Original Article
Toxicology International, *Jan-April 2017 / Vol-24 / Issue-1, 74-79*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2017/v24/i1/149038

The effect of nitrate on some biochemical parameters of rabbits and ameliorate its effect by using vitamin E and Rosemary (Rosmarinus Officinalis)

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Abstract

This study was conducted to investigate the effect of nitrate in the feed of the rabbits on the total protein, albumin, globulin, cholesterol, triglyceride and urea in serum and to study ameliorate nitrate toxicity by using vitamin E and rosemary. Twenty-eight adult New Zealand rabbits were divided randomly into four groups, Group 1(G_2): fed basal ration as a control group. Group 2 (G_2): fed ration supplemented with nitrate (168 mg/kg B.W./day), Group 3 (G_3): fed ration supplemented with nitrate (168 mg/kg B.W./day) plus vitamin E (100 mg/kg B.W.).Group 4 (G4): fed ration supplemented with nitrate (168 mg/kg B.W./day) plus rosemary (500 mg/kg B.W.). The results showed a significant($P \leq 0.05$) decrease in total protein and globulin in the group that fed nitrate (G_2) compared with the control (G_1)and groups fed a mixture of vitamin E and rosemary with nitrate(G_3 and G4). The cholesterol, triglyceride and blood urea concentration showed a significant increase ($P \leq 0.05$)in the group that fed nitrate (G_2) compared with the control group (G_1), whereas the groups that fed vitamin E and rosemary with nitrate(G_3 and G_4) showed a significant ($P \leq 0.05$) decreased in the parameters measured compared with then itrate fed rabbits (G_2). The results illustrated above revealed a remarkable impact of nitrate supplementation causing a significant decrement in protein concentrations along with considerable rise in the cholesterol, triglyceride and blood urea levels which was partially neutralized by vitamin E and rosemary.

Keywords: Nitrate, Rabbit, Rosemary, Vitamin E.

Received: 14-08-2016 **Revised:** 26-11-2016 **Accepted:** 11-12-2016

Introduction

Nitrate poisoning is recorded in many studies accomplished in multiple animal species as well as humans.*N*-nitrosoandnitrite that form when nitrite binds to another component before or after ingestion are toxic and leading to severe pathologies in the body [1]. The exposure tonitrate-nitrogen isstrongly associated with many diseases, such as methemoglobinemia (blue baby syndrome), gastrointestinal cancer, thyroid disorders, reproductive problems and diabetes [2]. In addition, another research group had shown a positive correlation between the incidence of dependent diabetes and nitrate concentrations in the potable water [3]. Several clinical studies predicted hepatorenal and/or hepatotoxicity in humans linked to nitrate intake [4].Nitrites and nitrates are of abundant concern to humans and animals due to their carcinogenic, mutagenic and teratogenic activities[5]. Antioxidant substances are known to neutralize toxicity and this is why they are widely used.In the present study,a mixture of vitamin E and rosemary were used to ameliorate nitrate toxicity. Vitamin E is a very important feed supplement as an effective liposoluble antioxidant[6].

Vitamin E is a potent natural biological antioxidant, which playsvery important role in consolidating of the cellularmembranesdue to their activity as a scavenging of free radicals [7].Natural antioxidants are necessary forgeneral health because theydeactivateharmful

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free radicals resulted from various stress conditions. Rosemary is aromatic herb, which is commonly used as flavoring food, also has been widely used in traditional medicine, rosemary has been used as a stimulant and mild analgesic, and it effective herbs for treating headaches, poor circulation, inflammation [8]. It is a plant with a powerful antioxidant activity [8].It contains some antioxidant components that had been shown to provide a defending role against oxidizing agents generated by oxidative stress,which include carnosol, carnosic acid, ursolic acid, rosmarinic acid[8], and caffeic acid thus effectively participate in lipid peroxidation [9]. Loet al. [10] reported that carnosol, a natural polyphenol component of rosemary leaves, illustrated effective anti-oxidative activity against free radicals.

Material and methods

Twenty-eight adult New Zealand female rabbits aged 10-14 months and approximately 1800 – 2200 grams weight. These rabbits were maintained under suitable environmental conditions,20-25°C,12 hours light and 12 hours dark throughout the experiment. Groups (7 rabbits in each group) were housed indoors inside well separated cages (2x3 meters) containing hard-wood chips as bedding. The rabbits were cared for a month prior to the experiment for adaptation and to estimate the average amount of daily food consumption rate. Rabbits were divided randomly into four groups: Group 1 (G_1): fed basal ration as a control group. Group 2 (G_2): fed ration supplemented with potassium nitrate (KNO_3) (168 mg / kg B.W. / day) according to Al-rawi[11]and named as nitrate treated group,Group 3 (G_3): given ration supplemented with a mixture of potassium nitrate (168 mg / kg B.W. / day) plus vitamin E (100 mg / kg B.W.) according to Alqayim and Asis,[12]. Group 4 (G_4): fed ration complemented with potassium nitrate (168 mg / kg B.W. / day) plus rosemary (500 mg / kg B.W.) according to Andon et al.[13]who determined the lethal dose 50 (LD_{50}) which is greater than 2,000 mg/kg body weight. Through the experiment, blood samples were collected from heart at zero, eight, twelve and sixteen weeks intervals. Total protein concentrations were measured following spectrophotometric method (Biolabokit) along with albumin, globulin,cholesterol, triglyceride and blood urea levels in serum according to Tietz[14].

Statistical analysis: The data were presented as Mean and Standard Error (Mean±SE) and subjected to statistical analysis using Two-way analysis of variance (ANOVA). In addition, post hoc test was used to find out the least significant differences (LSD) between different means (P≤0.05). Statistical Package for the Social Sciences program (SPSS) version 21 package was used for this purpose[15].

Results

There was a negative impact of nitrate supplementation on the total protein concentration in nitrate fed rabbits (G_2),which demonstrated a significant (P≤0.05) decrease compared with control group (G_1), and groups that fed mixture of nitrate plus vitamin E(G_3) and nitrate plus rosemary(G_4) during last period of experiment (16^{th} week) (Table 1). Albumin concentration revealed slight fluctuations in all groups, but it was lowest in nitrate fed animals (G_2) (Table 2). Globulin levels significantly declined(P≤0.05)in group that fed with nitrate (G_2),towards the end of the experiment specifically at 12^{th} and 16^{th} weeks of experiment(Table 3).Serum cholesterol and triglyceride levels soared significantly (P≤0.05) in group two (G_2)in comparison with the other groups (G_1, G_3 and G_4) (Tables 4 and 5). Interestingly, the effect of

Table 1. The effect of nitrate and vitamin E or rosemary on the serum total protein (g/dl)

Groups Time (week)	G_1 Control	G_2 Nitrate	G_3 Nitrate+Vit. E	G_4 Nitrate+Rosmary
Zero	6.94±0.21 Aa	7.07±0.08 Aa	6.98±0.18 Aa	6.99±0.22 Aa
8^{th}	6.81±0.17 Aa	6.89±0.07 Aab	6.96±0.20 Aa	7.05±0.25 Aa
12^{th}	6.92±0.12 a	6.49±0.13 Bb	7.08±0.08 Aa	6.98±0.20 Aa
16^{th}	7.10±0.13 Aa	6.42±0.22 Bb	7.21±0.09 Aa	7.23±0.8 Aa

The different capital letters refer to a significant difference between different groups at (P<0.05)
The different small letters refer to a significant difference between different times at (P<0.05)

nitrate on the blood urea was depicted in table (6) which clearly reveals its significant sharp rise (P≤0.05) in (G_2) during the late stages of experiment 12th and 16th weeks compared with control group (G_1).Finally, the data reported a significant (P≤0.05) increased blood urea levels within groups that fed nitrate (G_2) in 16th week compared with the values registered at the start(zero time).

Table 2. The effect of nitrate and vitamin E or rosemary on the serum albumin(g/dl)

Groups Time (week)	G_1 Control	G_2 Nitrate	G_3 Nitrate+ Vit. E	G_4 Nitrate+ Rosmary
Zero	3.88±0.17	3.52±0.21	3.69±0.11	3.63±0.09
8th	3.67±0.09	3.49±0.10	3.57±0.09	3.60±0.18
12th	3.97±0.16	3.42±0.16	3.47±0.08	3.34±0.23
16th	3.84±0.06	3.54±0.13	3.65±0.15	3.56±0.19

LSD= 0.35

Discussion

Nitrate supplementation has negatively influenced total protein concentrations (Table 1).This could be attributed to nitrate toxicity as many studies referred to this notion in humans and animals; therefore, nitrate classified as an oxidative agent [16].In line with above, excessive nitrate levels could harmfully manipulate food conversion efficiency and mediate endocrinological disturbances[17], thus it leads to an enormous reduction of nutrient digestibility and eventually loss of body weight [18]. In addition,nitrate-induced thyroid hormones deficiency could minimize body weight by inhibiting food intake through its impact on the nerve cells that could result in deprived appetite. Added to above, thyroid hormones (mainly thyroxin) deficiency inhibits the synthesis of growth hormone releasing factor (GHRF) in the hypothalamus and leads to growth retard symptoms, thus

Table 3. The effect of nitrate and vitamin E or rosemary on the serum globulin (g/dl)

Groups Time (week)	G_1 Control	G_2 Nitrate	G_3 Nitrate+Vit. E	G_4 Nitrate+Rosmary
Zero	3.05±0.08 Aa	3.35±0.26 Aa	3.29±0.10 Aa	3.35±0.22 Aa
8th	3.13±0.21 Aa	3.40±0.16 Aa	3.18±0.17 Aa	3.34±0.20 Aa
12th	3.14±0.07 ABa	3.06±0.27 Bab	3.60±0.09 Aa	3.64±0.30 Aa
16th	3.22±0.17 ABa	2.87±0.12 Bb	3.55±0.13 Aa	3.66±0.24 Aa

The different capital letters refer to a significant difference between different groups at (P<0.05)
The different small letters refer to a significant difference between different times at (P<0.05)

Table 4. The effect of nitrate and vitamin E or rosemary on the serum cholesterol (mg/dl)

Groups Time (week)	G_1 Control	G_2 Nitrate	G_3 Nitrate+Vit. E	G_4 Nitrate+ Rosmary
Zero	55.36±8.25 Aa	52.74±4.72 Ab	56.18±3.12 Aa	57.88±6.85 Aa
8th	62.84±7.24 Aa	56.82±7.36 Ab	55.38±4.16 Aa	55.92±3.97 Aa
12th	58.17±8.25 Aa	64.53±5.11 Aab	55.07±6.64 Aa	57.54±10.94 Aa
16th	59.91±6.01 Ba	72.81±8.68 Aa	58.22±5.62 Ba	56.14±8.06 Ba

The different capital letters refer to a significant difference between different groups at (P<0.05)
The different small letters refer to a significant difference between different times at (P<0.05)

Table 5. The effect of nitrate and vitamin E or rosemary on the triglyceride (mg/dl)

Groups Time (week)	G_1 Control	G_2 Nitrate	G_3 Nitrate+Vit. E	G_4 Nitrate+Rosmary
Zero	82.77±7.86 Aa	76.28±8.12 Ac	79.46±4.74 Aa	81.75±6.20 Aa
8th	84.14±11.32 Aa	89.67±6.94 Abc	81.03±5.38 Aa	81.21±10.29 Aa
12th	83.33±8.16 ABa	99.55±11.50 Ab	80.68±7.94 Ba	76.36±7.81 Ba
16th	82.19±3.85 Ba	118.60±9.14 Aa	77.94±7.30 Ba	72.12±9.51 Ba

The different capital letters refer to a significant difference between different groups at (P<0.05)
The different small letters refer to a significant difference between different times at (P<0.05)

Table 6. The effect of nitrate and vitamin E or rosemary on the blood urea (mg/dl)

Groups Time (week)	G_1 Control	G_2 Nitrate	G_3 Nitrate+Vit. E	G_4 Nitrate+Rosmary
Zero	45.12±4.90 Aa	45.97±5.57 Ab	46.23±5.40 Aa	47.01±11.84 Aa
8th	48.85±3.68 Aa	57.45±2.19 Aab	50.70±3.96 Aa	49.41±3.26 Aa
12th	46.05±3.92 Ba	58.24±4.96 Aa	49.94±5.38 ABa	52.04±5.83 ABa
16th	45.37±3.07 Ba	59.75±3.24 Aa	51.70±4.12 ABa	54.30±4.74 ABa

The different capital letters refer to a significant difference between different groups at (P<0.05)
The different small letters refer to a significant difference between different times at (P<0.05)

all these factors may interfere with the metabolic pathways causing sharp drop in the total protein concentrations. The paradox effect of vitamin E and/or rosemary which is the antitoxic activities could be implicated to the antioxidant function, rosemary is known as a biologically active phytochemical antioxidant as it contains phenolic compounds such ascarnosol, carnosic acid, isorosmanol, rosmanol, rosmadial,7-methyl-epirosmanol and caffeic acid[19].

Decreased albumin and globulin levels in (G_2)can be attributed to the same reasons mentioned above in addition to endocrine malfunctions and disorders as many studies highlighted that concept[16]. This may interpret why nitrate considered as a stress agent. The risk of nitrate toxicity mainly belongs to formation of reactive oxygen species, free radicals as well as other toxic transient compounds such as, peroxynitrite hydrogen peroxide and super oxide anion, which interrupt the equilibrium between oxidants and antioxidant, causing what so called oxidative stress [17], which results in an increase in cortisol hormone secretion (from adrenal gland) under oxidative stress, that leads to suppress immunity and reduces globulin concentrations. Furthermore, excess nitrite significantly harness B lymphocytes proliferation, thus interfere with antibody production [22].The findings in this study are in line with EL-Tahan et al. [19] who support the antagonistic effect on nitrate on the immune system. On the other hands, vitamin E and rosemary ameliorate the nitrate effect which are obviously observed in (G_3) and (G_4), by improving the globulin level compared with control group. This indicates thermostatically activity of vitamin E and rosemary [20]. Vitamin E is one of the most frequent studied nutrients presenting immunomodulation properties [21].Also, these results are consistent with Al-Sheyab et al. [22].Vitamin E is a natural antioxidant targeting cell membranes plus it enhances phagocytic activity of macrophages [23]. Rosemary activitywas described by Shokrollahi et al. [20] who used three concentrations (100, 200, 400 mg/kg/day) for 42 days showing remarkably increased globulin concentration in serum of goat kids which are nearly parallel to what I have found. I noted that nitrate caused an elevation of lipid profile in the serum, this might be due to impairment of liver function summarized by inhibition of enzymes responsible for conversion of cholesterol into bile acids. Sacharand Raina,[24] observed histological changes represented by hepatic severe degeneration to necrosis as a response to nitrate toxicity. I agreed with Azeezet al. [25] who reported that considerable rise in the concentration of creatinine and cholesterol in rats serum that fed nitrate. In contrast, the results revealed an obvious reduction in the toxic effect resulted from the nitrate administration by rosemary and vitamin E. This is consistent with many studies that interprets the antitoxic effect of rosemary and its extracts which are attributed to its antioxidant activity. Rosemary plays a protective role against hyper glycemia and hypercholesterolemia-induced by oxidative stress, thus keeps balanced blood stream[26].Sufficient vitamin E supplementation is recommended to protect from generation of toxic agents and free radicals damage. In addition, vitamin E effectively prevents lipid per oxidation [27].

Urea concentrations increment in nitrate fed rabbits (G_2) table (6), this was because the metabolic dysfunction, that is to say, the major portion of the ingested nitrate is excreted through urine as a crude nitrate, urea or ammonia, while fecal excretion is negligible thus this process participate negatively in urea synthesis. In the same way, nitrate has nephrotoxic effect, which agreed with Shour et al. [28]who reported that drenching rabbits with 25, 75, 150 and 300 mg $NaNO_3$ /kg b.w. for 30 days resulted in higher serum urea levels at 1.45, 1.78, 1.70, and 1.61 rates greater than the control level. A mixture of vitamin E and rosemary decreased the nitrate toxic effect, might be by protecting renal tissue because vitamin E was

reported as an excellent antioxidant that protects cells and tissues from harmful lipoperoxidation damage. Rosemary contains poly phenolic flavonoids compounds such asrosmaridiphenol, rosmadial, carnosol, carnosic and rosmarinic acid[29], these active compounds act to inhibit carnosic and rosmarinic acid in pulmonary,hepatic and renal cells[30].

Conclusion

To sum up, the prolonged exposure of rabbits to nitrate causes significant drop in the total protein and globulin concentrations, and increases cholesterol, triglyceride and urea in the serum. Toxic levels of nitrate can be resolved by supplementing vitamin E and rosemary to the diet which is strongly recommended to combat the toxic effect of nitrate.

Conflict of Interest

None Declared

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