EFFECT OF NITRATE ADMINISTRATION ON SERUM PROTEIN, SOME ENZYMES AND WEIGHT OF RABBITS*

ملخص البحث

في هذا البحث تم دراسة تأثير إعطاء تركيزات مختلفة من نترات الصوديوم يوميا عن طريق الفم مددا مختلفة على تركيز بروتينات سيرم الدم وعلى نشاط بعض الأنزيمات مثل ALT, AST وأنزيم الفوسفاتيز القلوى وعلى معدل الزيادة في وزن الأرانب. وقد أوضحت الدراسة بجلاء أن معدل الزيادة في وزن الأرانب قد تناقص معنويا خلال فترة الدراسة وان إعطاء الأرانب جرعة يومية قدرها ٣٠٠ ملجم $NaNO_3$/كجم من وزن الجسم لمدة ٣٠ يوماً أدى إلى نقص معدل الزيادة في الوزن بنسبة ٢٧,١٩ % مقارنة بحيوانات الكنترول . وقد تأثر أيضا تركيز البروتين الكلى في سيرم دم الأرانب المعاملة ، وأظهرت انخفاضاً واضحاً عند ٢٧,٧١ ، ٣٢,١١ ، ٢٣,٨٥ ، ١٥,٠٤ %نتيجة لتعاطي ٢٥ ، ٧٥ ، ١٥٠ ، ٣٠٠ ملجم $NaNO_3$/كجم من وزن الجسم ولمدة ٢٠ يوماً. وظهر كذلك تطابق زيادة تركيز الألبيومين مع زيادة جرعات النترات المعطاة بينما ظهر انخفاضا واضحا فى تركيز الجلوبيولين بعد ٢٠ يوما من اخذ الجرعة. ومن ناحية اخرى فان تعاطى ٧٥ و ١٥٠ ملجم $NaNO_3$/كجم من وزن الجسم ولمدة ٣٠ يوما قد سبب زيادة نشاط إنزيم ALT بحوالى ٣٣% بمقارنتهم بأرانب الكنترول. لقد زاد نشاط إنزيم الفوسفاتيز القلوى زيادة ملحوظة كنتيجة لتعاطى جرعات ٧٥ و ١٥٠ و ٣٠٠ ملجم $Na NO_3$/كجم من وزن الجسم ولمدة ٢٠ يوم بمقدار ٧,٦٧ و ٤٢,٤٩ و ٣٠,٨٨ على التوالى.

Abstract

The effect of daily oral administration of nitrate on serum total protein, albumin, globulin, transaminases (AST and ALT) and alkaline phosphatase (AP) in rabbits blood serum and body weight increment rate at various intervals during 30 days were studied. The data showed clearly that rabbits body weight increment rate decreased significantly in thirty days of inoculation as a result of nitrate oral administration. Administration of 300mg $NaNO_3$ /kg b.w. caused a decrease in the body weight increment rate by 27.19%compared with the control group. Total protein concentration in rabbits serum also affected by sodium nitrate daily administration, hence, it decreased significantly by 27.71, 32.11, 23.85 and 15.04% in response to the treatment by 25,75,150 and 300 mg $NaNO_3$ / kg b.w. for 20 days . It was obvious that the increment in albumin level coincided with the increment dose of sodium nitrate. However, a significant decrease in globulin concentration was observed after 20 days of nitrate administration. On the other hand, Administration of 75 or 150 mg $NaNO_3$/kg b.w. daily for 30 days caused an increase in the activity of ALT by about 33% compared with the control rabbits. The activity of AP increased, in general, significantly in response to sodium nitrate administration at levels of 75, 150 and 300 mg/kg b.w. for 20 days by 7.67, 42.49 and 30.88%, respectively.

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INTRODUCTION

Nitrate contamination of drinking water is a serious and growing problem that places thousands of people at several acute risks. Drinking water containing nitrate -nitrogen in excess of 10 mg / L can cause a sometimes fatal blood disorder called methemoglobinemia in infants under the age of six months. On the other hand, children exposed to excessive nitrate in their diet can have slightly retarded body growth and slower reflexes, but it has no apparent short term effects on adults (Terblanche, 1991). Consumption of nitrate in drinking water increases the risk of gastric cancer (Gilli *et al.*, 1984) and caused a genotoxic risk for humans as indicated by increased HPRT variant frequencies (Van Maanen *et al.*, 1996) and by endogenous formation of carcinogenic N-nitroso compounds from nitrate - derived nitrite (Kleinjans *et al.*, 1991 and Van Maanen *et al.*, 1996).

In accordance with the studies of Van Maanen *et al.*, (1994 and 1996), in human populations, on the effects of the consumption of drinking water with high nitrate level on the thyroid , drinking water nitrate contamination caused a dose - dependent increase in 24 hr urinary nitrate excretion and in increased salivary nitrate and nitrite levels. On the other hand, Timofeeva *et al.*, (1995) reported that, the daily administration of 150 mg sodium nitrate / kg body weight for 7 days caused a reduction in the activity of the digestive enzymes and a heterogeneous response of the enzyme systems of the liver and kidney of rats.

Nitrate per se is not toxic, but is the precursor of nitrite which is produced through microbial reduction of nitrate in the intestine or in food preparations and which causes the potentially deadly methemoglobinemia (Terblanche, 1991).

Nitrite derived from nitrate may react in vivo with amines and amides to form N-nitroso compounds, which may have carcinogenic properties. A nitrite intake has been positively associated with stomach cancer risk (Boeing, 1991).

The growing need for increasing the crop production to meet the increase in the population, the Gaza Strip is the most over populated area in the world, and producing the crop in the markets earlier than it's right season; made the farmers use the N-fertilizers excessively and in an irresponsible way. For the same reason out of their fear and to protect their crops from pests; they used the pesticides in the same irresponsible way casting a side all the usage prescriptions of the manufacturers . So, that could result in a leakage of nitrates and to a lesser extent pesticides (U.S. EPA, 1990) into underground water.

The chemical monitoring of the ground water in the Gaza strip - a narrow strip of 365 Km^2 , located in an arid to semi-arid region, along the eastern coast of the Mediterranean Sea - showed that it is highly contaminated by nitrate. Detected levels of nitrate in drinking water in the Gaza strip vary between 15 mg /L and 500 mg /L. The higher levels were recorded, especially, in Khan Younis Governorate, (Palestinian Ministry of Health, 1997 and GEP Gaza, 1996). However, the World Health Organization drinking water guideline value for nitrate has been set at a value of 45 mg/L (WHO, 1985). In the European Union, the maximum admissible nitrate level in the drinking water has been set at 50 mg/L (Roux, 1995), which means that over 900,000 people at the Gaza Strip receiving a water supply that exceeds these limits from municipality water systems for human consumption, where all potable water supplies in the Gaza strip are obtained from aquifer sources.

This study was conducted to investigate the effects of daily administration of sodium nitrate on the weight increment rate, soluble protein, transaminases and alkaline phosphatase.

MATERIALS AND METHODS

i- Experimental Animals:

Twenty five healthy male rabbits of similar age (28-30 days) and weight (ca. 800 gm) were caged in the animal experimental laboratory, College of Education, Gaza, Gaza Strip. Rabbits were fed on a commercial balanced diet prepared especially for rabbits (Anber). The diet and tap water (containing ca. 25 mg /L NO_3^-) were offered ad libitum all over the experimental period. Rabbits were divided into five groups, each contains 5 rabbits. Sodium nitrate 25, 75, 150 and 300 mg / Kg of body weight (dissolved in 3 ml distilled water) were introduced daily by means of a stomach tube to rabbits in groups 2, 3, 4 and 5, respectively, for 30 days, started 4 days after delivery. Rabbits in group (1) acted as a control and received 3 ml distilled water as the other groups. Blood samples were collected at zero time, 10, 20 and 30 days after inoculation.

ii-Blood sampling:

Blood samples were drawn from the retrobulber venous plexus of each rabbit through a heparinized capillary glass tube (Abd Rabo *et al.*, 1992). Clear serum samples were separated by centrifugation at 3000 r.p.m.for 20 min and then collected and stored in a deep freeze at (-20 C) for different biochemical analysis. However, determination of total protein, and enzymes were carried out on fresh serum samples.

iii-Chemical analysis:

Serum total protein was determined according to the method described by Weichselbaum, (1946). Serum albumin was determined using bromocresol green method according to

Doumas *et al.*, (1971). Serum globulin was calculated by subtracting serum albumin from serum total protein. On the other hand, serum transaminases and alkaline phosphatase activities were determined as recommended by Reitman and Frankel, (1957) and Tietz *et al.*, (1983), respectively.

RESULTS AND DISCUSSION

The mean values of total protein, albumin, globulin, AST, ALT, and alkaline phosphatase in rabbits blood serum and body weight increment rate at various intervals during 30 days as affected by daily oral administration of nitrate are summarized at tables 1,2 and 3.

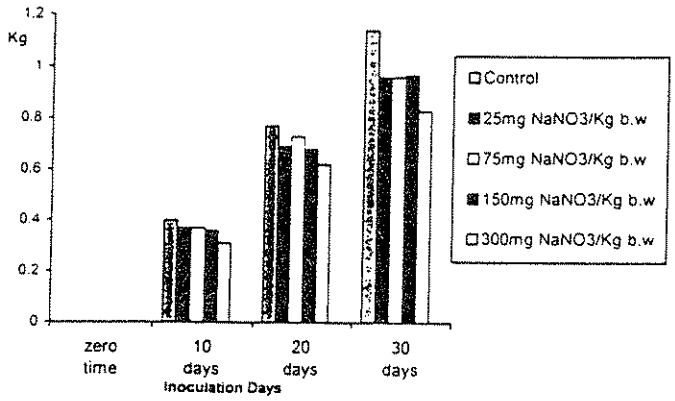
The data in table (1) clearly, showed that the daily oral administration of nitrate causes an obvious decrease in the rabbits body weight increment rate of the treated animals in comparison with the control ones, at the various intervals of the experimental period. Rabbits body weight increment rate decreased significantly in the thirtieth day of inoculation as a result of nitrate oral administration. The daily administration of sodium nitrate at levels of 25, 75 and 150 mg/kg body weight for 30 days decreased the weight increment rate by about 15.79%. While administration of 300 mg/kg caused a decrease by 27.19% as compared with control, (Fig.1). The decrease in the treated rabbits weight agreed with the observation of Terblanche, (1991) that children exposed to excessive nitrate in their diet can have slightly retarded body growth and slower reflexes.

Total protein concentration in rabbits serum also was affected by sodium nitrate daily administration, hence, it is decreased significantly by 27.71, 32.11, 23.85 and 15.04% in response to the treatment by 25, 75, 150 and 300 mg $NaNO_3$ / kg b.w. for 20 days, respectively, (table 2) . That decrease ceased and began to increase to the control level in the thirtieth day of inoculation. The decrease observed in total serum

Table (1) : Effect of the daily oral administration of nitrate on rabbits weight increment rate (kg).

Sampling date (after inoculation)	Experimental Groups				
	Control	25 mg $NaNO_3$ /Kg b.w	75 mg $NaNO_3$ /Kg b.w	150 mg $NaNO_3$ /Kg b.w	300mg $NaNO_3$ /Kg b.w
10 Days	0.4 ± 0.01	0.37 ± 0.02	0.37 ± 0.03	0.36 ±0.01	0.31 ± 0.04
20 Days	0.77 ± 0.01	0.69 ± 0.05	0.73 ±0.07	0.68 ± 0.04	0.62 ± 0.06
30 Days	1.14 ± 0.02	0.96 ± 0.4 *	0.96 ±0.03 *	0.97 ±0.02 *	0.83 ±0.08 *

All values expressed as mean ± SE
* Significant differences at $p < 0.05$



Fig(1) :Effect of the daily oral administration of nitrate on rabbits weight increment rate (Kg)

Table (2) : Effect of the daily oral administration of nitrate on rabbits serum protein

Parameters	Sampling date (after inoculation)	Experimental Groups				
		Control	25 mg $NaNO_3$ /Kg b.w	75 mg $NaNO_3$ /Kg b.w	150 mg $NaNO_3$ /Kg b.w	300mg $NaNO_3$ /Kg b.w
Total protein (gm/dl)	ZeroTime	5.33 ± 0.11	5.15 ± 0.20	4.85 ± 0.29	5.22 ± 0.17	5.40 ± 0.21
	10 Days	5.12 ± 0.27	5.20 ± 0.19	5.23 ± 0.19	5.43 ± 0.32	5.57 ± 0.24
	20 Days	5.45 ± 0.18	3.94 ± 0.25 *	3.70 ± 0.13 *	4.15 ± 0.07*	4.63 ± 0.07 *
	30 Days	5.76 ± 0.41	5.63 ± 0.35	5.85 ± 0.27	5.81 ± 0.30	5.97 ± 0.27
Albumin (gm/dl)	ZeroTime	3.15 ± 0.30	3.03 ± 0.15	2.81 ± 0.35	3.12 ± 0.44	3.25 ± 0.34
	10 Days	3.29 ± 0.15	3.18 ± 0.24	3.04 ± 0.10	3.28 ± 0.18	3.20 ± 0.20
	20 Days	2.62 ± 0.26	2.75 ± 0.31	2.89 ± 0.15	3.00 ± 0.12	3.36 ± 0.10
	30 Days	3.36 ± 0.16	3.40 ± 0.12	3.65 ± 0.26	3.78 ± 0.13	3.82 ± 0.26
Globulin (gm/dl)	ZeroTime	2.18± 0.24	2.12 ± 0.27	2.04 ± 0.40	2.10 ± 0.17	2.15 ± 0.20
	10 Days	1.83 ± 0.29	2.17 ± 0.30	2.19 ± 0.14	2.15 ± 0.33	2.38 ± 0.18
	20 Days	2.83 ± 0.41	1.19 ± 0.20 *	0.81 ± 0.16 *	1.15 ± 0.20*	1.27 ± 0.44*
	30 Days	2.41 ± 0.45	2.23 ± 0.25	2.20 ± 0.33	2.03 ± 0.37	2.15 ± 0.29

All values expressed as mean ± SE

* Significant differences at $p < 0.05$

protein is probably concordant with some disorders related with building new proteins by the liver or may be due to the disruption of hepatic cells during the inoculation.

Vice versa was noticed in the response of Albumin to nitrate administration. Albumin concentration in all groups was similar to the control level after 10 days of inoculation then began to increase. It could be noticed that the increment of albumin level coincided with the increment dose of sodium nitrate. In view of the consideration that the liver is the site of albumin production (Rossan, 1960), the formation or derangement in synthesis of albumin is due to a damaged liver. The increase observed in the albumin concentration in the present study is probably due to some faulty reabsorption in the kidneys or disruption of hepatic cell during the inoculation.

Globulin concentration in the treated four groups was low when compared with the control group consequence to the nitrate administration for 20 and 30 days. The higher and significant decrease was observed after 20 days of inoculation. Nitrate may stimulate the production of antigen - specific antibodies of different classes through activation of immune system responses, since globulins, particularly the gamma fraction, plays a special role in protecting the body against infection and toxicity, thus providing the body with the necessary immunity.

Data in table (3), represent the effect of nitrate administration on the activities of rabbit serum glutamate oxaloacetate transaminase (Aspartate Transaminase, AST), glutamate pyruvate transaminase (Alanine Transaminase, ALT), and alkaline phosphatase (AP).

The AST and ALT activities in serum of control rabbits increased to more than 2 fold after 10 days of inoculation compared with that at zero time, then remained fairly constant. No obvious change was observed in the activity of ALT through

Table (3) : Effect of the daily oral administration of nitrate on rabbits serum ALT, AST and Alkaline phosphatase activities (AP).

Parameters	Sampling date (after inoculation)	Experimental Groups				
		Control	25 mg $NaNO_3$ /Kg b.w	75 mg $NaNO_3$ /Kg b.w	150 mg $NaNO_3$ /Kg b.w	300mg $NaNO_3$ /Kg b.w
ALT (IU/ml)	ZeroTime	16.29 ± 1.16	20.93 ±3.49	18.04±2.54	20.04±3.86	16.83±3.82
	10 Days	35.00 ±4.43	28.67 ±6.06	37.33±2.67	40.50±6.44	30.50±2.72
	20 Days	35.33 ±4.84	32.67 ±3.84	39.67±5.78	33.25±2.69	28.50±1.85
	30 Days	40.50 ±2.06	43.00 ±5.51	54.00 ± 2.85*	53.00 ±2.38*	45.50 ±2.60
AST (IU/ml)	ZeroTime	11.65±0.66	10.90±2.13	13.13±1.66	17.07±3.44	14.27±3.33
	10 Days	32.00±4.92	32.33±4.67	42.50±0.29*	44.67±2.85*	43.00±1.63*
	20 Days	37.33±6.36	33.00±7.37	32.67±4.70	36.00±1.43	40.75±5.74
	30 Days	40.75±1.11	39.00±5.20	43.67±1.86	38.00±1.47	35.75±2.02
AP (IU/ml)	ZeroTime	110.33 ± 17.82	107.83 ± 8.08	113.00 ± 16.50	104.00 ±13.30	118.01±4.01
	10 Days	160.00 ± 4.04	130.50 ± 12.50	164.75 ± 17.57	164.50 ±6.37	160.50 ±10.09
	20 Days	104.13 ± 1.52	94.65 ±7.93	112.12 ±3.09	148.37±13.53*	136.29 ±5.93*
	30 Days	155.30 ± 6.77	146.37 ±9.20	146.87 ± 16.26	163.57 ±6.45	151.30 ±2.40

All values expressed as mean ± SE

* Significant differences at $p < 0.05$

the first 20 days of inoculation but a significant elevation, in general, was obvious in the thirtieth day of inoculation. Administration of 75 or 150 mg $NaNO_3$ /kg body weight daily for 30 days caused an increase in the activity of ALT by about 33% as compared with the control rabbits. On the other hand, the daily administration of 75, 150 and 300 mg $NaNO_3$ /kg body weight for 10 days resulted in a significant increase in the activity of AST (table 3). That elevation of AST activity in response to nitrate administration began to decline, approximately, to the control level in the thirtieth day of inoculation.

The elevation in serum transaminase activities may be attributed to some cell damage leading to leakage of the enzymes into the blood. Cornelius *et al.*, (1959), Wroblewski, (1958) and Lagace *et al.*, (1961) pointed out that, the damaged cells are often considered to be a cause of the increased level of enzyme in the sera.

Serum alkaline phosphatase activity (table 3) increased, in general, significantly in response to sodium nitrate administration at levels of 75, 150 and 300 mg/kg b.w. for 20 days by 7.67, 42.49 and 30.88%, respectively. That increase then declined approximately to the control level in the thirtieth day of inoculation. Bell and Freeman, (1971) reported that a high alkaline phosphatase activity is known to occur in cells or tissues exhibiting either a high turnover or a high anabolic rate.

The above mentioned data revealed, without any doubt, that the daily oral administration of sodium nitrate at the different tested levels influenced badly on the function of some organs in the rabbits body .So several attempts must be done to improve technical methods to remove nitrate from drinking water with emphasizing on reducing the irresponsible use of agrochemical, especially, N-fertilizers which could be a cause of the high levels of nitrate in drinking water in the Gaza Strip.

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