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Effect of nitrate poisoning on some biochemical parameters in rats

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Abstract

The present study was conducted to investigate the toxicity of potassium nitrate on glucose, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the possible ameliorative effect of ascorbic acid (Vitamin C). Male Wister rats are used as experimental model divided into three groups (each of 6-8 rats) and treated for six weeks as follows: Group 1: served as control; Group 2: received 2 % potassium nitrate added to the forage and Group 3: received 2 % potassium nitrate together with 1 % ascorbic acid added to rat's forage. Nitrate treatment in group 2 leads to high significant increase levels of glucose in 3rd, 4th, and 5th weeks, cholesterol level increased significantly in both 4th and 5th weeks, while ALT levels increased in the 4th, 5th and 6th weeks, and AST increased significantly in the 5th and 6th weeks. Addition of ascorbic acid with potassium nitrate, lead to reverse all the parameters nearly to normal. It was concluded that potassium nitrate causes significant toxic effect on some biochemical parameters which was ameliorated by ascorbic acid.

Keywords: Nitrate toxicity; Ascorbic acid; Biochemical parameters.
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تأثير التسمم بالنترات في بعض القيم الكيموحيوية في الجرذان

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الخلاصة

صممت تجارب هذه الدراسة لاختبار التأثير السمي لنترات البوتاسيوم على الكلوكوز، الكولسترول، خميرة ALT، خميرة AST، ودراسة أمكانية تقليل هذه التأثيرات السمية من خلال استخدام فيتامين C. استخدمت ذكور جرذان من نوع Wister تجريبياً حيث قسمت إلى ثلاث مجاميع (٦-٨ جرذان لكل مجموعة): المجموعة الأولى: تركت دون معاملة وعدت مجموعة سيطرة، المجموعة الثانية: أضيف ٢% من نترات البوتاسيوم إلى العلف، المجموعة الثالثة: أضيف ٢% نترات البوتاسيوم مع ١ % من فيتامين C إلى العلف. أظهرت النتائج أن نترات البوتاسيوم أحدثت زيادة معنوية في تركيز الكلوكوز في الأسابيع الثالث والرابع والخامس، وزيادة معنوية في تركيز الكولسترول في الأسبوعين الرابع والخامس، بينما ازداد تركيز خميرة ALT في الأسابيع الرابع والخامس والسادس، وخميرة AST في الأسبوعين الخامس والسادس. وعند إضافة فيتامين C إلى العلف المضاف له نترات البوتاسيوم لم تظهر أي تأثيرات معنوية وعادت كل القيم إلى مستويات قريبة من الطبيعية. أوضحت نتائج الدراسة الحالية إلى أن نترات البوتاسيوم أحدثت تأثيرات سمية من خلال تغير بعض القيم الكيموحيوية والتي تحسنت من خلال إضافة فيتامين C.

Introduction

Nitrate poisoning has been recorded in several studies (1) and it can occur in all animals (2). Nitrate themselves are not very toxic but nitrite which they converted to are ten fold more toxic than nitrate. In human nitrate is reduced to nitrite before ingestion in saliva and in the gastrointestinal tract (3,2). In ruminants such as cattle, sheep, and goat, the conversion of nitrate to nitrite is carried out by rumen bacteria (4).

Nitrate poisoning affect several biochemical parameters. A previous study indicates that nitrate poisoning cause decreased levels of glucose and alkaline phosphatase in sheep (5), while in study of (6) in sheep reported an increase in ALT, AST, AP. and glucose. Also (7) reported that an increase in levels of glucose, cholesterol, creatinine, lactate dehydrogenase, AST, and ALT in rats.

Vitamin C (Ascorbic acid) are known to be potent antioxidant (8,9), and may augment the function of endogenous free radical scavengers.

The objective of this study was to investigate the nitrate poisoning by potassium nitrate on some biochemical parameters in rats as experimental model, and the effect of ascorbic acid when used with nitrate.

Materials and methods

Male Wister rats age 3-4 months and 210-275 gm of body weight were housed in hanging cages and maintained under laboratory controlled of temperature (25 ± 2) and light (14 hour light and 10 hour dark), palleted food as concentrated forage and tap water were given.

The animals divided into 3 groups each of 6-8 rats. Group 1: left as control group; Group 2: Potassium nitrate (KNO_3) (Gerhard Bochman Tuttingreen, Germany) 2% (10) were added to the concentrated forage; Group 3: Coadminstration of Potassium nitrate 2% and ascorbic acid (Vitamin C) 1% (11) were added to the same forage. All treatments were for 6 weeks.

Blood samples were collected every week from the orbital plexus of vein into clear dry centrifuge tubes, allowed to clot; serum was separated after centrifugation at 1500 rpm for 15 minute (12). Serum Glucose, cholesterol, ALT, and AST levels were measured using colorimetric assay kits (Bicon Diagnostic GmbH Burbach, Germany).

All data analyzed by one way analysis of variance, the specific group differences were determined using Duncan multiple range test; the accepted level of significance was $P<0.05$ (13).

Results

After 6 weeks of experiment potassium nitrate 2 % given to rat's forage lead to significantly increases in levels of glucose in 3rd, 4th, 5th weeks (Table 1), cholesterol levels was increased in both 4th, and 5th weeks (Table 2).

While levels of ALT increased in 4th, 5th, 6th weeks (Table 3), and AST levels increased significantly in 5th, 6th weeks (Table 4).

When we add ascorbic acid at a dose 1 % to the diet containing nitrate 2 %, all parameters reverse nearly to the normal when compared with control, so there are no significant increase in all parameters (Tables 1-4).

Table 1: Effect of Nitrate poisoning alone and with ascorbic acid on glucose level (mg/dl).

Groups	weeks						
	0	1	2	3	4	5	6
Control	D 90.16 ± 2.76	D 93.14 ± 3.19	D 92.88 ± 2.01	D 96.81 ± 3.76	D 91.07 ± 2.14	D 93.81 ± 3.58	D 92.11 ± 1.85
KNO_3 2 %	D 94.51 ± 3.73	D 94.03 ± 5.02	CD 99.53 ± 2.4	AB 111.94 ± 2.07	A 116.59 ± 7.52	ABC 109.28 ± 6.08	BCD 102.58 ± 5.11
KNO_3 2 % + Vit. C 1 %	D 93.81 ± 5.11	CD 98.25 ± 2.92	D 96.22 ± 2.44	CD 97.84 ± 2.49	D 95.47 ± 3.54	D 96.61 ± 4.20	D 94.50 ± 4.78

Value is expressed as means ± SEM of 6-8 rats/ group.
Different letters indicate significant differences between groups horizontally and vertically at $P<0.05$.

Table 2: Effect of Nitrate poisoning alone and with ascorbic acid on Cholesterol level (mg/dl).

Groups	weeks						
	0	1	2	3	4	5	6
Control	D 138.48 ± 6.31	D 134.46 ± 8.15	CD 140.61 ± 9.22	CD 142.19 ± 4.39	CD 139.86 ± 2.98	CD 144.58 ± 5.20	CD 141.21 ± 4.74
KNO_3 2 %	CD 141.83 ± 6.58	CD 145.73 ± 3.46	ABCD 153.94 ± 7.62	ABC 158.7 ± 7.77	A 170.75 ± 5.13	AB 168.68 ± 3.55	BCD 151.16 ± 6.15
KNO_3 2%+ Vit. C 1 %	D 136.36 ± 8.62	D 134.84 ± 5.41	CD 141.21 ± 4.74	CD 143.72 ± 4.84	CD 139.82 ± 3.80	D 137.34 ± 7.10	D 134.63 ± 3.27

Value is expressed as means ± SEM of 6-8 rats/ group.
Different letters indicate significant differences between groups horizontally and vertically at $P<0.05$.

Table 3: Effect of Nitrate poisoning on ALT level (IU/L).

Groups	weeks						
	0	1	2	3	4	5	6
Control	D 19.04 ± 0.90	D 19.02 ± 1.38	D 19.82 ± 1.43	D 20.63 ± 0.97	D 20.15 ± 1.28	D 20.16 ± 1.14	D 19.29 ± 1.35
KNO_3 2 %	D 20.67 ± 0.87	D 20.25 ± 1.20	D 19.50 ± 1.33	BC 23.20 ± 2.49	A 30.74 ± 3.13	A 31.23 ± 2.43	AB 26.78 ± 1.92
KNO_3 2%+ Vit. C 1 %	BC 22.15 ± 4.01	D 19.48 ± 3.4	BC 21.96 ± 3.08	BC 23.53 ± 2.66	BC 23.94 ± 5.79	BC 24.36 ± 2.88	BC 22.83 ± 2.67

Value is expressed as means ± SEM of 6-8 rats/ group.
Different letters indicate significant differences between groups horizontally and vertically at P<0.05.

Table 4: Effect of Nitrate poisoning on AST level (IU/L).

Groups	weeks						
	0	1	2	3	4	5	6
Control	B 80.74 ± 2.53	B 85.09 ± 3.24	B 82.23 ± 2.44	B 82.74 ± 1.92	B 84.30 ± 1.90	B 83.36 ± 2.98	B 82.38 ± 4.03
KNO_3 2 %	B 79.86 ± 2.70	B 78.44 ± 2.18	B 79.59 ± 2.50	B 84.61 ± 3.83	B 89.24 ± 1.76	A 99.64 ± 2.97	A 101.10 ± 3.76
KNO_3 2%+ Vit. C 1 %	B 82.22 ± 4.01	B 85.36 ± 3.40	B 86.48 ± 3.05	B 84.32 ± 2.66	B 84.62 ± 5.74	B 87.11 ± 2.88	B 85.14 ± 2.67

Value is expressed as means ± SEM of 6-8 rats/ group.
Different letters indicate significant differences between groups horizontally and vertically at P<0.05.

Discussion

Significant increase in glucose level was observed at 3rd, 4th and 5th weeks in rat feeding at diet containing 2% of KNO_3, these results are consistent with those of the previous study of (7) in rats and human, and (14) in rats, but our result don't agree with the results of (5) in sheep. This may be due to stimulation of the rate of gluconeogenesis (14).

ALT and AST levels increased significantly in 4th, 5th, 6th and 5th, 6th, weeks respectively, similar result reported by (6) that reported increases in all parameters during 5th weeks of nitrate treatments.

The table 2 showed that cholesterol levels increases significantly in 4th, and 5th weeks of nitrate poisoning. All the changes of glucose, ALT, AST and cholesterol levels can be due to that liver is the major organ that affected directly by nitrate, also the pathologic changes and furetimal state of liver play a major role in prognosis of nitrate poisoning in animals (1,15). Also liver plays an active important role in the metabolism of cholesterol, and an increase level of cholesterol and other parameters in state of poisoning (16,17). Nitrate cause hypoxia (6) lead to increase of activity of ALT, because hypoxia cause hepatocellular injury (18).

In our study Vit. C administration to KNO_3-treatrd rats produced no significant changes in all biochemical parameters levels and returns nearly to normal levels. The results of this study agree with those of (19) in catfish, and (20) in humans. One of the established mechanisms of toxicity of nitrate is their ability to induce oxidative stress through the generation of free radicals (21,22). Vit. C is known to be potent antioxidant (8,9), thus its administration may augment the function of endogenous free radicals scavengers, decrease the deleterious effects of nitrates on body cells (23).

In conclusion, the results obtained from our study that toxic effects of potassium nitrate on some biochemical parameters were significant ameliorative effect by Vit. C by returning this parameters back to nearly to normal.

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