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Toxicity of Water Contaminated with Nitrate on the Guinea Pigs Treated with Cimetidin

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Abstract:

Background: The reduction of nitrate (NO_3^-) to toxic nitrite (NO_2^-) via bacterial enzymes is highly dependent on gastric pH. At specific medical conditions such as hypochlorhydria or achlorhydria (less acidic, high gastric pH), there is a significant risk of bacterial overgrowth that contributed to increase the conversion of nitrate to the nitrite in the stomach. Young monogastric animals (alike to human infants) are more sensitive to nitrate toxicity due to high gastric pH.

Aims: This study aimed to evaluate the toxicity of nitrate under higher gastric pH condition following cimetidin treatment. Moreover, it used Guinea pigs as a surrogated model for monogastric digestive system to assess nitrate toxicity under specific gastric medical problems.

R**esults**: The administration of 50 mg/kg.bw of cimetidin leads to an increase in stomach pH in Guinea pig as monogastric model following 6 weeks of the experimental period. The severity of the histopathological changes of stained stomach, liver, kidney, spleen and lung sections were higher in G2 (potassium nitrate + cimetidin-treated Guinea pigs) than in G3 (nitrate-toxicated Guinea pigs).

Conclusions: Under high gastric pH, the pathological changes were significantly higher in G2 (nitrate and cimetidin-treated group) than in the other groups. Furthermore, Guinea pigs represented a valuable monogastric surrogate model for both human and small animals to assess nitrate toxicity under specific gastric medical problems.

Keyword**:** Nitrate toxicity, cimetidin, high gastric pH, monogastric

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Introduction:

Nitrate toxicity is one of the main factors impacting the water quality. Around 63% of the water wells are contaminated with nitrate in Sulaimaniyah city, Iraq (Mustafa & Ahmad, 2008). Moreover, ground water well samples from Baghdad city, Iraq indicated high concentration of nitrate in Abu-Ghreeb and Diyala Ridge areas. The sources of nitrate are fertilizers, landfills and sewage (Al-Paruany *et al.,* 2016). High concentrations of nitrate in rivers and lakes affect water quality - making it unsuitable for drinking water (Singh *et al.*, 2022). In some cases, the concentrations of nitrate in the drinking water has been risen dramatically and exceeded the safety limit of 50 mg/L (Ramalingam *et al.*, 2022). High concentrations of nitrate are regularly linked with agriculture activities (Soltaninia et al., 2025).

Medical conditions such as hypochlorhydria or achlorhydria (less acidic gastric pH) pose a significant risk of bacterial overgrowth that contributed to increase the conversion of nitrate to the nitrite in the stomach (Sarker *et al.*, 2017). The elevation of blood nitrite levels leads to tissue hypoxia and Methemoglobinemia (MetHb, blue baby syndrome) (Ma *et al.*, 2018). Notably, young monogastric animals (alike to human infants) are more sensitive to nitrate toxicity due to high gastric pH (Ozmen *et al.,* 2005). Cimetidin medication can be defined as a histamine H2 receptor antagonist that used to reduce gastric acid. Moreover, cimetidin is associated with bacterial overgrowth and increasing intragastric nitrite (Snepar *et al.*, 1982). This study aimed to evaluate the toxicity of nitrate under higher gastric pH condition following cimetidin treatment. Moreover, it used Guinea pigs as a surrogated model for monogastric digestive system to assess nitrate toxicity under specific gastric medical problems.

Materials and Methods:

Potassium nitrate (500 mg KNO3, Sigma-Aldrich), (KNO3) was dissolved in 1000 ml of water. Cimetidin was dissolved in water according to the manufacturer instructions (50 mg/kg/b.w., Tagamet brand HB).

Experimental animals:

Cavia porcellus male guinea pigs (n=24), about 13 weeks of age were housed in the animal house at the College of Veterinary Medicine, University of Baghdad. These animals were equally divided into 4 groups. The first group considered as control group (G1). The second group (G2) was given 500 mg/L of potassium nitrate with 50 mg/kg.bw of cimetidin via water. The third group (G3) was given 500 mg/L of potassium nitrate only via drinking water. The fourth group administrated 50 mg/kg.bw of cimetidin only. The experimental period was 6 weeks and the treatments were administered daily through water.

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Stomach pH measurements:

Following 6 weeks of the experimental period, all animals were anesthetized using xylazine and ketamine, I/M injection (Holve et al., 2013), and then euthanized by cervical dislocation as reported in the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Stomach then was removed from each animal and the pH values were measured using a digital meter (TPS Pty Ltd, China) (Veterini et al., 2023). The data (mean) for the pH measurement was analyzed statistically using one-way ANOVA followed by (Tukey's multiple comparisons test). The statistical significance was determined at $p \leq 0.05$ using GraphPad Prism program (version 9.1.0).

Histopathological Evaluation :

To assess the histopathological changes, tissue specimens from stomach, liver, kidney, spleen and lungs were collected from each animal in different groups following euthanizing. After fixation process using 10% neutral buffer formalin, all samples were dehydrated using alcohol (variable increasing concentration), then embedded in paraffin wax and sectioned at 4-5um thickness prior to staining with H&E stain (Feldman & Wolfe, 2014; Sabeeh & Al-Awadi, 2023).

Ethics approval: Ethical approval was granted through local committee of the animal's care and use at the College of Veterinary Medicine, University of Baghdad (Approval Number: 828).

Results:

Stomach pH values

Stomach pH values were measured in control G1 and treatment groups (G2, G3 and G4, respectively) as illustrated in Fig. (1). The results showed that administration of cimetidin (50 mg/kg.bw) caused significant increase in pH values of the guinea pigs stomach (3.38±0.41 and 3.3±0.35) in G 2 and G4, respectively, compared to control group G1 (1.7±0.2). There was no significant increase in pH values of guinea pigs stomach in G 3 (1.76± 0.35) in comparison to the controls.

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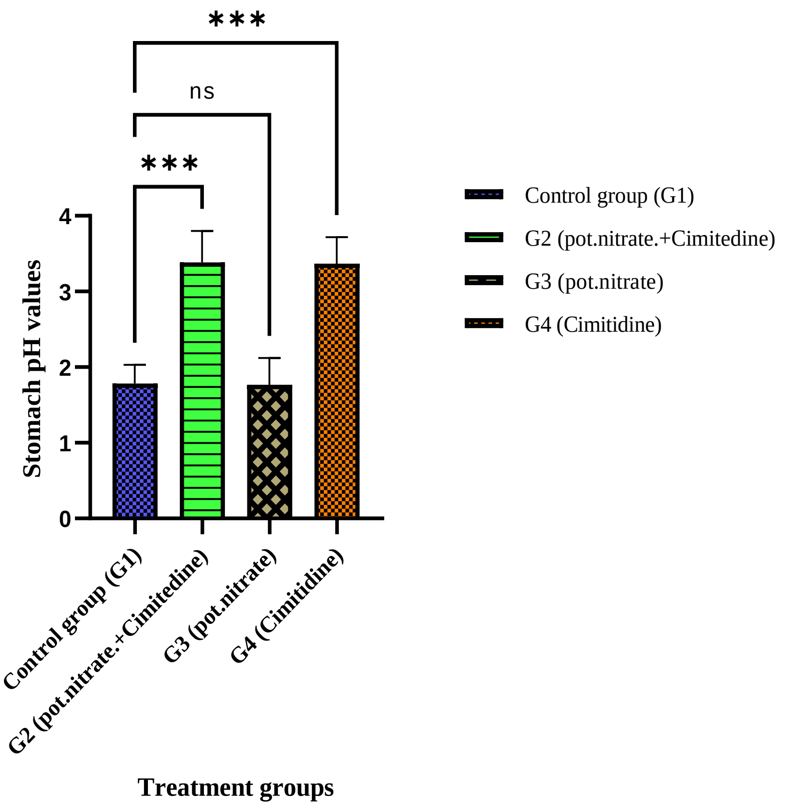

Fig. (1): Stomach pH. The experimental groups are shown in this figure as the followings: Control (G1) and treatment groups (G2, G3 and G4). Data represented as mean ± standard deviation (SD) of the Means (n=6/group).

Histopathological changes

In order to evaluate nitrate toxicity under less acidic gastric pH condition, tissue specimens from stomach, liver, kidney, spleen and lungs were collected from the experimental animals following 6 weeks of treatment.

Histopathological study of stomach sections showed mucosal layer ulceration and slight infiltration of inflammatory cells in the sub-mucosal layer and oedema in G2 animals (treated with potassium nitrate + cimetidin, Fig. 2B). Moreover, the pathological changes in G3 animals (treated with KNO_3 only) showed hyperplasia of the mucosal layer, congested blood vessels in the sub mucosal layer and marked oedema (Fig. 2C). However, normal gastric glands and mucosal layer structure were seen in G4 animals (treated with cimetidin only) and in control group (Fig. 2D, 2A).

The pathological alteration in liver sections in all treatment groups following six weeks were revealed in Fig. 3. There is marked dilatation and congestion of portal veins with periportal cellular infiltration in G2 (with potassium nitrate plus cimetidin-treated animals, Fig 3B). Several ballooned

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hepatocytes and vacuolated cytoplasm were seen in G3 (potassium nitrate-treated animals) (Fig. 3C). Furthermore, animals of G4 and control group (G1) showed normal central vein and hepatocytes arrangement (Fig. 3D, 3A, respectively).

In the kidney, marked renal tubules degeneration and vacuolated cytoplasm were seen in G2 (Fig. 4B) In addition, animals that treated with potassium nitrate only (G3) showed hyaline cast formation in the renal tubules. Group G4 (animals treated with cimetidin only) and G1 (control group) showed normal renal tubules structure (Fig. 4C, 4D).

Sections of spleens of potassium nitrate and cimetidin- treated Guinea pigs (G2) showed marked depletion of the white pulp with the loss of the germinal centre and necrotic areas in the lymphoid follicle (Fig. 5B). Additionally, G3 (potassium nitrate- treated animals) displayed reduced white pulp section, thinner marginal zone and loss of the germinal centre (Fig. 5C). However, G4 and G1 revealed normal white pulp and red pulp structure (Fig. 5D, 5A).

Histopathological sections of lungs showed normal alveolar wall structure in G1 and G4 (Fig. 6A, 6D). On the other hanh, G2 showed bronchiole wall destruction, bronchial lumen obstructed with red blood cell, mucous exudate, necrotic fragments and inflammatory cells (Figure 6B). Moreover, potassium nitrate-treated animals showed thickening of the alveolar wall which filled with pink material exudate, RBCs and inflammatory cells (Fig. 6C).

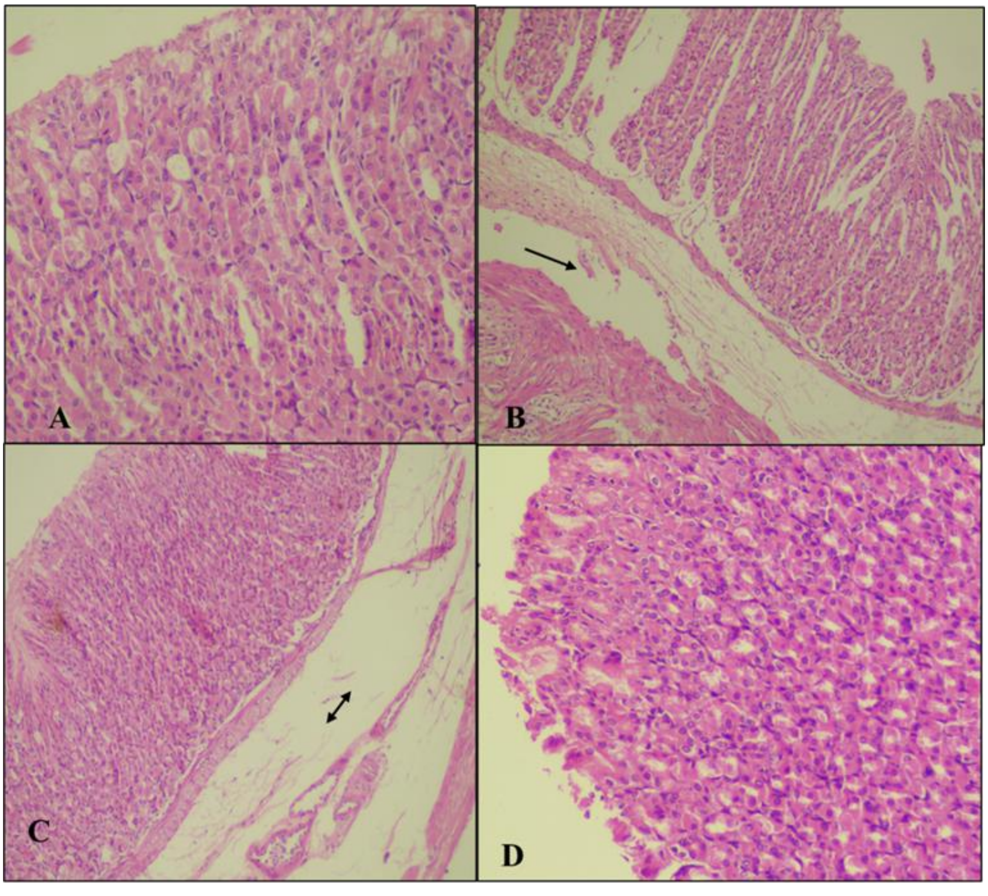

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Fig. (2): Histopathological Guinea pigs stomach sections (H & E stained) from normal controls and treatment groups following six weeks of experimentation. A: Control group (G1) shows normal histological structure of the mucosal layer of the glandular stomach (gastric glands) 20X. B: Group 2 (G2, animals treated with potassium nitrate and cimetidin) shows mucosal layer ulceration and slight infiltration of inflammatory cells in the sub mucosal layer and oedema (arrow) 10X. C: Group 3 (G3, animals treated with potassium nitrate only) shows hyperplasia of the mucosal layer and congested blood vessels in the sub mucosal layer and marked oedema (double-headed arrow) 10X. D: Group 4 (G4, animals treated with cimetidin only) shows normal gastric glands and mucosal layer structure 20X.

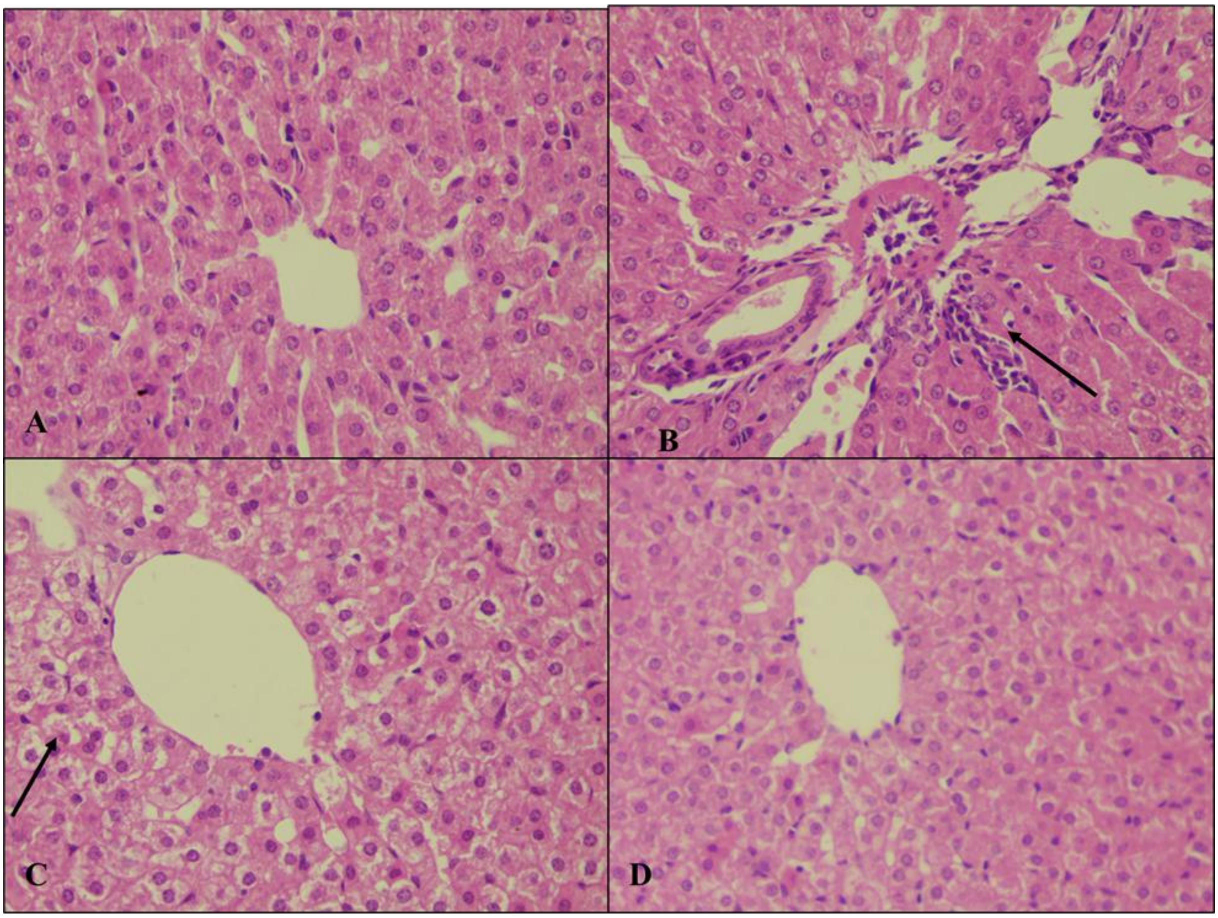

Fig. (3): Histopathological (H & E stained) sections of Guinea pigs liver from control and treatment groups following six weeks treatment. A: Control group (G1) shows normal histological structure of liver parenchyma, central vein and hepatocytes 40 X. B: Group 2 (G2, guinea pigs treated with potassium nitrate and cimetidin) shows marked dilatation of the portal vein with periportal cellular

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infiltration (arrow) 40 X. C: Group 3 (G3) shows several ballooned hepatocytes and vacuolated cytoplasm (arrow) 40X. D: Group 4 (G4, animals treated with cimetidin only) shows normal central vein and hepatocytes arrangement 20X.

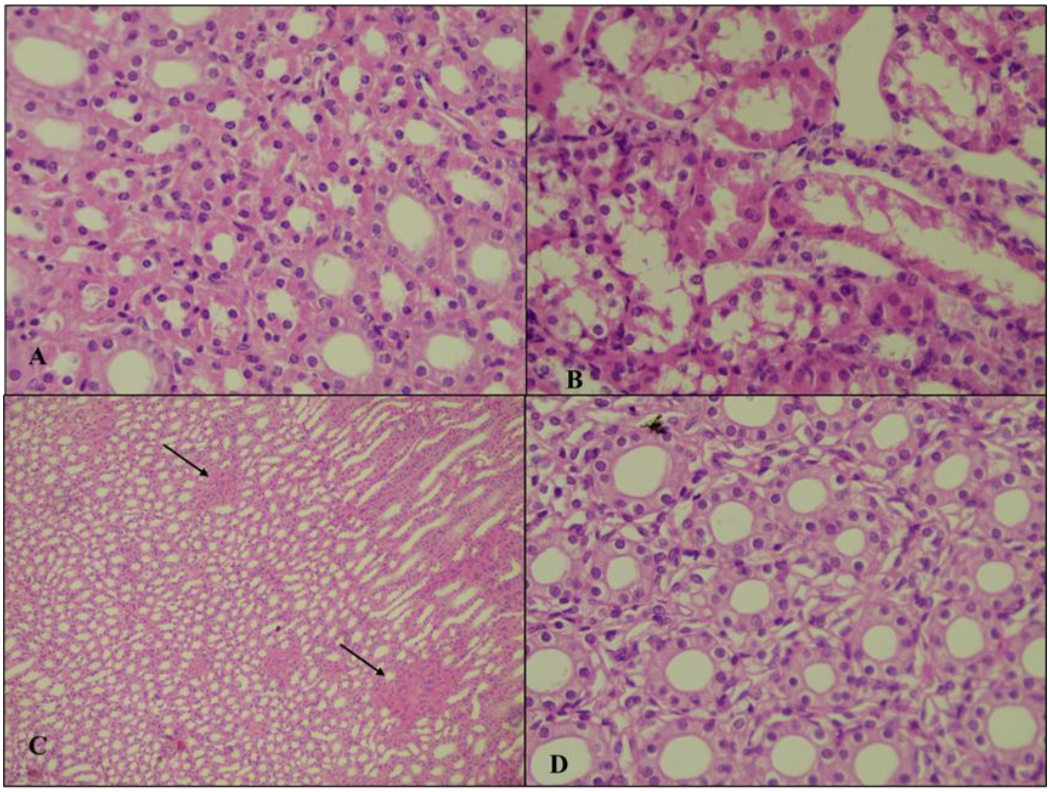

Fig. (4) Histopathology of Guinea pigs kidney sections from the control and treatment groups following six weeks of treatment. A: Control group (G1) shows normal histological structure of renal tubules 40 X. B: Group 2 (G2, Guinea pigs treated with potassium nitrate and Cimetidin) shows marked renal tubules degeneration, sloughing of some epithelium lining the renal tubules and vacuolated cytoplasm 40X. C: Group 3(G3, animals treated with potassium nitrate only) shows red blood cast formation in the renal tubules (arrow) 20X. Group 4 (G4, animals treated with cimetidin only) shows normal renal tubules structure 40 X.

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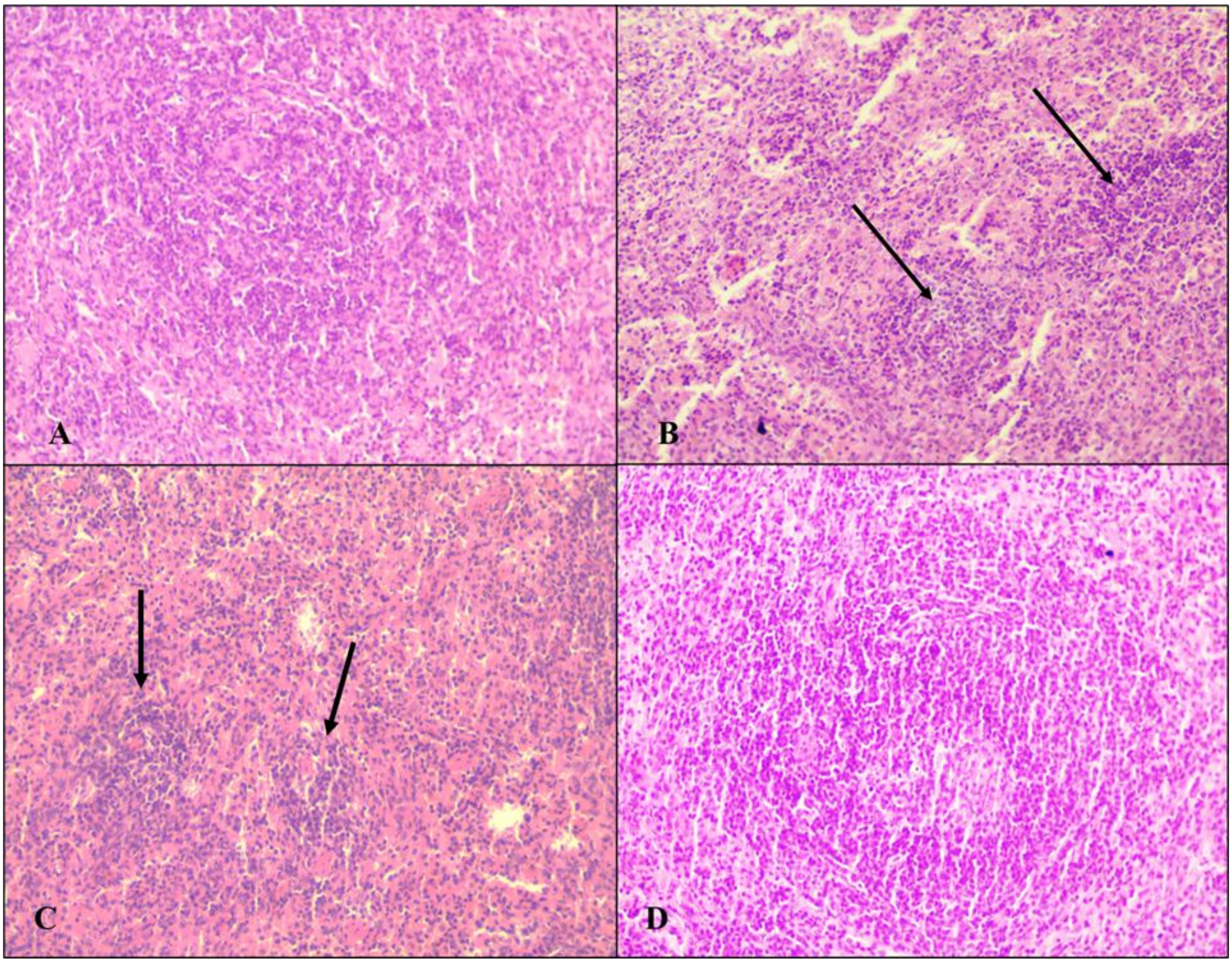

Fig. (5) Histopathology of stained Guinea pigs spleen sections from the control and treatment groups following six weeks (H & E stain). A: Control group (G1) shows normal histological structure of spleen 40 X. B: Group 2 (G2, Guinea pigs that treated with potassium nitrate and cimetidin) shows marked depletion of the white pulp with the loss of the germinal center and necrotic areas in the lymphoid follicle (arrows) 20X. C: Group 3 (G3, animals treated with potassium nitrate only) shows reduced white pulp section (white pulp depletion) (arrows), thinner marginal zone and absence of a germinal center 20X. Group 4 (G4, animals treated with cimetidin only) shows normal white pulp and red pulp structure 40 X.

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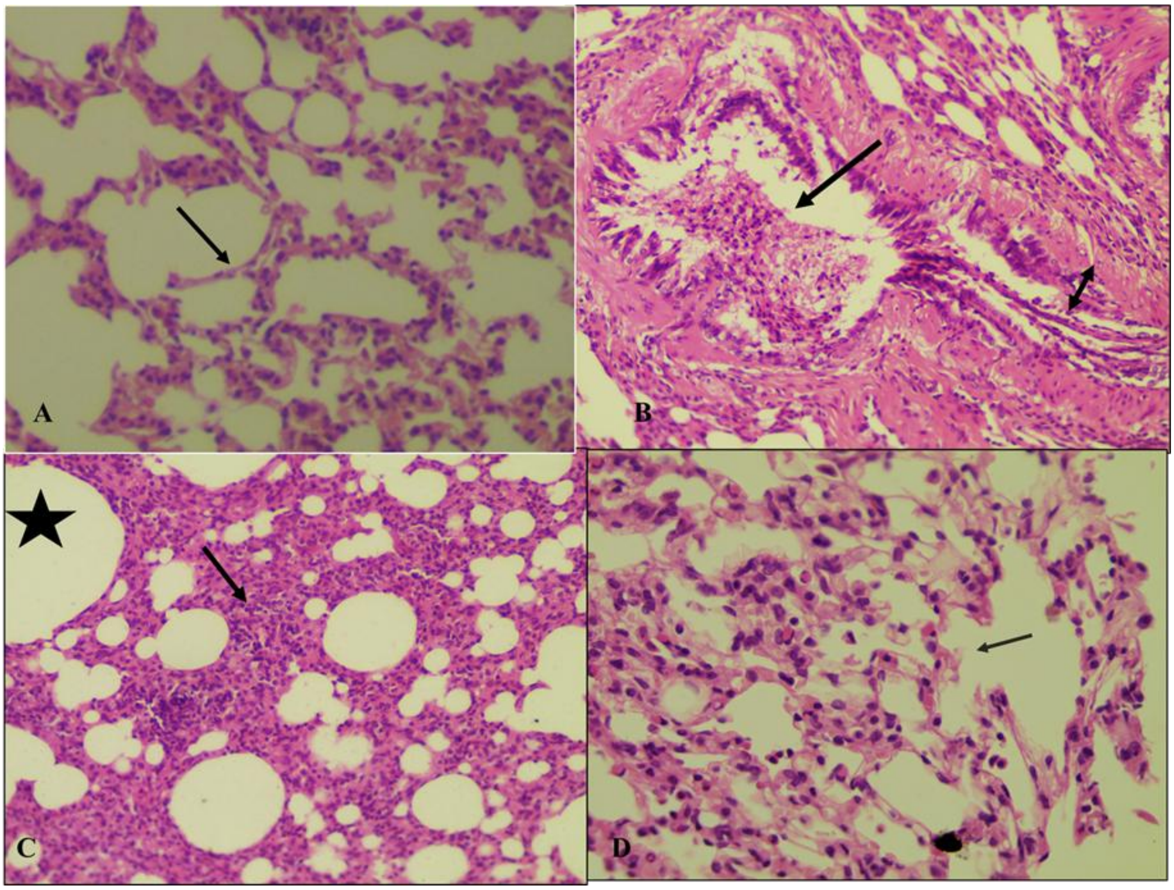

Fig. (6) Histopathological lung sections of control and six weeks treated Guinea pigs (H & E stain). A: Control group (G1) shows normal alveolar wall structure (arrow) 40 X. B: Group 2 (G2, Guinea pigs treated with potassium nitrate and cimetidin) shows hyperplasia and sloughing of bronchiole epithelium (double-headed arrow). Bronchiole wall destruction, bronchial lumen obstructed with red blood cell, mucous exudate, necrotic fragments and inflammatory cells (arrow) 40X. C: Group 3 (G3, animals treated with potassium nitrate only) shows thickening of the alveolar wall (filled with pink material exudate, RBCs, inflammatory cells (arrow) and emphysema (star) 20X. Group 4 (G4, animals treated with cimetidin only) shows normal alveolar wall (arrow) 40 X.

Discussion

The reduction of nitrate to nitrite depends on nitrate-reducing bacteria. However, in the medical conditions such as hypo-or a chlorhydria, gastric cancer, gastritis and peptic ulcer treatment, the gastric pH can be increased significantly to allow bacterial overgrowth (Sung *et al.*, 2018). Several researches focused on the problems of low gastric acidity and the ability of bacteria to be colonized in the stomach and small intestine (Fiorani *et al.*, 2023; Sarker *et al.*, 2017). In order to enhance bacterial overgrowth and to reduce gastric acid in Guinea pig model, cimetidin treatment was used. Of note, Guinea pigs as monogastric animals played as an excellent model for human infants and small animal (such as young pigs and un-weaned farm animals). It is also important to

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note that young monogastric animal have more sensitivity towards nitrate poisoning (Thakur et al., 2025).

Cimetidin medication can be defined as a histamine H2 receptor antagonist that used to reduce gastric acid (Thorens *et al.*, 1996). The results of this study confirmed the ability of cimetidin to increase the pH values of the guinea pigs' stomach in G 2 (3.38±0.41) and G4 (3.3±0.35), respectively, in comparison to the control group G1 (1.7±0.2) and G3 (1.76± 0.35), respectively. Higher gastric pH was essential in this project to enhance bacterial overgrowth and study the histopathological changes following nitrate toxicity in less acidic environment. The severity of the histopathological changes of stained stomach, liver, kidney, spleen and lung sections was higher in G2 (Guinea pigs treated with potassium nitrate + Cimetidin) than G3 (Guinea pigs toxicated with nitrate only). These results may be due to the high pH gastric value that measured in the G2 than in G3 (Fig. 1). All the histopathological changes that described in the results of this study were reported previously (Amira et al., 2023; Kattaia et al., 2017; Ogur et al., 2005). The pathological alterations in the liver tissues (Fig. 3B and 3C) are in agreement with González Delgado et al. (2018) who reported degenerative changes and necrosis of the hepatocytes in rats treated with high doses of sodium nitrate. Moreover, the results of this study are in accordance with Bouaziz-Ketata *et al.* (2014) who demonstrated hepatotoxicity in rats treated with nitrate (inflammatory cell infiltration and necrosis) due to increase transaminases and lactate deshydrogenase activities. Generally, administration of nitrite induced hypoxic nephrotoxicity (Zaidi, 2020) which may explain the pathological changes that seen in the kidney sections (Fig 4B ,4C). Furthermore, the pathological changes in the spleen tissues (Fig. 5B, 5C) are in agreement with Amira et al. (2023) that observed significant changes in the lymphoid tissue such as necrosis and depletion of the white pulp following administration of sodium nitrite in rats.

Conclusions:

Under high gastric pH, the pathological changes were significantly observed in G2 that treated with high concentrations of nitrate and 50 mg/kg.bw of cimetidin drug via water. The administration of 50 mg/kg.bw of cimetidin leads to an increase in stomach pH and potentially speeding the conversion of nitrate to toxic nitrite. Furthermore, guinea pigs play as valuable monogastric surrogate model for both human and young animals to assess nitrate toxicity under specific gastric medical problems**.**

Conflict of Interest:

Author declares no conflicts of interest

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