

## **Histological, Histochemical and Immunohistological Comparative Study between Ranitidine and Rebamipide in Gastric Mucosal Protection after Dexamethasone Induced Injury in Adult Male Albino Rats**

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### **ABSTRACT**

**Background:** Gastritis can be caused by many factors, one of them is drugs, and among these drugs is dexamethasone that has many uses in medicine. Dexamethasone prevent synthesis of gastric mucous barrier by surface epithelial cells, so the stomach wall will be injured by digestive enzymes and stomach HCl. Treatment of gastritis can be done by antisecretory drugs like H<sub>2</sub> receptors blocker as ranitidine or by drugs that stimulate prostaglandins synthesise by surface epithelial cells of gastric mucosa to form the mucous barrier like rebamipide.

**Aim of the work:** Comparison between ranitidine and rebamipide to detect which mechanism is better in gastric mucosal protection after dexamethasone administration.

**Material and methods:** Twenty male albino rats were included in this study, they were divided into four groups, control group, dexamethasone administrated group, dexamethasone and ranitidine administrated group, dexamethasone and rebamipide administrated group, each group contained five rats.

The examined samples were stained with hematoxylin & eosin stain, PAS & Alcian blue stains and TdT reaction, all of the results were statistically analyzed.

**Results:** The result showed improvement of the gastric mucosa by using both of ranitidine and rebamipide as protective agents against injury induced by dexamethasone but the improvement was better in the group that was administrated rebamipide as indicated by better number of healthy cells, low numbers of damaged cells and better formed mucous barrier. **Conclusion:** The drug that stimulates mucous barrier formation is better than antisecretory drug in gastric mucosa protection.

**Keywords:** Ranitidine, Rebamipide, Gastric Mucosal Protection, Dexamethasone.

### **INTRODUCTION**

Many injurious factors can cause gastritis followed with complication like ulcers bleeding and cancers<sup>(1, 2, 3)</sup>. Among these factors is corticosteroid like dexamethasone which has wide range in medical use as anti allergic and anti inflammatory agent<sup>(4, 5)</sup>. Corticosteroids inhibit synthesis of prostaglandins essential for mucous barrier building up so the stomach wall will be susceptible to injury by digestive enzyme and stomach HCl<sup>(6, 7)</sup>. Many drugs are used in the treatment of gastritis such as H<sub>2</sub> receptor blocker like ranitidine decrease HCl secretion by blocking histamine receptors on the parietal cells so it prevent the activation of the pepsinogen enzyme to pepsin<sup>(8)</sup>. The use of ranitidine leads to increased pH of the stomach. This condition is known as hypochlorhydria which results in many side effects including interference with normal absorption of iron and calcium leading to iron

deficiency anaemia and osteoporosis respectively in addition to decreased ability of stomach in food sterilization so the liability in GIT infection increase<sup>(9,10,11)</sup>. Another line of treatment is building up of the mucous barrier through using prostaglandin stimulators like rebamipide. This drug has no effect on pH so hypochlorhydria does not occur with that drug<sup>(12, 13,14)</sup>.

The aim of this work was to compare between ranitidine and rebamipide to evaluate which mechanism is better in protection of the gastric mucosa after administration of dexamethasone by studying the histological aspects using computerized image analysis and statistical analysis of the results.

### **MATERIALS AND METHODS**

Twenty males of adult albino rat were raised up in faculty of medicine Al-Azhar University in the

period between March and April 2015. Their weights ranged from 220 to 300 grams and their ages ranged from 3 to 6 months. They were housed in cages at room temperature in clean condition. They were fed on balanced diets containing 50% carbohydrates, 25% proteins and 25% fat and were provided with clean water that was continuously changed.

They were divided into four groups each group contained five rats as follows:

1- G1: the control group that was our references for the histopathological changes.

2- G2: the group that received dexamethasone only for four days in order to induce gastritis<sup>(15, 22)</sup>.

3- G3: the group that received dexamethasone and ranitidine together for four days attempting for gastric mucosal protection<sup>(17)</sup>.

4- G4: the group that received dexamethasone and rebamipide together for four days in attempting for gastric mucosal protection<sup>(16, 13)</sup>.

#### **Doses of the used drugs**

Dexamethasone (4 mg/ kg) given by intraperitoneal route as single dose each day<sup>(15)</sup>.

Rebamipide dose (100 mg/kg) given by oral route as two divided doses each day<sup>(16)</sup>.

Ranitidine dose (20 mg/kg) given by intraperitoneal route as single dose each day<sup>(17)</sup>.

At the 5<sup>th</sup> day of the experiment the animals were anaesthetized by diazepam then their abdomens were sectioned and their stomachs were taken. Finally the stomachs of the rats were fixed in neutral buffered formol saline and they were processed by paraffin technique<sup>(18)</sup>, to be subjected to:

a) Haematoxylin & eosin stains to show the morphology of the tissue and to detect of the numbers of vesicular nuclei within the high power field<sup>(19)</sup>.

b) Periodic acid Schiff (PAS) and Alcian blue stains to show the mucous barrier layer of the stomach mucosa for measuring its thickness and optical density<sup>(20)</sup>.

c) Terminal deoxynucleotidyl transferase (TdT) reaction

#### **Detection of apoptosis by TUNEL method**<sup>(21)</sup>:

a- Sections were dewaxed with xylene and hydrated

b- Sections were treated with proteinase K (20 µg/ml) at room temperature for 15 min.

c- Endogenous peroxidase activity was quenched with 3% hydrogen peroxide solution for 5 min.

d- Each section was incubated with 75 µl of equilibration buffer for 10 min.

e- Working strength TdT enzyme was applied in a humidified chamber at 37°C for 70 min.

f- Sections in jar containing working strength stop/wash buffer were agitated for 15 sec, and then incubated for 10 min at room temperature.

g- Anti-digoxigenin peroxidase conjugate was applied for 30 min in a humidified chamber at room temperature.

h- After washing in phosphate-buffered saline enough, DAB solution was applied to cover the specimens completely.

i- Staining was performed for 5 min at room temperature.

j- After washing in distilled water, sections were counterstained in 0.5% hematoxylin for 5 min.

The obtained results were statistically analyzed.

#### **Statistical analysis:**

1- The numbers of vesicular nuclei were detected in each group in the sections prepared from both the body and pylorus. Vesicular nuclei were used as an indicator of the healthy cells.

2- The mean optical density of the TdT reaction was estimated using the computer program "ImageJ" v.1.48 by The National Institutes of Health, USA.

3- The thickness of the mucous barrier layer was measured in micrometer and its optical density was estimated by using of the program (ImageJ) The obtained results were statistically analyzed and the following values were estimated:

a- Standard error of mean by the following formula:

Standard error of the mean = standard deviation of mean /  $\sqrt{\text{sample number}}$

b- P value

It was estimated in comparison with the control group by using Excel (Microsoft Corporation 2010), taking in consideration that a value less than 0.05 is a significant result

#### **RESULTS**

A- Hematoxylin & eosin stain results

##### **1- In the stomach body:**

In G1 under the low power (figure 1a) the mucosa was healthy. There were no ulcers no hemorrhage and no blood congestion. Under the

high power in both the upper surface of the mucosa (figure 2 a) and lower part of the mucosa (figure 3a) the glands boundaries are intact. The cells of the glands have vesicular nuclei. The lamina propria was rich in eosinophils (figure 3 a). The average number of vesicular nuclei per high power field was 26 cells in the upper surface of the mucosa and 29 cells in the lower part of the mucosa.

In G2 under the low power (figure 1b) there was marked blood vessels dilatation and congestion. Under the high power in both the upper surface of the mucosa (figure 2 b) and lower part of the mucosa (figure 3b) there was marked loss of the normal shape of the glands. The cells showed vaculation and nuclear pyknosis. There was disappearance of eosinophils from the lamina propria (Figure 3b). The average number of vesicular nuclei per high power field was 8 cells in the upper surface of the mucosa and 7 cells in the lower part of the mucosa.

In both G3 (figure 1 c) and G4 (figure 1d) under the low power the mucosa looks healthy.

In G3, under the high power in both the upper surface of the mucosa (figure 2 c) and lower part of the mucosa (figure 3c) the glands start to retain their shape but there were some cells in the glands still showing vaculation and nuclear pyknosis. There was also disappearance of eosinophils from the lamina propria. (Figure 3c). The average number of vesicular nuclei per high power field was 14 cells in the upper surface of the mucosa and 13 cells in the lower part of the mucosa.

In G4, under the high power in both the upper surface of the mucosa (figure 2d) and lower part of the mucosa (figure 3d) the glands boundaries were intact. It contained healthy cells with vesicular nuclei but there were scattered cells with pyknotic nuclei. There were also some dilated blood capillaries and some lymphocytic infiltration. It is noted that in the base of the mucosa there were disappearance of eosinophils from lamina propria (figure 3d). The average number of vesicular nuclei per high power field was 18 cells in the upper surface of the mucosa and 20 cells in the lower part of the mucosa.

#### 2- In the pylorus:

In G1 under low power (figure 4a) the mucosa was healthy. There were no ulcers no hemorrhage and no blood vessels congestion.

By high power in both the upper surface of the mucosa (figure 5 a) and lower part of the mucosa (figure 6 a) the glands boundaries are intact there were no inflammatory cells infiltrations. The cells of the glands have vesicular nuclei. The lamina propria was rich in eosinophils (figure 6 a). The average number of vesicular nuclei in high power field was 26 cells in the upper surface of the mucosa and 32 cells in the lower part of the mucosa.

In G2 under low power (figure 4b), there were marked blood vessels dilatation and congestion and interstitial hemorrhage in the submucosa.

Under the high power in both the upper surface of the mucosa (figure 5 b) and lower part of the mucosa (figure 6b) there was great loss of the normal shape of the glands. The cells showed vaculation and nuclear pyknosis. It is noted that in lower part of the mucosa (Figure 6b) there was blood extravasation in the lamina propria in addition to disappearances of eosinophils. The average number of vesicular nuclei in high power field was 9 cells in the upper surface of the mucosa and also 9 cells in the lower part of the mucosa.

Under low power in both G3 (figure 4 c) and G4 (figure 4d), the mucosa looks healthy.

In G3 under the high power in both the upper surface of the mucosa (figure 5c) and lower part of the mucosa (figure 6c) the glands started to retain their shape but there were some cells in the glands still showing vaculation and nuclear pyknosis. There were also disappearances of eosinophils from the lamina propria (Figure 3c). The average number of vesicular nuclei in high power field was 13 cells in the upper surface of the mucosa and also 13 cells in the lower part of the mucosa.

In G4 under high power in both the upper surface of the mucosa (figure 5d) and lower part of the mucosa (figure 6d) the glands boundaries were intact. It contained normal cells with vesicular nuclei but there were scattered cells with pyknotic nuclei. There were also some dilated blood capillaries and some lymphocytic infiltration. It was noted that in the base of the mucosa there were disappearance of eosinophils from lamina propria (figure 6d). The average number of vesicular nuclei in high power field

was 17 cells in the upper surface of the mucosa and also 17 cells in the lower part of the mucosa.

**B- TdT reaction results:**

The TdT reaction was estimated in the body in both the upper surface (figure 7) and lower part of the mucosa (figure 8) and also estimated in the pylorus in both of the upper surface (figure 9) and lower part of the mucosa (figure 10)

In G1 (figures 7 a, 8a, 9a and 10a) there was mild scattered TdT reaction. In G2 (figures 7 b, 8b, 9b and 10b), there was a marked scattered TdT reaction. In G3 (figures 7c , 8c, 9c and 10c), there was a moderate scattered TdT reaction, while in G4 (figures 7 d , 8d, 9d and 10d), there were mild scattered TdT reaction.

**C- PAS & Alcian blue stain results:**

In the stomach body (figure 12)

In G1 (figure 11a) the surface of the mucosa was covered with well formed PAS positive layer without interruptions. In G2 (figure 11b) There were remnants of PAS positive layer that cover the surface of the mucosa. In G3 (figure 11c). There were more remnants of PAS positive layer that cover surface of the mucosa. In G4 (figure 11d) the surface of the mucous was covered with well-formed PAS positive layer except for few interrupted area.

In the pylorus (figure 12)

In G1 (figure 12a) the surface of the mucosa was covered with well formed PAS positive layer without interruptions. In G2 (figure 12b). There was complete loss of the PAS positive layer that cover the surface of the mucosa. In G3 (figure 12c) there were remnants of PAS positive layer. In G4 (figure 12d) the surface of the mucosa was covered with interrupted area of PAS positive layer.

**Statistical analysis**

1- The mean number of vesicular nuclei in the upper surface of mucosa of the body is illustrated in table-1 and chart-1. It was noted that the highest number in vesicular nuclei was in G1 then G4 then G3, and the least number of vesicular nuclei was in G2.

2 – The mean number of vesicular nuclei in the lower part of mucosa of the body is illustrated in

table-2 and chart-2. It was noted that the highest number in vesicular nuclei was in G1 then G4 then G3, and the least number in vesicular nuclei was in G2.

3- The mean number of vesicular nuclei in the upper surface of mucosa of the pylorus is illustrated in table-3 and chart-3. It was noted that the highest number in vesicular nuclei was in G1 then G4 then G3, and the least number in vesicular nuclei was in G2.

4- The mean number of vesicular nuclei in the lower part of mucosa of the pylorus is illustrated in table-4 and chart-4. It was noted that the highest number in vesicular nuclei was in G1 then G4 then G3, and the least number in vesicular nuclei was in G2.

5- The mean optical density of TdT reaction in the upper surface of mucosa stomach body is illustrated in table-5 and chart-5. It was noted that the weakest reaction was in G1 then G4 then G3, and the strongest was in G2.

6- The mean optical density of TdT reaction in the lower surface of mucosa of stomach body is illustrated in table-6 and chart- 6. It was noted that the weakest reaction was in G1 then G4 then G3, and the strongest was in G2.

7- The mean optical density of TdT reaction in the upper surface of mucosa stomach pylorus is illustrated in table-7 and chart-7. It was noted that the weakest reaction was in G1, then G4 then G3, and the strongest was in G2.

8- The mean optical density of TdT reaction in the lower part of mucosa stomach pylorus is illustrated in table-8 and represented in chart-8. It was noted that the weakest reaction was in G1 then G4 then G3, and the strongest was in G2.

9 - The mean thickness of mucosal barrier in the stomach body is illustrated in table-9 and chart-9. It was noted that the thickest barrier was in G1, then G4 then G3, and the thinnest barrier was in G2.

10 – The mean thickness of mucosal barrier in the stomach pylorus is illustrated in table-10 and

represented in chart-10. It was noted that the thickest barrier was in G1, then G4 then G3, while the barrier was lost completely in G2.

11- The mean optical density of mucosal barrier in the stomach pylorus is illustrated in table-11 and chart-11. It was noted that the strongest reaction was in G1 then G4 then G3, and the weakest reaction was in G2.

12- The mean optical density of mucosal barrier in the stomach pylorus is illustrated in table-12 and chart-12. It was noted that the strongest reaction was in G1 then G4 then G3, while the reaction is absent in G2 due to barrier loss .

## DISCUSSION

Gastritis and its complication like ulcer bleeding, anaemia and even cancer can be caused by many agents; one of them is drugs <sup>(1, 2, 3)</sup>. One of those drugs is dexamethasone that has many indications in medicine like treatment of rheumatoid arthritis and bronchial asthma <sup>(4, 5)</sup>. Dexamethasone block the synthesis of prostaglandin by surface epithelial cells of the stomach mucosa leading to defect in mucous barrier formation, so the gastric mucosa becomes liable to damage by HCl and digestive enzymes leading to erosions and ulceration <sup>(6)</sup>. The drugs which are used in treatment of gastritis may work in different mechanisms, ranitidine blocks H<sub>2</sub> receptors of the parietal cells that secrete HCl, stomach pH will be elevated and will not be suitable for activation of pepsinogen. Rebamipide is another drug used in the treatment of gastritis; its action depends on stimulating the synthesis of prostaglandins by surface epithelial cells to build up the mucous barrier protecting the stomach wall from damage by HCl and pepsin <sup>(13, 14)</sup>. This work aimed to compare between the ranitidine and rebamipide to evaluate which mechanism is better for treatment and protection of gastric mucosa from dexamethasone.

In this work, we noticed disappearance of eosinophils from the lamina propria in all the groups that were injected by dexamethasone. So, it was a sign for us that the dose of dexamethasone was sufficient as previously reported <sup>(23)</sup>.

In our work, G2 showed damage of the mucous barrier, decrease in its density and its thickness in comparison to G1 as demonstrated by PAS & Alcian blue stain. This was accompanied with wide spread cell damage as detected by TdT in addition to decreased number of healthy cells, and marked congestion up to interstitial hemorrhage as demonstrated by hematoxylin and eosin stains. These findings showed the importance of mucous barrier in saving the stomach wall from serious damage as mentioned before <sup>(7)</sup>. By administrating ranitidine to protect the gastric mucosa in G3, we found improvement in the mucous barrier in the form of increasing its thickness and its density as demonstrated by PAS & Alcian blue stain. This was accompanied with improvement in the number of healthy cells with vesicular nuclei, as demonstrated by hematoxylin and eosin stains. There was also decreased number of damaged cells as demonstrated by TdT reaction. These results suggest that the ranitidine provided protection against dexamethasone induced gastritis as reported before <sup>(8)</sup>.

The most important improvement was in G4 by adding rebamipide as protective agent in which there was much increase in the thickness of mucous barrier and increases its density as demonstrated by PAS & Alcian blue stain. There was also increased number of healthy cells with vesicular nuclei, as demonstrated by hematoxylin and eosin stains. The damaged cells decreased as demonstrated by TdT reaction. Our findings suggest that rebamipide is more effective in stomach wall protection than ranitidine <sup>(13, 14)</sup>. This result indicates that building of the mucous barrier is more important in stomach wall protection than prevention of stomach HCl secretion.

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A- Hematoxyline and eosin

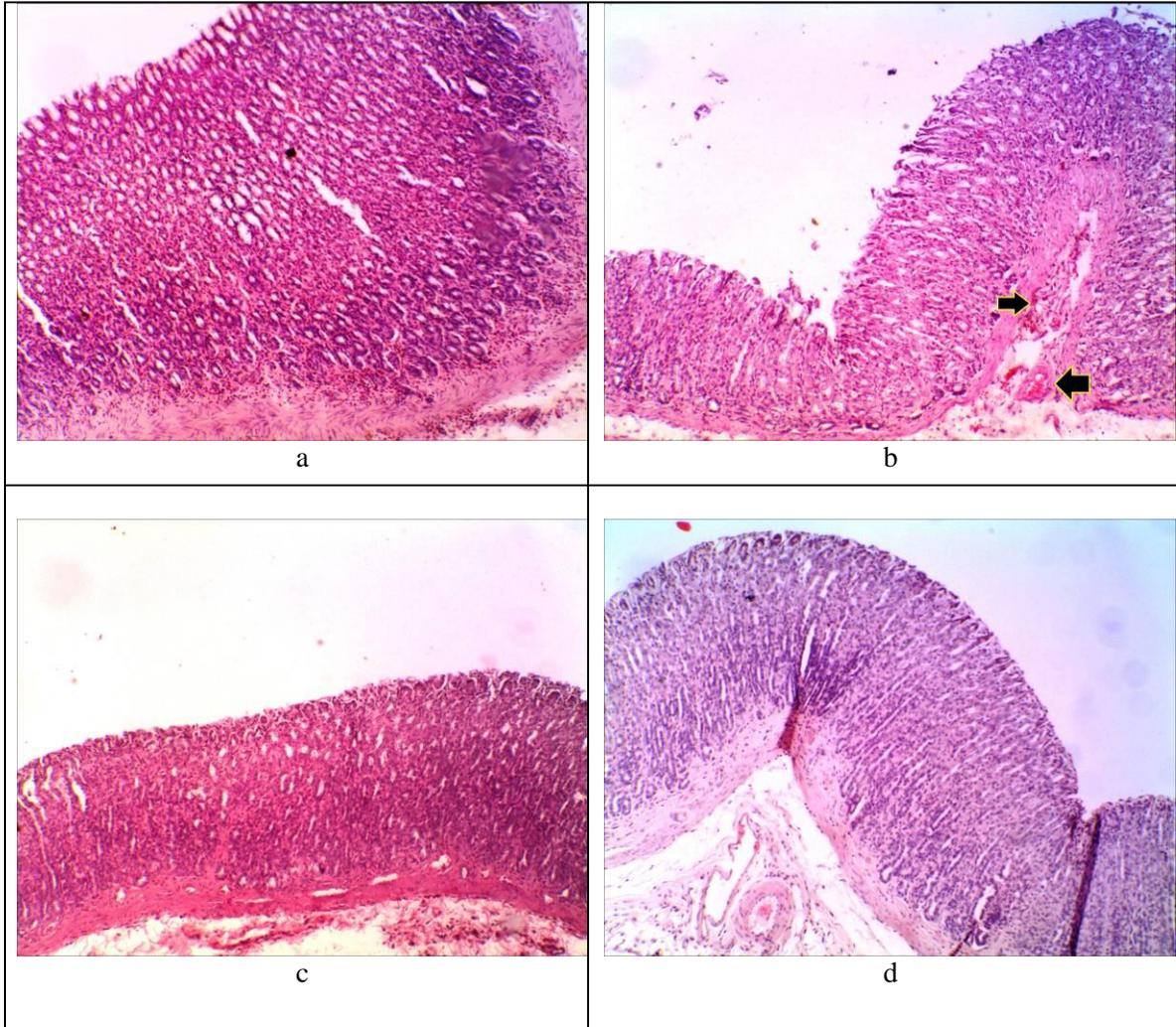


Figure 1: Photomicrographs of a section in the mucosa stomach body of rats, the mucosa is intact in G1 (1a), marked blood vessels congestion (black arrows) in the submucosa. In G2 (1b), the mucosa appear intact in both G3 (1c) and G4 (1d). x100 H & E.

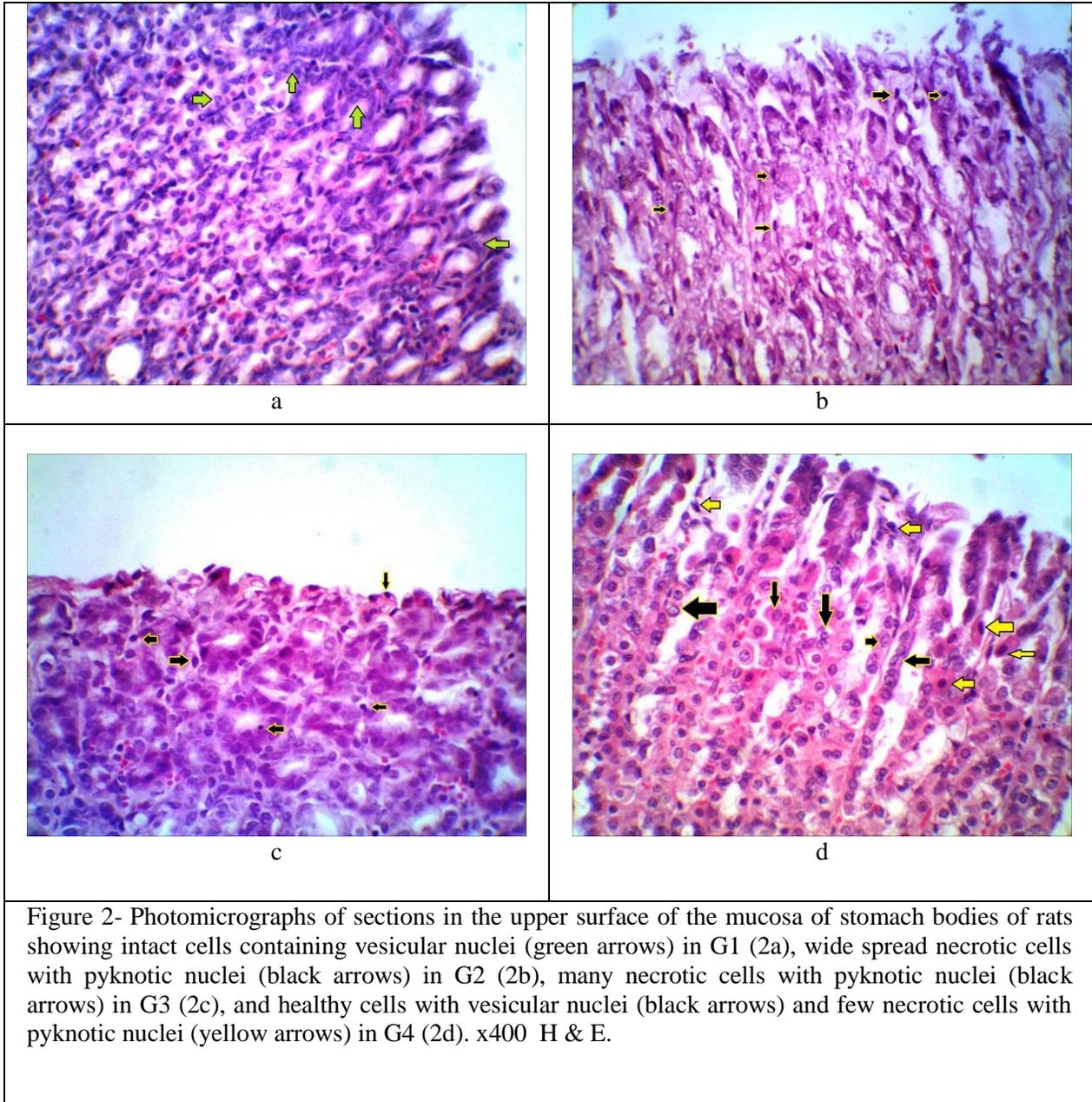


Figure 2- Photomicrographs of sections in the upper surface of the mucosa of stomach bodies of rats showing intact cells containing vesicular nuclei (green arrows) in G1 (2a), wide spread necrotic cells with pyknotic nuclei (black arrows) in G2 (2b), many necrotic cells with pyknotic nuclei (black arrows) in G3 (2c), and healthy cells with vesicular nuclei (black arrows) and few necrotic cells with pyknotic nuclei (yellow arrows) in G4 (2d). x400 H & E.

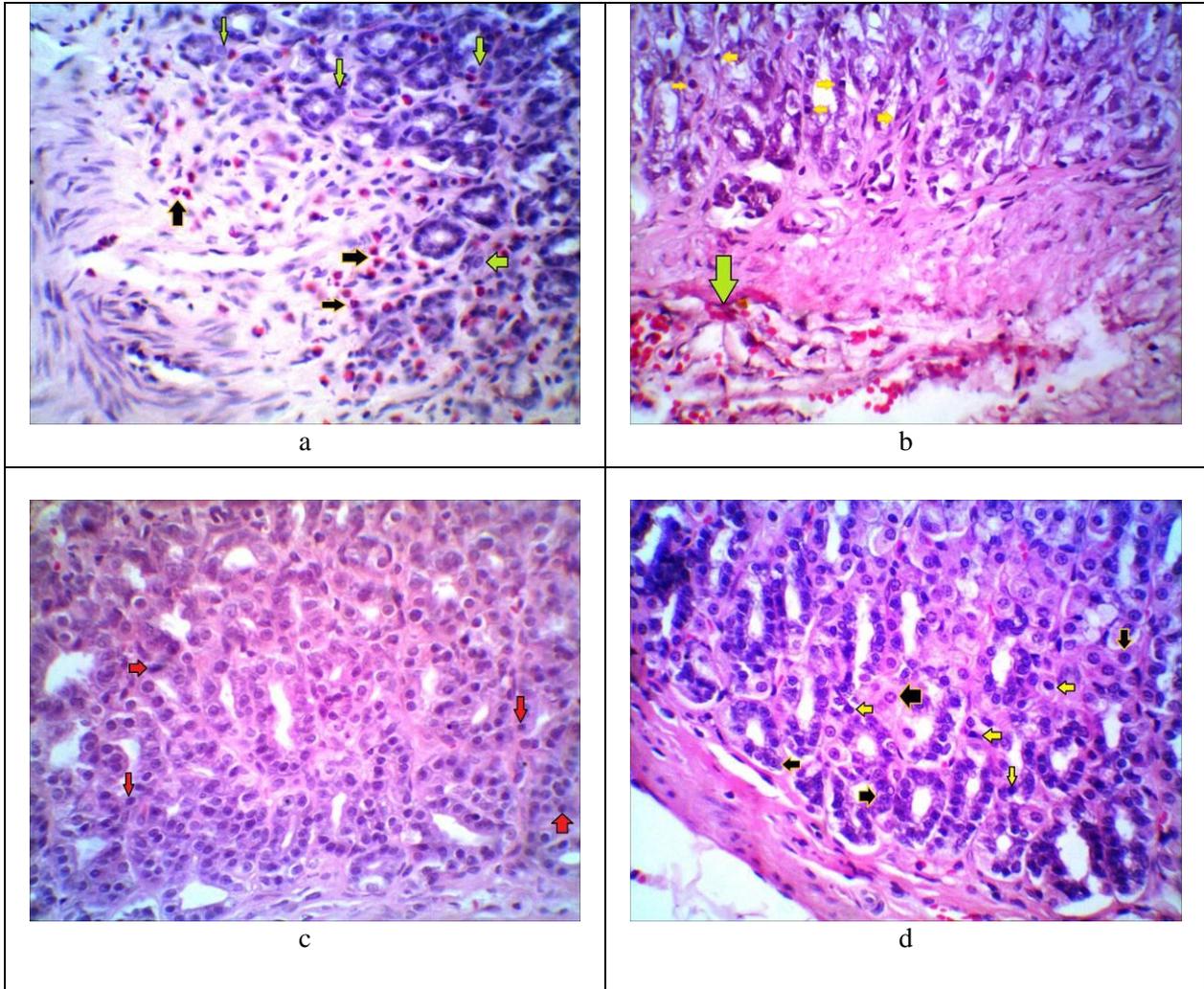


Figure 3- Photomicrographs of sections in the lower part of the mucosa of stomach bodies of rats showing intact cells containing vesicular nuclei (green arrows). The submucosa is rich in eosinophils (black arrows) in G1 (3d), wide spread necrotic cells with pyknotic nuclei (yellow arrows) and marked congested blood vessel (green arrow) in the submucosa in G2 (3b), many necrotic cells with pyknotic nuclei ( red arrows) in G3 (3C), and normal cells with vesicular nuclei( black arrows) and few necrotic cells with pyknotic nuclei (yellow arrows) in G4 (4d), it is noted that absence of eosinophils in the submucosa in G2, G3 and G4 . x400 H & E.

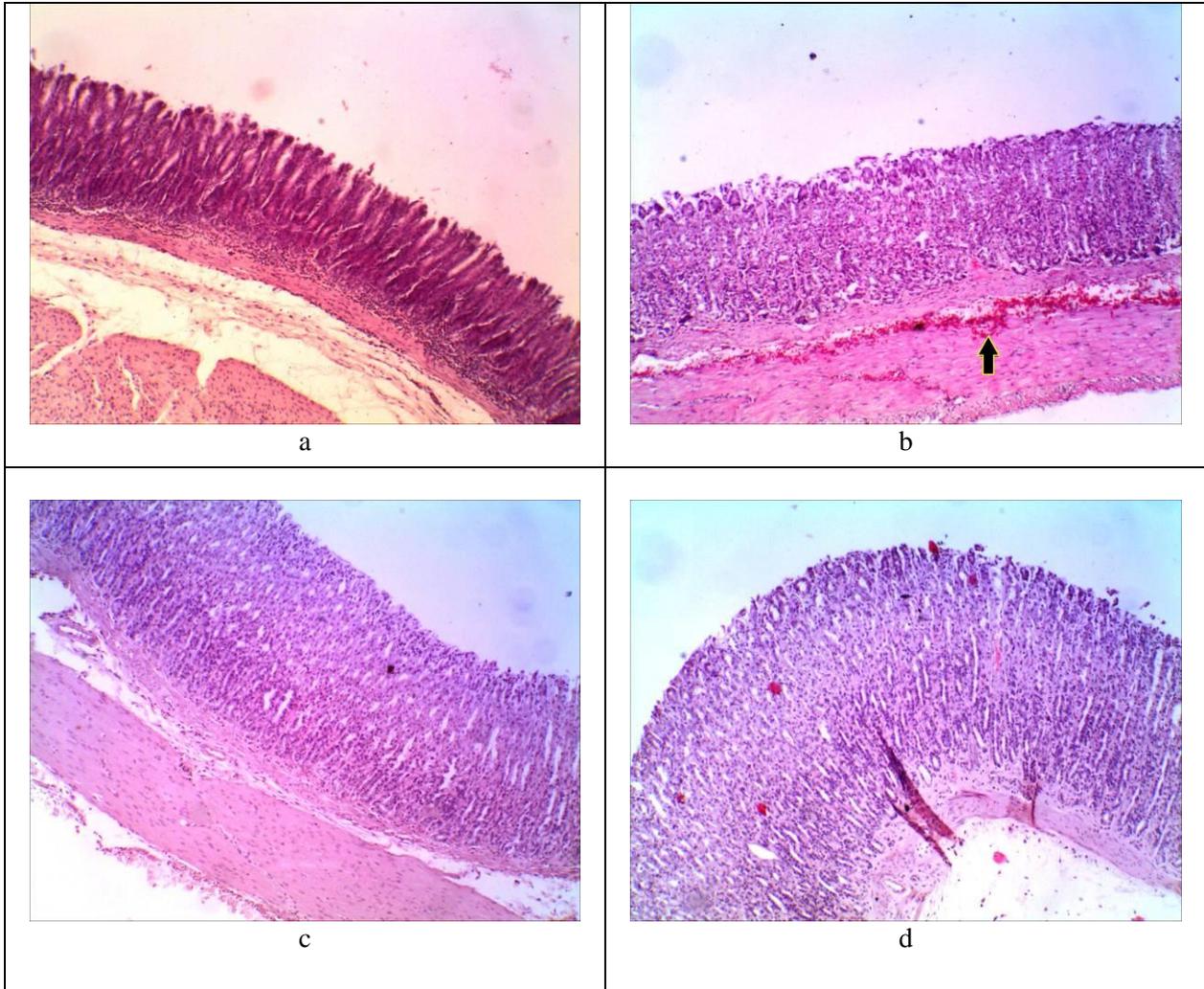


Figure 4- Photomicrographs of sections in the pylorus of rats showing normal mucosa without ulcers or blood vessels congestion in G1 (4a), marked blood vessels congestion with interstitial hemorrhage (black arrow) in the submucosa in G2 (4b), the mucosa appear intact without ulcers or blood vessels congestion in G3 (4c) and G4 (4d). x100 H & E.

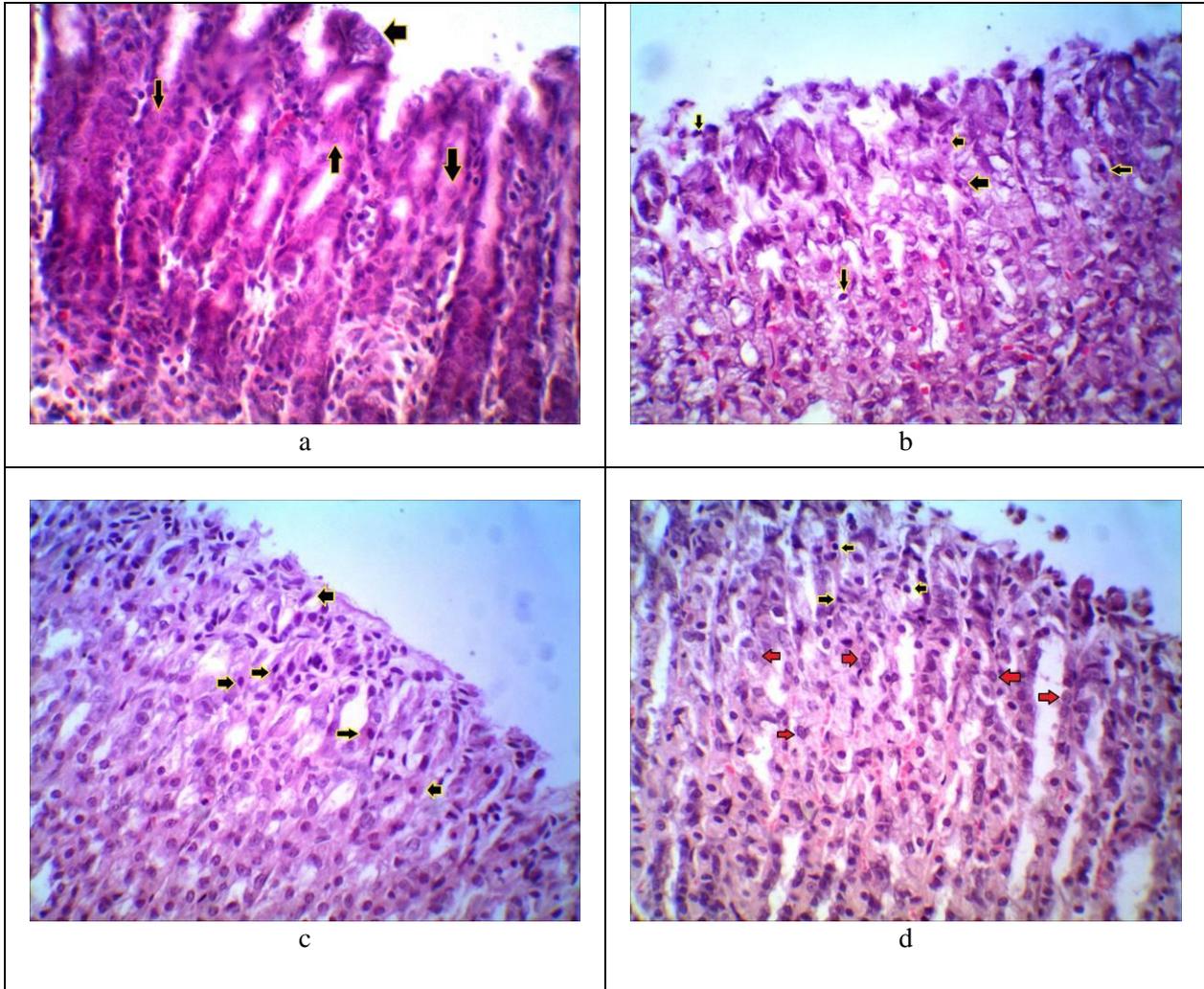


Figure 5- Photomicrographs of sections in the upper surface of the mucosa of stomach pylorus of rats showing intact cells containing vesicular nuclei (black arrows) in G1 (5a), wide spread necrotic cells with pyknotic nuclei (black arrows) in G2 (5b), many necrotic cells with pyknotic nuclei (black arrows) in G3 (5c), and healthy cells with vesicular nuclei (red arrows) and few necrotic cells with pyknotic nuclei (black arrows) in G4 (5d). x400 H & E.

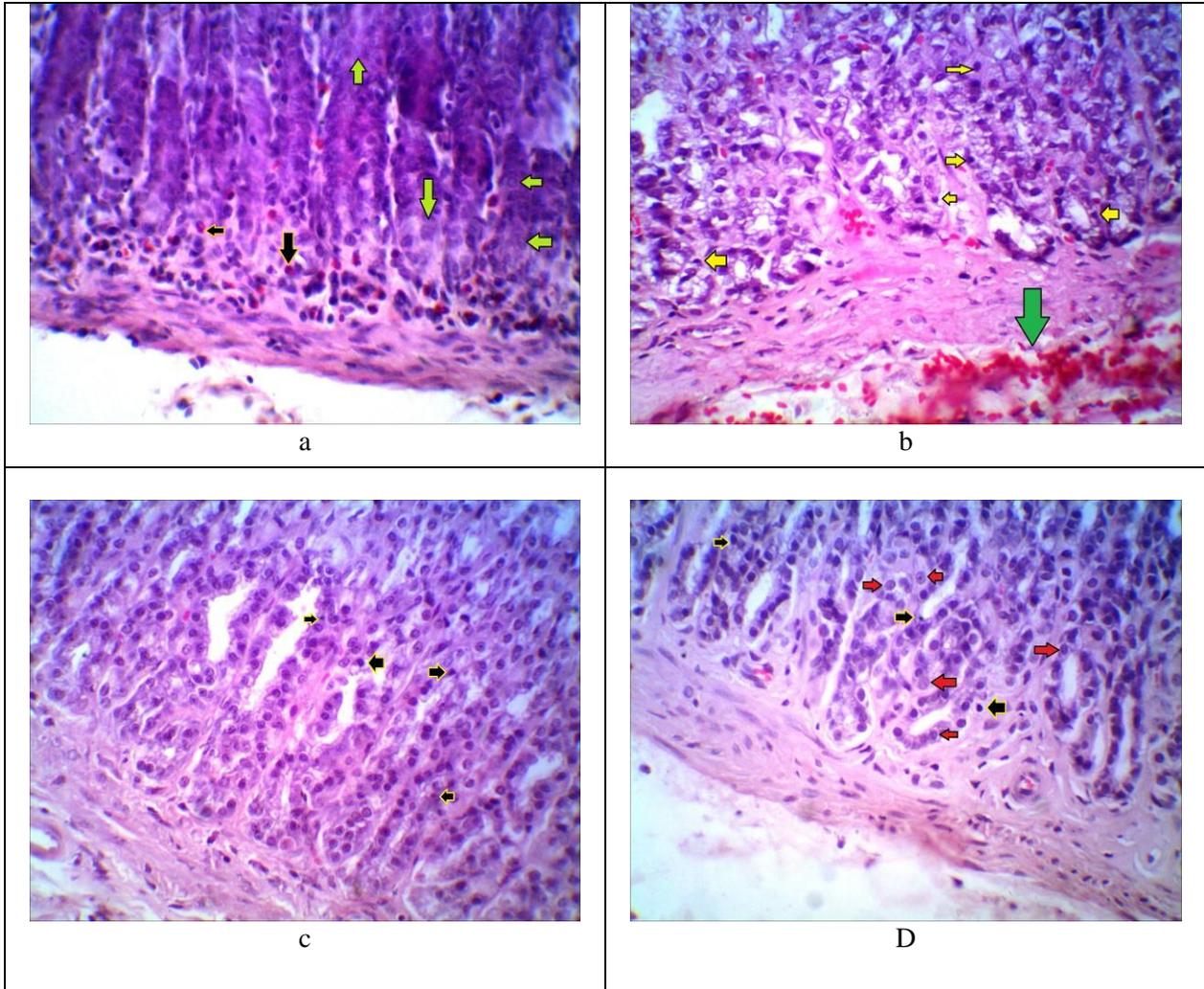


Figure 6- Photomicrographs of sections in the lower part of the mucosa of stomach pylorus of rats showing intact cells containing vesicular nuclei (green arrows). The submucosa is rich in eosinophils (black arrows) G1 (6a), there are wide spread necrotic cells with pyknotic nuclei (yellow arrows) and marked congested blood vessel (green arrow) with interstitial hemorrhage in the submucosa in G2 (6b), many necrotic cells with pyknotic nuclei ( black arrows) in G3 (6C), and healthy cells with vesicular nuclei( red arrows) and few necrotic cells with pyknotic nuclei ( black arrows) in G4 (6d), it is noted the absence of eosinophils in the submucosa in G2, G3 and G4. x400 H & E.

B- TdT reaction

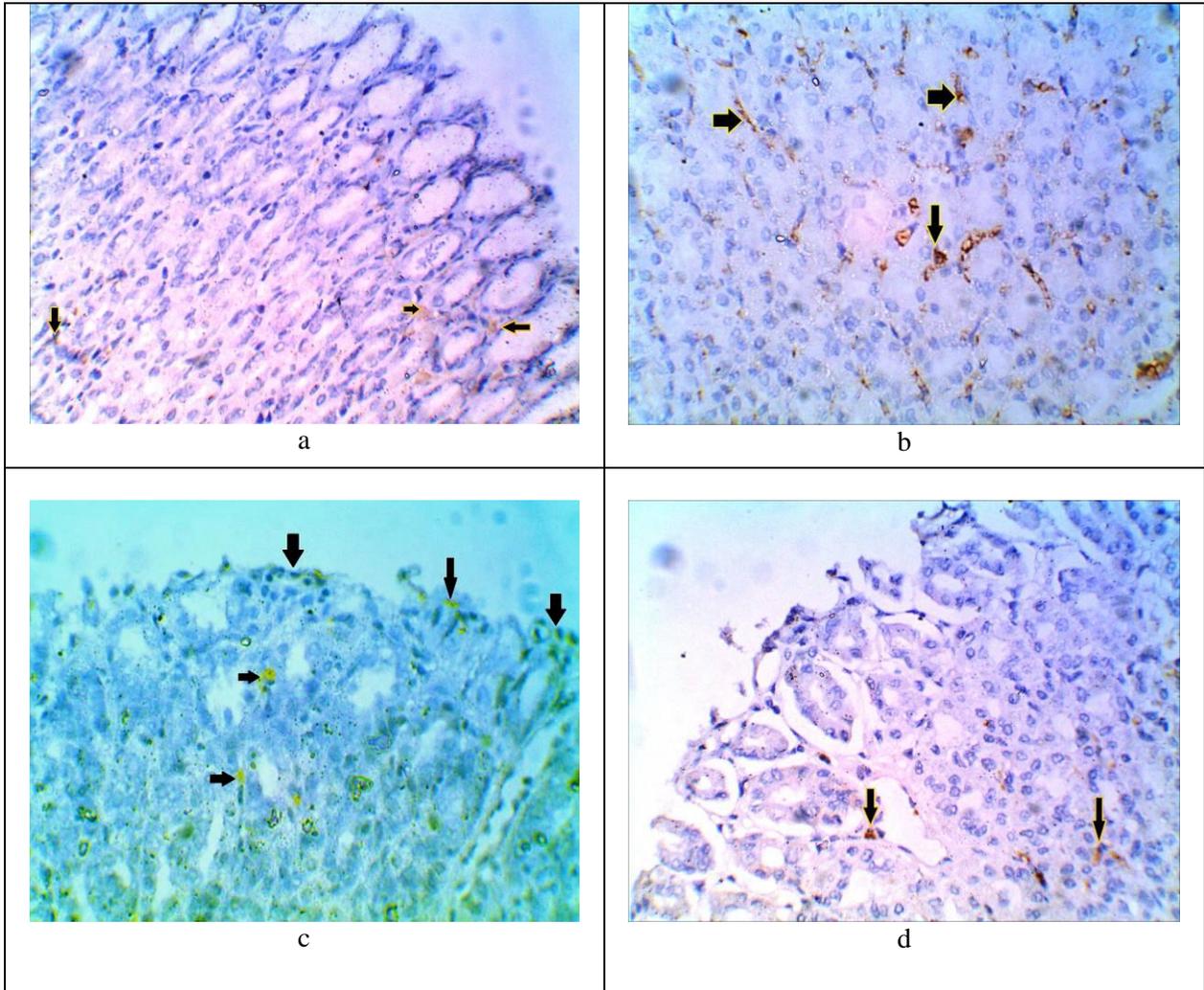


Figure 7- Photomicrographs of sections in stomach body of rats showing upper surface of the mucosa, it appears with mild scattered TdT reaction ( black arrows) in G1 (7a) , wide spread intense TdT reaction (black arrows) in G2 (7b), moderate scattered TdT reaction (black arrows). In G3 (7c) and mild scattered TdT reaction (black arrows) in G4 (7d). x400 TdT reaction.

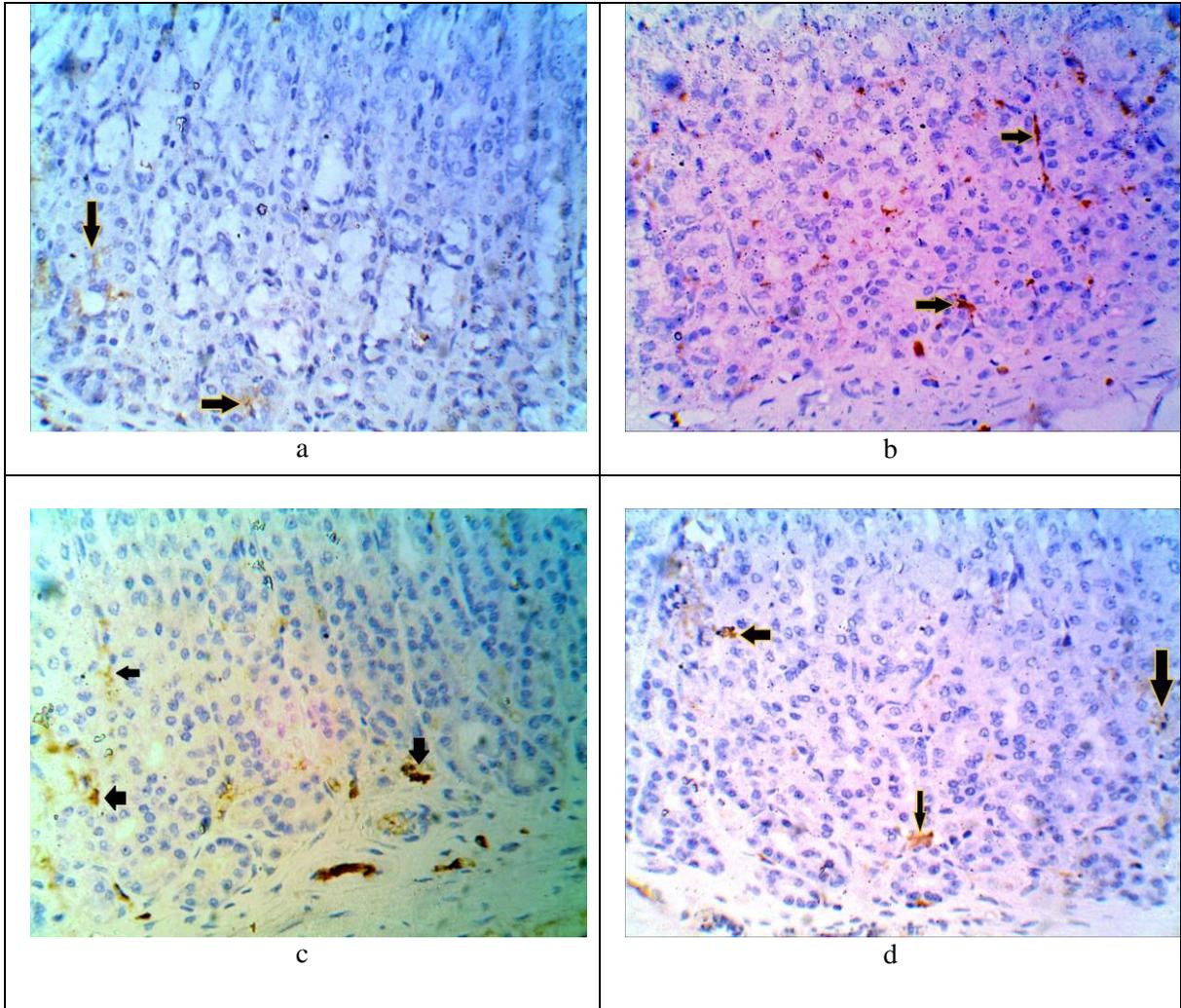


Figure 8- Photomicrographs of sections in stomach body of rats showing the lower part of the mucosa with very mild scattered TdT reaction (black arrows) in G1 (8a), wide spread intense TdT reaction (black arrows) in G2 (8b), moderate scattered TdT reaction (black arrows) in G4 (8c) and mild scattered TdT reaction (black arrows) in G4 (8d). x400 TdT reaction.

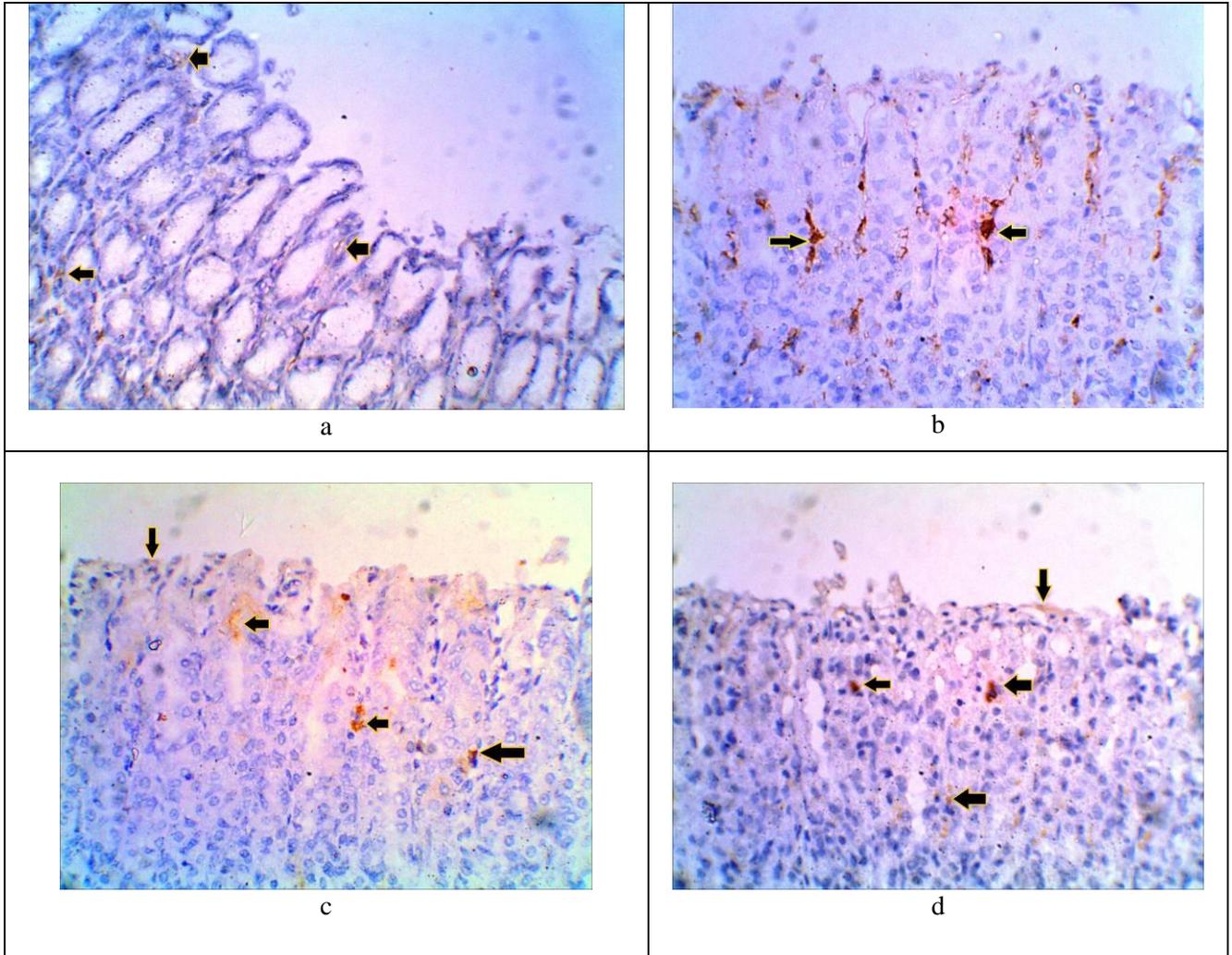


Figure 9- Photomicrographs of section in stomach pylorus of rats showing upper surface of the mucosa with mild scattered TdT reaction (black arrows) in G1 (9a). Wide spread intense TdT reaction (black arrows) in G2 (9b), moderate scattered TdT reaction (black arrows) in G3 (9c) and mild scattered TdT reaction (black arrows) in G4 (9d). x400 TdT reaction.

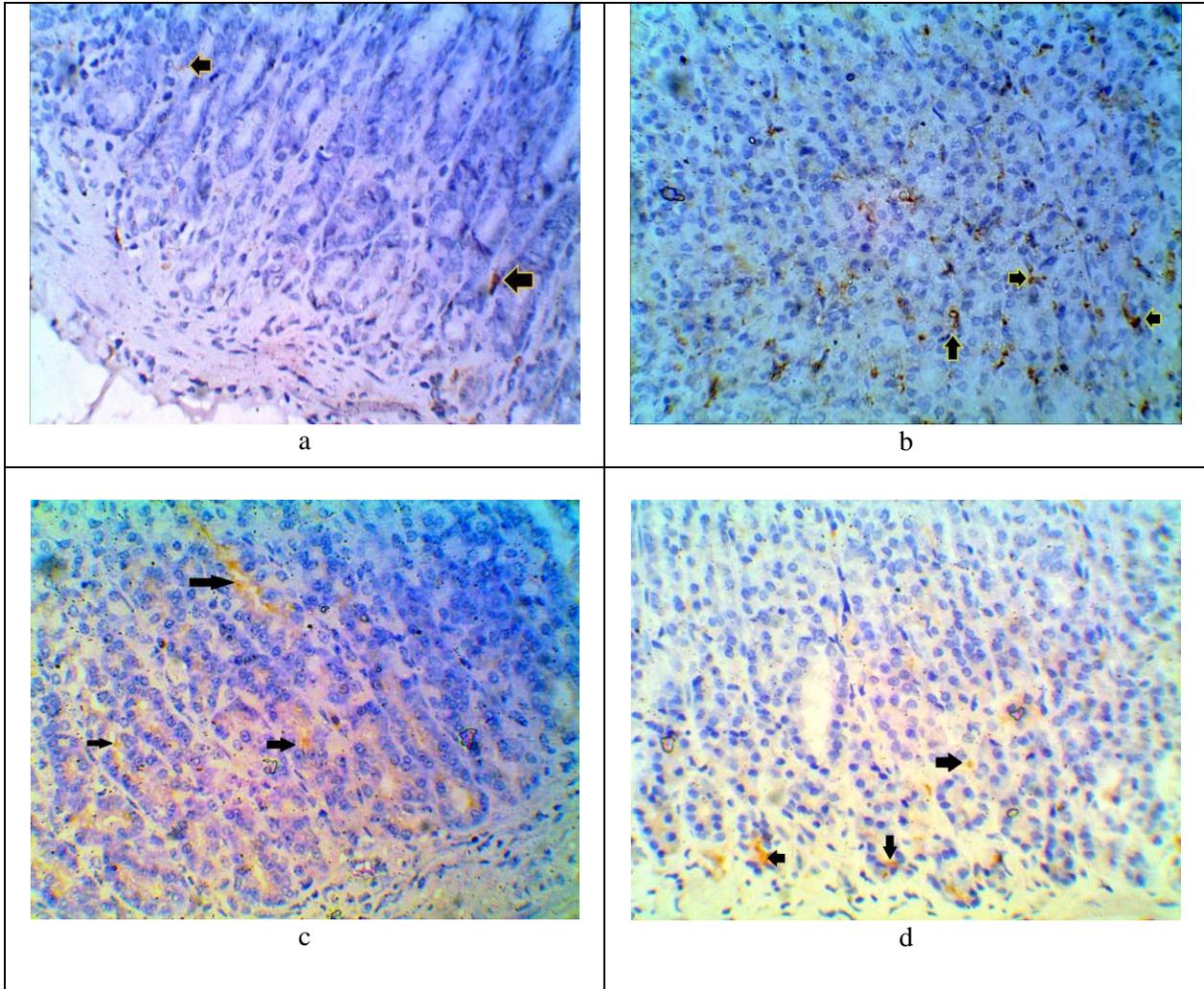


Figure 10- Photomicrographs of sections in stomach pylorus of rats showing the lower part of the mucosa with very mild scattered TdT reaction (black arrows) in G1 (10a), wide spread intense TdT reaction (black arrows). In G2 (10b), moderate scattered TdT reaction (black arrows) in G3 (10c) and mild scattered TdT reaction (black arrows) in G4 (10d). x400 TdT reaction.

- C- PAS & Alcian Blue.

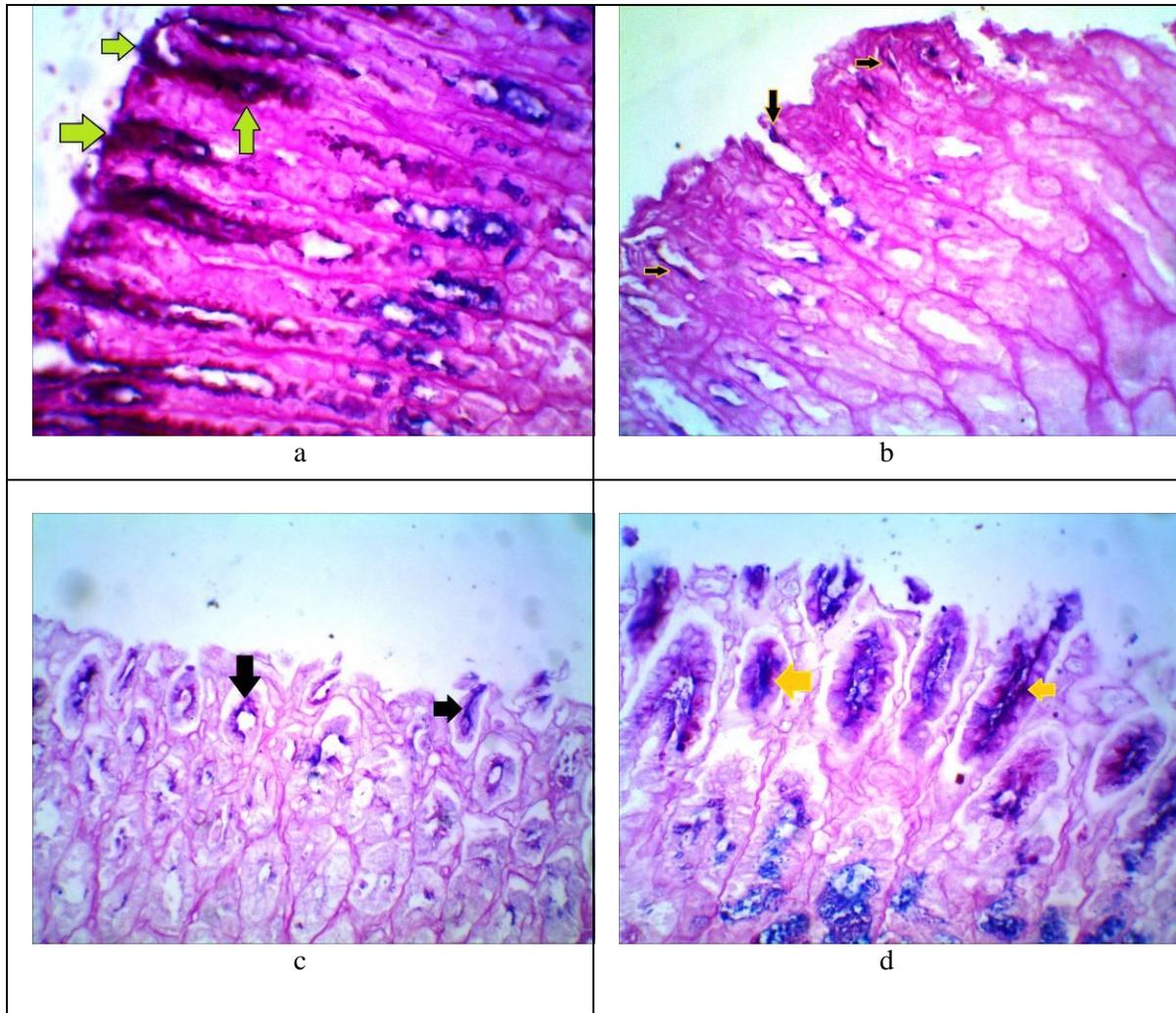


Figure 11- Photomicrographs of sections in stomach body of rats showing PAS positive mucous barrier layer (green arrows) in G1 (11a), remnants of PAS positive mucous barrier layer (black arrows) in G2 (11b). The remnants is increased in G3 (11c) and well-formed PAS positive mucous barrier layer (yellow arrows) with interrupted areas in G4 (11d). x400 PAS & Alcian Blue.

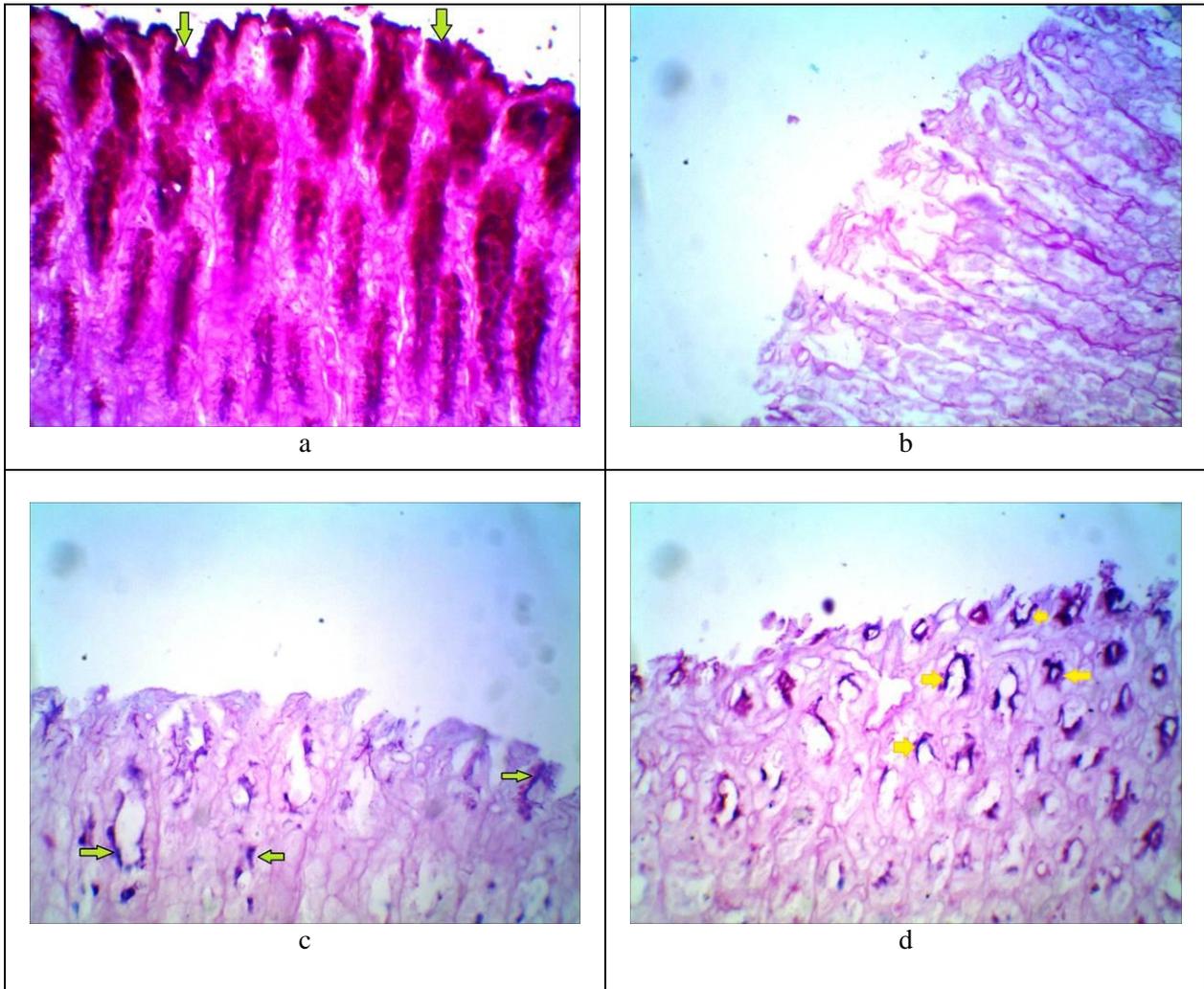


Figure 12- Photomicrographs of sections in the pylorus of the rat showing PAS positive mucous barrier layer (green arrows) in G1 (12a), absence of PAS positive mucous barrier layer in G2 (12b), remnants of PAS positive mucous barrier layer (green arrows) in G3 (12c) and interrupted areas of PAS positive mucous barrier layer (yellow arrows) in G4 (12d). x400 PAS & Alcian Blue.

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean number of vesicular nuclei ± Standard error of mean	26 ± 4.72	8± 2.05	14 ± 2.25	18 ± 2.17
p-value		P < 0.05	P < 0.05	P < 0.05
Significance		Significant	Significant	Significant

Table-1 The mean number of vesicular nuclei in the upper surface of mucosa of the body

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean number of vesicular nuclei ± Standard error of mean	29 ± 1.23	7± 1.34	13± 1.12	20± 4.73
p-value		P < 0.05	P < 0.05	P < 0.05
Significance		Significant	Significant	Significant

Table-2 The mean number of vesicular nuclei in the lower part of mucosa of the body

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean number of vesicular nuclei ± Standard error of mean	26± 1.18	9± 0.97	13 ± 0.65	17 ± 1.84
p-value		P < 0.05	P < 0.05	P < 0.05
Significance		Significant	Significant	Significant

Table-3 The mean number of vesicular nuclei in the upper surface of mucosa of the pylorus

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean number of vesicular nuclei ± Standard error of mean	32± 2.09	9± 2.65	13± 3.83	17 ± 2.63
p-value		P < 0.05	P < 0.05	P < 0.05
Significance		Significant	Significant	Significant

Table-4 The mean number of vesicular nuclei in the lower part of mucosa of the pylorus

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean optical density ± Standard error of mean	0.22 ± 0.01	0.56 ± 0.1	0.40 ± 0.05	0.34 ± 0.02
p-value		P < 0.05	P < 0.05	P < 0.05
Significance		Significant	Significant	Significant

Table-5 The mean optical density of TdT reaction in the upper surface of mucosa stomach body

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean optical density ± Standard error of mean	0.31 ± 0.01	0.59 ± 0.1	0.47 ± 0.11	0.42 ± 0.07
p- value		P < 0.05	P < 0.05	P < 0.05
Significance		Significant	Significant	Significant

Table-6 The mean optical density of TdT reaction in the lower surface of mucosa stomach body

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean Optical density $\pm$ Standard error of mean	0.29 $\pm$ 0.04	0.70 $\pm$ 0.15	0.50 $\pm$ 0.04	0.45 $\pm$ 0.14
p-value		P < 0.05	P < 0.05	P < 0.05
Significance		Significant	Significant	Significant

Table-7 The mean optical density of TdT reaction in the upper surface of mucosa stomach pylorus

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean optical density $\pm$ Standard error of mean	0.22 $\pm$ 0.01	0.63 $\pm$ 0.06	0.40 $\pm$ 0.04	0.35 $\pm$ 0.02
p-value		P < 0.05	P < 0.05	P < 0.05
significance		Significant	Significant	Significant

Table-8 The mean optical density of TdT reaction in the lower part of mucosa stomach pylorus

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
mean thickness of mucosal barrier $\pm$ Standard error of mean	8.93 $\pm$ 2.82	2.64 $\pm$ 0.63	3.35 $\pm$ 1.91	6.43 $\pm$ 1.59
p- value		P < 0.05	P < 0.05	P < 0.05
significance		Significant	Significant	Significant

Table-9 The mean thickness of mucosal barrier in the stomach body

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean thickness of mucosal barrier $\pm$ Standard error of mean	7.66 $\pm$ 1.37	0	3.63 $\pm$ 1.11	5.07 $\pm$ 0.53
p-value		P < 0.05	P < 0.05	P < 0.05
significance		Significant	Significant	Significant

Table-10 The mean thickness of mucosal barrier in the stomach pylorus

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean Optical density $\pm$ Standard error of mean	1.56 $\pm$ 0.16	0.85 $\pm$ 0.07	0.97 $\pm$ 0.11	1.1 $\pm$ 0.07
p-value		P < 0.05	P < 0.05	P < 0.05
significance		Significant	Significant	Significant

Table-11 The mean optical density of mucosal barrier in the stomach body

	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Mean Optical density $\pm$ Standard error of mean	1.46 $\pm$ 0.34	Not detected	0.98 $\pm$ 0.11	1.14 $\pm$ 0.1
p-value			P < 0.05	P < 0.05
Significance			Significant	Significant

Table-12 The mean optical density of mucosal barrier in the stomach pylorus

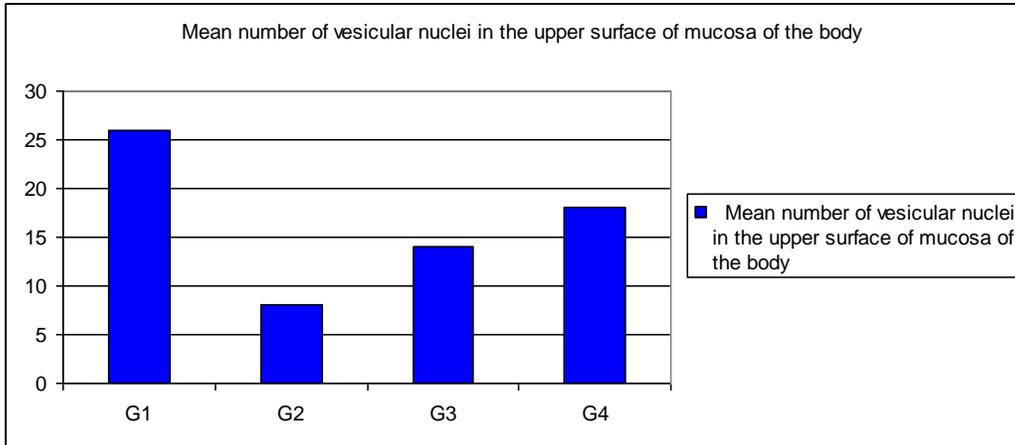


Chart-1 The mean number of vesicular nuclei in the upper surface of mucosa of the body

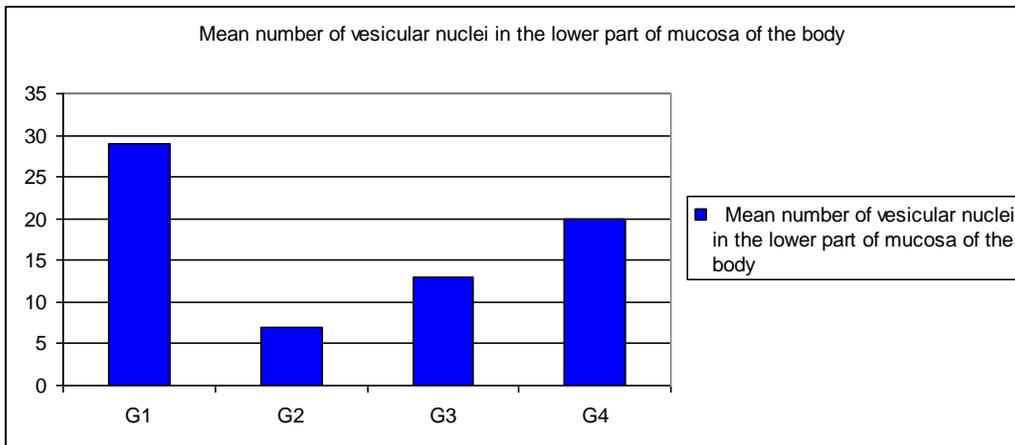


Chart-2 The mean number of vesicular nuclei in the lower part of mucosa of the body

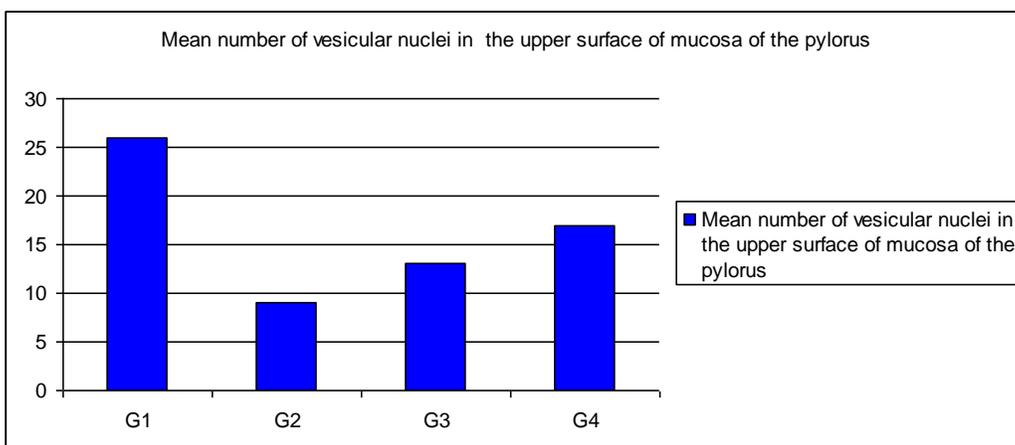


Chart-3 The mean number of vesicular nuclei in the upper surface of mucosa of the pylorus

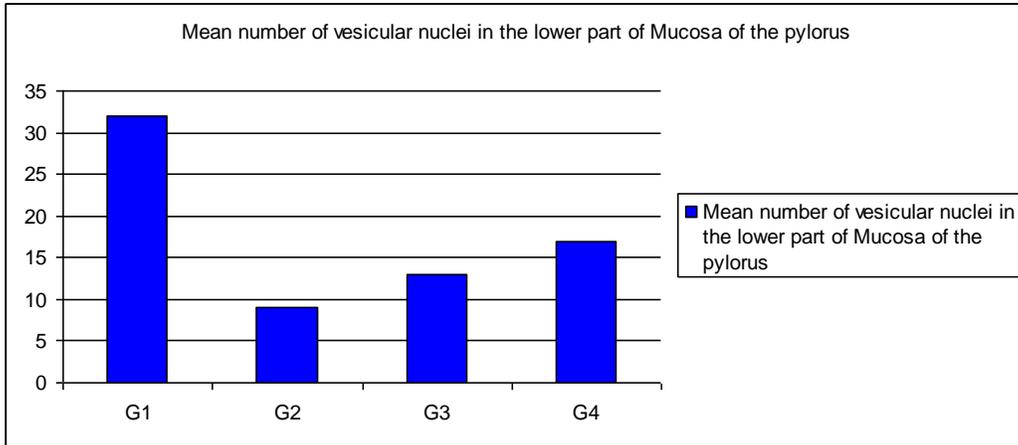


Chart-4 The mean number of vesicular nuclei in the lower part of mucosa of the pylorus

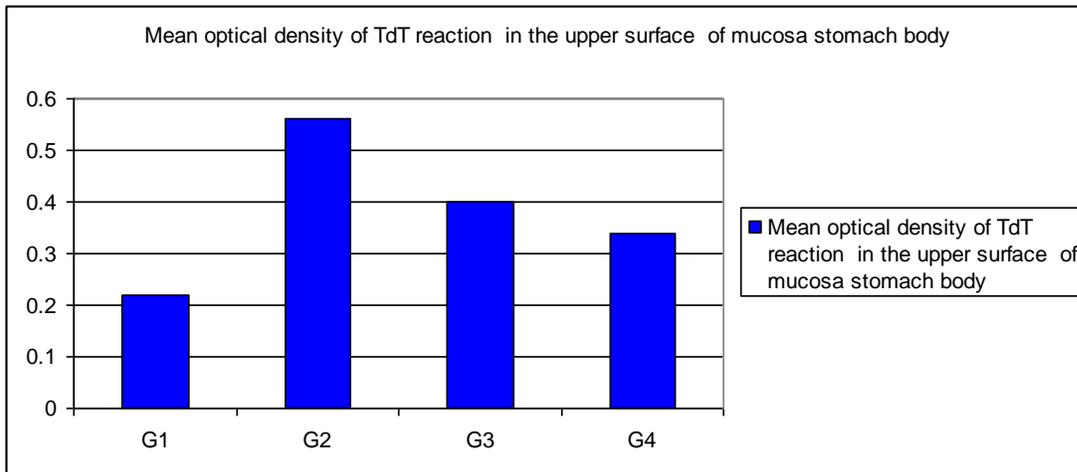


Chart-5 The mean optical density of TdT reaction in the upper surface of mucosa of stomach body

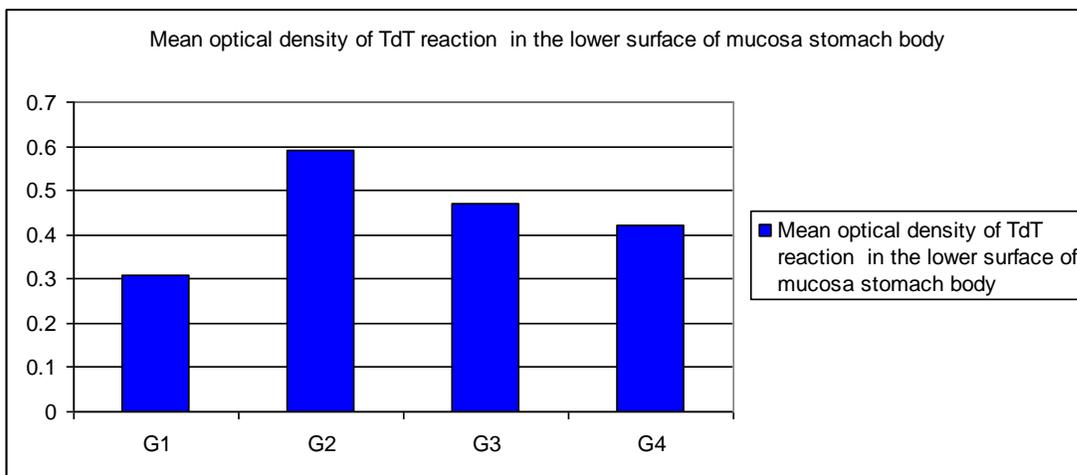


Chart-6 The mean optical density of TdT reaction in the lower surface of mucosa of stomach body

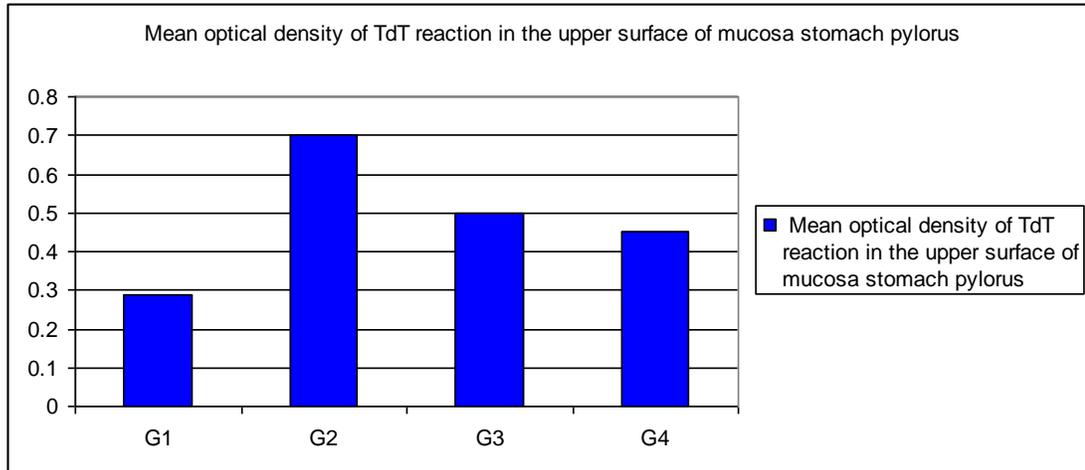


Chart-7 The mean optical density of TdT reaction in the upper surface of mucosa of stomach pylorus

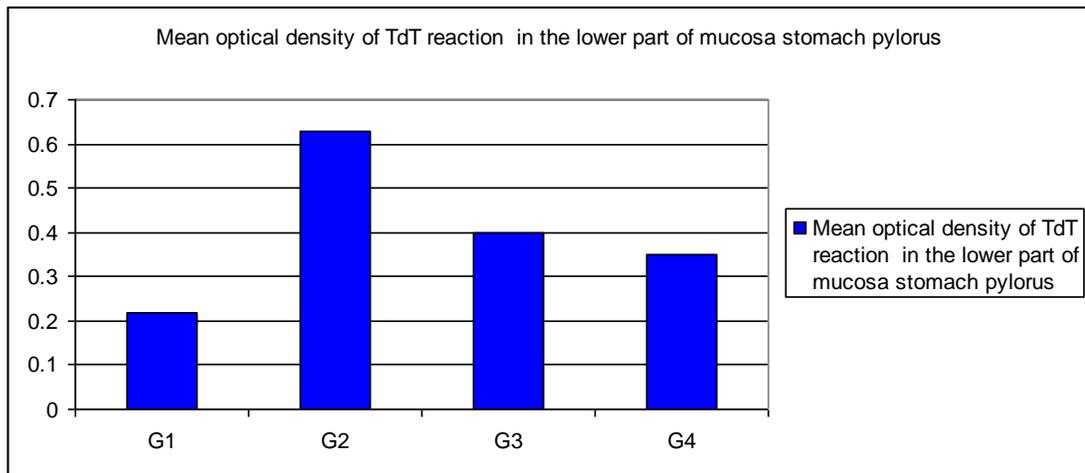


Chart-8 The mean optical density of TdT reaction in the lower part of mucosa stomach pylorus

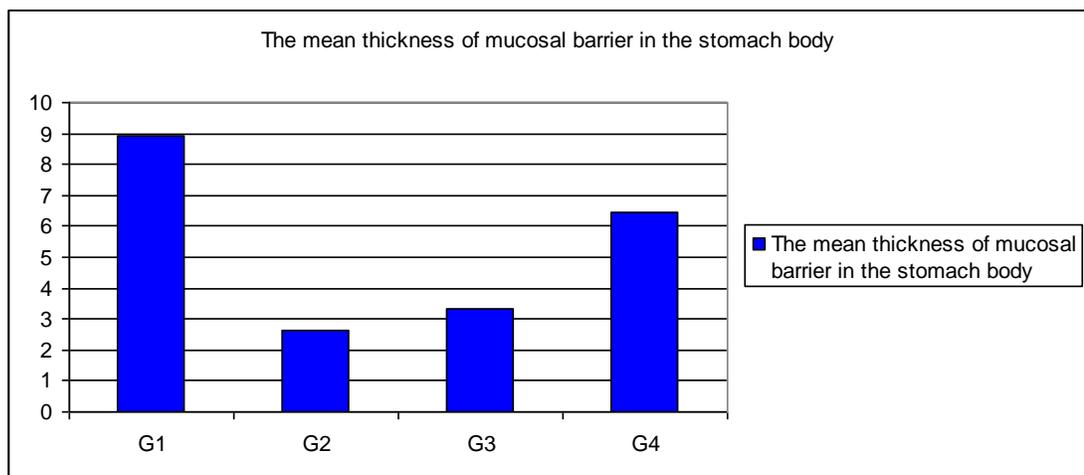


Chart-9 The mean thickness of mucosal barrier in the stomach body

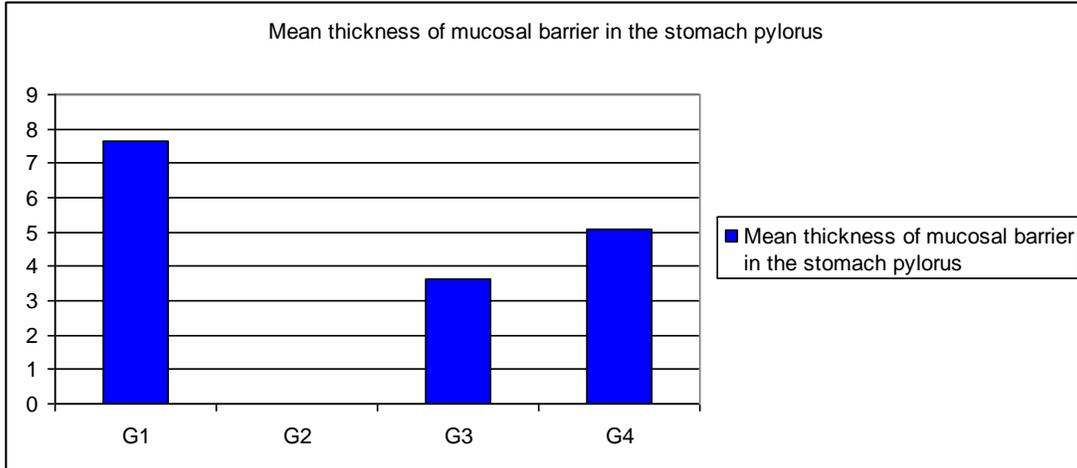


Chart-10 The mean thickness of mucosal barrier in the stomach pylorus

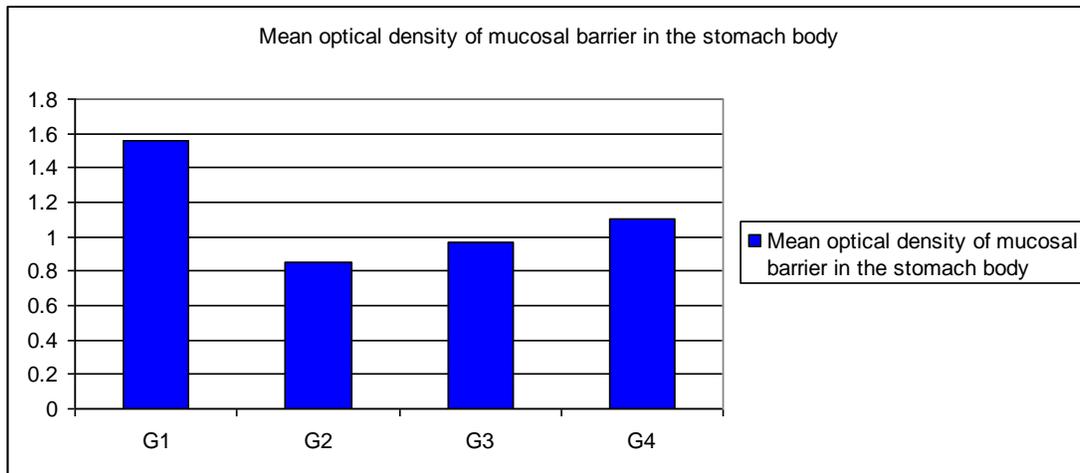


Chart-11 The mean optical density of mucosal barrier in the stomach body

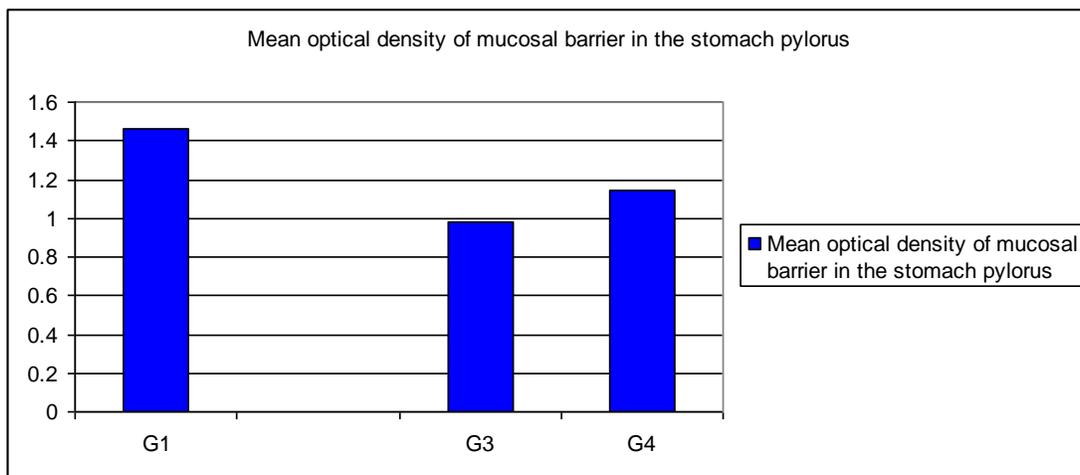


Chart-12 The mean optical density of mucosal barrier in the stomach pylorus