Supplementation of α -Tocopherol Attenuates Minerals Disturbance, Oxidative Stress and Apoptosis Occurring in Favism

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Abstract The favism is a metabolic disease that characterized with an acute hemolytic anemia where α -tocopherol is a type of tocopherol accumulated inside the human body. The objective of such a study was established to evaluate the effect of α -tocopherol in favism disorders. A total of 75 human cases were divided into 5 groups as follow; group 1 normal cases without any treatment and group 2 normal cases orally administrated α -tocopherol (200 mg/kg) once a day over 30 days period. Group 3 favism patients without any treatment. Groups 4 and 5 favism patients orally administrated 100 and 200 mg α -tocopherol/kg, respectively once a day over 30 days period. The results obtained revealed that oral administration of α -tocopherol into normal cases over 30 days period did not induce any biological change. In favism, hemoglobin, hematocrit, red and white blood cells, serum glucose, glucose-6-phosphate dehydrogenase, total protein, albumin, globulin, aspartate and alanine aminotransferases, blood glutathione, superoxide dismutase, glutathione peroxidase and serum calcium, phosphorous, sodium, potassium and chloride levels were significantly decreased. On the other hand, serum alkaline phosphatase, bilirubin, selenium, zinc, manganese, copper and iron, malondialdehyde levels showed significant increase in favism. Supplementation with α -tocopherol into favism restores all the above mentioned parameters to approach the normal levels. Also, α -

tocopherol has anti-apoptotic effect in favism. In conclusion, α -tocopherol attenuates minerals disturbance, oxidative stress and apoptosis occurring in favism.

Keywords Favism · α -Tocopherol · Anemia · Liver · Antioxidants · Apoptosis

Introduction

Favism is an acute hemolytic anemia that occurs in glucose 6-phosphate dehydrogenase (G6-PD) deficient individuals following faba beans ingestion [1]. Much eating of fresh faba beans, blood in urine was appeared, dizziness, headache, appetite lost, fatigue, eye jaundice, hemolytic anemia, increased bilirubin amount in urine, G6-PD deficient in 8-year-old male patient [2]. The hydrolysis of favism inducing factors (vicine and convicine) produced divicine and isouramil through microflora effect in the human gastrointestinal tract and then these favism inducing factors transfer into the blood stream [3]. Divicine and isouramil are highly reactive compounds that generate oxidative stress and free radicals [4], which interact with abundant amounts of blood oxygen molecules to produce super-oxide radicals which induce cell damage and lead to favism-like signs [5, 6]. The cleavage amplified polymorphism markers methods are used to produce faba beans cultivars with low vicine and convicine content and enhanced nutritional use of faba beans [7]. Antibiotic neomycin counteracts the acts of favism inducing factor “vicine” in rats [8]. Furthermore, favism induced oxidative stress and genotoxicity which abolished by anise oil treatment [9].

α -Tocopherol, is one type of tocopherol, is main food source found mainly in the European diet especially in olive and sunflower oils [10, 11]. Furthermore, α -

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tocopherol transfer protein is ~32 kDa protein and this protein is to bind to α -tocopherol molecule to form a complex compound that attached to the cell membrane [12]. Moreover, α -tocopherol can combine with oseltamivir to produce antiviral compound that decreased mortality rate and increasing of survival time by 3.2–4 days in Influenza virus infection [13]. Additionally, α -tocopherol co-administrated with retinol and ascorbic acid showed decreasing in body temperature, improved heat stress, and enhances productivity of pullets reared under critical thermal environment conditions [14]. Finally, α -tocopherol has antibacterial effect that attributed to a direct effect on the bacterial cell [15].

The aim of this study is to investigate the effect of orally administered α -tocopherol to rebalance the body homeostasis interrupted by the free radicals produced in favism.

Subjects and Methods

Materials

α -Tocopherol was purchased from Pharco pharmaceutical Company, Egypt (each capsule contained 100 mg α -tocopherol acetate). All kits reagents were of analytical grade and were purchased from Bio-diagnostic kits reagents, United Kingdom.

Subjects

A total of 75 human subjects were divided into 30 normal healthy cases and 45 favism patients. All subjects were males with age 25–47 years old. The research was performed after approval from the ethics committee of the National Research Centre, Cairo, Egypt and in accordance with NIH publication no 85:23 revised 1985.

Experimental Design

The human subjects were divided into 5 equal groups each of 15 cases as follows: (1) *Control group* Normal control subjects without any treatment for 30 days period. (2) *α -Tocopherol group* Normal control subjects daily and orally administrated with α -tocopherol (200 mg/kg) once a day over 30 days period. (3) *Favism group* favism patients without any treatment. (4) *Favism + α -tocopherol (100 mg/kg) group* favism patients administered daily and orally once a day with 100 mg/kg bw of α -tocopherol over 30 days period. (5) *Favism + α -tocopherol (200 mg/kg) group* favism patients administered daily and orally once a day with 200 mg/kg bw of α -tocopherol over 30 days period. The tocopherol doses were chosen followed therapeutic doses protocol used. The human subjects were

observed daily for any abnormal clinical signs and death during the study period.

Blood Sampling and Handling

After 1 month of α -tocopherol supplementation, a collection of 5 ml of blood samples from each human subject was occurred. Amount from this blood sample was collected in EDTA as an anticoagulant to evaluate blood parameters and antioxidants. The other amount of blood sample was allowed to coagulate and centrifuged at 4000 rpm for 15 min to separate blood serum which stored at -20°C for other parameters evaluation.

Determination of Blood Parameters, Serum Glucose and Glucose-6-Phosphate Dehydrogenase

Hemoglobin (Hb) content, white and red blood cells (WBCs and RBCs), hematocrit (Hct) count, serum glucose and glucose-6-phosphate dehydrogenase (G6-PD) levels were determined colorimetrically using kits instructions.

Determination of Liver Function and Antioxidants Parameters

Serum aspartate and alanine aminotransaminases (AST and ALT), alkaline phosphatase (ALP), total bilirubin, total protein, albumin levels were determined colorimetrically but serum globulin content was calculated by subtracting serum albumin from serum total protein. Blood glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and serum malondialdehyde (MDA) as lipid peroxidation were determined colorimetrically by using kits instructions.

Determination of Serum Minerals

Serum calcium (Ca), phosphorous (P), sodium (Na), potassium (K), chloride (Cl), selenium (Se), zinc (Zn), manganese (Mn), copper (Cu), and iron (Fe) were determined colorimetrically by following kits instructions.

Evaluation of Apoptotic Cells

The apoptosis in blood cells was occurred by TUNEL method using kits reagents obtained from Roche Diagnostic, Mannheim, Germany. Simply, blood samples were placed on HTC-labeled slides. These slides were washed with 1% phosphate buffer (PBS), permeabilized using 0.1%, Triton X-100, 0.1% sodium citrate in 1X PBS, then incubated at room temperature for 10 min. The slides were let in 1 h in dark room temperature, adding 1% PBS then slides analyzed by using fluorescent microscope.

Statistical Analysis

Results were tabulated as mean $\pm$ standard deviation (SD) where the variances between groups were evaluated by ANOVA test using SPSS 13 software program. Posthoc testing was applied for inter-group comparisons using the least significant difference (Tukey) test where significance occurred at P values ≤ 0.05 .

Results

The daily oral administration with α -tocopherol into normal healthy subjects did not produce any observable changes in food consumption, water intake and body weight. There is an increase in food consumption, water intake and body weight after administration with both doses of α -tocopherol into favism compared with favism.

Table 1 revealed the effect of oral administration with α -tocopherol into favism on blood parameters, serum glucose and G6-PD levels. The data showed that daily administration with α -tocopherol for 30 days into normal cases revealed Hb, Hct, RBCs, WBCs, serum glucose and G6-PD levels almost around the control normal levels. On the other hand, in favism showed a significant decrease in Hb, Hct, RBCs, WBCs, serum glucose and G6-PD levels as compared to control group. Meanwhile the favism treated with either dose of α -tocopherol showed increased in Hb, Hct, RBCs, WBCs, serum glucose and G6-PD levels compared to favism to approach the normal levels where higher dose of α -tocopherol was more effective than lower dose.

Table 2 revealed the effect of either dose of α -tocopherol into favism on liver function. It is clear that daily administration with α -tocopherol for 30 days into normal cases showed serum AST, ALT, ALP, bilirubin, protein, albumin, and globulin almost around the normal values. On the contrary, in favism there was a significant decrease in serum AST, ALT, total protein, albumin, and globulin but serum ALP and bilirubin showed a significant increase compared to control cases. On the other side, daily administration with both doses of α -tocopherol for 30 days into favism exhibited a significant increase in serum AST, ALT, total protein, albumin, and globulin levels but serum ALP and bilirubin showed a significant decrease levels compared to favism to approach the normal cases where higher dose of α -tocopherol was more effective.

Table 3 revealed the effect of oral administration with α -tocopherol into favism on blood antioxidants parameters. It is obvious that daily administration with α -tocopherol for 30 days into normal cases exhibited blood GSH, SOD, GPx and MDA levels almost around the control levels. On the other side, in favism showed a significant decrease in blood GSH, SOD and GPx levels but an increase in serum MDA levels as compared to control cases. Meanwhile the favism treated with either dose of α -tocopherol showed increases in blood GSH, SOD and GPx levels but a decrease in serum MDA level compared to favism to approach the control group where higher dose of α -tocopherol was more effective than lower dose.

Table 4 arranged the effect of oral administration with α -tocopherol into favism on serum minerals. The daily administration with α -tocopherol for 30 days into normal cases exhibited serum Ca, P, Na, K, Cl, Se, Zn, Mn, Cu and Fe levels almost around the control levels. In the contrary,

Table 1 Effect of α -tocopherol on hematological parameters, serum glucose, and G6-PD in favism

Parameters	Groups				
	Control	α -Tocopherol (200 mg/kg)	Favism	Favism + α -tocopherol (100 mg/kg)	Favism + α -tocopherol (200 mg/kg)
Hb (g/dl)	13.79 $\pm$ 0.45	13.28 $\pm$ 0.39	8.69 $\pm$ 0.46*	12.38 $\pm$ 0.55 ^a	13.17 $\pm$ 0.49 ^b
Hct (%)	43.81 $\pm$ 1.50	42.96 $\pm$ 1.62	30.19 $\pm$ 1.82**	38.25 $\pm$ 1.24 ^a	41.67 $\pm$ 1.54 ^b
RBCs (10^6 cells/mm ³)	5.90 $\pm$ 0.46	5.95 $\pm$ 0.51	3.65 $\pm$ 0.13***	4.86 $\pm$ 0.75 ^b	5.14 $\pm$ 0.61 ^b
WBCs (10^3 cells/mm ³)	9.45 $\pm$ 0.75	9.45 $\pm$ 0.90	6.85 $\pm$ 0.65*	8.25 $\pm$ 0.58 ^a	9.32 $\pm$ 1.09 ^b
Serum glucose (mg/dl)	108.1 $\pm$ 15.25	106.9 $\pm$ 15.90	76.5 $\pm$ 16.12**	98.2 $\pm$ 14.32 ^a	106.8 $\pm$ 12.36 ^b
Serum G6-PD (U/L)	34.32 $\pm$ 3.58	33.89 $\pm$ 3.82	19.25 $\pm$ 2.61**	29.98 $\pm$ 3.81 ^b	33.16 $\pm$ 3.51 ^b

Results were expressed as mean $\pm$ SD and significant difference according to control at $p \leq 0.05$, ANOVA showed a highly significant difference between all groups at $p \leq 0.0001$

* $p \leq 0.05$ significant difference compared to control

** $p \leq 0.01$ highly significant difference compared to control

*** Very highly significant at $p \leq 0.001$

^a $p \leq 0.05$ significant difference compared to favism

^b $p \leq 0.01$ highly significant difference compared to favism

Table 2 Effect of α -tocopherol on liver function in favism

Parameters	Groups				
	Control	α -Tocopherol (200 mg/kg)	Favism	Favism + α -tocopherol (100 mg/kg)	Favism + α -tocopherol (200 mg/kg)
Serum AST (U/L)	19.81 $\pm$ 3.95	19.26 $\pm$ 3.58	5.14 $\pm$ 1.35***	18.20 $\pm$ 3.51 ^b	19.36 $\pm$ 2.92 ^b
Serum ALT (U/L)	9.27 $\pm$ 3.23	9.12 $\pm$ 3.42	6.28 $\pm$ 2.35*	8.95 $\pm$ 2.23 ^a	9.12 $\pm$ 3.19 ^b
Serum ALP (U/L)	210.5 $\pm$ 6.81	209.7 $\pm$ 6.19	231.2 $\pm$ 7.14*	208.1 $\pm$ 6.87 ^b	209.8 $\pm$ 7.26 ^b
Serum total bilirubin (mg/dl)	0.56 $\pm$ 0.08	0.56 $\pm$ 0.09	0.75 $\pm$ 0.05**	0.52 $\pm$ 0.08 ^b	0.54 $\pm$ 0.07 ^b
Serum total protein (g/dl)	8.72 $\pm$ 0.54	8.31 $\pm$ 0.58	6.59 $\pm$ 0.55**	8.37 $\pm$ 0.49 ^b	8.54 $\pm$ 0.48 ^b
Serum albumin (g/dl)	5.08 $\pm$ 0.49	5.02 $\pm$ 0.67	4.84 $\pm$ 0.82	4.86 $\pm$ 0.52	4.96 $\pm$ 0.65
Serum globulin (g/dl)	3.64 $\pm$ 0.54	3.56 $\pm$ 0.38	1.75 $\pm$ 0.89**	3.51 $\pm$ 0.91 ^b	3.58 $\pm$ 0.74 ^b

Results were expressed as mean $\pm$ SD and significant difference according to control at $p \leq 0.05$, ANOVA showed a highly significant difference between all groups at $p \leq 0.0001$

* $p \leq 0.05$ significant difference compared to control

** $p \leq 0.01$ highly significant difference compared to control

*** Very highly significant at $p \leq 0.001$

^a $p \leq 0.05$ significant difference compared to favism

^b $p \leq 0.01$ highly significant difference compared to favism

Table 3 Effect of α -tocopherol on blood antioxidants in favism

Parameters	Groups				
	Control	α -Tocopherol (200 mg/kg)	Favism	Favism + α -tocopherol (100 mg/kg)	Favism + α -tocopherol (200 mg/kg)
Blood GSH (mg/dl)	38.12 $\pm$ 3.54	38.04 $\pm$ 3.71	25.81 $\pm$ 3.63**	33.69 $\pm$ 3.12 ^b	37.56 $\pm$ 3.76 ^b
Blood SOD (U/ml)	260.4 $\pm$ 8.39	260.2 $\pm$ 8.16	234.9 $\pm$ 7.86**	258.4 $\pm$ 9.07 ^b	259.6 $\pm$ 9.07 ^b
Blood GPx (U/L)	6250 $\pm$ 82.95	6255 $\pm$ 78.62	5985 $\pm$ 80.73*	6240 $\pm$ 79.51 ^a	6245 $\pm$ 80.74 ^a
Serum MDA (nmol/dl)	18.12 $\pm$ 2.46	18.07 $\pm$ 2.51	30.24 $\pm$ 3.62***	20.14 $\pm$ 3.51 ^b	19.53 $\pm$ 2.82 ^b

Results were expressed as mean $\pm$ SD and significant difference according to control at $p \leq 0.05$, ANOVA showed a highly significant difference between all groups at $p \leq 0.0001$

* $p \leq 0.05$ significant difference compared to control

** $p \leq 0.01$ highly significant difference compared to control

*** Very highly significant at $p \leq 0.001$

^a $p \leq 0.05$ significant difference compared to favism

^b $p \leq 0.01$ highly significant difference compared to favism

favism induced a highly significantly decreased in serum Ca, P, Na, K, Cl levels while serum Se, Zn, Mn, Cu and Fe levels showed a significant increase in favism. While, the favism treated with either dose of α -tocopherol showed a significant increase in serum Ca, P, Na, K, Cl levels but serum Se, Zn, Mn, Cu and Fe levels showed a significant decrease compared to favism to approach the normal cases where higher dose of α -tocopherol was more effective.

Fig 1 showed the blood of control and α -tocopherol treated groups with no apoptotic pattern (Fig. 1a, b), while apoptosis appeared in blood in favism patients (Fig. 1c). Furthermore, α -tocopherol (100 and 200 mg/kg) treatment into favism patients showed no apoptotic

pattern like control (Fig. 1d, e) as detected using the TUNEL assay.

Discussion

The metabolic disease favism is an acute hemolytic anemia where favism caused a vigorous decrease in blood GSH in red blood cells which lead into animals or humans death [16], and this death related to increased production and hydrolysis of favism inducing factors (vicine and convicine) through intestinal microflora [5], to produce a highly free radical generating compounds (divicine and

Table 4 Effect of α -tocopherol on serum minerals in favism

Parameters	Groups				
	Control	α -Tocopherol (200 mg/kg)	Favism	Favism + α -tocopherol (100 mg/kg)	Favism + α -tocopherol (200 mg/kg)
Ca (mg/dl)	10.24 $\pm$ 0.67	10.04 $\pm$ 0.81	7.12 $\pm$ 0.81*	10.09 $\pm$ 0.82 ^a	10.16 $\pm$ 0.70 ^b
P (mg/dl)	8.62 $\pm$ 0.54	8.45 $\pm$ 0.39	4.64 $\pm$ 0.65**	8.56 $\pm$ 0.47 ^b	8.58 $\pm$ 0.49 ^b
Na (mmol/L)	154.60 $\pm$ 3.29	152.17 $\pm$ 3.42	110.24 $\pm$ 4.16**	149.26 $\pm$ 3.71 ^b	151.26 $\pm$ 3.56 ^b
K (mmol/L)	5.62 $\pm$ 0.19	5.49 $\pm$ 0.26	3.71 $\pm$ 0.28*	5.54 $\pm$ 0.19 ^a	5.57 $\pm$ 0.19 ^a
Cl (mmol/L)	102.83 $\pm$ 2.19	101.50 $\pm$ 2.35	81.47 $\pm$ 1.85**	99.98 $\pm$ 2.27 ^b	99.98 $\pm$ 2.18 ^b
Se (μ g/L)	60.0 $\pm$ 2.46	59.4 $\pm$ 2.71	73.5 $\pm$ 2.34*	62.0 $\pm$ 2.43 ^a	61.0 $\pm$ 2.51 ^a
Zn (μ g%)	135.9 $\pm$ 5.92	133.8 $\pm$ 6.12	158.1 $\pm$ 4.62*	132.8 $\pm$ 6.21 ^a	134.8 $\pm$ 6.04 ^a
Mn (mg/L)	1.09 $\pm$ 0.03	1.08 $\pm$ 0.05	3.40 $\pm$ 0.71**	1.09 $\pm$ 0.050 ^b	1.07 $\pm$ 0.038 ^b

Results were expressed as mean $\pm$ SD and significant difference according to control at $p \leq 0.05$, ANOVA showed a highly significant difference between all groups at $p \leq 0.0001$. * $p \leq 0.05$ significant difference compared to control, ** $p \leq 0.01$ highly significant difference compared to control, *** Very highly significant at $p \leq 0.001$. ^a $p \leq 0.05$ significant difference compared to favism. ^b $p \leq 0.01$ highly significant difference compared to favism

isouramil) which are the principle agents responsible for favism symptoms [17, 18].

In this study; oral administration with α -tocopherol into normal healthy cases for 30 days period did not induced any change in blood parameters, serum glucose, G6-PD, liver function, blood antioxidants and serum minerals. On the other hand, in favism had a significant decrease in Hb, Hct, RBCs, WBCs, serum glucose and G6-PD levels were observed as compared to control group. The decrease in Hb, Hct and RBCs parameters may be related to hemotoxicity of vicine and convicine which occurred in the premature disposal of damaged red blood cells by the spleen [1]. Also, a decrease in WBC count in favism may related to decrease in lymphocytes due to direct action of vicine and convicine on lymphatic tissue or it may be resulted from depressing effect of corticosteroids upon mitosis in the lymphatic tissue as reported by Kaneko [19]. Moreover, serum glucose level was decreased in favism patients and this related to liver function injuries which lead into decrease glucose and glycogenic hormones secretions such as insulin, glucagon and adrenocorticoid steroids or it may be due to injuries in the kidney tubules leading to decrease recapture of glucose from blood during glomerule filtration [20]. Furthermore, a highly significant decrease in the activity of serum G6-PD in favism as compared to control; this decrease may be attributed to a compensatory response to oxidative stress where the consumption of G6-PD enzyme was to maintain sufficient levels of NADPH in response to the oxidative stress [21]. Such observations are in agreement with that of many researchers observations [6, 17, 21]. On the contrary, favism treated with either dose of α -tocopherol showed increased in Hb, Hct, RBCs, WBCs, serum glucose and G6-PD levels to approach the normal levels. Such findings

are in agreement with Kasperczyk et al. [22] who found α -tocopherol treatment recovers protein metabolism process through decreasing protein catabolism, and increasing γ -globulin levels.

In this research, there was a significant decrease in serum AST, ALT, total protein, albumin, and globulin levels but serum ALP and bilirubin levels revealed a significant increase in favism compared to control group. The decrease in serum total protein and albumin levels due to toxic liver injury related with decrease serum albumin level as a result to decrease protein secretion and decrease in globulin level due to deteriorated hepatic activity as reported by Comporti [23]. Serum AST and ALT levels were decreased also to these aminotransferases enzymes are protein in nature; these enzymes were exposed to oxidative denaturation, decrease protein secretion by liver tissues. Such observations are in agreement with that of Koriem et al. [24] who attributed this decline in the level of serum albumin to enhanced degradation and increased loss of albumin through the gastrointestinal tract. On the other side, daily administration with both doses of α -tocopherol for 30 days into favism exhibited a significant increase in serum AST, ALT, total protein, albumin, and globulin levels but serum ALP and bilirubin showed a significant decrease levels to approach the normal levels. Such findings are in agreement with that of Wong et al. [25] who found that α -tocopherol reduced lipid accumulation, inflammatory infiltrates and plasma liver enzyme activities.

The favism showed significant decrease in blood GSH, SOD and GPx levels but significant increase was observed in serum MDA level as compared to control group. On the contrary, favism that treated with either dose of α -tocopherol showed an increase in blood GSH, SOD and GPx levels but a decrease in serum MDA level compared to

◀**Fig. 1** The hepatic cross section of the control with no apoptotic pattern (a), and α -tocopherol group with no apoptotic pattern like control (b) while apoptosis appeared in liver tissues in favism patients (c). Furthermore, α -tocopherol (100 and 200 mg/kg) treatment into favism patients showed no apoptotic pattern like control (d and e) as detected using the TUNEL assay

favism. Such observations are in agreement with that of Kim et al. [26] who found that D- α -tocopherol succinate significantly reduced the cisplatin-induced increase in reactive oxygen species (ROS). D- α -tocopherol succinate treatment induced a 15% reduction of ROS and 50% decrease in necrosis and late apoptosis as compared to cisplatin treatment.

In favism revealed a significant decrease in serum Ca, P, Na, K, Cl levels while serum Se, Zn, Mn, Cu and Fe levels exhibited a significant increase. These effects were related to decreased Na^+/K^+ -ATPase expression process with increased levels of vicine and convicine inside the biological system. Such minerals disturbance is greatly related to favism disorders. While, the favism patients treated with either doses of α -tocopherol showed a significant increase in serum Ca, P, Na, K, Cl levels but serum Se, Zn, Mn, Cu and Fe levels showed a significant decrease compared to favism patients. Such findings are in agreement with Shin et al. [27] who found that α -tocopherol protects brain cell membranes from lipid peroxidative damage during tissue hypoxia Na^+/K^+ -ATPase activity probably by being incorporated in cell membrane and also as circulating antioxidant.

In the current study, favism induced blood cell apoptosis where this effect is related to a decrease in G6-PD activity in favism in parallel with increased oxidative stress and DNA oxidative damage [28]. On the other hand, α -tocopherol has anti-apoptotic activity [29].

Conclusion

The daily supplementation of α -tocopherol for 30 successive days attenuates minerals disturbance, oxidative stress and apoptosis occurring in favism and this effect was dose dependent.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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