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In Vivo Experimental Study on Calcium Nitrate-Induced Oxidative Liver and Kidney Damage in Wistar Albinos Rats

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AS and SS performed and faciliatated the animal experiment, analyzed results and discussion of results. KF conceived the idea of study, supervised the research and manuscript. AS wrote the manuscript. All authors read and approved the final manuscript

Key words
Calcium nitrate, Renal oxidative stress, Wistar rats, Glutathione, Superoxide dismutase, Lipid peroxidation, Hepatic oxidative stress

ABSTRACT

Calcium nitrate is a common fertilizer in agriculture, however, the toxic effects limit its uses. This study, therefore, aimed to evaluate the harmful impact of calcium nitrate on oxidative stress markers of liver and kidney function in rats. Twenty-eight male Wistar albino rats were divided into a control group that received distilled water, and three treated groups received 200, 400, or 800 mg/kg body weight of calcium nitrate for 30 days. Liver and kidney specimens were obtained from rats of different groups immediately after sacrifice, stored at -20°C in the freezer for the subsequent preparation of the homogenate which was used for determination of oxidative stress parameters. Results revealed a significant dose-dependent increase in malondialdehyde content, a decrease in glutathione content, enzymatic activity of catalase activity, and glutathione peroxidase in liver and kidney homogenates. Conclusively, calcium nitrate caused the induction of liver and kidney oxidative injury in a dose-dependent manner, where the higher effects were noticed with the highest doses.

INTRODUCTION

Synthetic fertilizers are widely used in agriculture as a rapid and inexpensive source of nutrients for plants, leading to a significant increase in crop productivity (Ahmad *et al*., 2017). Nitrogen fertilizer application is one of the most important land management practices for improving crop and pasture productivity (Mazzetto *et al*., 2020). Calcium nitrate, as a nitrogen source (Goyal, 2012), is considered the best form of nitrogen fertilizer due to its effective synergy with K, Mg, and Ca, explained by a concomitant uptake of cations and anions without antagonism, in addition to its beneficial effect in the plant rhizosphere (Dogbatse *et al*., 2024). Moreover, only one-third to one-half of the minerals applied as fertilizers are effectively absorbed by plants, but half or two-thirds of the remaining parts result in soil, water, and air pollution, causing considerable environmental and human health concerns (Sheahan *et al*., 2017; Elahi *et al*., 2019; Li *et al*., 2019). Additionally, studies investigating the toxic effects of repeated-low doses of inorganic fertilizers contaminated drinking water on albino rats have reported induction of serious health conditions, including anemia, leukocytosis, and liver and kidney impairments (Samia *et al*., 2022; 2023), leukopenia, and reproductive alteration (Ihedioha and Idika, 2007a, b). Nitrates, the end product of nitrogen fertilization, can cause considerable water contamination when it is not promptly absorbed by plant roots. Leafy green vegetables, such as lettuce, exhibit the highest nitrate levels (Liu *et al*., 2014). Nitrates serve as precursors to nitric oxide (NO), a molecule involved in various physiological functions, including neurotransmission (Garthwaite, 1991), immune regulation (Hibbs, 1991), smooth muscle vascular relaxation (Moncada *et al*., 1986), and inhibition of platelet aggregation (Radomski *et al*., 1987). However, nitrates can also impact mitochondrial functionality, increasing the production of reactive oxygen species (ROS) by mitochondrial complex III and other reactive molecules, such as superoxide anion (O^{2}•-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), and singlet oxygen ($1O^2$) (Akopova *et al*., 2016). As a result, cells possess an antioxidant defense system that can effectively

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alleviate oxidative stress leading to lipid peroxidation, protein carbonylation, amino acid modification, DNA damage, and protein S-nitrosylation (Kurutas, 2016). As reported, ROS can induce an antioxidant response as a defense mechanism, including increased glutathione (GSH) levels and overexpression of detoxification enzymes, such as superoxide dismutase (SOD) isoforms, catalase, thioredoxin, glutathione peroxidase, and glutathione transferase (He *et al*., 2017). Also, ROS can damage hepatocytes by inducing inflammation, fibrosis, necrosis, apoptosis, or even malignant transformation (Muntané *et al*., 2013). Furthermore, Nitric oxide (NO), a signaling molecule with diverse physiological functions, can also pose health risks under certain conditions. NO can react with oxyhemoglobin (HbO_2) to form methemoglobin (metHb), a form of hemoglobin that cannot transport oxygen effectively. Also, NO can react with secondary amines to produce N-nitroso compounds, some of which are known carcinogens (Volkmer *et al*., 2005). These N-nitroso compounds can also be hepatotoxic, causing liver fibrosis and tumors (Erkekoglu and Baydar, 2010). Thus, exposure to environmental pollutants can increase the risk of kidney disease (Calvert, 2016), through the accumulation of toxins in the renal tubules, resulting subsequently in the dysfunction or failure of the kidney (Al-Attar *et al*., 2017). In this regard, the present study aimed to investigate the impact of various doses of a commercial calcium nitrate named "CALNISOL®" on renal and hepatic oxidative stress markers in albino rats over a 30 days exposure period.

MATERIALS AND METHODS

Chemical materials

Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$) commercialized under the name Calnisol® (CAS No.: 10124-37-5) was purchased from Profert company, a fertilizer company in Bejaia, Algeria.

Animals

Twenty-eight male Wistar rats weighing 240±20 g, and obtained from the Pasteur Institute, Algiers, Algeria were housed in the animal house of our institution under standard laboratory conditions (T° 25±2°C, humidity 50±10%, and 12h/12h light/dark), and had free access to food and water before and during the experiment.

Experimental procedures

After three weeks of acclimatization, rats were divided into four groups of seven rats each. The first group was the control group received water as a vehicle, and the second, third, and fourth groups received, respectively via oral gavage 200, 400, and 800 mg of calcium nitrate/kg body weight for 30 days (Samia *et al*., 2023). At the end of the experimental period, the animals were fasted overnight and sacrificed by cervical decapitation. The liver and kidneys were removed and stored at -20°C for subsequent determination of oxidative stress parameters.

Determination of oxidative stress markers

For assessment of oxidative stress parameters one gram of liver and kidney tissues of control and treated rats were homogenized in 10 ml of phosphate-buffered saline (PBS: Tris 50 mM, NaCl 150 mM, pH 7.4), centrifuged at 9000 g, 4°C for 15 min, and the supernatant was aliquoted into Eppendorf tubes and stored at -20°C until use.

Malondialdehyde (MDA), a lipid peroxidation marker, reduced glutathione (GSH) content, and protein concentration were determined by spectrophotometry using the methods of Esterbauer *et al*. (1992) and Bradford (1976), respectively. Catalase (CAT) activity was estimated by the method of Aebi (1984), while glutathione peroxidase (GPx) activity was determined by the method of Flohé and Günzler (1984).

Statistical analysis

The results are provided as mean ± standard deviation (mean ± SD). Comparisons between groups were performed using one-way analysis of variance (ANOVA), using SPSS software (version 25).

RESULTS

Table I shows calcium nitrate-induced body weight changes. The final body weight and the body weight gain significantly ($p<0,01$) increased in calcium nitrate at 800 mg /kg bwt treated rats as compared to the control group.

Figure 1 shows calcium nitrate-induced changes in oxidative markers. Calcium nitrate at a dose of 800mg/kg significantly decreased the hepatic and renal GSH content, but not significantly at doses of 200 and 400 mg/kg as compared to the control group (Fig. 1A). Further, the MDA level in kidney and liver tissues increased significantly ($p<0.05$), highly significantly ($p<0.01$), and non-significantly respectively in treatment with calcium nitrate at doses of 400, 800, and 200 mg/kg compared to the control group (Fig. 1B). Treatment of rats with calcium nitrate at a dose of 800 mg/kg significantly decreased GPx activity in the liver and kidneys compared to control rats (Fig. 1C). Similarly, Figure 1D showed that the catalase activity decreased highly significantly ($p < 0.01$) in the liver tissue of rats treated with calcium nitrate at 800mg/kg bwt (G4), significantly ($p < 0.05$) in the kidney tissue, and non-significantly in rats treated with calcium nitrate at 200 mg/kg.

Table I. Changes in body weight of control, and treated animals with calcium nitrate at doses of 200, 400 and 800 mg/kg for 30 days.

Parameters	Treatments			
	Control	200 mg/kg	400 mg/kg	800 mg/kg
Initial body weight (g)	256.14±6.28	257.14±4.52	257.29±3.95	256.86±5.55
Final body weight (g)	289.43±6.24	389.29±3.35	291.86±5.58	300.14±3.67
Weight gain (%)	33.29±10.06	32.14±5.34	34.57±3.50	43.29±6.73

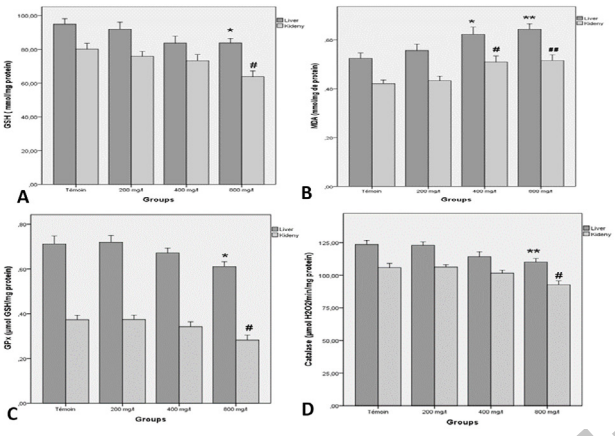

Fig. 1. Changes in hepatic and renal glutathione content (A), malondialdehyde content (B), glutathione peroxidase activity (C) and catalase activity (D) in control and rats treated with calcium nitrate at doses of 200, 400 and 800 mg/kg for 30 days.

DISCUSSION

The organism possesses a potential antioxidant defense, including enzymatic (e.g CAT, SOD, GPx, and GST), and non-enzymatic like GSH molecules acting effectively against the oxidative stress-mediated production of ROS (Halliwell, 1994; Banerjee *et al*., 2001). This study demonstrated that the treatment of rats with calcium nitrate induced a significant increase in body weight. This result contradicts the findings of Rouag *et al*. (2020), who reported a decrease in body weight following exposure of rats to sodium nitrate. In contrast, the work of Messaadia *et al*. (2013) showed no significant changes in the physiological growth of rats treated with ammonium nitrate compared to the control group. Similarly, Gueroui and Kechrid (2016) reported no significant change in body weight in rats exposed to silver nitrate-contaminated drinking water at a concentration of 20 mg/L for 3 months. This is likely due to the accumulation of this pollutant in the adipose tissues, leading consequently to increased protein catabolism to ensure sufficient energy during the detoxification process (Al-Ayed, 2000; Nujić and Habuda-Stanić, 2017). Our results revealed a significant increase in MDA along with a significant decrease in GSH content, and the enzymatic activity of catalase, GPx, and GST in the liver and kidneys of rats treated with calcium nitrate at 200, 400, and 800 mg/kg for 30 days. GSH is an important element that plays a crucial role in cell protection. It possesses a thiol group (SH) able to bind with the toxic metabolites. Also, it acts in synergy with other antioxidant enzymes such as GPx, CAT, and SOD to exert antioxidant effects (Saka *et al*., 2011). This decrease in GSH content is possibly due to its involvement in the detoxification reactions with free radicals (Kebièche *et al*., 2011). These results are similar to those obtained in a study conducted on rats treated with sodium nitrate at a dose of 400 mg/kg for 50 days (Bouaziz-Ketata *et al*., 2014), and those reported in female rats exposed to 500 mg/L NaNO3 for 28 days (Anwar and Mohamed, 2015). In this study, MDA content in the liver and kidney tissues was increased significantly in calcium nitrate-treated rats as compared with controls. This result is in line with that reported in a study investigating the effect of ammonium nitrate at different doses of 8, 24, and 40 mg/kg in rats (Messaadia *et al*., 2013), and that conducted on sodium nitrate-exposed rats, showing an increased MDA level, and a decreased GSH content (Rouag *et al*., 2020). The increased level of tissue MDA is strongly referred to as the increased oxidative damage associated with increased free radicals leading to increased lipid peroxidation (breakdown of the cell polyunsaturated fatty acids) (Ben-Saad *et al*., 2017). In fact, increased lipid peroxidation weakens the cell membrane function by losing its fluidity and the activity of membrane and cytoplasmic enzymes (Ben-Saad *et al*., 2017). In addition, catalase, GPx, and GST are considered the main enzymatic defense systems in the cell, whose activity variations indicate the extent of cytotoxic damage occurring in various tissues (Sies, 1993). Our findings revealed a clear reduction in the activity of these enzymes in the liver and kidneys of calcium nitrate-treated rats compared to controls. GPx is a unique selenoenzyme in mammalian cells, and its molecule has four selenium atoms in the active center making a form of selenocysteine

(Walczak *et al*., 1996). It breaks down H_2O_2 into water and lipid peroxides, primarily in the mitochondria and sometimes in the cytosol (Góth *et al*., 2004; Ighodaro *et al*., 2018). Moreover, GPx activity was significantly decreased in the liver and kidneys of calcium nitrate-exposed rats, and this result is similar to that found in the previous study of Rouag *et al*. (2020). The decreased activity of GSH-Px could be explained by an overproduction of H_2O_2 and depletion of selenium and glutathione (Aposhian, 1999; Shila *et al*., 2005) known as the substrate and cofactor of GSH-Px (Ramachandran and Saravanan, 2013). On the other hand, hyperglycemia can produce ROS, and inhibit the active sites of the antioxidant enzymes such as SOD, CAT, and GSH-Px (Sindhu *et al*., 2004). On top of that, CAT, a major component of the antioxidant enzyme system, utilizes iron or manganese as a cofactor and catalyzes in association with the degradation or reduction of water and molecular oxygen (Chelikani, 2004). In this regard, the obtained results revealed a significant decrease in CAT activity in the liver and kidney tissues of calcium-nitrated exposed rats. This finding is in line with that reported in the study by Dar *et al*. (2019) conducted on ammonium nitrate-exposed rats, proving that the excessive production of H_2O_2 can reduce CAT activity.

CONCLUSION

This study reported the dose-dependent toxic effect of calcium nitrate on liver and kidney function and consequently led to explore the dangers of excessive and uncontrolled use of chemical fertilizers on mammalian and human health.

DECLARATIONS

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Ethical statement

The experimental procedures were conducted in accordance with the National Institutes of Health guidelines for animal care and were approved by the ethics committee of our institution.

Generative AI and AI-assisted technology statement

The authors have declared that no generative AI or AI-assisted technologies were used to create this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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