

Histopathological Study of Kidney and Liver of Rats after Induction of Poisoning by Nitrate

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Abstract: The purpose of this research is to investigate the effects of nitrate as a calcium appearance on the hepatic and renal histology. Iraqi fertilizer firm in supplied the calcium nitrate tetrahydrate was used in this study. Twenty-four male albino wistar rats weighing 220 ± 25 g were kept under conditions of natural photoperiod, humidity $50 \pm 10\%$, and temperature $25 \pm 2^\circ$ C. These were divided into 3 groups: G1 control group, G2 received 200 mg/kg, G3 received 400 mg/kg. Rats were administered calcium nitrates orally for thirty days after the salts were dissolved in mineral water. Animals were then sacrificed by being beheaded in the neck region. Organs such as the kidneys and liver were taken for histological analysis. Liver and kidney samples were taken from rats that were given varying amounts of calcium nitrate for a duration of 30 days. The liver sections showed regular histological architecture with hexagonal lobules and visible capillary sinusoids. The livers of the 200 mg/kg group showed mild histological damage, while the 400 mg/kg doses showed significant vascular dilatation, congestion, hepatocyte degeneration, bleeding, and lipid vacuolation. The kidneys showed severe histopathological changes, including glomerular atrophy, intra-glomerular hemorrhage, and vascular congestion, compared to the G2 which showed mild renal lesions. In conclusion, nitrate induced significant alterations in the kidneys and liver architecture that were dose-dependent. Finally, greater dosages of calcium nitrate used excessively might be harmful to humans and other mammals.

Keywords: Liver, Kidney, Histopathology, Nitrate.

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INTRODUCTION

The use of synthetic fertilizers is prevalent in agriculture due to their affordability and the fact that they quickly and easily deliver plant nutrients. However, there are concerns about the potential negative effects on human and environmental health caused by contamination of soil, air, and water [1]. The control of plant growth and development is one of several biological activities that rely on calcium, an important macronutrient [2]. An increase in fruit calcium content is possible with the most common forms of calcium fertilisers, as stated in [3]. These include calcium nitrate as well as calcium chloride.

According to [4], crops that are experiencing fast development, those with a high calcium need, and those in fertigation systems may be supplied with calcium nitrate rapidly. If plants are unable to absorb

nitrates, a result of nitrogen fertilisation, the chemicals might be washed into water streams. Notably, the nitrate content is higher in green, leafy vegetables like lettuce. Agricultural soil management practices are associated with 60% of total nitrous oxide emissions [5].

Methemoglobinemia, GIT, liver as well as lungs cancers [6], and non-Hodgkin's lymphoma are among the health problems that can develop in humans after consuming nitrates or nitrite in water and food. Moreover, nitrate reductase enzymes are produced by the bacterial flora of the mouth. As a result, about 25% of the nitrate that is consumed might be converted to NO₂ in the mouth. Based on the gastric content and redox environment, nitrous acid may be converted into other nitrogen oxides, such as nitrogen dioxide (NO₂) and dinitrogen trioxide (N₂O₃), due to the acidic pH of the stomach.

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After absorption in the colon, nitrate undergoes enterosalivary circulation, where it is converted to nitrite in the mouth and then the cycle resumes [7]. Notably, nitrate is formed when the body metabolizes nitrosamine and nitric oxide; this compound is known to be a highly carcinogenic agent that may cause cancers of the stomach, liver, and esophagus [8]. Animals have been the subject of several investigations on the impact of nitrate-induced changes on biochemical markers of the liver and kidney [9]. Consequently, the purpose of this research is to investigate the effects of nitrate as a calcium appearance on the hepatic and renal histology.

MATERIALS AND METHODS

Chemical Material: Iraqi fertilizer firm in supplied the calcium nitrate was used in this study.

Animals:

In plastic cages, twenty-four male albino wistar rats weighing 220 ± 25 g were kept under conditions of natural photoperiod, humidity $50 \pm 10\%$, and temperature $25 \pm 2^\circ$ C. The animals were given pellets and water from the faucet on an as-needed basis. These were divided into 3 groups:

G1 control group, G2 received 200 mg/kg, G3 received 400 mg/kg.

Rats were administered calcium nitrates orally for thirty days after the salts were dissolved in mineral water. Animals were then sacrificed by being beheaded in the neck region. Organs such as the kidneys and liver were taken for histological analysis.

The experimental rats' liver and kidney were taken out, cleaned with PBS, then fixed in a Bouin's solution for a whole day, and then embedded in paraffin wax. After that, the tissue sections were divided into 5 μ m-thick slices using a rotary microtome, and they were stained with hematoxylin and eosin [10]. After being dried, the preparations were examined under an optical microscope.

RESULTS

Sections of the liver and kidneys of rats that were given dosages of 200, 400, and mg/kg of calcium carbonate for 30 days showed clear histological changes. There were clear capillary sinusoids and hexagonal lobules in the liver's normal histological architecture, as seen in the section (Figs. 1).

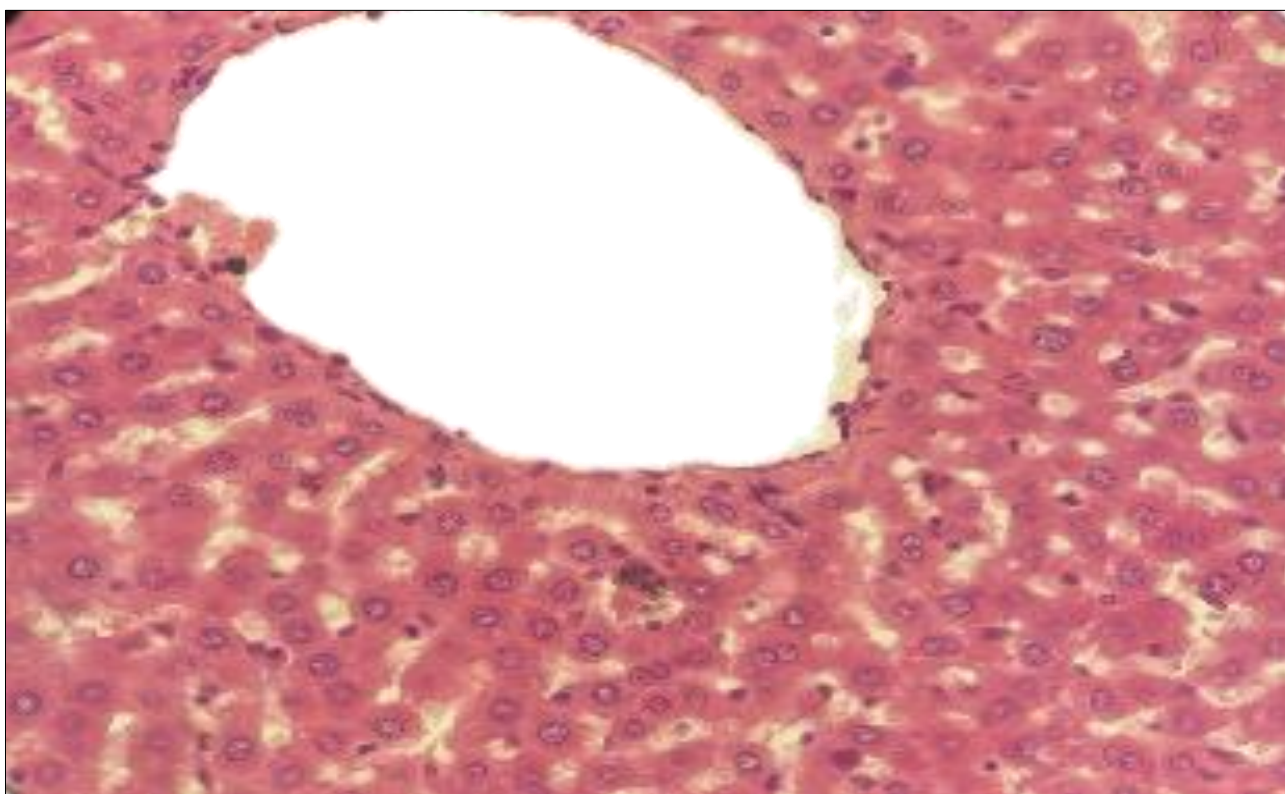


Figure 1: Photomicrograph of liver from rat of control group shows normal tissue architecture. H&E, X10.

When comparing the livers of the G2 to the control group, mild histological damage was discovered (Fig. 2). However, the 400 mg/kg higher doses (G3) showed mild inflammations, hepatocyte degradation,

haemorrhage, vascular dilatation and congestion, and lipid vacuolation in comparison to the control group (Fig. 3).

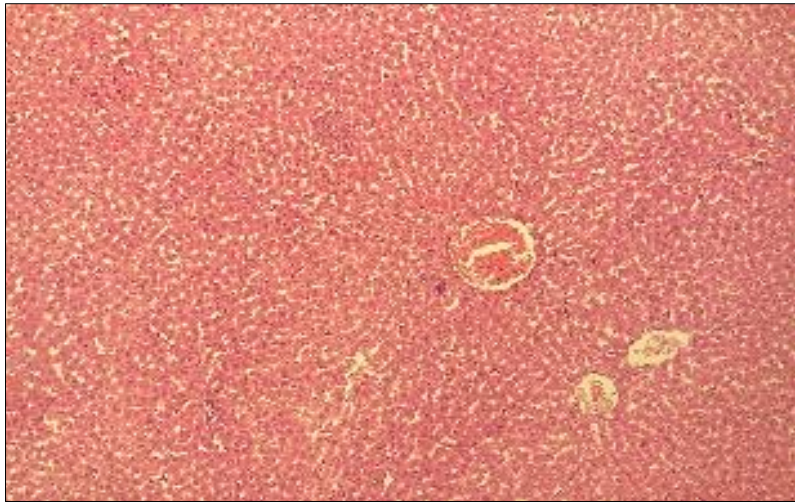


Figure 2: Photomicrograph of liver from rat of 200 mg/kg shows mild blood vessels congestion, fatty changes and inflammatory cells infiltrating. H&E, X10.

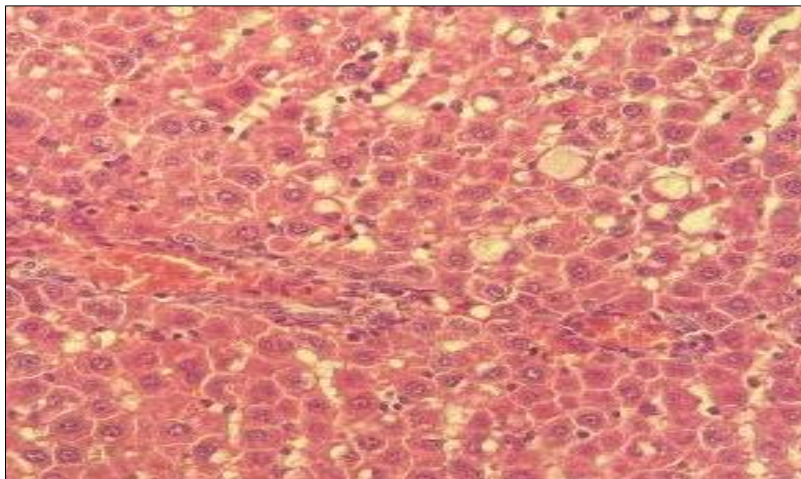


Figure 3: Photomicrograph of liver from rat of 400 mg/kg shows blood vessels congestion, fatty changes and inflammatory cells infiltrating (red arrow). H&E, X40.

Moreover, microscopic examinations of the renal tissues in rats treated with nitrate revealed severe histopathological alterations, such as glomerular atrophy, Bowman's space dilation, haemorrhage of intraglomerulari, as well as vascular congestion (Fig. 6), in

contrast to G2, which showed mild lesions (Fig. 5). This was in contrast to the kidneys of control rats, which showed normal renal parenchyma with clearly defined renal glomeruli and tubules (Fig. 4).

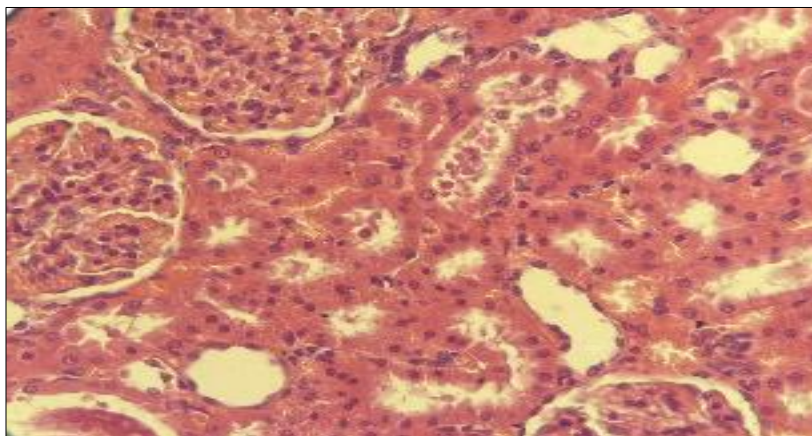


Figure 4: Photomicrograph of kidney from rat of control group shows normal tissue architecture. H&E, BX40.

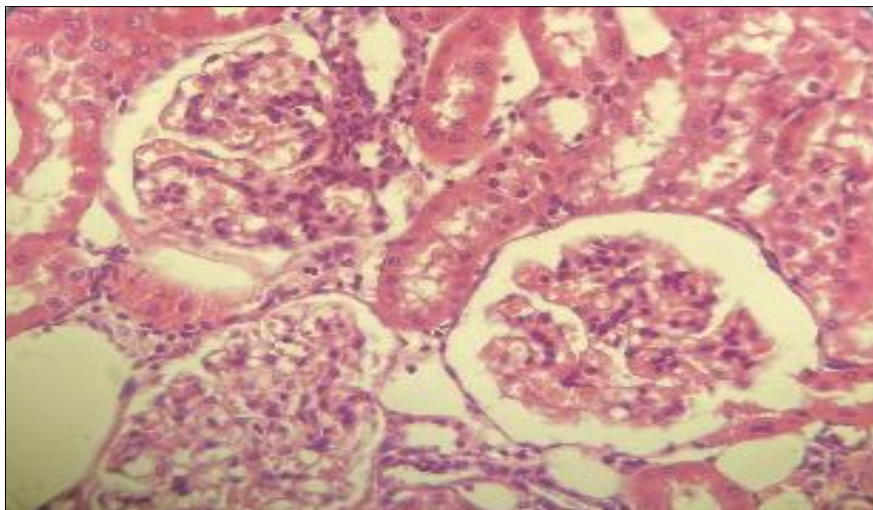


Figure 5: Photomicrograph of kidney from rat G2 shows mild glomerular atrophy and vacuolation. H&E, X40.

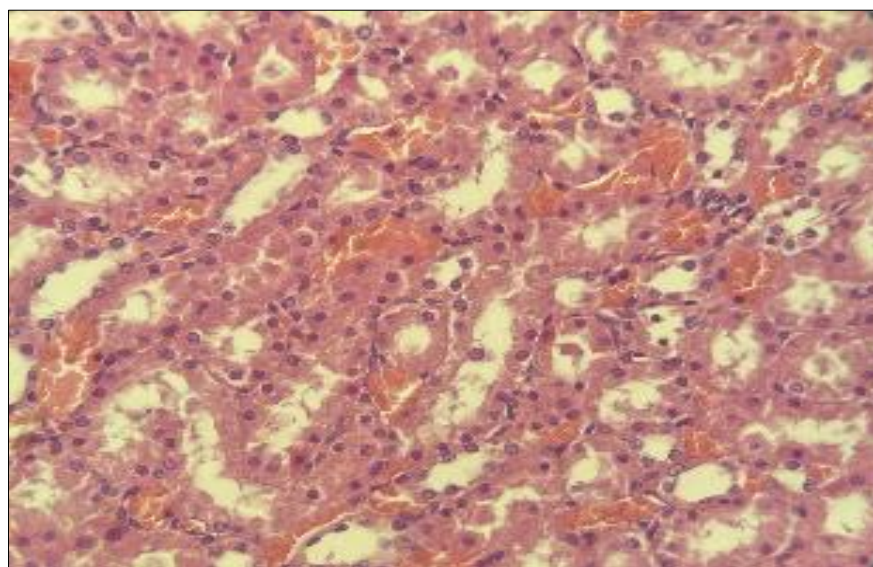


Figure 6: Photomicrograph of kidney from rat of toxin group shows blood vessels congestion and hemorrhage (black arrow) Mesangial cell hyperplasia and inflammatory cells infiltrating (red arrow). H&E, X40.

DISCUSSION

Numerous health issues for people are caused by the increased use of synthetic fertilisers, particularly nitrate fertilisers [11]. The processes of food absorption, digestion, and metabolic utilisation can all be impacted by the chemical makeup of fertilisers [12]. Groundwater may get polluted with nitrates from fertilisers used on soil or animal dung, which can harm people and other animals [13].

Ca nitrate induces histological alterations in the liver, as shown by the present research. These alterations include hepatocyte cytoplasmic vacuolation, mononuclear cell infiltration, venous congestion, and fatty degeneration. Similar histological changes were documented in a few earlier studies [14-16]. The administration of calcium nitrate treatment caused the renal tubules to dilate, the glomeruli to atrophy, and leukocytes to infiltrate. Anwar and Mohamed [17], observed that rats given 500 mg/L NaNO₃ for different

periods (3, 4 and 6 weeks) experienced renal blood vessel congestion and glomerular shrinkage in their kidneys.

Rats given NaNO₃ showed a necrosis of the cells and mononuclear cell infiltration in the portal canals, especially in the lobules of the liver. These alterations may be the consequence of nitrate-induced changes in membrane dispersion. The earlier research by Ogur *et al.*, [18], revealed comparable alterations in the adult rats' liver tissue. Indeed, hepatic degeneration and cell necrosis, ranging from increased intercellular space to hydropic degeneration, were seen in the livers of rats treated with nitrate.

Furthermore, glomerular tuft atrophy and renal blood vessel congestion were seen upon microscopic inspection of renal tissue in the group treated with nitrate (Anwar and Mohamed, 2015).

CONCLUSION

Nitrate induced significant alterations in the kidneys and liver architecture that were dose-dependent. Finally, greater dosages of calcium nitrate used excessively might be harmful to humans and other mammals.

REFERENCES

- Ahmed, M., Rauf, M., Mukhtar, Z., & Saeed, N. A. (2017). Excessive use of nitrogenous fertilizers: an unawareness causing serious threats to environment and human health. *Environmental Science and Pollution Research*, 24, 26983-26987.
- Meriño-Gergichevich, C., Alberdi, M., Ivanov, A. G., & Reyes-Díaz, M. (2010). Al³⁺-Ca²⁺ interaction in plants growing in acid soils: al-phytotoxicity response to calcareous amendments. *Journal of soil science and plant nutrition*, 10(3), 217-243.
- Lanauskas, J., Kviklienė, N., Uselis, N., Kviklys, D., Buskienė, L., & Staugaitis, R. M. (2012). The effect of calcium foliar fertilizers on cv. Ligol apples.
- Martínez Martínez, L., Velasco Velasco, V. A., Ruiz Luna, J., Enríquez-del Valle, J. R., Campos Ángeles, G. V., & Montañó Lugo, M. L. (2013). Effect of calcium nitrate and substrates on tomato yield. *Revista mexicana de ciencias agrícolas*, 4(spe6), 1175-1184.
- Vitale, L., Polimeno, F., Ottaiano, L., Maglione, G., Tedeschi, A., Mori, M., De Marco, A., Di Tommasi, P., & Magliulo, V. (2017). Fertilizer type influences tomatoyield and soil N₂O emissions. *Plant Soil Environ*, 63, 105-110. <https://doi.org/10.17221/678/2016-PSE>.
- Espejo-Herrera, N., Cantor, K. P., Malats, N., Silverman, D. T., Tardón, A., García-Closas, R., ... & Villanueva, C. M. (2015). Nitrate in drinking water and bladder cancer risk in Spain. *Environmental research*, 137, 299-307.
- Pereira, C., Ferreira, N. R., Rocha, B. S., Barbosa, R. M., & Laranjinha, J. (2013). The redox interplay between nitrite and nitric oxide: From the gut to the brain. *Redox biology*, 1(1), 276-284.
- Kim, H. J., Chang, W. K., Kim, M. K., Lee, S. S., & Choi, B. Y. (2002). Dietary factors and gastric cancer in Korea: A case-control study. *International journal of cancer*, 97(4), 531-535.
- Azzez, O. H., Mahmood, M. B., & Hassan. (2011). Effect of nitrate poisoning on some biochemical parameters in rats. *Iraqi J Vet Sci*, 25(2), 47-50.
- Hould, R. (1984). Techniques d'histopathologie et de cytopathologie. *Ed Maloine*, 19(21), 225-227.
- WHO. (2006). World Health Organization, Nitrate and potential endogenous formation of N-nitrosocompounds Food Additives Series: no 50 Who. Geneva, P1-9 24. Kostogrys, R. B Pisulewski.
- Organization for Economic Cooperation and Development (OECD). 2000. Guidance Notes for the Analysis and Evaluation of Repeat Dose Toxicity Studies. Environment, Health and Safety Publications Series on Testing and Assessment, Environment Directorate, OECD, Paris, 27-72.
- Lockhart, K. M., King, A. M., & Harter, T. (2013). Identifying sources of groundwater nitrate contamination in a large alluvial groundwater basin with highly diversified intensive agricultural production. *Journal of contaminant hydrology*, 151, 140-154.
- Kattaia, A. A., Abd El-Baset, S. A., Mohamed, E. M., Abdul-Maksou, R. S., & Elfakharany, Y. M. (2017). Molecular mechanisms underlying histological and biochemical changes induced by nitrate in rat liver and the efficacy of S-Allylcysteine. *Ultrastructural Pathology*, 41(1), 10-22.
- Delgado, M. G., Zamora, A. G., Gonsebatt, M. E., Mata, E. M., Vargas, G. G., Rincón, E. C., & Morales, R. P. (2018). Subacute intoxication with sodium nitrate induces hematological and biochemical alterations and liver injury in male Wistar rats. *Ecotoxicology and Environmental Safety*, 166, 48-55.
- Ikele, C. B., Okwuonu, E., & Ijem, A. N. (2021). Further studies on evaluation of the toxicity potential of Terminalia catappa Lin. Combretaceae leaf extract: effects on the histology, liver enzymes, and haematology profile of albino rats. *Jordan Journal of Biological Sciences*, 14(5).
- Ogur, R., Coskun, O., Korkmaz, A., Oter, S., Yaren, H., & Hasde, M. (2005). High nitrate intake impairs liver functions and morphology in rats; protective effects of α -tocopherol. *Environmental toxicology and Pharmacology*, 20(1), 161-166.
- Anwar, M. M., & Mohamed, N. E. (2015). Amelioration of liver and kidney functions disorders induced by sodium nitrate in rats using wheat germ oil. *Journal of Radiation Research and Applied Sciences*, 8(1), 77-83.