

**ROLE OF RIBOFLAVIN AND ITS SUPPLEMENT ON INDOMETHACIN-INDUCED  
ULCER IN ALBINO RATS**Adebayo-Gege GI<sup>2</sup>, Igbokwe VU<sup>1</sup>, \*Seriki SA<sup>2</sup> and Olufeagba M.<sup>2</sup><sup>1</sup>Department of Physiology, Faculty of Medical Sciences Nnamdi Azikwe University, Nigeria<sup>2</sup>Department of Physiology, Faculty of Basic Medical Sciences, Bingham University, Nigeria**\*Corresponding Author: Seriki SA**

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**ABSTRACT**

**Background/Aim:** Vitamins are vital nutrients required by organisms for proper growth and sustenance. They are organic compounds and vital amines required in small quantities which are not synthesizable in adequate quantities by the living organisms. Riboflavin, also known as Vitamin B<sub>2</sub>, has been known to cause overproduction of prostaglandin E<sub>2</sub> in the gastric mucosa, which in turn causes suppression of gastric acid production. This study investigates the role of riboflavin and its supplement on indomethacin-induced ulcer in albino rats. **Method:** Thirty six Swiss albino male rats (120-180g) were randomly divided in to six (6) groups; group I (control), group II (indomethacin treated, 40mg/kg), group III (0.3mg/kg of yeast), group IV (low dose of Vit.B<sub>2</sub>, 0.065mg/kg), group V (medium dose of Vit.B<sub>2</sub>, 0.13mg/kg), group VI (high dose of Vit.B<sub>2</sub>, 0.26mg/kg). The animals were pre-treated with yeast and Vit.B<sub>2</sub> for 14 days and fasted for 24hours before ulcer induction. Ulcer was induced using indomethacin (40mg/kg) and sacrificed after 16hrs. Ulcer was scored and the stomach was fixed in 10% formalin for H&E processing for parietal cell count. Test of statistical significant difference between treatments were done using the student's t-test for paired observation, p<0.05. **Result:** In the group III (pre-treated with yeast), the ulcer score was 11.40 ± 1.72 while group II (indomethacin -treated), 17.80± 1.39; the score was significant lower in the yeast pretreated animals compared to the group treated with indomethacin, p<0.05. In group IV (low dose of vit.B<sub>2</sub> (0.065mg/kg), the ulcer score was 15.20± 1.72; compared to the group II, there was no significant differences. The group V and group VI ulcer score was 7.00±0.55 and 4.60±0.93, p<0.05 was significant reduced as compared with group II (indomethacin-induced ulcer). Mucous cell count for the yeast pretreated, group III, V and VI was 3928.2±447.06/mm<sup>2</sup>, 1384,8±102.85/mm<sup>2</sup>, 3113.2±174.54/mm<sup>2</sup>, 4164.2±171.24/mm<sup>2</sup> as compared with the group II (indomethacin, induced ulcer) 1925±81.06/mm<sup>2</sup>, p<0.05 were significant respectively. The group IV mucous cell count was 1384±102.85/mm<sup>2</sup> was significantly lower as compared with the indomethacin treated group II. The parietal cell count in the group III (yeast treated animals) as compared with the control shows no significant difference (27.48±1.22/mm<sup>2</sup> vs 24.8± 1.29/mm<sup>2</sup>). The group IV, V and VI (24.86 ± 1.33/mm<sup>2</sup>, 28.86±1.33/mm<sup>2</sup>, 26.76±0.74/mm<sup>2</sup>), there was no significance as compared with the group II, and group I. **Conclusion:** Riboflavin and yeast are potent anti-ulcer drugs by its inhibitory actions on the ulcer formation, downstream of parietal counts and upregulation of mucous cell count (responsible for mucous secretion, a defense system).

**KEYWORDS:** Vitamin B<sub>2</sub>, Parietal cells, Mucous cell, Ulcer score, Indomethacin.**INTRODUCTION**

Peptic ulcer is the most common gastrointestinal disorder in clinical practice.<sup>[1]</sup> It is a conglomerate of heterogenous disorders, which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid and/or pepsin. Various factors could contribute to the formation of peptic ulcer such as the infection of stomach by *Helicobacter pylori*<sup>[2]</sup>, the frequent use of non-steroidal anti-inflammatory drugs (NSAIDs)<sup>[3]</sup> and consumption of alcohol, stress, smoking.<sup>[4]</sup> Duodenal ulcers are more common in adult males. Gastric ulcers occur commonly at old age and in lower socio-economic class of individuals. Although a number of anti-ulcer drugs such as H<sub>2</sub> receptor antagonists, proton pump

inhibitors<sup>[5]</sup> and cytoprotectants are available for ulceration, all these drugs have side effects and limitations. Its pathogenesis is influenced by factors such as acid-pepsin secretion, mucosal barrier, mucous secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermal growth factor).<sup>[6]</sup> Gastric ulcers emanate from an imbalance between aggressive and protective factors.<sup>[7]</sup> The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs.<sup>[8]</sup>

The search for an ideal anti-ulcer drug continues and has been extended to other diets supplement for their better protection, easy availability, low cost, and safety. Vitamins are organic compounds required by organisms as vital nutrients (vital amines) in limited amounts.<sup>[9]</sup> They are not synthesizable in adequate quantities by living organisms, and thus, must be obtained from diet.<sup>[9]</sup> They are classified on the basis of their biological and chemical activity as opposed to their structure. They can also be loosely classified on the basis of solubility properties. Based on their solubility properties, they are broadly classified into water-soluble and lipid-soluble vitamins. Thirteen vitamins are universally recognized at present.<sup>[10]</sup> The lipid-soluble vitamins are Vitamins A, D, E and K, while the water soluble vitamins comprise only Vitamins B-complex and C. Vitamin B-Complex refers to the group of B-vitamins that play very important roles in cellular metabolism. They were once thought to be a single vitamin referred to as Vitamin B. Later research evidenced the existence of chemically distinct vitamins that often co-exist in the same types of food.<sup>[9]</sup> In general, supplements containing all eight B-vitamins are referred to as Vitamin B-complex. Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) is a water soluble vitamin present in a wide variety of foods. It was initially isolated, although not purified, from milk whey in 1879 and given the name lactochrome. It can be crystallized as orange-yellow crystals and in its pure form is poorly soluble in water. Its most important biologically active forms, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), participate in a range of redox reactions, some of which are absolutely key to the function of aerobic cells.<sup>[11]</sup>

This study investigates the gastro-protective role of riboflavin and its supplement on indomethacin-induced ulcer in rat's stomach.

## MATERIALS/METHOD

**Drugs and chemicals:** Riboflavin and yeast were both purchased from Sigma (St. Louis, MO, USA). The solvents and other chemicals of analytical grade were used and obtained from the institute's central store. Indomethacin was purchased from Medrel pharmaceuticals (India) PVT Limited.

**Animals and Grouping:** Thirty six (36) rats were purchased from the Nigerian Institute of Trypanosomiasis Research in Vom, Plateau state. They were divided into six (6) groups with six (6) in each group. The animals were acclimatized to laboratory conditions for a week before the commencement of the experiment, and were allowed free access to standard dry pellet diet and water *ad libitum*. The experiment protocol was carried out with the approval of Bingham University Animals Ethics Committee. The animals were kept under a controlled temperature atmosphere of 24-27°C and a 12-hour light/dark cycle. They were fasted overnight with access to water prior to each experiment.

## Animal Grouping

Group I - Normal Control (vehicle only i.e. 0.1% Sodium Bicarbonate)  
 Group II - Indomethacin with vehicle (40mg/kg)  
 Group III - Naturally Occurring Riboflavin (Yeast)  
 Group IV - Low Dose Riboflavin Tablets  
 Group V - Medium Dose Riboflavin Tablets  
 Group VI - High Dose Riboflavin Tablets

## Ulcer studies

40mg/kg Indomethacin was administered after two weeks pre-treatment with different doses of riboflavin and yeast. About 16 hours post administration of indomethacin. The animals were sacrificed under proper anaesthesia and their stomachs were dissected out along their greater curvatures and cleaned with normal saline solution to remove any outstanding debris. The stomachs were exposed on cork boards and carefully examined to establish the integrity of the eroded mucosal lining using a magnifying glass. The mucosal lesions were scored by three observers. The gastric ulcer lesions were scored/graded according to the method described by Alphin and Ward<sup>[12]</sup> which depends on the calculation of a lesion index using a 0-2 scoring system based on the length of each lesion.

A score for ulceration was made as follows

**Table 1: Ulcer Scoring Criteria<sup>[12]</sup>**

Ulcer Score	Criteria
0	Normal Stomach
0.5	Punctuate hemorrhage or pin-point ulcer
1	Two or more hemorrhagic ulcers
2	Ulcers >3mm in diameter

## Determination of Parietal cell count and mucous cell count

After the animals are sacrifice, the stomach was excised and cut at the greater curvature. Then washed and transferred in to 10% formalin. Sections were therefore prepared from strips removed from the fundic area of the stomach and stained using the method of Marks and Drysdale<sup>[13]</sup> using the Hematoxylin and Eosin stain PARS staining for mucous cell. The nuclei of the parietal cells were stained blue-purple while the mucous cells appear magenta red. Parietal cell population was counted as number of cells/field as described by Perraso.<sup>[14]</sup>

## Statistical Analysis

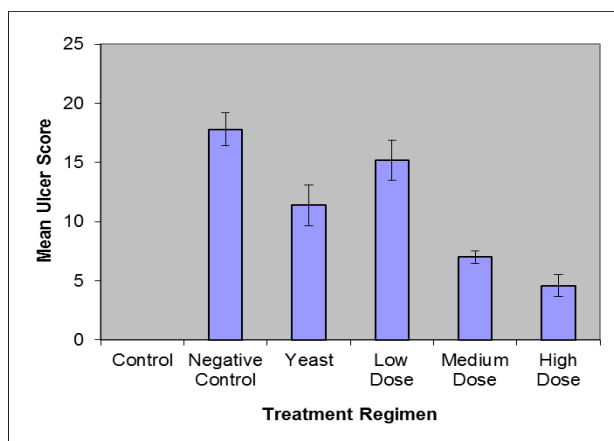
Test of statistical significant difference between treatments were done using the student's t-test for paired observation. The data collected from the pre-performed experiments was analyzed by one-way analysis of variance followed by multiple comparison t-tests for evaluation of differences between groups. The results were expressed as the mean and the standard error of mean. P < 0.05 was considered to be statistically insignificant.

## RESULTS

**Effects of Riboflavin Pre-treatment on Mean Ulcer Scores:** The Mean Ulcer Score for the animals in Group III (naturally occurring riboflavin-yeast was  $11.40 \pm 1.7$  as compared with the group II, there was a significant difference between the two groups ( group II =  $17.80 \pm 1.39$  vs Group III=  $11.40 \pm 1.72$ ,  $p < 0.05$ ).

In Group IV (Low Dose Riboflavin), the Mean Ulcer Score was  $15.20 \pm 1.72$  as compared with the group II in which ulcer was induced, there was no significant difference between the two groups ( Group II =  $17.80 \pm 1.39$  VS Group IV =  $15.20 \pm 1.72$ ,  $p < 0.05$ ).

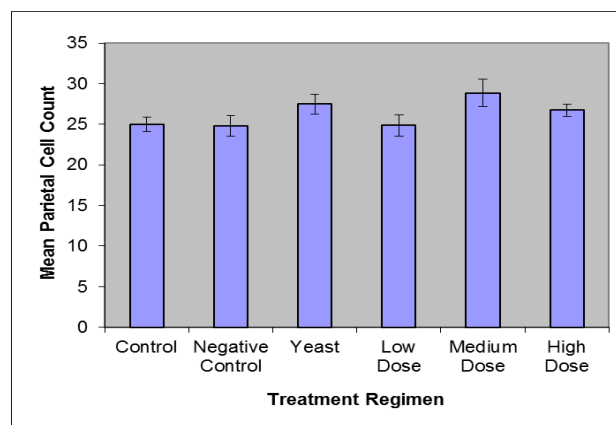
The Group V (Medium/Normal Dose Riboflavin) was  $7.00 \pm 0.55$ . When compared with the group II in which ulcer was induced, there was a significant difference between the two groups (Ulcer score of group II =  $17.80 \pm 1.39$  Vs group V =  $7.00 \pm 0.55$ ,  $p < 0.05$ ). The Mean Ulcer Score for the animals in Group VI which were pretreated with high dose Riboflavin (0.260 mg/kg body weight) was determined to be  $4.60 \pm 0.93$ . When compared with group II, there was a significant difference between the two groups (Group II =  $17.80 \pm 1.39$  vs Group VI =  $4.60 \pm 0.93$ ,  $p < 0.05$ ).



**Figure 1: Effect of riboflavin on indomethacin induced ulcer,  $p < 0.05$**

### Effects of Riboflavin on Parietal Cell Counts

The administration of riboflavin produced no significant change in the average number of parietal cells per unit area in the various groups. From figure1, the Mean Parietal Cell Count in , group I was  $24.98 \pm 0.92$  cells/field , as compared with Group II had a Mean Parietal Cell Count of  $24.80 \pm 1.29$  cells/field has no significant difference. For Group III, the Mean Parietal Cell Count was  $27.48 \pm 1.22/\text{mm}^2$  vs group II,  $24.80 \pm 1.29/\text{mm}^2$ , there was increase in the parietal cell counts ;  $p > 0.05$ . Group IV  $24.86 \pm 1.33/\text{mm}^2$  vs. group II,  $24.80 \pm 1.29/\text{mm}^2$  control;  $p > 0.05$ ,no significant differences .Group V, Parietal Cell Count of  $28.86 \pm 1.69/\text{mm}^2$  VS group II  $24.80 \pm 1.29/\text{mm}^2$  control; there was increase in number of parietal cell,  $p > 0.05$ . The group VI Mean Parietal Cell Count was calculated to be  $26.76 \pm 0.74/\text{mm}^2$  vs.  $24.80 \pm 1.29/\text{mm}^2$  control;  $p > 0.05$ .



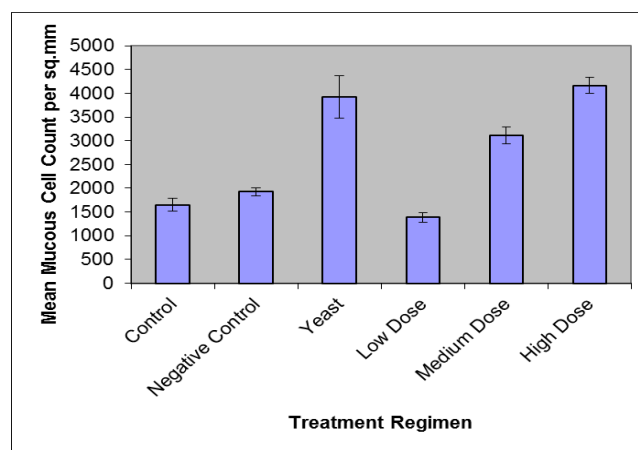
**Figure 2: Effect of riboflavin on mean parietal cell count,  $p < 0.05$**

### Effects of Riboflavin on Mucous Cell Count

Riboflavin pretreatment produced some very interesting significant changes evidenced in the microscopic and statistical analysis of the stomach mucosae of the rats especially in the groups in which yeast, medium and high dose riboflavin were administered.

From figure 3, mean mucous cell count in the group I was  $1653.0 \pm 137.06$  while in the group II, mean mucous cell count was estimated to be  $1925 \pm 81.06$ .

Figure 2 compares the treated groups: III, V, VI, with the group II, there was significant increase in the level of mucous cell count,  $p < 0.05$ . The mean mucous cell count in the respective treated groups: IV, V, VI was  $3928.2 \pm 447.06/\text{mm}^2$ ,  $3113.2 \pm 174.54/\text{mm}^2$ ,  $4164.2 \pm 171.24/\text{mm}^2$  Vs  $1925 \pm 81.06/\text{mm}^2$ . In the group IV, mucous cell count per unit area was significant low as compared with the group II ( $1384.8 \pm 102.85/\text{mm}^2$  vs.  $1925 \pm 81.06/\text{mm}^2$ ), this suggest that the riboflavin could be dose dependent.



**Figure 3: Effect of riboflavin on the mean mucous cell count,  $P < 0.05$**

## DISCUSSION

The effect of riboflavin and riboflavin-rich supplements such as yeast on indomethacin-induced was investigated in this study.

Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) commonly used for fever and pain, effectively induced acute ulcer in the rats.<sup>[15]</sup> It caused gastric injury by suppressing the formation of prostaglandins that is responsible for production of mucus, bicarbonate secretion, increases the mucosal blood flow, increases the resistance of epithelial cells to injury and inhibits release of inflammatory mediators such as tumor necrosis factor, interleukins.<sup>[16]</sup>

Results showed that riboflavin has a significant gastrointestinal protective activity against indomethacin-induced gastric ulceration. Riboflavin (vit.B<sub>2</sub>) administered in different doses (0.065mg/kg, 0.13mg/kg, 0.26 mg/kg) and riboflavin-rich supplement: yeast (0.3mg/kg) also significantly reduced the ulcer score in the different groups.

From figure 1, the group VI pretreated with high dose of riboflavin, showed a significant reduction in the ulcer score from  $17.80 \pm 1.39$  to  $4.60 \pm 0.93$ ,  $p < 0.05$  as compared with the untreated group II (indomethacin induced ulcer).

The ulcer score in the group VI ( $4.60 \pm 0.93$ ) treated with high dose of riboflavin was low compared to those of group V ( $7.00 \pm 0.55$ ) and IV ( $15.20 \pm 1.72$ ) treated with medium and low doses of riboflavin respectively, thereby suggesting that riboflavin pre-treatment role on ulcer is dose-dependent. There was also a significant reduction in ulcer score in Group III (treated with naturally occurring riboflavin: yeast, with  $11.40 \pm 1.7$  compared to the group II (given indomethacin only  $17.80 \pm 1.39$ ), indicating that yeast also has some gastrointestinal protective properties.

Riboflavin also increased the number of mucous cells in the experiment with dose (Fig 3) even as yeast also does, indicating that increased prostaglandin synthesis increases mucus and bicarbonate secretion, promote mucosal blood flow, increases the resistance of epithelial cells to cytotoxins-induced injury and suppress the recruitment of leukocytes into gastric mucosa. Prostaglandins also down regulate the release of a number of other inflammatory mediators that may contribute to the generation of gastric ulcer.<sup>[17,18]</sup>

This experiment also show yeast a rich naturally occurring riboflavin to have a good gastro protective effect and it is therefore suggested that it could carried out its activities by increasing prostaglandin synthesis and which increase the resistance of the mucosa to inflammatory mediator that may generate gastric ulcer.

## CONCLUSION

Riboflavin and yeast, a riboflavin supplement antagonize the aggressive factors like acid and pepsin that play an important role in the pathogenesis of gastric ulcers, by augmenting the defensive mucosal factors that protect the gastric mucosa from injury.

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