

EFFECT OF POTASSIUM NITRATE POISONING ON SOME BLOOD SERUM BIOCHEMICAL PARAMETERS IN RATS

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ABSTRACT

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The present study was conducted to investigate the toxicity of potassium nitrate on blood serum glucose, cholesterol levels, alanine aminotransferase (ALT), Aspartate aminotransferase (AST) activity levels, 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 concentrated forage and Group 3 : received 2 % potassium nitrate together with 1 % ascorbic acid added to rat's concentrated forage. Nitrate treatment in group 2 leads to high significant increase in serum levels of glucose on 3rd, 4th, and 5th weeks, cholesterol level increased significantly on both 4th and 5th weeks, while ALT activity levels increased on the 4th, 5th and 6th weeks, and AST increased significantly on 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 blood serum biochemical parameters which were ameliorated by ascorbic acid.

Key words: Potassium nitrate, blood serum, biochemical parameters, Rats

INTRODUCTION

Nitrate poisoning has been recorded in several studies (Muslih, 1991) and it can occur in all animals (Jan *et al.*, 2009). Nitrates themselves are not very toxic but nitrite which they converted to be tenfold more toxic than nitrate. In human, nitrate is reduced to nitrite before ingestion in saliva and in the gastrointestinal tract (Kyriakidis *et al.*, 1997 and Jan *et al.*, 2009). In ruminants such as cattle, sheep and goat, the conversion of nitrate to nitrite is carried out by rumen bacteria (Chemlnitskii *et al.*, 1987). Nitrate poisoning affects several biochemical parameters. A previous study indicate that nitrate poisoning cause decrease levels of glucose and alkaline phosphatase in sheep (Majid, 1996), while in study of (Al-kafajii, 1996) in sheep reported an increase in ALT, AST, AP, and glucose. Also (Boukereche, 2007) reported that an increase in levels of glucose, cholesterol, creatinine levels, lactate dehydrogenase, AST, and ALT activity levels in rats.

Vitamin C (Ascorbic Acid) is Known to be potent antioxidant (Ayo *et al.*, 2006 and Suteu *et al.*, 2007), 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 blood

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 ($25 \pm 2^\circ\text{C}$) and light (14 hour light and 10 hour dark), palliated food concentrated forage and tap water were given.

The animals divided into three groups each of 6-8 rats. Group 1 left as control group; Group 2: potassium nitrate (KNO_3) (Gerhard Bocham Tuttingreen, Germany) 2% (Til, 1985) were added to the concentrated forage; Group 3: Coadminstration of potassium nitrate 2% and ascorbic acid (Vitamin C) 1 % (Madhuban and Anilava, 2003) were added to the same forage. All treatment 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 (Fox *et al.*, 1984). Serum Glucose, cholesterol levels, ALT, and AST activity levels were measured using colorimetric

assay kits (Bicon Diagnostic GmbH Burbach, Germany).

All data analysed by one way analysis of variance, the specific group differences were determined using Duncan multiple range test; the accepted level of significance was $P < 0.05$ (Bruning and Kintz, 1977).

RESULTS

After 6 weeks of experiment potassium nitrate 2 % added to rat's forage lead to significantly increased levels of glucose on 3rd, 4th, 5th weeks (Table 1) while

cholesterol levels was increased on both 4th, and 5th weeks (Table 2).

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

When ascorbic acid was added 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 on all parameters (Table 1-4).

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

Groups	weeks						
	0	1	2	3	4	5	6
Control	D 90.1± 2.7	D 93.1±3.1	D 92.8±2.0	D 96.8±3.7	D 91.0 ± 2.1	D 93.8 ± 3.5	D 92.1 ±1.8
KNO ₃ 2%	D 94.5 ± 3.7	D 94.0 ± 5.0	CD 99.5 ± 2.4	AB 111.4 ± 2.0	A 116.5 ± 7.5	ABC 109.2 ± 6.0	BCD 102.5 ± 5.1
KNO ₃ 2% + Vit. C 1%	D 93.8 ± 5.1	CD 98.2 ± 2.9	D 96.2 ± 2.4	CD 97.8 ± 2.4	D 95.4 ± 3.5	D 96.6 ± 4.2	D 94.5 ± 4.7

Value is expressed 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 Potassium Nitrate poisoning alone and with ascorbic acid on blood Cholesterol level (mg/ dl)

Groups	weeks						
	0	1	2	3	4	5	6
Control	D 138.4±6.3	D 134.4 ± 8.1	CD 140.6 ± 9.2	CD 124.1 ± 4.3	CD 139.8± 2.9	CD 144.5 ± 5.2	CD 141.2 ± 4.7
KNO ₃ 2%	CD 141.8± 6.5	CD 145.7 ± 3.4	ABCD 153.9 ± 7.6	ABC 158.7 ± 7.7	A 170.7± 5.1	AB 168.6 ± 3.5	BCD 151.1 ± 6.1
KNO ₃ 2%+ Vit. C 1%	D 136.3 ± 8.6	D 134.8 ± 5.4	CD 141.2 ± 4.7	CD 143.7 ± 4.8	CD 139.8 ± 3.8	D 137.3 ± 7.1	D 134.6 ± 3.2

Value is expressed 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 Potassium Nitrate poisoning alone and with ascorbic acid on ALT activity level (IU/ L)

Groups	weeks						
	0	1	2	3	4	5	6
Control	D 19.0 ± 0.9	D 19.0 ± 1.3	D 19.8 ± 1.4	D 20.6 ± 0.9	D 20.1 ± 1.2	D 20.1 ± 1.1	D 19.2 ± 1.3
KNO ₃ 2%	D 20.6 ± 0.8	D 20.2 ± 1.2	D 19.5 ± 1.3	BC 23.2 ± 2.4	A 30.7 ± 3.1	A 31.2 ± 2.4	AB 26.7 ± 1.9
KNO ₃ 2% + Vit. C 1%	BC 22.1 ± 4.0	D 19.4 ± 3.4	BC 21.9 ± 3.0	BC 23.5 ± 2.6	BC 23.9 ± 5.7	BC 24.3 ± 2.8	BC 22.8 ± 2.6

Value is expressed 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 alone and with ascorbic acid on AST activity level (IU/ L)

Groups	weeks						
	0	1	2	3	4	5	6
Control	B 80.7 ± 2.5	B 85.1 ± 3.2	B 82.2 ± 2.4	B 82.7 ± 1.9	B 84.3 ± 1.9	B 83.3 ± 2.9	B 82.3 ± 4.0
KNO ₃ 2%	B 79.8 ± 2.7	B 78.4 ± 2.1	B 79.5 ± 2.5	B 84.6 ± 3.8	B 89.2 ± 1.7	A 99.6 ± 2.9	A 101.1 ± 3.7
KNO ₃ 2% + Vit. C 1%	B 82.2 ± 4.1	B 85.3 ± 3.4	B 86.4 ± 3.6	B 84.3 ± 2.6	B 84.6 ± 5.7	B 87.1 ± 2.8	B 85.1 ± 2.6

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

DISCUSSION

Significant increases in glucose level were observed at 3rd, 4th, and 5th weeks in rats feeding on diet containing 2 % of KNO₃. These results are consistent with those of the previous study of Boukerche *et al.* (2007) in rats and human, and Wiechetek *et al.* (1993) in rats, but not agree with Majid (1996) in sheep. This may be due to stimulation of the rate of gluconeogenesis (Wiechetek *et al.*, 1993).

Activity of ALT and AST levels increased significantly on 4th, 5th, 6th and 5th, 6th, weeks respectively. Similar result reported by Al-kafajii (1996) reported increases in all parameters during 5th week of nitrate treatments.

Table 2 showed that serum cholesterol levels increased significantly on 4th, and 5th week 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. The pathologic

changes and functional state of liver play a major role in prognosis of nitrate poisoning in animals (Muslih, 1991 and Hassan, 1991). Also liver plays an active important role in the metabolism of cholesterol, and an increase level of cholesterol and other parameters in the state of poisoning (Chatterjea and Shinde, 2005 and Bishop *et al.*, 2005). Nitrate causes hypoxia (Al-kafajii, 1996) lead to increase of activity of ALT, because hypoxia causes hepatocellular injury (Thrall *et al.*, 2004).

In present study Vitamin C administration to KNO₃-treated rats produced no significant changes in all biochemical parameters levels in comparison to control levels and recorded levels return nearly to normal levels. The results of this study agree with those of Saha and Kaviraj (2009) in catfish, and Bassenge *et al.* (1998) in humans. One of the established mechanisms of toxicity of nitrate is their ability to induce oxidative stress through the generation of free radicals (Singhal *et al.*, 2001 and Manassaram *et al.*, 2006). Vit. C is known to be

potent antioxidant (Ayo *et al.*, 2006 and Suteu *et al.*, 2007), thus its administration may augment the function of endogenous free radicals scavengers, decrease the deleterious effects of nitrate on body cells (Isyaku, 2009).

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

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تأثير التسمم بنترات البوتاسيوم على بعض القيم الكيموحيوية في مصل دم الجرذان

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صممت تجارب هذه الدراسة لاختبار التأثير السمي لنترات البوتاسيوم على الكلوكوز ، الكولسترول ، نشاط خميرة ALT، وخميرة AST ودراسة امكانية تقليل هذه التأثيرات السمية من خلال استخدام فيتامين C. استخدمت ذكور جرذان من نوع Wister تجريبيا حيث قسمت الى ثلاث مجاميع (٦-٨ جرذان / مجموعة) : المجموعة الاولى : تركت دون معاملة وعدت مجموعة سيطرة ، المجموعة الثانية : اضيف ٢% من نترات البوتاسيوم الى العلف ، المجموعة الثالثة: اضيف ٢ % نترات البوتاسيوم مع ١ % من فيتامين C الى العلف. أظهرت النتائج أن نترات البوتاسيوم أحدثت زيادة معنوية في مصل الدم لكل من تركيز الكلوكوز في الاسبوع الثالث والرابع والخامس ، وزيادة معنوية في تركيز الكولسترول في الاسبوعين الرابع والخامس، بينما ازداد تركيز نشاط خميرة ALT في الاسبوع الرابع والخامس والسادس ، وخميرة AST في الاسبوعين الخامس والسادس. وعند اضافة فيتامين C الى العلف المعامل بنترات البوتاسيوم لم تظهر أي تأثيرات معنوية وعادت كل القيم الى مستويات قريبة من الطبيعية. أوضحت نتائج الدراسة الحالية الى ان نترات البوتاسيوم أحدثت تأثيرات سمية من خلال تغير بعض القيم الكيموحيوية والتي تحسنت من خلال اضافة فيتامين C.