Effect of Vitamin E and C Supplementation on Liver Enzymes of Mice Exposed to Sodium Nitrate

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Abstract

Background: The liver is the major organ responsible for metabolism, detoxification, and secretory functions in the body. Vitamin C is an essential co-factor, acting as a reducing agent and may have heaptoprotective property. Vitamin E is an antioxidant factor. Sodium nitrate is a salt and an anti-oxidant that is used as a preservative.

Objective: To evaluate the effect of sodium nitrate, vitamin E and vitamin C administration on liver enzymes, alanine transaminase, aspartate transaminase and alkaline phosphatase.

Materials and Methods: Sixty healthy adult male mice divided randomly into six groups, group 1, received vitamin E solution 0.5 ml / liter distal water daily for 2 weeks. group2, received vitamin C solution 0.5 gm / liter distal water daily for 2 weeks. Group 3, received sodium nitrate solution 0.5gm/liter distal water daily for 2week. Group 4, received sodium nitrate solution orally in dose170 mg/kg body weight and received vitamin E solution 0.5 ml / liter distal water daily for 2 weeks. Group 5, received sodium nitrate solution orally in dose170 mg/kg body weight and received vitamin C solution 0.5 gm / liter distal water daily for 2 weeks. Group 6 (Control), receive distal water daily for 2 weeks. After the last day of administration, the animals were euthanized and blood samples were drawn from the heart by cardiac puncture into plain tubes, and then subjected to biochemical assays.

Results: A significant difference in alanine transaminase level was reported between control group and group of mice received vitamin E (P-value = 0.001), vitamin E+ NaNO3 (P value =0.015). No significant difference in alanine transaminase level was reported between control and other groups. Significant difference in aspartate transaminase level reported between control group and group of mice received vitamin E (P-value = 0.000202573), vitamin C (P value = 0.00143), NaNO3 (p value=0.008076). No significant difference in aspartate transaminase level was reported between control and group of mice received (vitamin E+ NANO3) (vitamin C+NANO3) groups. Significant difference in alkaline phosphatase level reported between control group and group of mice received vitamin E (P-value = 4.5E-13), vitamin C (P value = 4.45E-18), (p value=6.17E-06), vitamin E+ NANO3 (p-value=3.68E-15). No significant difference in aspartate transaminase level was reported between control and group received (Vitamin C+ NANO3), p-value = (0.091718).

Conclusion: Vitamin E and C have the ability to ameliorate the effect of sodium nitrate in exposed groups although the effect of vitamin E more obvious due to pharmaceutical formulation, finally antioxidant effects of vitamins leads to modulation the nitrate toxicity and hepatoprotective effect.

Key worlds: Sodium nitrate, vitamin E, vitamin C, liver enzymes

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Introduction

Vitamin E is a natural component of the membrane lipid bilayer and thus helps to maintain membrane stability [1]. The molecular and cellular effects of vitamin E have been explained either by acting as an antioxidant preventing damage to membranes or proteins and regulating their activity by specifically scavenging reactive oxygen species [2], or by interacting and regulating specific enzymes and influencing cellular structures such as membranes and lipid domains[3] . Vitamin C which was discovered by Szent-Gyorgyi (1928)[4], is a six-carbon compound structurally related to glucose, consisting of two inter-convertible compounds: L- ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L dehydroascorbic acid. Vitamin C is found in citrus, soft fruits and leafy green vegetables. Kidney and liver are good animal-derived sources of vitamin C[5]. Vitamin C is an essential co-factor involved in many biochemical functions and acts as an electron donor or reducing agent. Vitamin C has been reported by researchers to have heaptoprotective property [6].

The liver is the major organ responsible for metabolism, detoxification, and secretory functions in the body. Hence, it regulates various important metabolic functions in mammalian systems. Hepatic damage is associated with the distortion of these metabolic functions. The liver tissue is reported to be one of the tissues with a high regenerative capacity [7]. The liver functions includes: Bile synthesis and secretion; bilirubin conjugation and excretion. Storage of glycogen; vitamins (A, D, E, K, B12) and metals (iron, copper). In addition to metabolism of protein (plasma protein synthesis; urea formation; amino acid interconversions - deamination) [8].

The toxic effects of nitrates and nitrites are well documented in mammalians including impairment of reproductive function, hepatotoxicity, dysregulation of inflammatory responses and tissue injury, growth retardation, and endocrine disturbance [9]. It inhibits a number of anti-tumor cytotoxic effector cell types as natural killer cells against pathogens and tumor cells [10]. Sodium nitrite exerts its effect by generation of free radicals that impair oxidant / antioxidant balance [11]. Nitrite, in high concentrations, is undoubtedly toxic to humans. Acute effects have been observed from accidental ingestion, for example in contaminated drinking water, sausages, and medicines [12].

The principal toxic effect is oxidation of oxyhemoglobin to ferrihemoglobin, leading to methemoglobinaemia. This can be fatal, particularly in newborn infants in which the methemo-globin-reducing capacity is low, leading to so-called `blue baby syndrome [13]. Excessive levels of nitrate can be reduced to nitrite which couples with oxyhaemoglobin NaNO3 resulting in formation of MetHb.

So this study aim to determine the serum level of alanine transaminase (ALT) ,aspartate transaminase (AST) and alkaline phosphatase (ALP) in mice exposed to vitamin E, Vitamin C and sodium nitrate

Materials and Methods

Experimental design

Sixty healthy adult male mice were kept in well-aerated laboratory cages at Department of physiology and pharmacology; College of veterinary medicine; University of Diyala (all were healthy adult albino male mice obtained from ministry of health, drug investigation department) during the period from 1st October, 2014 to 1st October, 2015.

.They were allowed to acclimatize to the laboratory environment for one week before the study commenced. They were maintained on standard animals feeds and drinking distillated water and kept in room of 20-25°C, with half day light.

Sixty healthy adult male mice divided randomly into six groups. Group1, received vitamin E solution 0.5 ml / liter distal water daily for 2 weeks. Group2, received vitamin C solution 0.5 gm / liter distal water daily for 2 weeks. Group 3, received sodium nitrate solution 0.5gm /liter distal water daily for 2week. Group 4, received sodium nitrate solution orally in dose 170 mg/kg (0.1cc orally via gavage needle) and received vitamin E solution 0.5 ml / liter distal water daily for 2 weeks. Group 5, received sodium nitrate solution orally in dose 70 mg/kg body weight (0.1cc orally via gavage needle) and received vitamin C solution 0.5 gm / liter distal water daily for 2 weeks. Group 6 (Control) receive distal water daily for 2 weeks.

Vitamins

Vitamin E and C were obtained from local market of Baqubah. The vitamin E which is given as solution prepared by dissolving 0.5 ml on 1 liter distal water according to manufacturer (AVICO- Jordan). The vitamin C which is given as solution prepared by dissolving 0.5 gm on 1 liter distal water according to manufacturer (vitacost-USA).

Sodium nitrate

Sodium nitrate (riedeldehaen - Germany) as solution prepared by dissolving 0.5 gm on 1 liter distal water ,while the dose via gavage needle 0.1cc (70mg/kg body weight) according to [14]. With modification

Samples collection

After the last day of administration, the animals were euthanized and blood samples were drawn from the heart of each by cardiac puncture into plain tubes, allowed to clot and the serum separated by centrifugation at 3000 r.p.m for 15 minutes and the serum collected and then subjected to biochemical assays .Serum was separated and stored at -20 °C until analyzed.

Serum Enzymes Analysis

Serum samples were used for determination of alanine transaminase (ALT), using Randox - Uk (catalogue NO.AL146) and aspartate transaminase (AST) using Randox - Uk (catalogue NO.AS147), alkaline phosphatase (ALP), from BioMérieux Diagnostics, France, Cat. No.61511, in mice exposed to vitamin E, vitamin C and sodium nitrate along period of experiment.

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 Vassar Stats Website for Statistical Computation software [15]. A significant level of ($P<0.05$). The least significant differences (LSD) used to identify significant differences.

Results

The value of ALT (mean ± SD) (U/L) among different mice groups illustrated in Table (1). High level of ALT reported in mice received vitamin E (63.80 ± 5.43) (U/L) ,followed by mice exposed to vitamin E+ NaNO3 (59.81 ±3.94) (U/L). Low level of ALT reported in mice received vitamin C (50.72 ±3.38) (U/L). As shown in table (2), significant difference in ALT level reported between control group and group of mice exposed to vitamin E (P-value = 0.001), vitamin E+NaNO3 (P-value = 0.015). No significant difference in ALT level was reported between control and other groups.

Table (1): Descriptive statistic of serum ALT level in all groups.

Parameter	N	Minimum	Maximum	Mean± SD
ALT(control)	10	45.80	62.60	53.29 ± 6.58
ALT(Vitamin E)	10	58.00	72.00	63.80 ± 5.43
ALT(Vitamin C)	10	45.66	55.40	50.72 ±3.38
ALT(NaNO3)	10	48.90	60.40	55.82 ±4.05
ALT(Vitamin E + NaNO3)	10	53.45	64.90	59.81 ±3.94
ALT(Vitamin C+ NaNO3)	10	49.30	56.60	53.27 ±2.46

Table (2): Difference in serum ALT level between study groups and control group.

ANOVA compared with control group						
Parameter		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
ALT (U/L) (vitamin E)	Between Groups	552.3005	1	552.3005	15.16177	0.001064
	Within Groups	655.689	18	36.42717		
	Total	1207.99	19			
ALT(U/L) (vitamin C)	Between Groups	32.97312	1	32.97312	1.203551	0.287073
	Within Groups	493.13736	18	27.39652		
	Total	526.11048	19			
ALT (U/L) (NaNO3)	Between Groups	32.0045	1	32.0045	1.07129	0.31436
	Within Groups	537.745	18	29.87472		
	Total	569.7495	19	569.7495		
ALT(U/L) (vitamin E+ NaNO3)	Between Groups	212.552	1	212.552	7.221159	0.015054
	Within Groups	529.823	18	29.43461		
	Total	742.375	19	742.375		
ALT (vitamin C+ NaNO3)	Between Groups	0.001805	1	0.001805	7.31E-05	0.993272
	Within Groups	444.3759	18	24.68755		
	Total	444.3777	19	444.3777		

The value of AST (mean ± SD) (U/L) among different mice groups illustrated in Table (3). High level of AST reported in mice exposed to NaNO3 (304.20 ±16.55) (U/L), followed by control group (285.19 ±11.56) (U/L) .Low level of AST reported in mice exposed to Vitamin E (265.08 ±7.34) (U/L). As shown in table (4), significant difference in AST level reported between control group and group of mice exposed to vitamin E (P-value =0.000202573) , vitamin C (P-value =0.00143),NaNO3 (p value=0.008076). No significant difference in AST level was reported between control and group of mice exposed to (vitamin E+ NaNO3) (vitamin C+ NaNO3) groups.

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Table (3): Descriptive statistic of AST level in all groups

Parameter	N	Minimum	Maximum	Mean ± SD
AST **(U/L)** control	10	269.00	303.90	285.19 ±11.56
AST **(U/L)** vitamin E	10	253.60	273.40	265.08 ±7.34
AST **(U/L)** vitamin C	10	248.00	278.20	265.10 ±12.30
AST**(U/L)** (NaNO3)	10	282.00	328.00	304.20 ±16.55
AST**(U/L)** (vitamin E +NaNO3)	10	267.80	295.35	284.64 ±8.03
AST **(U/L)** (vitamin C + NaNO3)	10	270.50	303.10	284.65 ±11.10

Table (4): Difference in AST level between study groups and control**.**

ANOVA compared with control group						
Parameter		Sum of Squares	df	Mean Square	F	Sig.
AST (U/L) (vitamin E)	Between Groups	2022.0605	1	2022.0605	21.5499368	0.000202573
	Within Groups	1688.965	18	93.8313889		
	Total	3711.0255	19			
AST(U/L) (vitamin C)	Between Groups	2018.041	1	2018.041	14.14719	0.00143
	Within Groups	2567.629	18	142.6461		
	Total	4585.67	19			
AST(U/L) (NaNO3)	Between Groups	1806.9005	1	1806.901	8.863513	0.008076
	Within Groups	3669.449	18	203.8583		
	Total	5476.3495	19			
AST(U/L) (vitamin E+ NaNO3)	Between Groups	1.5125	1	1.5125	0.015261	0.903052
	Within Groups	1783.948	18	99.10822		
	Total	1785.4605	19			
AST(U/L) (vitamin C+ NaNO3)	Between Groups	1.458	1	1.458	0.011345	0.916352
	Within Groups	2313.194	18	128.5108		
	Total	2314.652	19			

Table (5) shown the value of alkaline phosphatase (mean ± SD) (U/L) among different mice groups. High level of alkaline phosphatase reported in mice exposed to vitamin E+NaNO3 (75.60±4.27) (U/L) ,followed by mice received vitamin C (69.80 ±1.47) (U/L) and vitamin E (69.50 ± 4.85) .Low level of alkaline phosphatase reported in mice exposed to vitamin C + NaNO3 (35.20 ±4.54) (U/L).

Significant difference in Alkaline phosphatase level reported between control group and group of mice received vitamin E (P value = .5E-13), vitamin C (P-value =4.45E-18), NaNO3 (p-value = 6.17E-06), vitamin E+NaNO3 (p-value=3.68E-15) as shown in table (6). No significant difference in AST level was reported between control and group of mice exposed to (vitamin C+ NaNO3), p-value = (0.091718).

Table (5): Descriptive statistic of alkaline phosphatase in all groups.

Parameter	N	Minimum	Maximum	Mean ±SD
Alkaline phosphatase **(U/L)** (control)	10	35	41	38.10 ±2.42
Alkaline phosphatase **(U/L)** (vitamin E)	10	65	78	69.50 ±4.85
Alkaline phosphatase **(U/L)** (vitamin C)	10	68	72	69.80 ±1.47
Alkaline phosphatase **(U/L)** (NaNO3)	10	47	82	68.60 ±15.12
Alkaline phosphatase **(U/L)** (vitamin E+ NaNo3)	10	70	81	75.60 ±4.27
Alkaline phosphatase **(U/L)** (vitamin C+NaNo3)	10	30	40.	35.20 ±4.541

Table (6): Difference in alkaline phosphatase between study groups and control.

ANOVA compared with control group						
Parameter		Sum of Squares	df	Mean Square	F	Sig.
Alkaline phosphatase **(U/L)** (vitamin E)	Between Groups	4929.8	1	4929.8	334.3497	4.5E-13
	Within Groups	265.4	18	14.74444		
	Total	5195.2	19			
Alkaline phosphatase **(U/L)** (vitamin C)	Between Groups	5024.45	1	5024.45	1247.45	4.45E-18
	Within Groups	72.5	18	4.027778		
	Total	5096.95	19			
Alkaline phosphatase **(U/L)** (NaNO3)	Between Groups	4651.25	1	4651.25	39.65448	6.17E-06
	Within Groups	2111.3	18	117.2944		
	Total	6762.55	19			
Alkaline phosphatase **U/L)** (vitamin E+ NaNO3)	Between Groups	7031.25	1	7031.25	582.4321	3.68E-15
	Within Groups	217.3	18	12.07222		
	Total	7248.55	19			
Alkaline phosphatase **(U/L)** (vitamin C+ NaNO3)	Between Groups	42.05	1	42.05	3.173585	0.091718
	Within Groups	238.5	18	13.25		
	Total	280.55	19			

Discussion

In the present study, significant increase in value of ALT in mice received vitamin E alone and other group received vitamin E and NaNO3.

The enhancement in activates of ALT could be attributed to antioxidant nature of vitamin E in the management of hepatotoxicity due to oxidative stress was reported by previous researches [16-18]. Vitamin E can play a role in protecting cells from injury caused by reactive oxygen species and lipid peroxidation[19]. Vitamin E has already been shown to decrease cellular injury[18]. And improve liver function[20]. Anti-oxidant role of Vitamin E proved in previous studies via repairing of the genotoxicity and improves the hematological and biochemical changes [21]. High level of AST showed in mice exposed to NaNO3 the increased activities could be attributed to the toxic effect of nitroso-compound formed in the acidic environment of the stomach, causing's severe hepatic necrosis[22].

In current study ,the observed elevation in the activity of liver enzymes in NaNO3 treated group come in agreement with the Bansal *et al* ,(2005) and El Gendy *et al*., (2007) [23, 24]. Such elevation in the activity of these enzymes could be attributed to the toxic effect of nitroso-compounds causing severe hepatic injury and necrosis [25]. The results of the study revealed no

significant difference in AST concentration in control; vitamin E and Vitamin C groups respectively the antioxidants protect the cells from oxidative stress by using both enzymatic and non-enzymatic strategies. Carotenoids, vitamin E and vitamin C may protect against free radicals and lipid peroxidation thereby reducing macro-vascular complications [26], or due to vitamin E is non-enzymatic membrane antioxidant and it is less specific in reaction with the free oxygen than enzymatic antioxidants but it is more universal and therefore, it can play a preventive role in the free radical reaction[19]. The antioxidant function of vitamin C is related to its reversible oxidation and reduction characteristics. Thus, vitamin C may particularly prevent certain types of hepatic cellular damage [27].

The statistical analysis of Alkaline phosphatase level showed significant differences in all groups except group of mice received (Vitamin C and NaNO3),p value=(0.091718) when compared with control group, these enzymes are usually liver makers whose plasma concentration above homeostatic limit could be associated with various forms of disorders which affect the functional integrity of the liver[28]. Vitamin C has a role to improve the liver function under the effects of toxin [29] .

In conclusions. This study revealed that Vitamin E and Vitamin c have the ability to ameliorate the effect of sodium nitrate in exposed groups although the effect of vitamin E more obvious due to pharmaceutical formulation, finally antioxidant effects of vitamins leads to modulation the nitrate toxicity and hepatoprotective effect.

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