Effect Of Oral Administration Of Nitrate On Serum Glucose, Some Lipids, And Non-protein Nitrogen Constituents

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ملخص البحث

ـدف هـذه الدراسة ا تقدير تأث اعطاء تراكيز تلفة من نترات الصوديوم يوميا عن طريق الفم دة 30 يوما على كل من تركيز سكر الغلوكوز وبعض اللبيدات وا كونات النيتروجينية غ ا وتينية س م دم الأرانب.

وقد تب أن إعطاء الأرانب نترات صوديوم دة 30 يوما بتراكيز 25 و75 و150 و300 مللغرام/كيلو غرام من وزن ا سم أدت ا زيـادة ملموسـة تركيز ض البوليك بنسبة 43.68 و68.97 و51.72 و75.86% على الترتيب مقارنة يوانات موعة ا قارنة. كما أن النترات زادت تركيز اليوريا س م حيوانات التجربة ، وذلك اليوم العشرين و اليوم الثلاث أيضا من بداية التجربة . ومن ناحية أخرى فإن إعطاء الأرانب جرعة 150 و 300 مللغرام نترات صوديوم/كغم من وزن ا سم دة 30 يوم سببت زيادة ملموسة تركـيز الكرياتـ سـ م الارانب ا اضعة للتجربة . أما سكر الغلوكوز فقد ارتفع بشكل عام س م الأرانب نتيجة إعطاء ا رعات ا خـتلفة مـن النترات ، وخلال الفترات الزمنية ا ختلفة ا أخذ العينات فيها ، أما تركيز الغلسريدات الثلاثية فقد ا فض نتيجة اعطاء النترات وذلك اليوم العشرين و اليوم الثلاث من بداية التجربة . وقد كان رعات النترات تأث قليل على مستوى الكولسترول س م الارانب . و كن القول أن للنترات بشكل عام حـ التركيزات الضعيفة منها (25 مللغرام نترات صوديوم/كيلوغرام من وزن ا سم) تأث ا ضاراً على الكبد والكلية وأيض الكربوهيدات واللبيدات .

Abstract

An evaluation of the effects of daily oral administration of sodium nitrate for 30 days on serum non-protein nitrogen constituents, e.g., urea, uric acid and creatinine , glucose, cholesterol and triglycerides was carried out. The daily oral administration of NaNO3 for 30 days at levels of 25, 75,150 and 300 mg/kg b.w. increased significantly the concentration of uric acid by 43.68, 68.97, 51.72 and 75.86% , respectively compared to the control level. Nitrate administration raised up the concentration of urea in rabbit's blood serum, in general, at the twentieth and thirtieth days of inoculation. Administration of 150 and 300 mg NaNO3/kg b.w. for 30 days caused a significant increase in the creatinine level when compared with the control level. In general, glucose content of rabbit's blood serum, increased significantly in response to the administration of NaNO3 levels at the different tested intervals within the thirty days of inoculation. Serum triglycerides content, of the rabbits treated with NaNO3 at the different levels, decreased significantly at the twentieth and thirtieth days of inoculation. Nitrate administrations for 30 days, at the different tested levels, have little effect on the cholesterol concentration. In the twentieth and thirtieth day of inoculation, cholesterol concentration slightly decreased in the serum of treated rabbits in response to the different levels of NaNO3. Finally, nitrate administration for 30 days, even at the lowest tested level,i.e. , 25 mg /kg b.w , had harmful effects on liver , kidney , carbohydrate and lipid metabolisms .

Keywords: Administration of sodium nitrate -serum non-protein nitrogen constituents - urea - acid - creatinine - glucose -cholesterol - triglycerides.

INTRODUCTION

The growing need for increasing crop production to meet the increase in the population in the Gaza Strip , the most overpopulated area in the world, and producing the crop in the markets earlier than its right season ; led farmers to use the N-fertilizers excessively and in an irresponsible way.

To protect their crops from pests ; they used pesticides in the same irresponsible way in complete neglect of all the usage prescriptions of the manufacturers . That kind of practice could result in the leakage of nitrates and to a lesser extent pesticides (U.S. EPA ,1990) into underground water . Although the use of chemicals has increased the efficiencies of crop production , the general population is increasingly concerned about potential environmental effects . Ground water contamination is one of these concerns. Contamination of drinking water is a growing problem in the Gaza Strip that is a narrow strip of 365 Km^2 , located in an arid to semi-arid region along the eastern coast of the Mediterranean Sea . Nitrate is one of the undesirable drinking water contaminants that place thousands of people at several acute risks.

In a geomedical study on the geographical distribution of Cancer in the Gaza Strip, Abu Mayla (1995) concluded the presence of a relationship between Cancer incidence and many environmental factors, especially, the pollutants of underground water.

Nitrate per se is not toxic, but is the precursor of nitrite that is produced through microbial reduction of nitrate in the intestine or in food preparations and is linked with the potentially deadly methemoglobinemia in infants (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 reports of the Palestinian Ministry of Health(1997) and GEP Gaza (1996), for the chemical analysis of the ground water in the Gaza strip showed that it is highly contaminated by nitrate . Detected levels of nitrate in drinking water-all potable water supplies in the Gaza strip are obtained from aquifer sources-vary between 15 mg /L and 500 mg /L . The higher levels were recorded , especially, in khan Younis Governorate. However, the World Health Organization drinking water guideline value for nitrate has been set at 45 mg/L (WHO,1985). In the European Union, the maximum admissible nitrate level in drinking water has been set at 50 mg/L (Roux, 1995), which means that over 900,000 people at the Gaza Strip are receiving a water supply that exceeds these limits from municipality water systems for human consumption .

Drinking water containing nitrate -nitrogen in excess of 10 mg / L can cause sometimes fatal blood disorder called methemoglobinemia in infants under the age of six months but it has no apparent short term effects on adults (Terblanche,1991). Consumption of nitrate in drinking water increased 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.

Few or probably no investigations have been conducted on the effects of sodium nitrate administration on animal physiology, especially in the Gaza Strip . So this investigation is an attempt to evaluate the effects of daily oral administration of sodium nitrate at levels of 25 , 75 , 150 and 300 mg / Kg of body weight for 30 days on serum glucose ,Lipids, and non-protein nitrogen constituents , i.e. urea ,uric acid and creatinine.

MATERIALS AND METHODS

i- **Experimental Animals**:

Twenty-five healthy 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 . The 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. The group of rabbits was divided into five subgroups, each containing 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 the rabbits in subgroups 2, 3, 4 and 5, respectively, for 30 days, starting 4 days after delivery from the farm. The rabbits in subgroup (1) acted as a control and received 3 ml distilled water as the other subgroups. Blood samples were collected at zero time, 10, 20 and 30 days after administration.

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 analyses.

iii-Chemical analysis:

Serum samples were analyzed for glucose , triglycerides and total cholesterol by the methods described by Trinder (1969) , Fossati and Prencipe (1982) and Allain (1974) ,respectively. Non-protein nitrogen constituents were determined by the methods of Mackay and Mackay (1927) for urea , Fossati *et al.,* (1980) for uric acid and Bartels and Bohmer (1972) for creatinine.

iv- The statistical analysis:

The statistical analysis for T- test was performed by using SPSS.

RESULTS AND DISCUSSION

Results in table (1), represent the effect of daily oral administration of sodium nitrate for 30 days on the non-protein nitrogen constituents of rabbit's serum ,i.e. urea , uric acid and creatinine .

Uric acid concentration in the serum of treated rabbits in the tenth and twentieth days of inoculation resembled the control level. However, the daily oral administration of $NaNO_3$ for 30 days at levels of 25, 75,150 and 300 mg/kg b.w. increased significantly the concentration of uric acid by 43.68, 68.97, 51.72 and 75.86% in comparison with the control level, respectively. Uric Acid is the end product of the catabolism of tissue nucleic acid ,i.e. purine and pyrimidine bases metabolism (Wolf *et al*.,1972). In the present work , the serum uric acid levels exhibited significant increment in the inoculated rabbit for 30 days. This may be due to degradation of purines and pyrimidines or to an increase of uric acid level by either overproduction or inability of excretion (Wolf *et al*.,1972).

In general, nitrate administration raised up the concentration of urea in rabbit's blood serum at the twentieth and thirtieth days of inoculation table (1) . Urea concentration in rabbit's serum treated with 25, 75, 150 and 300 mg $NaNO_3$ /kg b.w. for 30 days were 1.45 , 1.78, 1.70, and 1.61 times greater than the control level (Fig.1). Urea is the principal end product of protein catabolism .Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea. On the other hand, the elevated serum

urea levels may be due to the destruction of red cells during inoculation. The presence of some toxic compounds might increase blood urea and decrease plasma protein (Varely , 1976). In addition , the elevation of blood urea is a good indicator for kidney diseases .

Inspection of the data in table (1) revealed that the concentrations of creatinine in the four subgroups treated with sodium nitrate were approximately similar to the control level, in the tenth and twentieth days of inoculation. However, administration of 150 and 300 mg $NaNO_3$/kg b.w. for 30 days caused a significant increase in the creatinine level when compared with the control level. Creatinine is the least variable nitrogenous constituent of the blood, it is more readily excreted by the kidneys than urea and uric acid . Serum creatinine concentration is only elevated when kidney function is seriously impaired. About 50% of kidney function must be lost before a rise in the serum concentration of creatinine can be detected (Kaptan and Szabo , 1983). Thus a rise in the serum creatinine concentration occurs with cases of nephritis.

In general, the glucose content of rabbit's blood serum , table (2), increased significantly in response to the administration of $NaNO_3$ at the different tested intervals within the thirty days of inoculation. The highest increase was observed in the twentieth day of inoculation. Administration of $NaNO_3$ at 25, 75 150 and 300 mg/kg b.w. for twenty days increased serum glucose contents of the treated rabbits by 21.78, 17.63, 15.45 and 12 %, respectively, when compared with the control group, (Fig.2). Nitrates may directly or indirectly play a specific role in pancreatic secretions , gluconeogenesis process, glycogen metabolism or glucose oxidation . It means that there was a disturbance in carbohydrate metabolism of rabbits by the daily oral administration of nitrates.

The statistical analysis of the data, table (2), showed that serum triglycerides content of the rabbits treated with $NaNO_3$ at the different levels, decreased significantly at the twentieth and thirtieth days of inoculation . Nitrate administration at levels 25, 75, 150 and 130 mg / kg b.w for 20 days caused a decrease in the serum triglycerides content by 34.84, 38.98 , 22.88 and 15.99% , respectively, compared with the control, (Fig.2). In the thirtieth day of inoculation the decrease was by 26.79, 24.58, 22.68 and 21.73 % ,respectively. The decrease of serum triglycerides contents resulting from administration 25 and 75 mg $NaNO_3$ / kg b.w was more pronounced than that resulting from 150 and 300 mg $NaNO_3$ /kg b.w either in the twentieth or thirtieth day of inoculations .

The data in table (2) showed that, the daily oral administration of

sodium nitrate for 30 days ,at the different tested levels has little effect on the cholesterol concentration. In the twentieth and thirtieth days of inoculation, cholesterol concentration decreased slightly in the serum of treated rabbits in response to the different levels of $NaNO_3$ compared to the control level (Fig.2). The observed changes in serum triglycerides and cholesterol contents take place in the liver due to imbalance between the normal rates of lipid synthesis, utilization and secretion (Glaser and Mager ,1972).

It was noticed from the data in tables (1) and (2) that, there was no specific trend in serum glucose, cholesterol, triglycerides creatinine and uric acid content of control subgroup during the experimental period, similar to that observed by Abd Rabo *et., al* (1992) in glucose and cholesterol in the serum of rabbits.

Finally, from the previous results one can easily conclude that nitrate administration for 30 days, even at the lowest tested level, i.e., 25 mg /kg b.w, had harmful effects on liver, kidney, carbohydrate and lipid metabolisms. Consequently, the municipalities of the Gaza Strip should spare no efforts to supply people with water for human consumption containing less than the maximum admissible nitrate level in the drinking water set by the European Union or better that set by the World Health Organization. Furthermore, effects of nitrate administration for long periods on the body defense mechanisms of the human body need further investigations.

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