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Sodium nitrite-induced hepatotoxicity in male Wistar rats and its attenuation by silymarin: Biochemical, oxidative stress, and histopathological evidence

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Abstract

In cattle and other ruminants, nitrate poisoning is a potentially fatal toxicological problem because nitrate is readily reduced to nitrite in rumen, leading to systemic toxicity. This study was designed to evaluate the toxicity of sodium nitrite in terms of hepatic oxidative status, hepatic functions, and hepatic histopathological architecture in male Wistar albino rats and concurrent potential protective effects of silymarin. Forty rats were procured for the study and randomly assigned to five treatment groups, with eight animals per group. The treatments were – control, sodium nitrite (30 mg/kg/day), sodium nitrite (60 mg/kg/day), sodium nitrite (30 mg/kg/day) + silymarin (150 mg/kg/day), and sodium nitrite (60 mg/kg/day) + silymarin (150 mg/kg/day). The treatments were administered to rats orally for a period of 60 days. The results revealed a significant ($P \leq 0.05$), dose dependent sodium nitrite-induced reduction in the activities of serum superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) compared with the control. On the other hand, sodium nitrite exposure significantly increased serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alpha-fetoprotein (AFP); however, alanine aminotransferase (ALT) increased significantly only at higher dose (60 mg/kg/day). Liver histopathology exhibited dose-dependent hepatocellular degeneration, dilation of sinusoids, infiltration of inflammatory cells, vascular congestion, and necrotic spots. The co-administration of silymarin resulted in significant improvement of SOD and CAT activities and reduction of ALT, ALP, and AFP levels, along with a considerable reversal of sodium nitrite-induced hepatic histopathological changes. In conclusion, significant hepatic oxidative and histopathological damage was induced by sodium nitrite, whereas co-administration of silymarin exerted a considerable ameliorative effect.

1. Introduction

Sodium nitrite ($NaNO_2$) is an inorganic compound routinely used in the food industry as an antimicrobial agent, preservative, and colour stabilizer. It maintains product quality, particularly processed ones, by inhibiting lipid oxidation and microbial growth (Reynolds et al. 2021; Taus 2021). Furthermore, sodium nitrite acts as chemical intermediate and has anti-corrosive properties and therefore has found extensive applications in other industries, including pharmaceutical, metallurgical, textile, and chemical industries (Shakil et al. 2022). However, exposure to sodium nitrite has emerged as an important environmental and public health concern because it causes oxidative stress, methemoglobinemia, and multi-organ toxicity. It facilitates the formation of reactive nitrogen species (RNS) and cancer causing N-nitroso compounds, leading to oxidative damage of organic molecules and disruption of cellular homeostasis and normal physiological functions (Sorour et al. 2023; Chaudhary et al. 2023). In addition to its ramifications in human medicine, nitrate and nitrite toxicity is equally challenging in veterinary medicine. Excess accumulation of nitrate has been reported in grasses, pastures, silage, and drinking water due to excess application of nitrogen fertilizer, drought conditions, reduced sunlight, etc. (Reynolds and Drewnoski 2022; Abdelbagi et al. 2023). Among livestock, ruminants are highly susceptible because of rapid conversion of nitrate to nitrite by rumen microbes, resulting in acute nitrite accumulation due to impaired ammonia conversion. The rapid flux of nitrite into the circulation results in methemoglobinemia, which reduces the oxygen-carrying capacity of the blood and results in systemic tissue hypoxia (Oliynyk 2024; Abdelbagi et al. 2023). Clinically, nitrate poisoning in cattle is characterized by breathing difficulty, cyanosis, loss of appetite, decreased production, reproductive disorders, and sudden death. However, chronic exposures have been associated with compromised growth, immunity, and hepatic function, along with significant economic losses (Reynolds and Drewnoski 2022; Oliynyk 2024).

Although the classical hallmark of nitrate poisoning is methemoglobinemia, current research suggests oxidative stress as a principal mechanism behind the nitrite-induced toxicity. This toxicity is mediated by excessive generation of RNS and reactive oxygen species (ROS), which compromises the body antioxidant defenses and initiates lipid peroxidation, mitochondrial dysfunction, DNA damage, and apoptosis (Chaudhary et al. 2023; Hassan et al. 2024). The liver is one of the most vulnerable organs affected by nitrite poisoning, which can cause disruption of membrane integrity of hepatocytes and impairment of hepatic functions (Soliman et al. 2021; El-Nabarawy et al. 2020). The exposure to sodium nitrite has been reported to increase serum aminotransferases, oxidative stress biomarkers, compromise endogenous antioxidant enzyme activities, and induce histopathological lesions (Soliman et al. 2021; El-Nabarawy et al. 2020; Khassaf 2024). Depletion of serum superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) is regarded as reliable indicators of hepatoxicity mediated by oxidative stress. Similarly,

elevation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are indicators of hepatocellular membrane disruption and impaired liver functions (Soliman et al. 2021; Shakil et al. 2022). Furthermore, alpha-fetoprotein (AFP), an oncofetal protein and tumor biomarker, has recently been categorized as a hepatocellular injury marker, which gets activated on repeated exposure to toxicity (Chaudhary et al. 2023; Sorour et al. 2023).

Silymarin is a naturally occurring hepatoprotective agent, which has generated considerable interest among researchers as a results of its strong antioxidant, anti-inflammatory, and membrane-stabilizing properties. Silymarin has been reported to exert significant hepatoprotective action by effective scavenging of free radicals, improving cellular antioxidant pool, preserving mitochondrial integrity, suppressing inflammatory mediators, and hepatocellular regeneration (Wadhwa et al. 2022; Amien et al. 2015). Nitrite toxicity has been extensively studied; however, very few studies have adopted an integrated approach of combining oxidative stress, biochemical, and histopathological evaluations to assess the hepatoprotective efficacy of silymarin with respect to nitrate poisoning in cattle. Considering the economic impact of nitrate poisoning in cattle and role of oxidative stress in nitrite-induced tissue injury, experimental rodent models may provide valuable insights into the development of effective antioxidant-based therapeutic strategies to prevent nitrite poisoning (Oliynyk 2024; Reynolds and Drewnoski 2022). Therefore, the present study aimed to investigate hepatic oxidative stress, liver function, and histopathological alterations in male Wistar rats on exposure to different doses of sodium nitrite by evaluating antioxidant enzymes (SOD, CAT, GPx), hepatic biomarkers (ALT, AST, ALP, and AFP), and hepatic histology. The study further evaluated the hepatoprotective role of silymarin in mitigating the sodium nitrite-induced hepatotoxicity.

2. Materials and Methods

2.1 Experimental animals and husbandry

The study was conducted between 28 October 2025 and 28 December 2025. A total of forty adult male Wistar albino rats, weighing 200–250 g and aged 12–14 weeks, were procured from the Animal House Facility of the College of Veterinary Medicine, Tikrit University, Iraq. Animals were housed in standard polypropylene cages under controlled laboratory conditions maintained at 22 ± 2 °C with a relative humidity of 50–60% and proper ventilation. Animals were under constant observation for health status and clinical signs. They were provided *ad libitum* access to standard pellet feed and clean drinking water throughout the experimental period.

2.2 Ethical approval

All the experimental procedures involving animals were carried out according to ARRIVE 2.0 guidelines for the care and use of laboratory animals. All the protocols used in this study were reviewed and approved by the Ethics Committee of the College of Education for Pure Sciences, Tikrit University, Iraq (Ethical Approval No. 2026-12). Every effort was made to reduce the number of animals used and minimize the suffering of animals.

2.3 Chemicals

Analytical-grade sodium nitrite (purity ≈ 99%), manufactured in Germany, was supplied by Sekama Chemical Supplies, Iraq and administered orally to rats at the dose rate of 30 and 60 mg/kg/day in 1 mL of distilled water. The dose rates of sodium nitrite were chosen as sublethal (1/6th of LD_{50}) and median lethal (1/3rd of LD_{50}) dose to represent moderate and severe sub-chronic environmental exposure, respectively (El-Nabarawy et al. 2020; Merinas-Amo et al. 2025). Silymarin was procured from Global Pharmaceutical Industries, Egypt and administered orally at a dose rate of 150 mg/kg body weight. The dose rate of silymarin was selected based on the recommendation of previous empirical studies (Amien et al. 2015; Khassaf 2024).

2.4 Experimental design

Following simple randomization, the animals were equally divided into five treatment groups (n = 8) after an acclimatization period of one week. The pattern of treatment groups was as follows: Group I (control) – received only distilled water, Group II – received sodium nitrite at 30 mg/kg body weight/day, Group III – received sodium nitrite at 60 mg/kg body weight/day, Group IV – received sodium nitrite at 30 mg/kg body weight/day + silymarin at 150 mg/kg body weight/day, and Group V – received sodium nitrite at 60 mg/kg body weight/day + silymarin at 150 mg/kg body weight/day. The oral administration of treatments was done for a period of 60 days. The doses of sodium nitrite were based on previous toxicological studies, and the silymarin dose was selected based on its published hepatoprotective studies (Khassaf 2024; Merinas-Amo et al. 2025; Amien et al. 2015; El-Nabarawy et al. 2020).

2.5 Sample collection

The animals were subject to an overnight fasting and anesthetized with isoflurane at the end of experimental period. Blood samples were collected directly from the heart by cardiac puncture using sterile syringes and transferred into tubes containing clot-separating gel and centrifuged at 3,000 rpm for 15 min. The serum was separated and stored at – 20 °C in properly labelled Eppendorf tubes until biochemical analysis. The livers of euthanized animals were carefully excised, washed with ice-cold normal saline to remove leftover blood, placed on filter paper for drying, and finally fixed in 10% formalin in properly labelled clean plastic containers for histopathological examination.

2.6 Serum biochemistry

The harvested serum samples were subjected to biochemical analyses of hepatic function and oxidative stress biomarkers. Antioxidant enzyme activities – SOD, CAT, and GPx (Colorimetric Assay Kit, Elabscience BT Co. Ltd., Wuhan, China) were assayed using commercially available assay kits following manufacturer's instructions. Measurements of serum ALT, AST (Enzymatic Kinetic Kit, Randox Laboratories Ltd., Crumlin, UK), ALP (p-NPP Colorimetric Kit, Randox Laboratories Ltd., Crumlin, UK), and AFP (Rat AFP ELISA Kit, Cusabio Technology LLC, Houston, TX, USA) were done using commercially available kits following manufacturers' instructions. All the analyses were performed in duplicate.

2.7 Histopathological examination

The liver samples were fixed in 10% neutral buffered formalin for 24–48 h, dehydrated through a series of graded ethanol (70% - 100%), cleared in xylene, and embedded in paraffin blocks. Tissue sections of 5 µm thickness were made using a rotary microtome (Leica RM2235, Germany) and stained with hematoxylin and eosin (H&E) (Drury and Wallington 1980). Histopathological evaluation was performed in a blinded manner by an experienced pathologist. The stained tissue sections were examined using a light microscope (Olympus BX53, Tokyo, Japan) equipped with a digital camera (Olympus DP27) and

representative photomicrographs were analyzed by CellSens image analysis software.

2.8 Semi-quantitative histopathological assessment

To assess the extent of hepatic damage, histopathological lesions were evaluated semi-quantitatively. Ten randomly selected microscopic fields per tissue section were evaluated under ×200 magnification by a pathologist in a completely blinded manner. Five primary histopathological parameters used for grading the degree of hepatic damage were hepatocellular necrosis, inflammatory cell infiltration, hydropic degeneration, vascular congestion, and sinusoidal dilation. The lesions were graded using a 5-point semi-quantitative scoring scale as follows:

Grade 0: Normal architecture / no detectable lesions (<5% affected area)

Grade 1: Minimal / mild lesions (5% - 25% affected area)

Grade 2: Moderate lesions (26% - 50% affected area)

Grade 3: Severe lesions (51% - 75% affected area)

Grade 4: Profound / diffuse lesions (>75% affected area)

2.9 Statistical Analysis

Data were analyzed using IBM SPSS Statistics software (Version 26.0; IBM Corp., Armonk, NY, USA). The normality of data distribution and homogeneity of variances were assessed using the Shapiro-Wilk test and Levene's test, respectively. Experimental results are expressed as the Mean ± standard error of the Mean (SEM). Differences among the five experimental groups were evaluated using one-way analysis of variance (ANOVA). Upon obtaining significant ANOVA results, Tukey's HSD post-hoc test was applied for pairwise multiple comparisons between groups. Statistical significance was established at $P \leq 0.05$.

3. Results

3.1 Antioxidant enzyme activities

Serum antioxidant enzyme activities under the influence of sodium nitrite exposure and silymarin treatment are given in Fig. 1, Fig. 2, and Fig. 3. A significant decrease ($P \leq 0.05$) in SOD concentration was recorded in both nitrite-treated groups compared with control, with significantly greater reduction in the group receiving 60 mg/kg sodium nitrite compared with the group receiving 30 mg/kg sodium nitrite. Co-administration of silymarin demonstrated a significant recovery in SOD activities compared with the corresponding sodium nitrite-treated groups ($P \leq 0.05$). However, enzyme activity could not be restored to the normal level observed in the control group (Fig. 1). A similar trend was observed in CAT activity with the exception that CAT activity reduction was statistically similar between two sodium nitrite exposed groups (30 mg/kg vs. 60 mg/kg) (Fig. 2). Serum GPx activity significantly ($P \leq 0.05$) declined in sodium nitrite-exposed groups compared with control and decline was significantly higher in 60 mg/kg body weight with respect to 30 mg/kg group. However, it was observed that concurrent administration of silymarin did not significantly restore the GPx activity of corresponding sodium nitrate-treated groups (Fig. 3). Overall, it was observed that sodium nitrate exposure significantly suppressed the endogenous SOD, CAT, and GPx activities; and silymarin supplementation neutralized the negative effects of nitrate poisoning on SOD and CAT activities but not GPx

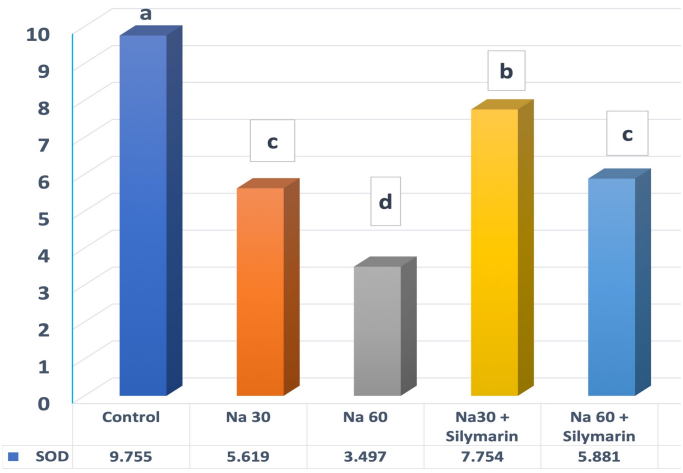

Fig. 1. Effect of treatments on serum SOD concentrations (U/mL). Different superscripts indicate significant differences

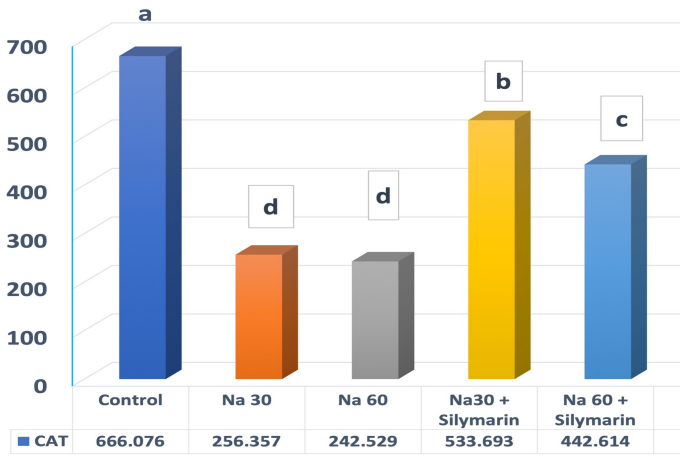

Fig. 2. Effect of treatments on serum CAT concentrations (U/mL). Different superscripts indicate significant differences

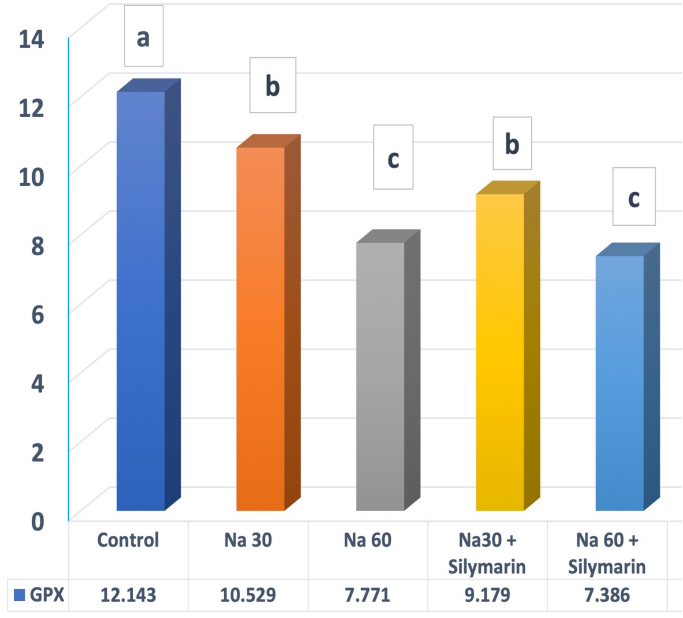

Fig. 3. Effect of treatments on serum GPx concentrations (U/mL). Different superscripts indicate significant differences

activity.

3.2 Liver function biomarkers

Nitrite-treated groups exhibited a significant ($P \leq 0.05$) elevation in serum AST activity compared with control, with no significant difference between the two sodium nitrite levels (Fig. 4). Silymarin co-supplementation in both the nitrate-treated groups revealed no significant effect on the AST activity in these groups. The serum ALT activity significantly ($P \leq 0.05$) increased in the group treated with sodium nitrite at 60 mg/kg body weight compared with the control group but no significant change was observed in 30 mg/kg body weight group compared with the control. Furthermore, silymarin co-supplementation revealed significant ($P \leq 0.05$) reduction of ALT activity compared with their corresponding nitrite-treated groups and was below the control group activity (Fig. 5). Similarly, serum ALP was significantly ($P \leq 0.05$) elevated in both sodium nitrite-treated groups compared with control group, with no significant difference between the two sodium nitrite levels. Co-administration of silymarin resulted in significant ($P \leq 0.05$) reduction of ALP activity in both nitrite-treated groups and the activity was significantly lower than the control group as well (Fig. 6). Serum AFP concentration was significantly ($P \leq 0.05$) elevated in nitrite-treated groups in a dose-dependent manner compared with the control. However, co-administration of silymarin significantly decreased the serum AFP levels compared with the corresponding nitrate-treated groups; however, the AFP levels remained higher than that observed in the control group (Fig. 7). Collectively, the results indicated that sodium nitrite exposure significantly altered the serum biomarkers of hepatic function, whereas co-administration of silymarin mitigated these alterations to a considerable extent.

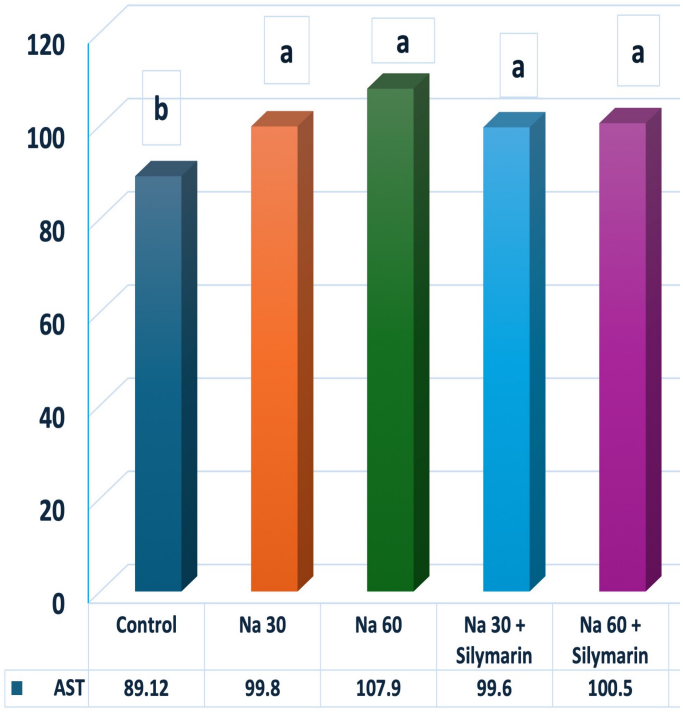

Fig. 4. Effect of treatments on serum AST concentrations (U/mL). Different superscripts indicate significant differences

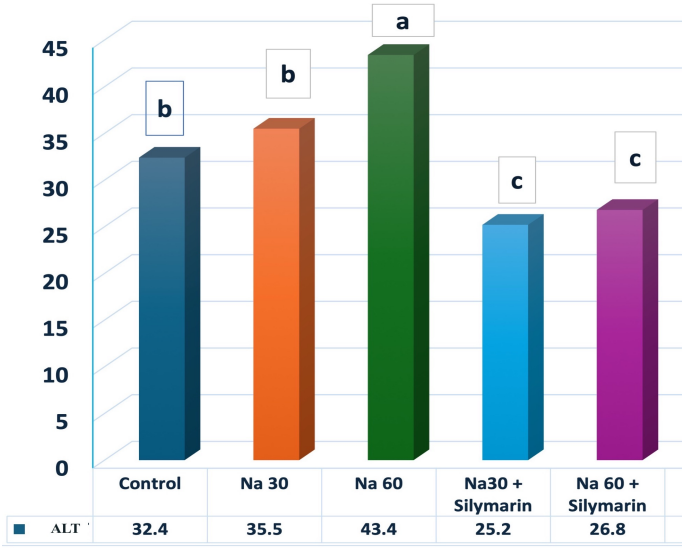

Fig. 5. Effect of treatments on serum ALT concentrations (U/mL). Different superscripts indicate significant differences

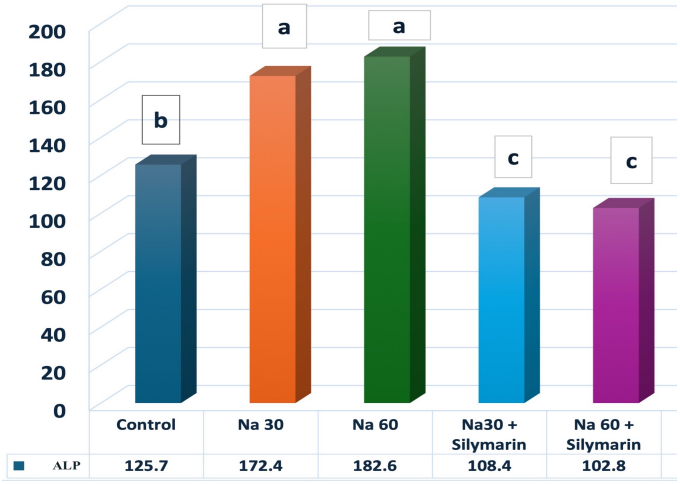

Fig. 6. Effect of treatments on serum ALP concentrations (U/mL). Different superscripts indicate significant differences

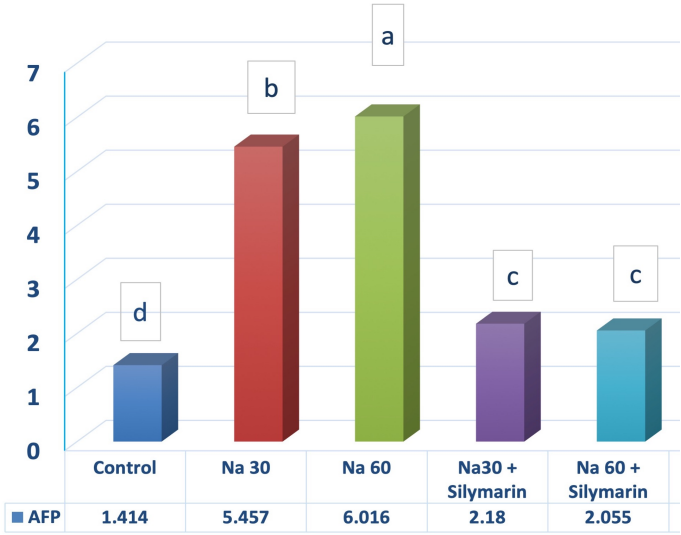

Fig. 7. Effect of treatments on serum AFP concentrations (ng/mL). Different superscripts indicate significant differences

3.3 Hepatic histopathology

The illustration of histopathological changes in the liver tissue in response to exposure to sodium nitrite and co-administration of silymarin is shown in Fig. 8. Hepatic cross-sections from control group revealed normal architecture, with intact hepatocytes with distinct nuclei and normal portal vein, well-organized hepatic cords, evenly distributed narrow sinusoids, and without any inflammatory lesions (Fig. 8A; Fig. 8B). In contrast, the hepatic cross-sections from sodium nitrite-exposed animals revealed dose-dependent histopathological changes. Administration of sodium nitrite at 30 mg/kg body weight resulted in moderate hepatocellular degeneration, dilated sinusoids, vascular congestion, and mononuclear inflammatory cell infiltration around the portal vein (Fig 8C; Fig. 8D). However, the lesions became more pronounced in animals administered sodium nitrite at 60 mg/kg body weight. The hepatocellular degeneration, mononuclear inflammatory cell infiltration, vascular congestion, dilation of sinusoids, and diffuse hepatocellular necrosis were more severe (Fig. 8E; Fig. 8F). Co-administration of silymarin resulted in a significant reduction in the severity of hepatic degeneration induced by sodium nitrite. Considerable preservation of normal hepatic architecture with mild vascular congestion and inflammatory cell infiltration was observed in animals exposed to sodium nitrite at 30 mg/kg body weight. However, silymarin co-administration in animals exposed to sodium nitrite at 60 mg/kg body weight resulted in a mild improvement of hepatic architecture, with reduced hepatic degeneration and lower inflammatory cell infiltration (Fig. 8G; Fig. 8H). Overall, the results revealed significant, dose-dependent sodium nitrite-induced hepatocellular degeneration and co-administration of silymarin markedly reduced the severity of the induced hepatic lesions.

4. Discussion

The results of the present study demonstrated dose-dependent sodium nitrite-induced hepatic oxidative stress and liver injury, as reflected by the alterations in antioxidant enzyme activities, serum hepatic biomarkers, histopathology. On the other hand, co-administration of silymarin partially restored these biochemical and structural changes, which suggests the hepatoprotective potential of silymarin against nitrite-induced toxicity. These results are particularly relevant to cattle, which are highly susceptible to nitrite poisoning because of rapid reduction of nitrate in rumen to toxic nitrite, which is responsible oxidative stress, generalized hypoxia, and multi-organ dysfunction (Oliynyk 2024; Reynolds and Drewnoski 2022; Abdelbagi et al. 2023). This study demonstrated significant reduction of serum SOD, CAT, and GPx activities in experimental rats exposed to sodium nitrite,

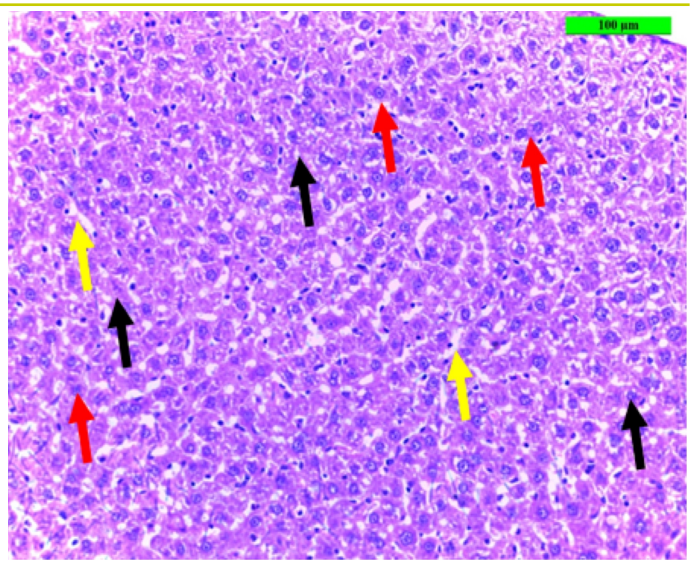

Fig. 8B. *The histological section of the control group displays normal hepatic lobular architecture; hepatocytes are arranged in an orderly fashion (black arrow), characterized by a polygonal shape and centrally located, rounded nuclei (red arrow). Hepatic sinusoids appear narrow and evenly distributed (yellow arrow). H & E 100X*

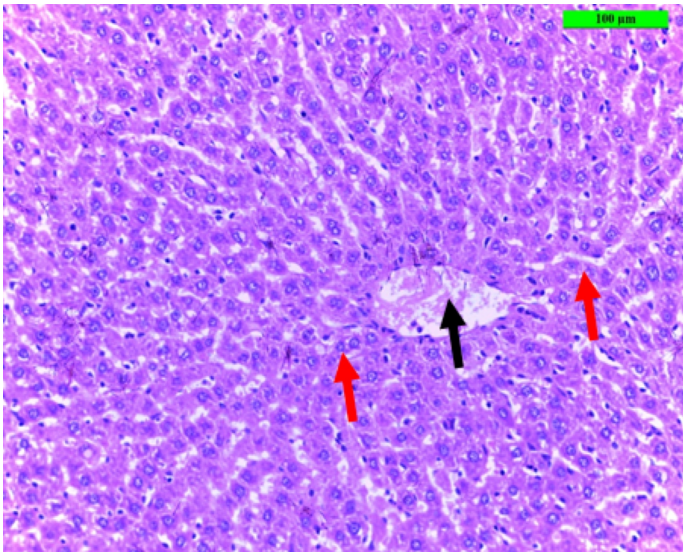

Fig. 8A. *The histological section from the control group shows an intact portal triad, including a normal portal vein (black arrow). The surrounding hepatocytes maintain a normal appearance without degeneration (red arrow). H & E 100X*

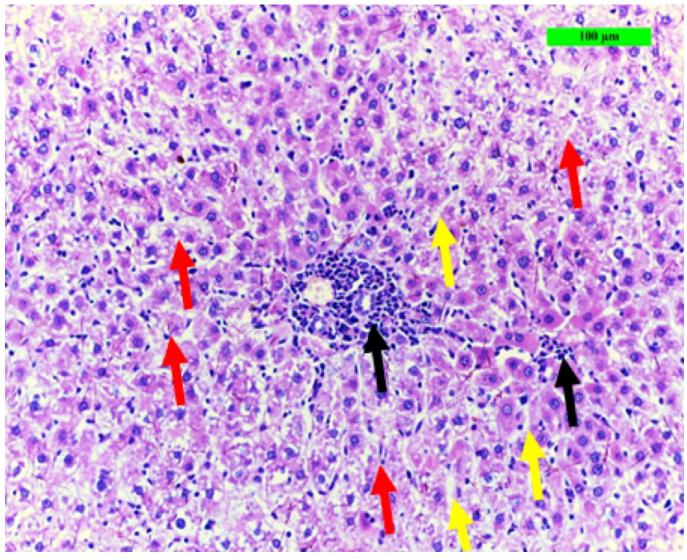

Fig. 8C. *The section from the low-dose sodium nitrite group shows hepatic parenchyma with a focal aggregation of mononuclear inflammatory cells within the lobule (black arrow). Surrounding hepatocytes exhibit mild cytoplasmic vacuolation (red arrow), and hepatic sinusoids appear slightly dilated (yellow arrow). H & E 100X*

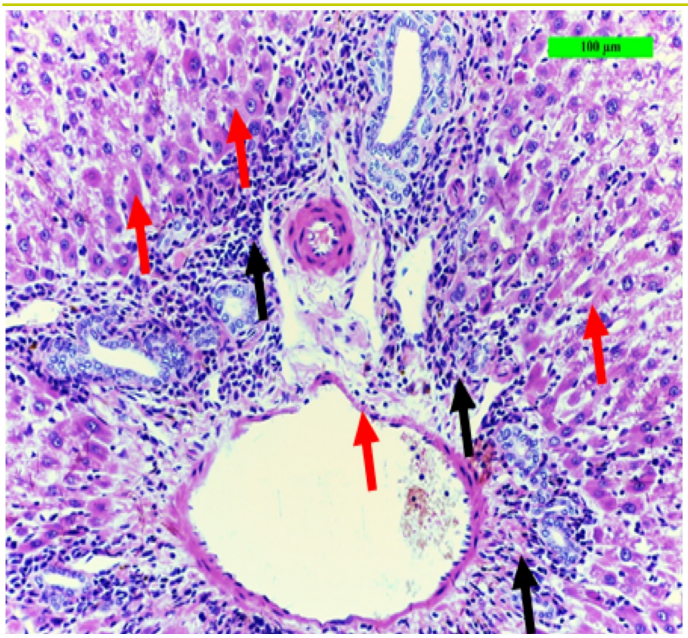

Fig. 8D. *The section from the low-dose sodium nitrite group shows the portal tract with marked mononuclear inflammatory cell infiltration surrounding the portal vein (black arrow). Mild hepatocellular vacillation is observed in the adjacent parenchyma (red arrow). H & E 100X*

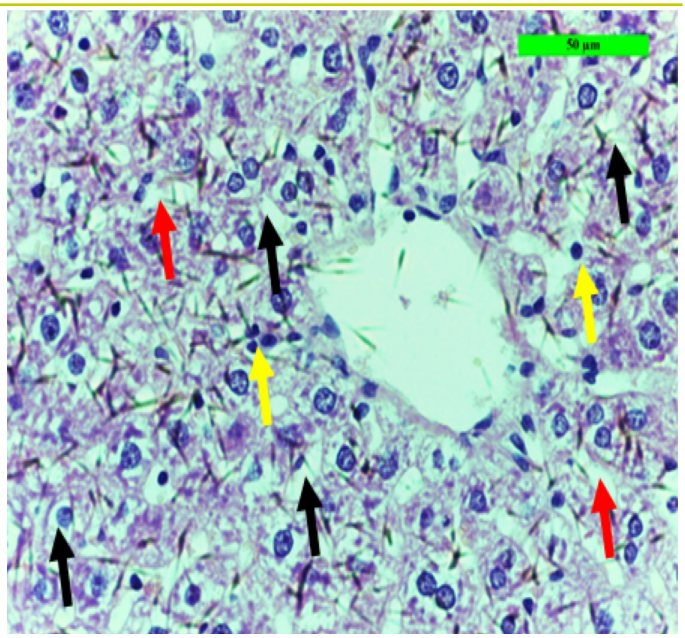

Fig. 8F. *A section from the sodium nitrite-treated group shows moderate hydropic degeneration in scattered hepatocytes. Pyknotic nuclei are observed in a few instances (black arrow), the hepatic sinusoids appear slightly dilated (red arrow), and inflammatory cell infiltration is noted (yellow arrow). H & E 100X*

suggesting oxidative stress as a primary underlying mechanism of nitrite toxicity. The exposure to sodium nitrite potentially causes excess production of ROS and RNS, which result in rapid depletion of cellular antioxidant enzyme pool. These enzymes are involved in maintaining intracellular redox homeostasis by mediating detoxification of free radicals, thereby preventing oxidative injury (Manirafasha et al. 2019; Handy et al. 2021; Chidambaram et al. 2024). Decline in SOD, CAT, and GPx activities have been reported earlier, which suggest that oxidative stress induced by nitrite overweighs the antioxidant defense system and causes hepatic oxidative injury (Shakil et al. 2022; Soliman et al. 2021; Khassaf 2024). Previous reports suggest that silymarin reduces oxidative injury and prevents depletion of cellular antioxidant enzyme pool by scavenging free radicals, maintaining mitochondrial integrity,

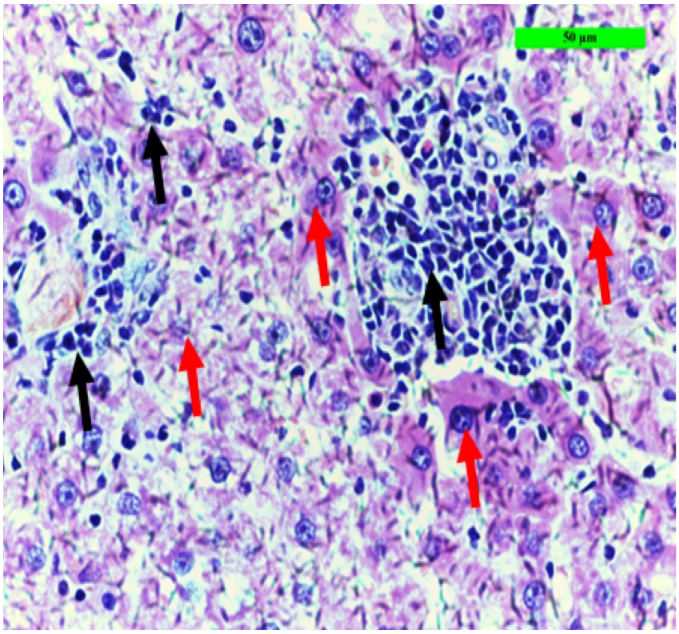

Fig. 8E. *The image shows the hepatic parenchyma of the group receiving the high-dose of sodium nitrite, displaying a dense focal aggregation of mononuclear inflammatory cells within the lobular region, forming an inflammatory infiltrate (black arrow), alongside mild degenerative changes (red arrow). H & E 100X*

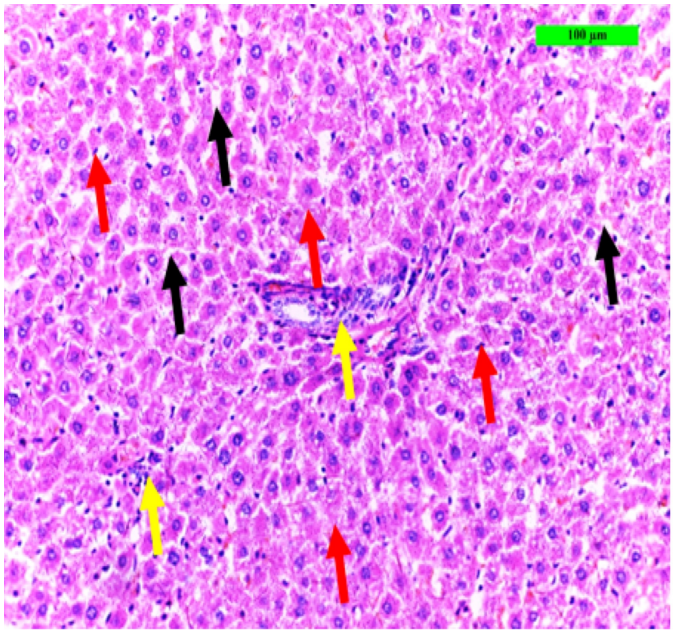

Fig. 8G. *In the group treated with a low dose of sodium nitrite and silymarin, moderate sinusoidal dilation is observed between the hepatic cell cords (black arrow). Some hepatocytes exhibit cytoplasmic swelling (red arrow), and mild inflammatory cell infiltration is also noted (yellow arrow). H & E 100X*

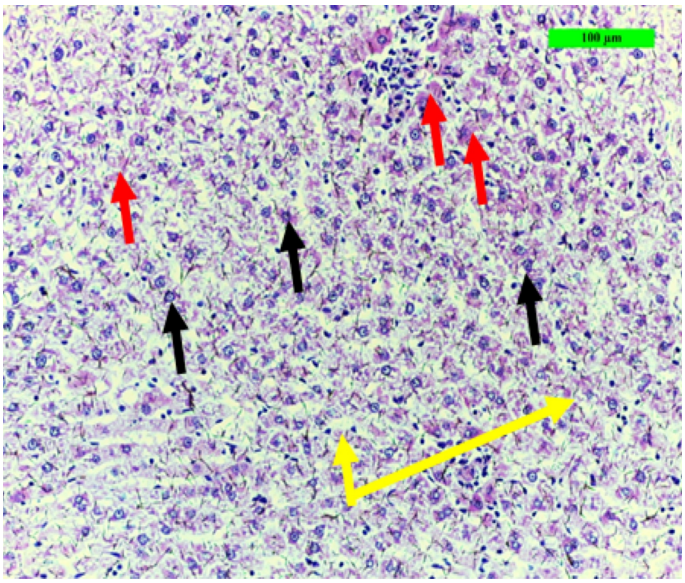

Fig. 8H. *In the group treated with the double dose of sodium nitrite and Silymarin, slight histological improvement is observed, although extensive hepatocellular injury persisted. Many hepatocytes exhibit nuclear pyknosis (black arrow). Cytoplasmic eosinophilia is increased in necrotic cells (red arrow), and the hepatic cords lose their linear arrangement (yellow arrow). H & E 100X*

enhancing intracellular antioxidant capacity, and stabilizing cellular membranes (Amien et al. 2015; Wadhwa et al. 2022). The present study demonstrated significant improvement in serum SOD and CAT activities in rats co-administered silymarin compared with their corresponding nitrite-treated rats; however, the enzyme activities could not be restored to the level observed in the control group. On the other hand, no significant improvement in GPx activity was observed upon silymarin co-administration in rats, indicating preferential preservation of SOD and CAT enzymatic pathways over glutathione-dependent antioxidant pathways.

In this study, serum hepatic function biomarkers – AST, ALT, ALP, and AFP demonstrated significant sodium nitrite-induced increase, indicating hepatocellular membrane injury and disruption of normal liver functions. AST and ALT are intracellular enzymes and nitrate toxicity induces their release into the circulation due to cellular damage and increased membrane permeability (Shakil et al. 2022). Similarly, the elevated levels of serum ALP are associated damage to hepatobiliary membranes. Earlier studies have reported elevated serum hepatic enzymes due to sodium nitrate-induced hepatotoxicity (El-Nabarawy et al. 2020; Soliman et al. 2021). In this study, co-administration of silymarin demonstrated no amelioration of elevated serum AST levels, whereas significant declines in serum ALT and ALP were observed, such that their levels were significantly lower than the control group. These observations indicate that the hepatoprotective effects of silymarin are more pronounced in terms of normalization of ALT and ALP levels, rather than AST levels. This differential effect could be attributed to the presence of AST in several extrahepatic tissues and continued higher levels indicate systemic tissue damage and slower recovery following nitrate toxicity. Hence, the present study suggests that silymarin markedly ameliorated the nitrite-induced hepatic degeneration; however, complete restoration of normal levels of all biochemical markers could not be achieved.

The present study revealed a dose-dependent significant increase in serum AFP levels, which was ameliorated by silymarin co-administration; however, the levels still remained above the control levels. Though AFP is traditionally recognized as an oncofetal protein, there is growing evidence supporting its use as an indicator of hepatocellular injury followed by regenerative activity (Chaudhary et al. 2023; Sorour et al. 2023). Elevated serum levels of AFP potentially indicate activation of regenerative as a response to hepatocellular injury induced by sodium nitrate rather than neoplastic transformation. Partial return of serum AFP towards normal level due to silymarin co-administration corresponds with the improved hepatic function biomarkers and histological parameters.

The present study revealed a dose-dependent sodium nitrite-induced hepatocellular degeneration, dilatation of sinusoids, infiltration of inflammatory cells, focal necrotic lesions, whereas co-administration of silymarin markedly reduced the severity of these hepatic lesions. The histopathological observations made in this study strongly correlate with the biochemical results. Earlier studies have reported such oxidative stress-induced hepatic lesions in different toxicological investigations (El-Nabarawy et al. 2020; Soliman et al. 2021; Khassaf 2024). The disruption of membrane integrity as a result of lipid peroxidation induced by oxidative stress results in compromised hepatocellular architecture and eventual cell death. Histopathological analysis in this study revealed substantial reduction in the severity of hepatic lesions in response to co-administration of silymarin, which is consistent with its antioxidant and membrane-stabilizing properties. However, it is worthy mentioning that although significant protection against nitrate-induced toxicity was conferred by silymarin, complete recovery from hepatic injury was not observed. The trend of hepatic histopathological recovery was closely paralleled by the restoration of antioxidant enzyme pool and hepatic function biomarkers, which establish the hepatoprotective role of silymarin.

The results of the present study have strong implications in veterinary medicine. In certain regions excess accumulation of nitrate in forage and drinking water makes cattle highly susceptible to nitrate poisoning because of rapid conversion of nitrate to nitrite in rumen, resulting in oxidative stress and methemoglobinemia (Oliynyk 2024; Reynolds and Drewnoski 2022; Abdelbagi et al. 2023). The rat model used in this study to investigate the sodium nitrite-induced oxidative stress and hepatocellular changes provides mechanistic insights into nitrate poisoning in ruminants. Hence, silymarin administration can be investigated as an antioxidant-based intervention for mitigating nitrate poisoning in livestock. However, future investigations should include additional oxidative stress biomarkers and molecular pathways for better understanding of mechanisms of action and standardization of the silymarin dose.

5. Conclusions

The results demonstrated that prolonged exposure of adult male Wistar albino rats to sodium nitrite caused dose-dependent hepatotoxicity. The hepatotoxicity was demonstrated by depletion of antioxidant enzyme pool (SOD, CAT, and GPx), elevation of serum hepatic biomarkers (AST, ALT, ALP, and AFP), and hepatocellular degeneration. Oxidative stress was identified as a major underlying mechanism of hepatic injury induced by nitrite poisoning. All these nitrite-induced adverse effects were ameliorated by silymarin co-administration to a considerable extent; however, complete recovery was not achieved. This study

highlights the hepatoprotective potential of silymarin against nitrite-induced oxidative hepatic degeneration in rat model. However, further investigations are required to establish silymarin as a potential antioxidant-based therapeutic strategy in nitrate poisoning in cattle with an expanded panel of oxidative stress biomarkers and standardization of its dose for complete recovery of hepatic damage.

Declarations

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References

Abdelbagi M, Ridwan R, Fitri A, Nahrowi, Jayanegara A. (2023). Performance, methane emission, nutrient utilization, and the nitrate toxicity of ruminants with dietary nitrate addition: A meta-analysis from in vivo trials. Tropical Animal Science Journal 46(1): 74–84. https://doi.org/10.5398/tasj.2023.46.1.74

Amien AI, Fahmy SR, Abd-Elgleel FM, Elaskalany SM. (2015). Renoprotective effect of *Mangifera indica* polysaccharides and silymarin against cyclophosphamide toxicity in rats. The Journal of Basic & Applied Zoology 72: 154–162. https://doi.org/10.1016/j.jobaz.2015.09.006

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D, Sharifi-Rad J. (2023). Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Frontiers in Chemistry 11: 1158198. https://doi.org/10.3389/fchem.2023.1158198

Chidambaram SB, Anand N, Varma SR, Ramamurthy S, Vichitra C, Sharma A, Mahalakshmi AM, Essa MM. (2024). Superoxide dismutase and neurological disorders. IBRO Neuroscience Reports 16: 373–394. https://doi.org/10.1016/j.ibneur.2023.11.007

Drury RA, Wallington EA. (1980). Carleton's Histological Technique. 4th ed. Oxford University Press, New York.

El-Nabarawy NA, Gouda AS, Khattab MA, Rashed LA. (2020). Effects of nitrite graded doses on hepatotoxicity and nephrotoxicity, histopathological alterations, and activation of apoptosis in adult rats. Environmental Science and Pollution Research 27(12): 14019–14032. https://doi.org/10.1007/s11356-020-07901-6

Handy DE, Joseph J, Loscalzo J. (2021). Selenium, a micronutrient that modulates cardiovascular health via redox enzymology. Nutrients 13(9): 3238. https://doi.org/10.3390/nu13093238

Hassan HA, Ahmed HS, Hassan DF. (2024). Free radicals and oxidative stress: Mechanisms and therapeutic targets. Human Antibodies 32(4): 151–167. https://doi.org/10.3233/HAB-240011

Khassaf HK. (2024). Sodium nitrite effects on some blood and biochemical parameters in glutathione treated male rats. Basrah Journal of Veterinary Research 23(1): 90–96. https://doi.org/10.23975/bjvr.2024.183016

Manirafasha C, Oyenihi OR, Brooks NL, du Plessis SS, Aboua YG. (2019). Potential antioxidative effects of kolaviron on reproductive function in streptozotocin-induced diabetic Wistar rats. In: Shalaby E, editor, Antioxidants. IntechOpen. https://doi.org/10.5772/intechopen.84822

Merinas-Amo T, Merinas-Amo R, Márquez Prados L, Font R, Celestino MDR, Alonso-Moraga Á. (2025). Bioassays to assess the safety of potassium and sodium nitrates and nitrites. Processes 13(2): 325. https://doi.org/10.3390/pr13020325

Oliynyk IY. (2024). From Chilean saltpeter to modern agriculture: navigating nitrate toxicity in ruminants through compartmental modeling. The Animal Biology 26(2): 11–18. https://doi.org/10.15407/animbiol26.02.011

Reynolds JG, Britton MD, Belsher JD. (2021). A review of sodium nitrite solubility in water and physical properties of the saturated solutions. Journal of Chemical & Engineering Data 66(8): 2931–2941. https://doi.org/10.1021/acs.jced.1c00175

Reynolds MB, Drewnoski ME. (2022). Is it time to rethink our one-size-fits-all approach to nitrate toxicity thresholds in forages? Translational Animal Science 6(1): txac023. https://doi.org/10.1093/tas/txac023

Shakil MH, Trisha AT, Rahman M, Talukdar S, Kobun R, Huda N, Zzaman W. (2022). Nitrites in cured meats, health risk issues, alternatives to nitrites: A review. Foods 11(21): 3355. https://doi.org/10.3390/foods11213355

Soliman MM, Aldhahrani A, Metwally MM. (2021). Hepatoprotective effect of *Thymus vulgaris* extract on sodium nitrite-induced changes in oxidative stress, antioxidant and inflammatory marker expression. Scientific Reports 11(1): 5747. https://doi.org/10.1038/s41598-021-85264-9

Sorour MA, Mehanni AHE, Mahmoud ESA, El-Hawashy RM. (2023). Nitrate, nitrite and N-nitrosamine in meat products. Journal of Sohag Agriscience 8(1): 121–135. https://doi.org/10.21608/jsasj.2023.316218

Taus F, Pigaiani N, Bortolotti F, Mazzoleni G, Brevi M, Tagliaro F, Gottardo R. (2021). Direct and specific analysis of nitrite and nitrate in biological and non-biological samples by capillary ion analysis for the rapid identification of fatal intoxications with sodium nitrite. Forensic Science International 325: 110855. https://doi.org/10.1016/j.forsciint.2021.110855

Wadhwa K, Pahwa R, Kumar M, Kumar S, Sharma PC, Singh G, Verma R, Mittal V, Singh I, Kaushik D, Jeandet P. (2022). Mechanistic insights into the pharmacological significance of silymarin. Molecules 27(16): 5327. https://doi.org/10.3390/molecules27165327

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Al-Kraie NIH, Khabbaz SN, Dalis NS, Obiadmm RM, Dalas IS. (2026). Sodium nitrite-induced hepatotoxicity in male Wistar rats and its attenuation by silymarin: Biochemical, oxidative stress, and histopathological evidence. Letters in Animal Biology 06(1): 138 – 145. *https://doi.org/10.62310/liab.v6i1.410*