production as well as providing ribose, an integral part of ATP cells natural fuel and source of energy.

The rate of patients with ulcer has greatly increased over the last couple of decades, and while this may be linked to a number of factors responsible, gastric acid secretion has been implicated as one of the factors responsible for ulcer. Studies have shown that glutathione promotes leukotrienes

*Corresponding author; e-mail: femiamole@yahoo.co.uk; Tel: 2348033327658

and prostaglandins biosynthesis and that prostaglandins have been associated with inhibition of gastric secretion, stimulate bicarbonate secretion and increases blood volume. Hence, there is a need for ongoing studies to explore the various glutathione enhancers readily available for prevention of peptic ulcer.

2 Materials and methods

2.1 Drugs and chemicals

Riboceine capsules (Max International Uttah, USA), ethanol (Severn Biotech Ltd, Worcestershine, England), doubled distilled water (Pharmaceutical chemistry Laboratory, College of Medicine, University of Lagos Nigeria), diclofenac and misoprostol were used.

2.2 Experimental animals

Rats (100-140g) of either sex used in this study were obtained from the Laboratory Animal Centre of the College of Medicine University of Lagos, Lagos, Nigeria (CMUL). The animals were housed in plastic cages at room temperature under standard environmental conditions, received rodents chow and had free access to drinking water *ad libitum*. All experiments were performed in compliance with institutional and ethical treatment of experimental animals as contained in United States National Institute for Health Guidelines [7]. Riboceine was administered at 7 mg/kg, 14 mg/kg, 28 mg/kg and standard group was administered with misoprostol 50 µg/kg.

2.3 Experimental design

After one week of acclimatization, the rats were divided into 5 groups (n=5), and treatment was carried out according to the group allotment. Group 1 (control) received doubled distilled water at 10 ml/kg body weight orally for 7 days. Groups 2-4 (riboceine treatment) were administered with 7 mg/kg, 14 mg/kg and 28 mg/kg body weight respectively for 7 days orally. Group 5 (standard treatment) were given with misoprostol 50 µg/kg for 7 days orally.

2.4 Anti-ulcer activity

2.4.1 Ethanol-induced ulcer

Animals were fasted for 24 hr after which they were administered with ethanol (1 mL). After one hour, the animals were sacrificed and the stomachs were removed and examined for lesions [8].

2.4.2 Diclofenac-induced ulcers

Animals were fasted for 24 hr after which they were ligated according to the method of Shayne et al. [9] under light anesthesia with care taken not to cause bleeding or to occlude blood vessels. Six hours after ligation, the animals were sacrificed by cervical dislocation. The stomachs were removed, contents collected, measured for stomach volume content, centrifuged and subjected to analysis for titration. In all the models, lesions were scored as follows: 0 = no lesion; 0.5 = hemorrhage; 1 = 1-3 small lesions; 2 = 1-3 large lesions; 3 = 1-3 thickened lesions; 4 = more than 3 small lesions; 5 = more than 3 large lesions; 6 = more than 3 thickened lesions [10].

The ulcer index was calculated according to the following formula:

Ulcer Index (UI) = (ADU x % RU) / 100

Where, % RU = percentage rats with ulceration (ulceration is defined as ulcer score of 1 and above); ADU = Average degree of ulceration for each group (i.e. mean ulcer score for each group).

The percentage inhibition was calculated by,

% Inhibition = [(UI control – UI treatment) / UI control] x 10

2.5 Antioxidant assay

Malondialdehyde (MDA), Glutathione s- Transferase (GST), Glutathione peroxidase (GP_X) were analyzed. The stomachs of the animals were washed in an ice cold 1.15 % KCl solution, blotted and weighed. They were then homogenized with 0.1 mL phosphate buffer (pH 7.2), putting the stomachs each into the mortar, laboratory sand was added to it (acid washed sand) and it was blended in the mortar and pestle together. The resulting homogenate was centrifuged at 2500 rpm for 15 min, and the supernatant was decanted and stored at – 20 °C until analysis.

2.5.1 Reduced glutathione (GSH) assay

The reduced glutathione (GSH) content of the tissue as non-protein sulphydryls was estimated according to the method described by [11]. A 1.0 mL of supernatant was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5, 5 dithiobisnitrobenzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate) and 3.0 mL of phosphate buffer (0.2 M, pH 8.0) and the absorbance was read at 412 nm [11]

2.5.2 Superoxide dismutase (SOD) assay

Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by [12]. The reaction mixture (3 mL) containing 2.95 mL of 0.05 M sodium carbonate buffer pH 10.2, 0.02 mL of the supernatant and 0.03 mL of epinephrine in 0.005 N HCl was used to initiate the reaction. The reference cuvette contained 2.95 mL buffer, 0.03 mL of substrate (epinephrine) and 0.02 mL of water. Enzyme activity was calculated by measuring the absorbance at 480 nm for 5 minutes by using extraction coefficient $\sum = 4020\ M^{-1}cm^{-1}$[13].

2.5.3 Catalase (CAT) enzyme assay

Catalase activity was determined according to previously published method [14]. It was assayed colorimetrically at

620 nm and expressed as μ moles of H_2O_2 consumed/min/mg protein at 25 ºC. The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M phosphate buffer (pH 7.0), 0.1 mL of supernatant and 0.4 mL of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 mL of dichromate acetic acid reagent (5 % potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). $\sum = 40\ M^{-1}\ cm^{-1}$ was used for the calculation [15].

2.5.4 Malondialdehyde (MDA) assay

Malondialdehyde (MDA), an index of lipid peroxidation, was determined using the previously published method [16]. A 1.0 mL of the supernatant was added to 2 mL of (1:1:1 ratio) TCA-TBA-HCL reagent (thiobarbituric acid 0.37 %, 0.2 N hydrochloric acid and 15 % tricarboxylic acid) and boiled at 100 °C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance was read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDA-TBA-complex of $1.56 \times 10^5\ M^{-1}cm^{-1}$ [17].

2.5.5 Glutathione-s-transferase assay

A 1.67 mL volume of sodium phosphate buffer (0.1 M pH 6.5) was added to the test tube containing 0.2 mL of 1 mM of homogenate in a total volume of 2 mL. The change in absorbance was recorded at 340 nm and the enzyme activity was calculated as nano moles of CDNB conjugates formed per min/mg protein using molar extinction coefficient of $9.6 \times 10^3\ M^{-1}\ cm^{-1}$.

2.6 Statistical analysis

Results obtained from the study were expressed as mean ± standard error of the mean (S. E. M). Statistical comparisons between the groups were analyzed using the one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test was performed for inter group comparison. Graphs were plotted using Graph Pad Prism 8. Results were considered to be significant at $p \leq 0.05$.

3 Results

3.1 Ethanol-induced ulcer model

As shown in figure 1, there was ulcer index of 6.5 ± 0.6325 in control rats. Riboceine produced a significant ($p < 0.05$) reduction in ulcer index with peak effect (60%) at the highest dose of 28 mg/kg which is less than misoprostol (50 μg/kg) whose protection was 96% as seen in figure 1.

3.2 Diclofenac-induced ulcer model

There was ulcer index of 3.3 ± 0.8 in control rats. Riboceine produced no significant reduction in ($p > 0.05$) ulcer index with peak effect (72.72%) at the highest dose of 14 mg/kg which is less than misoprostol (50 μg/kg) whose protection was 93.9% as seen in figure 2.

3.3 Pylorus ligation-induced ulcer model

Riboceine (7, 14, 28 mg/kg) produced a significant reduction ($p < 0.01$) in the ulcer index. The peak effect was at 7 mg/kg (% protection of 87.4) which is lesser than misoprostol (50 μg/kg) whose protection was 96.8% as seen in figure 3.

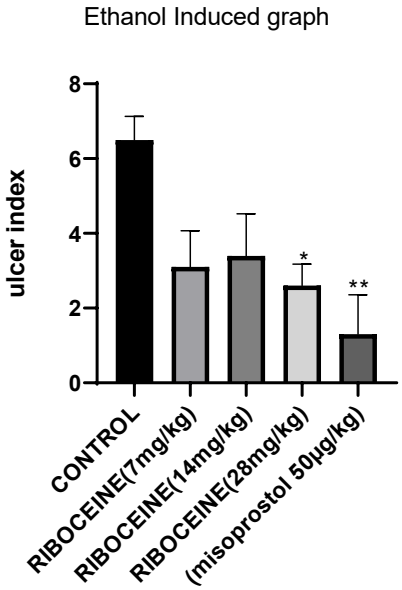

Figure 1: ethanol induced model showing the ulcer index. Bar chart represents mean±SEM. *P<0.05, **P<0.01 vs control (one way ANOVA followed by Turkey's multiple comparison test).

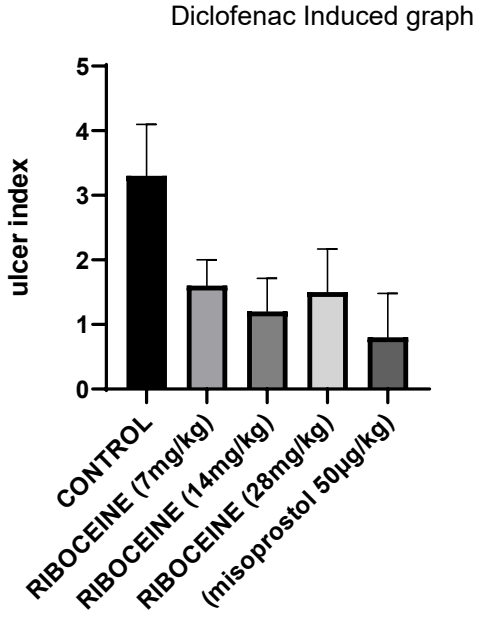

Figure 2. Diclofenac induced model showing the ulcer index. Bar chart represents mean±SEM.

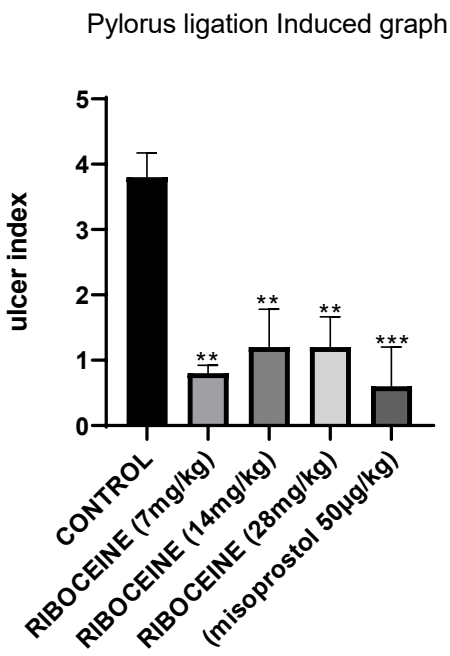

Figure 3. Pylorus ligation-induced model showing the ulcer index. Bar chart represents mean±SEM. **P<0.01, ***P<0.001 vs control (one way ANOVA followed by Tukey's multiple comparison test).

3.4 Effect of riboceine and misoprostol on antioxidants indices in rat stomach

Riboceine at 7 mg/kg, 14 mg/kg, 28 mg/kg doses produced a significant increase ($p < 0.05$, $p < 0.01$, $p < 0.01$) respectively in the level of GSH in the stomach compared to the control. Riboceine at 7 mg/kg, 14 mg/kg, 28 mg/kg doses produced no significant difference in the levels of the diverse antioxidants (SOD, CAT, MDA, GST, GPX). Misoprostol (50 µg/kg) produced no significant difference at all levels of the various antioxidants when compared with the control group, as seen in Table 1.

3.5 Histopathological analysis

The histopatholgical analysis of stomach tissues were given in Fig 4, Fig 5, Fig 6 and Fig 7.

4 Discussion

The etiology of peptic ulcer is unknown in most cases. Yet, it is generally accepted that it is from imbalance between aggressive factors and the maintenance of mucosal integrity via the endogenous defense mechanism [18]. To regain the balance, different therapeutic approaches have been tried. Riboceine formed other means of glutathione enhancement like N-acetylcysteine [19] helps in the biosynthesis of prostaglandins which has shown to inhibit acid secretion in

Table 1. Effect of riboceine and omeprazole on antioxidants indices in rat stomach.

Treatment	GSH ($\mu mol/ml$)	SOD (μmol/ml/min/mg protein)	CAT (μmol/ml/min/mg protein)	MDA ($\mu mol/ml$)	GST (μmol/ml/min/mg protein)	GPx(μmol/ml/min/mg protein)
Control	12.155± 1.275	4.240±0.740	25.240±1.380	1.325±0.095	1.160±0.110	0.525±0.105
Riboceine (7 mg/kg)	1.880 **±0.400	3.505±1.775	20.200±5.460	2.800±0.405	0.720±0.200	0.055±0.005
Riboceine (14 mg/kg)	3.765**±0.135	2.880±0.240	20.190±3.620	2.520±0.330	0.665±0.125	0.300±0.200
Riboceine (28 mg/kg)	5.375*±1.210	2.540±0.380	16.675±1.315	2.155±0.115	0.595±0.055	0.135±0.005
Misoprosto l (50 µg/kg)	11.215±1.005	2.91±0.08	14.91±5.49	2.7±0.48	0.845±0.065	0.300±0.070

Values are expressed as mean±SEM. (n=5). *p<0.05, **p<0.01 vs Control (One way ANOVA with Tukey's multiple comparison Test)

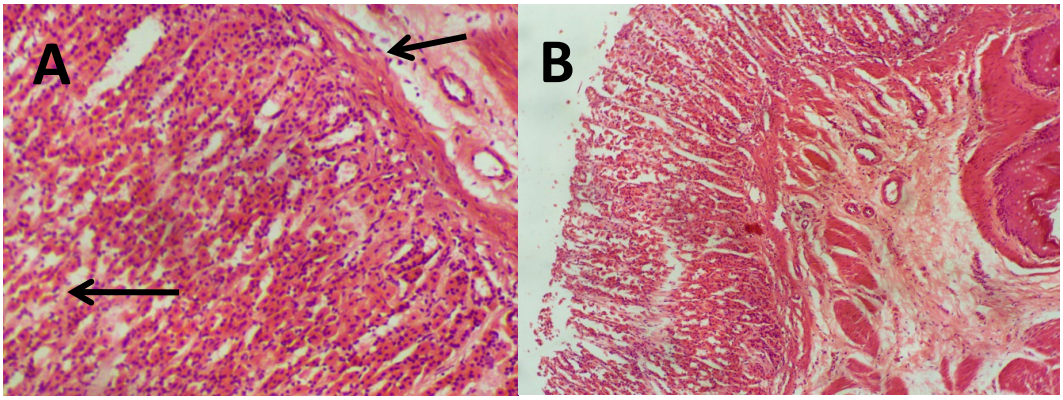

Figure 4. Histopathology diagrams showing the stomach for anti-ulcer activity study for (A) Control at 10mg/kg and (B) misoprostol (50 µg/kg)

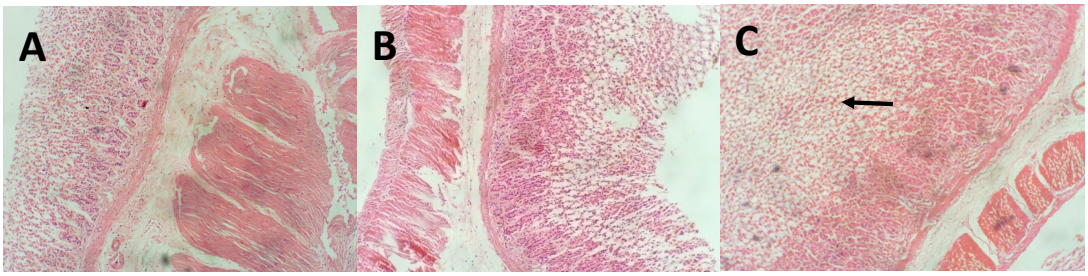

Figure 5. Histopathology diagram showing the stomach of rats for anti-ulcer activity study at (A) 7 mg/kg, (B) 14 mg/kg and (C) 28 mg/kg of riboceine

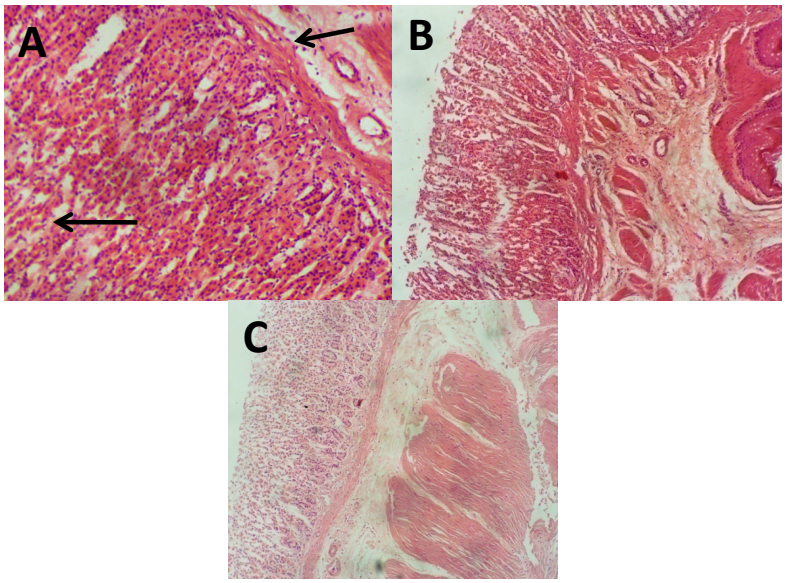

Figure 6. Histopathology result of stomach of rats after treated with Riboceine 7 mg/kg, Misoprostol (50 µg/kg) and control 10mg/kg. Control stomach (A) shows mild mucosal erosion. Misoprostol stomach (B) shows normal. No abnormalities seen in riboceine-treated animals (C)

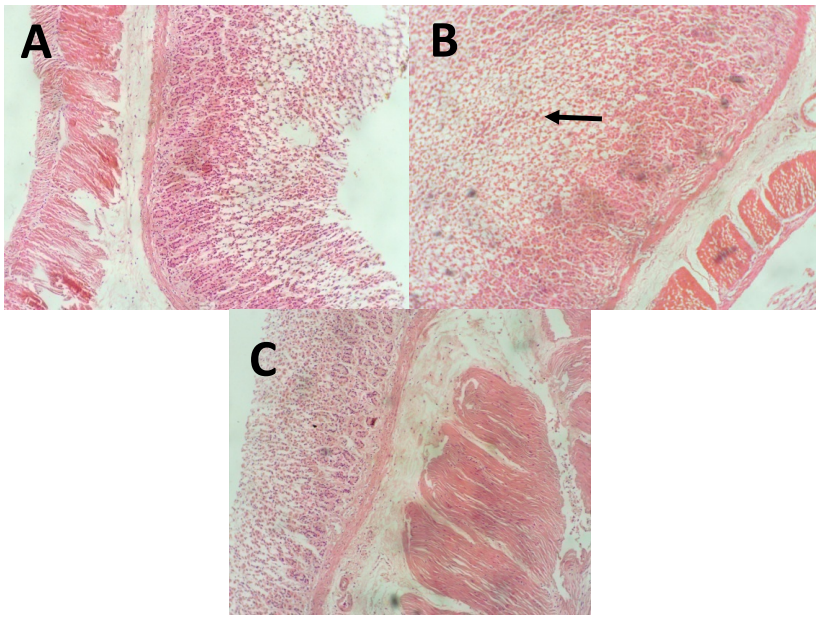

Figure 7. Interpretation of the histopathology result of riboceine 7 mg/kg, 14 mg/kg and 28 mg/kg administration on stomach of rats. Riboceine 14 mg/kg (A) and 28 mg/kg stomach (B) shows mucosal erosion and riboceine 7 mg/kg (C) shows no abnormalities

gastric mucosal. Its prophylactic property was investigated in this study using ethanol, diclofenac, pylorus ligation-

induced ulcer models in rats.

The induction of peptic ulcer lesions with the use of ethanol is thought to be due to disturbance in gastric secretion damage to the mucosa, alteration in permeability, gastric mucus depletion and free radical production [20]. This is attributed to the release of superoxide and hydroperoxy free radicals during metabolism of ethanol, as oxygen derived free radicals have been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa [21]. Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of hemorrhage and necrotic aspect of tissues injury. This leads to cell death and exfoliation in the surface epithelium [22]. In the course of the experiment, riboceine 28 mg/kg produced 60% and lowest numbers of lesions which was less than the effect (96%) by misoprostol 50 µg/kg , a significant reduction ($p < 0.03$) in the ulcer index was obtained at 28 mg/kg.

The induction of ulcer lesions with the use of diclofenac was thought to arise as a result of decreasing the mucus and bicarbonate secretion thereby decreasing the effectiveness of the juxtamucosal pH gradient in protecting the epithelium. Diclofenac is known to destroy the layer of surface-active phospholipids or the mucosal surface, independent of effects on prostaglandin synthesis [23]. Lastly, it diminishes the ability of epithelial growth factor to promote epithelial repair. Thus, inhibition of epithelial proliferation has been observed when the cells are exposed to NSAIDs and this appears to involve a reduction of epithelial growth factor signaling pathway [24] and inhibition of epithelial growth factors signaling pathway [25]. There was no significant reduction ($p > 0.05$) in the ulcer index in this model. Riboceine 14 mg/kg produce 72.72% (Fig 3) and the lowest number of lesions which was lesser than the effect (93.9 % by misoprostol 50 µg/kg (Fig 3)). The induction of ulcer lesion with the use of pylorus ligation may arise due to stress-induced increase in gastric hydrochloric acid secretion and (or) stasis of acid, and the role of secretion is also a vital factor in ulcer formation due to exposure of the unprotected lumen of the stomach which are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosa barrier [26]. These factors are associated with the development of upper gastrointestinal damage which include lesions, ulcers and life-threatening perforation and hemorrhage. It may be suggested that the riboceine inhibits acid production and a slight acid neutralizing effect as it probably protected the mucus membrane from further damage due to permeability of the mucus membrane. This model showed that riboceine 7 mg/kg produced a significant ($p < 0.01$) reduction (94.85%) while 14 mg/kg and 28 mg/kg produced (88%) protection which was lesser than the effect of 96.8 % by misoprostol 50 µg/kg (Fig 4).

Conclusion

The experimental findings in this study indicate that riboceine is effective in the prevention of ulcer and the possible mechanism of action may be by mucosal protection through its cytoprotective effect by stimulating prostaglandin synthesis leading to inhibition of acid secretion. This justifies its use in the prevention of ulcer.

Conflict of interest

None.

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