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Pathophysiological Effects of Vitamin C and E- Selenium Combination on Lipid Profile and Serum Glucose of Experimentally Induced Sodium Nitrate Intoxication in Mice

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ABSTRACT

To evaluate the effect of sodium nitrate (NaNo3), vitamin E - selenium combination and vitamin C administration on total serum bilirubin , triglyceride, cholesterol and blood glucose. Sixty healthy adult male mice divided randomly into six groups:- **Group1,**received vitamin E - selenium combination solution 0.5 ml / litter(L) distal water(D.W) daily for 2 weeks, **group2,** received vitamin C solution 0.5 gm / L (D.W) daily for 2 weeks, **group 3,** received NaNo3 solution 0.5gm/L (D.W) daily for 2 week, **group 4,** received 70 mg/kg NaNo3 solution via gavage needle and received vitamin E - selenium combination solution 0.5 ml / L (D.W) daily for 2 weeks . **Group 5,** received NaNo3 solution (170 mg/kg) via gavage needle and received vitamin C solution 0.5 gm / L (D.W) daily for 2 weeks, **group 6**(Control) receive (D.W) daily for 2 weeks. No significant difference in total serum bilirubin level was reported between control and other groups. Significant difference in total serum bilirubin level reported between (NaNO3) group and (NaNO3+ vitamin C) group (value =0.043813). Significant difference in triglyceride level reported between control group and all treated groups. Significant difference in triglyceride level reported between (NaNO3) group and all treated groups except (vitamin C+NaNO3), p value =0.316852. Significant difference in Cholesterol level reported between control group and all treated groups . Significant difference in Cholesterol level reported between (NaNO3) group and (vitamin C) treated group (p value=0.003725), (vitamin C+NaNO3), p value <0.0001.Significant difference in glucose level reported between control group and all treated groups. Significant difference in glucose level reported between (NaNO3) group and (vitamin C) treated group, (vitamin E - selenium combination +NaNO3), (vitamin C+NaNO3) treated group (p value<0.0001). This study revealed that administration of vitamin C and vitamin E - selenium combination have obvious effect on amelioration of toxic effects of NaNo3 on normal physiology of liver. Vitamin C decrease the level of total serum bilirubin and increase serum cholesterol level in mice treated with NaNO3. Vitamin E - selenium combination have the ability to decrease the level triglyceride in mice treated with NaNO3.

Keywords: Vitamin E , Vitamin C, lipid profile, sodium nitrate

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INTRODUCTION

In recent years, considerable attention has been paid to the problem of nitrate due to the intensive use of nitrates as agricultural fertilizers which reach to humans and animals by different routes [1] . Nitrate is a naturally occurring form of nitrogen and is an integral part of the nitrogen cycle in the environment. It is formed from fertilizers, decaying plants, manure and other organic residues. Nitrate is found in air, soil, water, and vegetables food and is produced naturally within the human body [2]. Due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture, vegetables and drinking water may contain higher concentrations of nitrate than in the past [3].

The major source of nitrate in the human body is through intake of food and water [4]. Vegetables may account for more than 70% of the nitrates in a typical human diet [4] . Drinking water may contain variable amounts of nitrates which accounts for up to 21% of total nitrates intake in a typical human diet [3] . The presence of nitrate in vegetable as in water and generally in other foods is a serious threat to man's health. Nitrate per se is relatively non-toxic[5] but approximately 5% of all ingested nitrate is converted by microflora in the gastrointestinal tract to the more toxic nitrite [6].Nitrite and N- nitroso compounds which form when nitrite binds to other substances before or after ingestion are toxic and can lead to severe pathologies in humans [7]. The ability of animals to resist the toxic effects of environmental agents is dependent on the detoxication and antioxidant systems. Recently, several nutrients and other chemicals are effective antioxidants, such as vitamins, trace elements, amino acids and their derivatives, fatty acids and plant phenolics [4]. The antioxidant vitamin C (ascorbic acid) and E (α, β, δ, and γ tocopherols and tocotrienols) are involved in protecting cellular organelles from oxidative damage [8, 9]. The aim of current study is to evaluate the effect of sodium nitrate ,vitamin E and vitamin C administration on total Serum Bilirubin , Triglyceride, Cholesterol and blood glucose .the second aim is to evaluate the Amelioratory effects of vitamin E and C in sodium nitrate treated mice and their effect on parameters under investigation .

MATERIAL AND METHODS

Sixty healthy adult albino male mice obtained from ministry of health, Drug investigation Department. Vitamin E (20 IU) + selenium (260 mcg) combination solution prepared by dissolving 0.5 ml / L(DW) [10] . Ascorbic acid (Vitamin C) solution prepared by dissolving 0.5 gm /L (DW)[11].Sodium nitrate solution was prepared by dissolving 0.5 gm /L (DW)[12].

Experimental Design:

Sixty healthy adult male mice were kept in standard conditions. They were maintained on standard animals feeds and drinking distillated water in room of 20-25°C, with half day light in animal house; college of veterinary medicine, university of Diyala. They were divided randomly into six groups:-

Group1, received vitamin E - selenium solution 0.5 ml /L (DW) daily for 2 weeks. Group2, received vitamin C solution 0.5 gm /L (DW) daily for 2 weeks. Group 3, received NaNo3 solution 0.5gm/L (DW) daily for 2 week. Group 4 ,received NaNo3 solution orally in dose 70 mg/kg and received vitamin E - selenium combination solution 0.5 ml / L (DW) daily for 2 weeks . Group 5 received NaNo3 solution orally in dose170 mg/kg and received vitamin C solution 0.5 gm / L (DW) daily for 2 weeks. Group 6 (Control) receive distal water daily for 2 weeks .

Statistical analysis :

Data were shown as the mean ± SD .Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a VassarStats Website for Statistical Computation software [13]. Significant level of ($P<0.05$). The least significant differences (LSD) was used to identify significant differences[14] .

RESULTS

The value of total serum bilirubin (TSB), expressed in (mean±SD) among different groups was illustrated in table (1). High level of TSB reported in mice exposed to vitaminE(0.6100± 0.07379),followed by NANO3 group (0.6000± 0.15635) , vitamin E - selenium combination +NaNO3(59.8100±3.94030).Low level of TSB reported in mice exposed to vitamin C+NaNO3 (0.4800± 0.07888) .As shown in table(2) , No significant difference in

TSB level was reported between control and other groups. Significant difference in TSB level reported between (NaNO3) group and (NaNO3+ vitamin C) group (value =0.043813).

Table(3)showed the value of triglyceride (mean±SD) (U/L) among different mice groups .High level of triglyceride reported in mice exposed to (Vitamin C+NaNo3) , (277.8000± 2.04396),followed by (NaNo3) group (269.7000± 24.81957).Low level of triglyceride reported among control group (105.0000± 4.21637) .

As shown in table (4) , significant difference in triglyceride level reported between control group and all treated groups . Significant difference in triglyceride level reported between (NaNO3) group and all treated groups except (vitamin C+NaNO3), p value =0.316852.

High level of Cholesterol reported in mice exposed to (NaNO3+vitamin C) (159.2000± 0.78881) ,followed by (vitamin C) group, (142.6000± 10.95648) and vitamin E - selenium combination (133.4000± 12.17648) as shown in Table(5) .Low level of Cholesterol reported among control (114.4000± 4.27395). As shown in table (6) , Significant difference in Cholesterol level reported between control group and all treated groups . Significant difference in Cholesterol level reported between (NaNO3) group and (vitamin C) treated group (p value=0.003725), (vitamin C+NaNO3), p value <.0001.

High level of Glucose reported in mice exposed to (NaNO3+vitamin C) (241.8000± 2.78089) ,followed by (NaNO3+VITE) group, (202.8000± 17.98641) and vitamin E - selenium combination (155.2000 ± 5.07280) .Low level of Cholesterol reported among (vitamin C) treated group (135.8000 ± 9.00370) as shown in Table (7). Significant difference in glucose level reported between control group and all treated groups. Significant difference in glucose level reported between (NaNO3) group and (vitamin C) treated group , (vitamin E - selenium combination +NaNO3) , (vitamin C+NaNO3) treated group (p value<.0001) as shown in table (8).

Table (1): Descriptive Statistic Of Total Serum Bilirubin Among Different Groups

Total Serum Bilirubin (mg/dL)	No.	Minimum	Maximum	Mean ± SD
Control	10	0.40	0.70	0.5100 ± 0.13703
Vitamin E- selenium combination	10	0.50	0.70	0.6100± 0.07379
Vitamin C	10	0.40	0.70	0.5667± 0.12247
NaNo3	10	0.40	0.80	0.6000± 0.15635
Vitamin E - selenium combination + NANO3	10	0.40	0.60	0.4900± 0.07379
Vitamin C+NaNO3	10	0.40	0.60	0.4800± 0.07888

Table (2): Comparison Of Total Serum Bilirubin Among Different Groups .

Total serum bilirubin (mg/dL)		ANOVA Compared with Control		ANOVA Compared with NaNo3	
		F	P value	F	P value
Vitamin E - selenium combination	Between Groups	4.12844	0.057197	0.03	0.864423
	Within Groups				
Vitamin C	Between Groups	1.445902	0.244767	1.69	0.210952
	Within Groups				
NANO3	Between Groups	1.874036	0.187856		
	Within Groups				
NaNO3+ vitamin E- selenium combination	Between Groups	0.165138	0.689261	4.05	0.059380
	Within Groups				
NaNO3+ Vitamin C	Between Groups	0.36	0.555985	4.7	0.043813
	Within Groups				

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Table (3): Descriptive Statistic Of Triglyceride Among Different Groups

Triglyceride (mg/dL)	No.	Minimum	Maximum	Mean ± SD
Control	10	100.00	110.00	105.0000± 4.21637
Vitamin E - selenium combination	10	185.00	211.00	192.6000± 9.86802
Vitamin C	10	185.00	264.00	205.3000 ± 31.42204
NaNo3	10	236.00	295.00	269.7000± 24.81957
Vitamin E - selenium combination +NaNo3	10	85.00	92.00	87.7000± 2.94581
Vitamin C+NaNo3	10	275.00	280.00	277.8000± 2.04396

Table (4): Comparison Of Triglyceride Among Different Groups.

Triglyceride (mg/dL)		ANOVA Compared with Control		ANOVA Compared with NaNo3	
		F	P value	F	P value
Vitamin E - selenium combination	Between Groups	666.3821	1.13E-15	83.33	<.0001
	Within Groups				
Vitamin C	Between Groups	100.0882	8.87E-09	25.87	<.0001
	Within Groups				
NANO3	Between Groups	427.9988	5.37E-14		
	Within Groups				
Vitamin E - selenium) +NaNO3	Between Groups	113.1294	3.43E-09	530.25	<.0001
	Within Groups				
Vitamin C+NaNO3	Between Groups	13600.13	2.29E-27	1.06	0.316852
	Within Groups				

Table (5): Descriptive Statistic Of Cholesterol Among Different Groups

Cholesterol (mg/dL)	No.	Minimum	Maxi-mum	Mean± SD
Control	10	110.00	120.00	114.4000± 4.27395
Vitamin E - selenium combination	10	119.00	148.00	133.4000± 12.17648
Vitamin C	10	128.00	152.00	142.6000± 10.95648
NaNO3	10	110.00	136.00	126.8000± 10.25020
NaNO3+ Vitamin E - selenium combination	10	128.00	136.00	130.8000± 2.97396
NaNO3+Vitamin C	10	158.00	160.00	159.2000± 0.78881

Table (6): Comparison Of Cholesterol Among Different Groups.

Cholesterol (mg/dL)		ANOVA Compared with Control		ANOVA Compared with NaNo3	
		F	P value	F	P value
Vitamin E - selenium	Between Groups	21.67734	0.000197	1.72	0.206176
	Within Groups				
Vitamin C	Between Groups	57.49647	5.21E-07	11.09	0.003725
	Within Groups				
NaNO3	Between Groups	12.46703	0.002387		
	Within Groups				
NaNO3+ Vitamin E- selenium	Between Groups	99.20656	9.5E-09	1.4	0.252116
	Within Groups				
NaNO3+Vitamin C	Between Groups	1062.551	1.85E-17	99.33	<.0001
	Within Groups				

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Table (7): Descriptive Statistic of Glucose among Different Groups

Glucose (mg/dL)	No.	Minimum	Maximum	Mean± SD
Control	10	142.00	150.00	146.8000 ± 3.01109
Vitamin E - selenium combination	10	148.00	160.00	155.2000 ± 5.07280
Vitamin C	10	119.00	142.00	135.8000 ± 9.00370
NaNO3	10	148.00	158.00	152.2000± 4.13118
NaNO3- Vitamin E - selenium combination	10	188.00	227.00	202.8000± 17.98641
NaNO3+Vitamin C	10	239.00	245.00	241.8000± 2.78089

Table (8): Comparison Of Glucose Among Different Groups

Glucose (mg/dL)		ANOVA Compared with Control		ANOVA Compared with NaNo3	
		F	P value	F	P value
Vitamin E - selenium combination	Between Groups	20.27586	0.000275	2.1	0.164498
	Within Groups				
Vitamin C	Between Groups	13.42456	0.001776	27.41	<.0001
	Within Groups				
NaNO3	Between Groups	11.15816	0.003642		
	Within Groups				
Vitamin E - selenium combination +NaNO3	Between Groups	94.29373	1.4E-08	75.18	<.0001
	Within Groups				
Vitamin C+NaNO3	Between Groups	5372.024	9.6E-24	3237.16	<.0001
	Within Groups				

DISCUSSION

The increase in total bilirubin level in the serum of the sodium nitrate treated mice in the present study could be attributed to the increase in the rate of red blood corpuscles destruction and/or damage of the liver tissue[15]. Antioxidant were found to reduce oxidative radical induced reactions and have protective effect on stabilization of metabolic process in erythrocytes that prevent the development of oxidative and hypoxia[16].It is generally believed that vitamin E scavenges free radicals[17] . The hypercholesterolemia due to mobilization of free fatty acids from the adipose tissue to the blood stream and increase of Acetyl-COA, leading to increase in the synthesis of cholesterol or due the synthesis of cholesterol or due to peroxidation of cell membrane lipids [18].Propolis therapy has been demonstrated to reduce the high levels of triglycerides, total cholesterol and esters of cholesterol, probably through the antioxidant mechanism exerted by the flavones in propolis [19]

The intensification of the oxidative stress enhances the influence of non-essential fatty acids which, in turn, increase the serum and tissue levels of cholesterol and triglycerides. It has been shown that antioxidants and flavonoids can act as inhibitors of lipid peroxidation by neutralizing the radicals of polyunsaturated fatty acids and by interrupting the chain reactions [20]

Lipid peroxidation is an important biological consequence of cellular oxidative stress and is one of the main causes of hepatic lesions produced by CCl4 mediated by the free radicals derived from this toxic substance [21]. significant increase in serum glucose level was observed in sodium nitrite intoxicated mice This finding suggests nitrite- stimulation of gluconeogenesis and glucose shift from tissue to blood or an impairment of glucose mobilization [22]. Furthermore, nitroso-compounds can alter the antioxidant system causing disturbance in the metabolic processes leading to hyperglycemia[23]. The obtained results go in parallel with those reported on the hyperglycemic effect of sodium nitrite in rats [24]

The hepatoprotective effect of vitamin C is said to be associated with it oxidative property. Vitamin C is a water-soluble antioxidant which decreases lipid peroxidation either directly or indirectly by regenerating vitamin E, the major lipid-soluble antioxidant [25]. Vitamin C was also reported to scavenge aqueous reactive oxygen species (ROS) by rapid election transfer that inhibits lipid peroxidation [25] .In this work it is ob-

served that vitamin C may have hepatoprotective effect. This hepatoprotective effect tends to increase synergistically when co administered with other agents precisely antioxidants.[25].

CONCLUSION

This study revealed that administration of vitamin C and Vitamin E - selenium combination have obvious effect on amelioration of toxic effects of NaNo3 on normal physiology of liver. Vitamin C decrease the level of total serum bilirubin and increase serum cholesterol level in mice treated with NaNO3. Vitamin E - selenium combination have the ability to decrease the level triglyceride in mice treated with NaNO3.

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