JJBS

Volume 16, Number 1,March 2023
ISSN 1995-6673
Pages 35 – 40
https://doi.org/10.54319/jjbs/160106

Jordan Journal of Biological Sciences

Calcium Nitrate Toxicity on Rat Liver and Kidney Functions: A Biochemical and Histopathological Evaluation

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Received: March 8, 2022; Revised: June 17, 2022; Accepted: June 30, 2022

Abstract

Calcium NitrateTetrahydrate is a wide-used nitrogen fertilizer in Algerian agriculture. The present study was aimed to examine the toxic effects of calcium nitrate on kidney and liver functional biochemical markers. Twenty-eight male albino *wistar* male rats were divided into three treated groups receiving orally 200, 400 and 800mg/kg of calcium nitrate, and one untreated control group. Results showed a dose- dependent increase in kidney and liver relative weights, serum levels of glucose, cholesterol, triglyceride, blood urea, creatinine, and uric acid, and enzymatic activity of transaminases and alkaline phosphatase. However, serum protein and albumin levels were significantly decreased in a dose dependent manner as compared with those of control group. In addition, hepatic and renal histological changes were evidenced by hepatocyte degeneration, necrosis, dilation and sinusoid congestion, atrophy of glomeruli, vascular congestion, and infiltration of inflammatory cells. It is noteworthy that these adverse stress effects were higher in 400 and 800 mg/kg calcium nitrate treated rats than those treated with 200mg/kg and control group. In conclusion, the study proved the effective ability of subacute exposure of calcium nitrate to induce liver and kidney stress dysfunctions.

Keywords: Calcium Nitrate; Histology; Liver and kidney stress dysfunctions; Rats

1. Introduction

Synthetic fertilizers are widely used in agriculture as a quick and less expensive source of delivering plant nutrients leading to increase productivity (Ahmad et *al*., 2017) and agricultural yield, but soil, air and water contamination effects on human and environmental health limit its use (Panico et *al*., 2020; Elahi et *al.*, 2019; Li et *al.*, 2019). Calcium is an essential macronutrient for a variety of biological functions (Meriño-Gergichevich et *al*., 2010), including regulation of plant growth and development (Hepler, 2004). Hence, calcium fertilizers such as calcium nitrate and calcium chloride, the most common calcium fertilizer forms, can be used to increase the calcium content of fruits (Lanauskas et *al.*, 2012). Calcium can be delivered quickly from calcium nitrate to crops in fertigation systems, as well as to those of rapid growth and those undergoing specific periods of high calcium demand(Martínez et *al.*, 2013).Further, nitrates, the end product of nitrogen fertilization, can be washed into water streams when it is not absorbed by plant roots, and, noteworthy, the green leafy foods like lettuce have the highest nitrate levels, the 60% of total nitrousoxide emissions could be linked to agricultural soil management practices (Vitale et al., 2017; Liu et *al*., 2014).Additionally, consumption of nitrates or nitrite in drinking water and food may result in the onset of health condition disorders, such as methemoglobinemia and cancers of the stomach, liver, colon, and lungs in humans, and particularly thyroid and kidney cancers, and non-Hodgkin's lymphoma (Espejo-Herrera et *al.*, 2015).Moreover, the nitrate reduction process starts with mouth bacterial flora producing nitrate reductase enzymes, and hence about 25% of ingested nitrate can be converted to NO_2 in the mouth. The acidic pH of the stomach promotes the formation of nitrous acid, which can be metabolized into various nitrogen oxides, including nitrogen dioxide (NO_2^{-2}) and dinitrogen trioxide (N_2O_3) depending on the redox environment and gastric content. In addition, enterosalivary circulation of nitrate has been described after its absorption in the intestine, where it is converted into nitrite in the mouth and, then the cycle is restarted (Pereira et *al*., 2013). Of note, the metabolism of nitric oxide and nitrosamine in the body results in the formation of nitrate which is reported as the highly carcinogenic agent leading to carcinoma of the stomach, liver, and esophagus (Kim et *al.*, 2002).Several studies have reported the effects of nitrate induced-alterations liver and kidney biochemical markers in experimental animals(Azzez et *al*., 2011).Therefore, the present work aims to study the biochemical and histological stress effects of calcium nitrate on hepatic and renal functions in rats.

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2. Materials and Methods

2.1. Chemical materials

Calcium nitrate tetra-hydrate ($Ca(NO_3)_2$-$4H_2O$) as Calnisol® (Case number: 10124-37-5) was purchased from a fertilizer company named Profert, Bejaia, Algeria.

2.2. Biological materials

Twenty-eight male albino wistar rats weighing 240 ± 20 g and obtained from the Pasteur Institute, Algiers of Algeria were housed on sawdust in plastic cages in an animal house of our Institution maintained with a temperature of 25±2° C, humidity 50±10%, and natural photoperiod. Animals were fed ad-libtium with sticks consisting of corn, barley, milk and vitamin supplements, and tap drinking water.

2.3. Experimental procedures

Rats were acclimated 21days before experiments, and then were divided randomly into three (seven rats/group) treated groups that received 200, 400, and 800mg/kg doses of calcium nitrate respectively, and one untreated control group of seven rats per group. Calcium nitrates were dissolved in mineral water and were given orally to rats for 30 days. Thereafter, animals were sacrificed by cervical decapitation, and blood samples were collected into heparinized tubes for the biochemical analyses. Liver and kidney organs were removed for histo-pathological evaluation.

2.4. Biochemical analyses

The blood samples were centrifuged for 15 minutes at 3000 rpm, and the resulting plasma was stored at -20°C to be used for the spectrophotometric evaluation of biochemical parameters (glucose, cholesterol, triglycerides, transaminases (ASAT and ALAT), alkaline phosphatase (ALP), total proteins, albumin, creatinine, urea and uric acid) using commercially available diagnostic kits (Diagnopharm, Bouira, Algeria;Réf, B07050012, B11010011, B16010011, B18005022, B17005022, B20005022,, B08030013, B24050012, B05020022, B10020024 and B01010011 respectively) using a fully automated chemistry analyzer.

2.5. Histological examination

The liver and kidney from the experimental rats were removed, rinsed with physiological water and fixed in Bouin's solution for 24 hours, and embedded in paraffin wax. The tissue sections were then cut into slices of 5 µm thickness by a rotary microtome, and stained with hematoxylin and eosin (Hould, 1984). The preparations were dried and observed with an optical microscope (OPTIKA brand).

2.6. Statistical analysis

Data were expressed as mean ± SD values. The comparison between control and treatments was tested by Student's t-test using IBM SPSS Statistics 23.0 software. Differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Physiological observations

The liver relative weights (bwt) per 100g of the animal body were significantly higher in 400 and 800 mg/kg calcium nitrate treated rats than those of 200mg/kg and controls since kidney relative weights showed no significant changes (Tab.1).

Table 1. Changes in hepatic and renal relative weights in control rats and treated rats (data are given as mean ± SD, 7rats/group).

Parameters	Treatments			
	Control	200mg /kg	400mg/ kg	800mg/kg
Relative liver weight (g/100 g b.w) Relative kidney weight (g/100 g b.w)	2.70±0.14 0.23±0.01	2.74±0.14 0.24±0.02	3.01±0.24* 0.24±0.02	3.06±0.17*** 0.24±0.02

*p <0.05; ***p < 0.001: Significant difference compared to the control group.

3.2. Biochemical results

The serum levels of glucose, cholesterol and triglycerides were significantly increased in treated groups compared with control group (Table 2). As indicated in Tables 3, calcium nitrate treatments caused a significant elevation in the enzymatic activity of transaminases (ASAT, ALAT) and alkaline phosphatase (ALP) enzymatic activities, along with a marked decrease in protein and albumin levels. In addition, the levels of serum blood urea, creatinine, and uric acid levels were higher in calcium nitrate treated rats compared to controls (Table.4).

Table 2. Lipid profiles in control and treated rats (Data are given as mean ± SD, 7 rats/group).

Parameters	Treatments			
	Control	200mg/kg	400mg/kg	800mg/kg
Glucose (g/l)	1.12±0.07	1.15±0.09	1.21±0.05**	1.25±0.05***
Cholesterol (g/l)	0.96±0.08	0.96±0.07	1.05±0.05*	1.11±0.05***
Triglycerides (g/l)	0.68±0.17	0.67±0.07	0.80±0.03*	0.86±0.04**

*p < 0.05; **p < 0.01; ***p < 0.001: Significant difference compared to the control group.

Table 3. Hepatic biochemical profiles in control and treated rats (data are given as mean ± SD, 7 rats/group).

Parameters	Treatments			
	Control	200mg/kg	400mg/kg	800mg/kg
ASAT (U/L)	114.61±16.29	111.73±10.30	123.37±5.35	134.67±5.29**
ALAT (U/L)	67.60±9.03	63.40±9.50	73.41±4.45	78.73±5.69**
ALP (U/L)	117.16±5.46	118.31±10.02	129.67±5.41	137.14±7.40**
Total protein (g/l)	71.17±5.18	64.37±8.17	60.87±4.09***	59.56±3.12***
Albumin (g/l)	36.00±1.85	35.04±1.14	33.98±1.70*	33.51±1.27**

*p < 0.05; **p < 0.01; ***p < 0.001: Significant difference compared to the control group.

Table 4. Renal biochemical profiles in control and treated rats (Data are given as mean ± SD, 7 rats/group).

Parameters	Treatments			
	Control	200mg/kg	400mg/kg	800mg/kg
Urea (mg/dL)	40±0.2	40±0.3	42±0.2	43±0.2**
Creatinine (mg/dL)	1.17±0.10	1.19±0.09	1.21±0.08	1.30±0.05**
Uric acid (mg/dL)	4.75±0.54	4.59±0.48	4.94±0.44	5.31±0.44*

*p < 0.05; **p < 0.01: Significant difference compared with the control group.

3.3. Histopathological observations

As shown in Figs 1 & 2, liver and kidney sections from rats treated with 200, 400, and 800 mg/kg doses of (Ca $(NO_3)_2 4H_2O$) for 30 days revealed marked histological alterations when compared with those of control rats, showing regular histological architecture with hexagonal lobules, and visible capillary sinusoids (Fig1A, B). No histological damage was found in the liver of the 200mg/kg (Ca $(NO_3)_2 4H_2O$) treated group compared with the control group, while the higher doses (400 and 800mg/kg) showed considerable vascular dilation and congestion, degeneration of hepatocytes, hemorrhage, and lipid vacuolation, and thus moderate inflammations compared to the control group (Fig 1, C – F). Moreover, Microscopic observations of renal tissues from (Ca $(NO_3)_2 4H_2O$) treated rats showed severe histopathological changes evidenced by glomerular atrophy associated with dilation of Bowman's space, intra-glomerular hemorrhage, and vascular congestion (Fig 2, B- D) when compared with kidney from control rats showing normal renal parenchyma with well-defined renal glomeruli and tubules (Fig 2A).

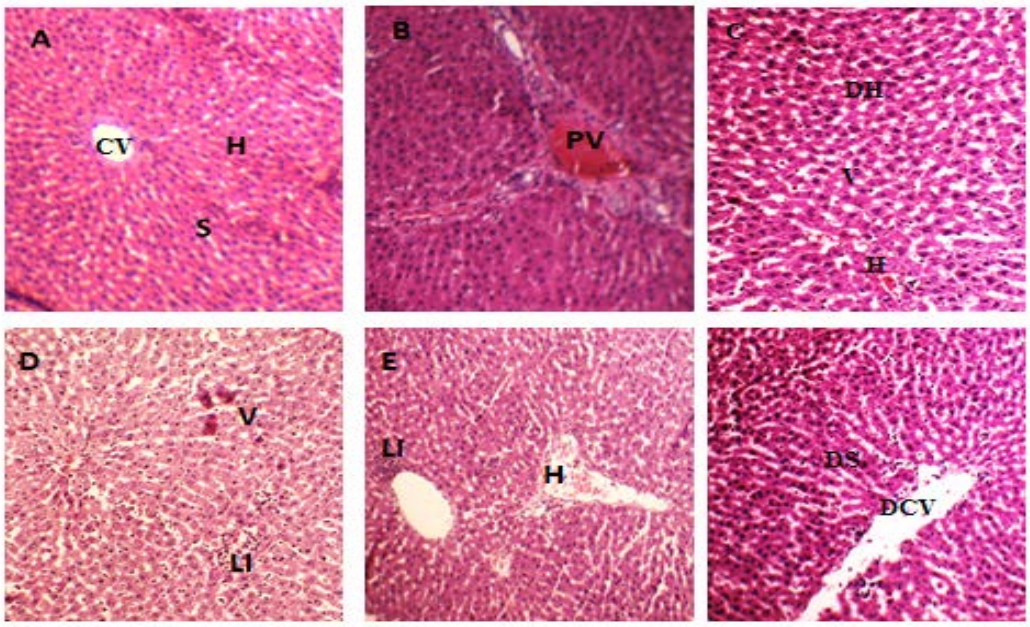

Figure 1. Photomicrograph of liver histology stained with H&E from control rats and rats treated with calcium nitrate for 30 days. The control group's liver sections revealed normal preserved histoarchitecture (A-B). The damage caused by calcium nitrate in liver sections (C–H) increases in a concentration-dependent manner.

CV, centralvein; S, sinusoids; H, hepatocyte;DH, degenerated hepatocyte; V,vacuoles; H, hemorrhage; LI, leucocyte infiltrations; DCV,dilated central vein; DS, dilatedsinusoid;

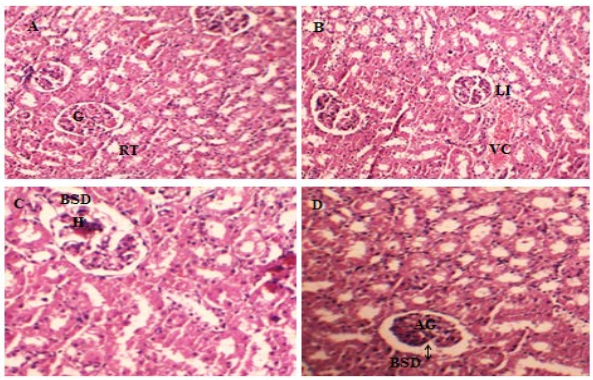

Figure 2. Photomicrographs of H&E-stained kidney histological sections from control and treated rats. The control group's kidney sections revealed normal preserved histoarchitecture (A). The damage caused by calcium nitrate in kidney sections (B–D) increases in a concentration-dependent manner.

AG, atrophy of glomerulus; BSD, Bowman's space dilatation; VC,vascular congestion; LI, lymphocyte infiltration; H, haemorrhage

4. Discussion

The intensified use of synthetic fertilizers, including nitrate fertilizers leads to multiple human health problems (WHO, 2006). The chemical components of the fertilizers can affect the digestion and absorption processes and the metabolic use of ingested food(Weil and McCollister, 1963; OCDE, 2000).Nitrates from fertilizers applied on soil surface or animal excrements contaminated drinking water reserves can seep into groundwater, and cause consequently human and animal health problems (Lockhart, 2013).Thus, the present study was conducted to evaluate the potential toxicity of (Ca $(NO_3)_2 4H_2O$), a nitrogen fertilizer, in rats. The organ weights are a valuable tool in assessing the in vivo toxicity of a xenobiotic (Bailey et *al.*, 2004; Sellers et *al.*,2007). In this regard, our results revealed significant increase in the liver relative weights. This result is in agreement with the study of Lee et al. (2020) reporting an increase in the hepato-somatic ratio in rats that received 1000mg/kg/day of ammonium nitrate, in addition to the result of Bouaziz-Ketata et *al.*, (2014) showing an increase in relative liver weight in rats treated with $NaNO_3$ for 7 weeks. This hepatomegaly can be explained by the over-espression of oxidative stress and over-excitation of the liver detoxification process in response to the harmful effect of xenobiotics (Guerriero et *al.*, 2014; Abdel-Gawad et *al.*, 2020).Furthermore, blood glucose level was significantly increased in treated rats compared with controls, and this concords with those previously reported (Azzez et *al*., 2011; Delgadoa et *al.*,2018).The hyperglycemia due to calcium nitrate treatment is likely explained by the activation of the glycogenolysis process, resulting in the release of glucose by glycogen phosphorylase under the action of amylase(Hijmans et *al.*, 2014).Several European research studies have attempted to discover a close association between nitrate concentration in drinking water and insulin-dependent diabetes by increasing blood glucose while decreasing hepatic glycogen (IDD), but the comparison revealed sparse and contradictory data(Moltchanova et *al.*,2004).On the other hand, results showed a very highly significant increase in the levels of cholesterol and triglycerides. This hyperlipidemia can be explained by hypothyroidism(Jublanc and Bruckert, 2004; Pearce, 2004).Previous *in vivo* experimental studies have proven that inorganic nitrate is a short-term goitrogenic agent causing hypertrophy of the epithelial cells of the thyroid gland(Boukerche et *al*., 2007; Gatseva and Argirova, 2008; Messaadia et *al*, 2013).On top of that, (Ca $(NO_3)_2 4H_2O$) treated rats for 30 days resulted in an elevation of enzymatic activity of transaminases (AST, ALT) and alkaline phosphatase (ALP) and bilirubin level, and a lowering of total protein and albumin levels. This result is in line with that previously reported (Messaadia et *al*., 2013; Fouad et *al.*, 2017; Kattiaa et *al.*, 2017). In addition, increased levels of ALT and AST in the blood indicate their increased release in blood following hepatocyte necrosis leading to liver impaired function (Krim et *al.*,2013). As previously reported, the concentration of transaminases (AST, ALT) may become high in ammonium nitrate (Messaadia et *al*., 2013) or sodium nitrate treated rats (Delgadoa et *al*.,2018). In this study, calcium nitrates induced a marked decrease in blood total protein levels. A similar result was reported in adult rats treated with sodium nitrate in drinking water at concentrations of 550 mg/L for four months (Azza *et al*., 2011). The decline in blood total protein levels is mainly due to the effects of nitrate on the liver, either through necrotic alterations or other mechanisms (Anthony et *al*., 1994). Additionally, waste products from protein metabolism that are eliminated by the kidneys, such as blood creatinine, urea, and uric acid concentrations, are usually regarded as indicators of kidney function (Tootian et *al*., 2012). Accordingly, our results showed a significant increase in blood urea, creatinine, and uric acid levels in response to ($Ca(NO_3)_2 4H_2O$)toxicity, similar to those previously reported (Messaadia *et al.*, 2013).The increased blood urea, uric acid, and creatinine levels suggested impaired renal function as evidenced by changes in reabsorption threshold, renal blood flow, and glomerular filtration. The results of the present work indicated that (Ca $(NO_3)_2 4H_2O$) induces histopathological alterations in the liver characterized by venous congestion, mononuclear cell infiltration, cytoplasmic vacuolation of hepatocytes, and fatty degeneration. These histological alterations were similarly reported in some previous studies (Bouaziz-Ketata et *al*., 2014; Kattaia et *al*., 2017; Delgadoa et *al*., 2018; Ikele et *al*., 2021), and in return, calcium nitrate treatment resulted in the dilation of the renal tubules, atrophy of the glomeruli, and leukocyte infiltration. In this regard, Anwar and Mohamed (2015) reported glomerular atrophy and renal blood vessel congestion in the kidneys of rats treated with 500 mg/L $NaNO_3$ for 4 and 6 weeks.

5. Conclusion

(Ca $(NO_3)_2 4H_2O$) caused marked dose-dependent changes in liver and kidney biochemical stress profiles and consequently led to renal insufficiency without hepatotoxicity. Conclusively, the excessive use of higher doses of calcium nitrate may place mammalian and human health at risk.

Acknowledgments

The results were obtained within the project ATRP/ code N°: D01N01UN410120180001"Origin and diversification of pollution (water, air and soil) by heavy metals of some biotopes by the use of bio-indicator models" funded by Directorate General for Scientific Research and Technological Development (DGRSDT)

Conflict of Interests

None.

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