



PROTECTIVE EFFECT OF VITAMIN E ON BIOMARKERS OF OXIDATIVE STRESS AND INFLAMMATORY RESPONSE IN A RAT MODEL OF ENDOTOXEMIA

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ABSTRACT

The present study aimed to investigate the protective effect of vitamin E on the oxidative stress induced by lipopolysaccharide (LPS, endotoxin) in rats. Male rats (n=80) were divided into four equal groups. Group I (control): rats administered corn oil (20 mg/kg/b.w/day) for 5 weeks. Group II, rats administered vitamin E orally (100 mg/kg/b.w/day) for 5 weeks. Group III, rats injected intraperitoneally (I/P) with LPS (20 mg/kg/b.w.). Group IV, rats administered vitamin E (100 mg/kg/b.w/day) for 5 weeks and then I/P with LPS (20 mg/kg/b.w.). Serum and liver samples were collected at 2 and 5 hours post-injection with endotoxin. Sera were assessed for glucose, uric acid, total cholesterol, triacylglycerols (TG), phospholipids, free fatty acids [FFA], nitric oxide (NO), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyltransferase (GGT), haptoglobin, α -1-acid glycoprotein (α -1-AGP), vitamin C, vitamin E, reduced glutathione (GSH) and L-malondialdehyde (L-MDA) in addition for determination of liver glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), vitamin C, vitamin E, GSH and L-MDA. The obtained results revealed that, endotoxemia potentially increased serum glucose, uric acid, TG, phospholipid, FFA, haptoglobin, AGP, GSH, L-MDA concentrations as well as ALT, AST and GGT activities. Moreover, the values of SOD, CAT activities and GSH, L-MDA concentrations in liver tissues were significantly decreased. Treatment with vitamin E decreased LPS-triggered pathogenic responses by mitigating liver damage and prevented the increase of L-MDA, glucose, uric acid, triacylglycerols, phospholipid, FFA, ALT, GGT, haptoglobin and AGP. Moreover, treatment with vitamin E in endotoxin injected rats ameliorates the oxidative stress by increasing the concentrations of serum and tissue GSH, SOD, CAT and Vitamin C. It could be concluded that, vitamin E protects against lipid peroxidation, oxidative stress and decrease the inflammatory response to endotoxin injection.

Key Words: Antioxidant status, Endotoxin, Inflammatory markers, Oxidative stress, Vitamin E

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1. INTRODUCTION

Lipopolysaccharide (LPS) is one component of Gram-negative bacterial cell wall, which released by destruction of cell wall and acts as a potent bacterial product in the induction of host inflammatory responses and tissue injury [47]. Increased oxidative stress following endotoxin administration is supported by the increased formation of lipid peroxidation products [19]. Moreover,

when bacteria are lysed by immune effector cells and molecules, surges of endotoxin may be released into the host, intensifying the inflammatory response and causing further activation of immune effector cells [43]. Endotoxin induces shock states in both humans and animals characterized by fever, hypotension, intravascular coagulation, and finally multi-organs failure system (MOFS) [74].

Detoxification of endotoxin is considered to be mediated mainly by the reticuloendothelial system (RES), particularly Kupffer cells, in the liver [73]. The lipid soluble α -tocopherol (vitamin E) is a chain-breaking, free radical trapping, nonenzymatic, naturally occurring antioxidant in cell membranes and plasma lipoproteins. Vitamin E is a fat-soluble vitamin necessary in the diet of many species for normal reproduction, normal development of muscles, normal resistance to erythrocytes to hemolysis, and various other biochemical functions. Chemically, it is α -tocopherol, one of the three tocopherols [α , β , and γ] occurring in wheat germ oil, cereals, egg yolk, and beef liver. It is also prepared synthetically. Tocopherols act as antioxidant [48].

Accordingly, this study was performed to investigate the protective effect of oral supplementation of vitamin E on lipid peroxidation, biomarkers of oxidative stress and some inflammatory markers in serum and tissues in an endotoxemic rat model.

2. MATERIALS AND METHODS

2.1. *Experimental animals*

Eighty white male albino rats of 8- 10 weeks old and weighing 150- 200 g were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied ad-libitum.

2.2. *Induction of Endotoxemia*

Endotoxemia was induced by injecting the rats intraperitoneally with a single dose of LPS (from *Escherichia coli*, serotype O26) in a dose 20 mg/kg/b.w. [17]. This strain was obtained from Egyptian National Institute of Animal Health and was extracted in the Department of Biotechnology, Faculty of Veterinary Medicine, Cairo University.

2.3. Preparation and Dosage of Vitamin E
Vitamin E was administered orally in a daily dose of 100 mg/kg/b.w. and prepared by dissolved in Corn oil for dilution [9].

2.4. *Experimental design*

Animals were randomly divided into 4 main groups (20 each) placed in separate cages and classified as follow:

Group I [control group]: Rats administered with corn oil orally at a daily dose of 20 mg/kg/b.w. for 5 weeks and served as normal group.

Group II [vitamin E normal treated group]: Animals received vitamin E (α -tocopherol), orally at a daily dose of 100 mg/kg/b.w for 5 weeks.

Group III [endotoxin group]: Rats injected intraperitoneally (I/P) with a single dose of LPS (20 mg / kg/b.w.).

Group IV (endotoxin pre- treated vitamin E group): Rats received vitamin E (α -tocopherol), orally in a daily dose of 100 mg/kg/b.w for 5 weeks before endotoxin injection. One hour after the last dose of vitamin E pretreatment rats were injected intraperitoneally (I/P) with a single dose of endotoxin (20 mg/ kg/b.w.).

2.5. *Sampling*

2.5.1. *Blood samples*

Blood samples for serum separation were collected by ocular vein puncture in dry, clean, and screw capped tubes after overnight fasting from all animals groups (control and experimental groups) twice along the duration of experiment at 2 and 5 hours from the onset of LPS injection. Serum was separated by centrifugation at 4000 rpm for 15 minutes. The clean, clear serum was separated by automatic pipette and received in dry sterile samples tube and proceed directly for glucose determination, then kept in a deep freeze at -20° C until used for subsequent biochemical analysis.

2.5.2. *Liver specimens*

Rats were killed by decapitation. The liver specimen quickly removed, cleaned by rinsing with cold saline and stored at -20°C. Briefly, liver tissues were minced into small pieces, homogenized with ice cold 0.05M potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were centrifuged at 4000 rpm for 15 minute at 4°C. The supernatant was used for the determination of L-malonaldehyde (L-MDA), vitamin E, vitamin C and antioxidant enzymes.

2.5.3. Biochemical analysis

Serum glucose, Uric Acid, Total cholesterol, TG, FFA, phospholipids, NO, AST, ALT, GGT, Haptoglobin, AGP, Vitamin C, Vitamin E, G-SH, L-MDA, SOD, GR, CAT and GPx were analyzed according to the methods described previously [3, 8, 11, 12, 21, 28, 33, 36, 45, 54, 57, 60, 62, 64, 66, 77, 83, 89]

2.6. Statistical analysis

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, Ver. 13.0, 2009). Values of $P < 0.05$ were considered to be significant.

3. RESULTS AND DISCUSSION

Free radicals play a role in the pathogenesis of various diseases (e.g., cardiovascular and cerebrovascular diseases, neurosensory disorders, Parkinson's disease, inflammation, cancer and others), and the pharmacological agents scavenging free radicals are considered important [10, 49]. LPS creates a status of oxidative stress which characterized by releasing reactive oxygen species (ROS), neutrophils, phagocytic cells and cytokines [82]. Reactive oxygen species (ROS) are formed as a normal product of aerobic metabolism in mitochondrial and microsomal enzymatic reactions [64]. However, these ROS can be

generated at elevated rates under pathophysiological conditions [4].

The obtained data showed in Table 1 revealed that, the mean value of serum glucose concentration significantly increased at 2 and 5 hours after endotoxin injection in rats when compared with control group. These results came in accordance with the recorded data of Yelich and Janusek [87] who found that, the administration of endotoxin caused hyperglycemia as initial response to endotoxin followed by hypoglycemia. These results may be related to that, hyperglycemia is associated with protein glycation and simultaneously, with the production of reactive oxygen species and has been implicated in free radical injury caused by hyperglycemia [37]. In addition, bacterial endotoxins induced pancreatitis which caused an increase in serum amylase associated with increase in glucose level. On the other hand, the presented data showed that vitamin E pretreatment endotoxin group significantly decreased serum glucose concentration at 5 hours after endotoxin injection when compared with rats injected with endotoxin only. These results are in a harmony with the recorded data of Kheir-Eldin *et al.*, [47] who mentioned that, the endotoxin elevated the blood sugar level and vitamin E pretreatment showed decrease effect on the elevated level of plasma glucose because vitamin E improves the free radical defense system potential and may have a beneficial effect in improving glucose transport and insulin sensitivity [25].

In addition, the mean value of serum uric acid concentration significantly increased in endotoxin injected rats (5hr.) when compared with control group (Table 1). These results are similar to the recorded data of Kahl and Elsasser, [46] who recorded that, uric acid concentration was increased after LPS challenge in rats treated for 12 days. This may be attributed to the uric acid is the product of the free radical generating xanthine oxidase

reaction, and enzymatic and non-enzymatic lipid peroxidation so that uric acid protects against oxidative damage of proteins and nucleobases by directly scavenging hydroxyl radical, singlet oxygen, hypochlorous acid, oxoheme oxidants, and hydroperoxyl radicals [25, 52]. In addition, the presented results demonstrated that, serum uric acid concentration decreased significantly in pretreatment vitamin E group when compared with LPS injected rats (5hr.). Similarly, Zoroglu *et al.*, [88] concluded that, decreased uric acid concentration after oral administration of vitamin E in rats injected with LPS. These results are mainly because vitamin E has been indicated as the major antioxidant that prevents the propagation of free radical damage in biological membranes of xanthine oxidase during conversion of xanthine to uric acid [84].

The obtained data in Table 1 showed that, no significant change in serum total cholesterol concentration in rats injected LPS (2hr. and 5hr.) group and in pretreatment vitamin E group when compared with control group. These results came in accordance with the recorded data of Lehr *et al.*, [51] who concluded that, no effect of endotoxins was observed on the serum total cholesterol levels and no change after administration of vitamin E. Furthermore, there was no change in hepatic cholesterol levels after LPS administration. Moreover, as both LDL receptor mRNA and protein levels were not significantly altered by LPS this finding suggests that a decrease in hepatic clearance of LDL does not contribute to LPS-induced hypercholesterolemia [27].

Regarding to serum triacylglycerol (TG), the mean value of serum TG concentration significantly increased in endotoxin injected rats when compared with control group (Table 1). These results agreed well with the recorded data of Adi *et al.*, [1] who reported that, the administration of endotoxin (LPS) has been used to mimic

infections. Both infections and LPS administration produce hypertriglyceridemia by increasing hepatic lipoprotein secretion and reducing lipoprotein clearance, presumably by decreasing lipoprotein lipase activity. It was proposed that, hypertriglyceridemia was due to the catabolic effects of a circulating factor on fat cells that directly caused cachexia. Serum TG concentration was significantly decreased in pretreatment vitamin E group when compared with endotoxin treated rats only (5hr.). These results came in accordance to Chunga *et al.*, [18] who recorded that, serum TG increased in response to LPS and these increases were, in part, reduced by α -tocopherol supplementation only. Additionally, vitamin E can reduce triglycerides. This may be attributed to vitamin E enable to compete with triglycerides in the blood stream, thereby reducing their impact. It may also reduce the incidence of comorbid diseases associated with high triglyceride levels, such as atherosclerosis [80].

Intraperitoneal injection of endotoxin to normal rats significantly increased serum phospholipids concentration at 5 hours after injection as compared with control group (Table 1). These results are similar to the recorded data of Gmeiner and Schlecht, [32] who stated that, the increase of LPS injection was paralleled by increasing amounts of phospholipid while the overall protein content in the outer membrane decreased in the rats. This is may be due to the increase of serum phospholipids after endotoxin which leads to a change in the activity of one or more phospholipid transfer proteins, with decreased transfer of phospholipids out of the plasma into tissues or increased transfer from tissues into plasma [85]. In addition, a significant decrease in the value of serum phospholipids concentration was reported in vitamin E pretreatment endotoxin group at 5 hours after endotoxin injection when compared with rats treated with endotoxin.

Protective effect of Vitamin E against Oxidative stress

Table 1 Effect of pretreatment with vitamin E on blood serum parameters at 2 and 5 hours after endotoxin injection in rats.

Parameter	Animal group					
	Control group	Endotoxin group		Endotoxin + vitamin E group		Vit. E group
		2 hrs	5 hrs	2 hrs	5 hrs	
Glucose(mg/dl)	92.33±7.64 ^d	132.17±7.26 ^{ab}	142.50±5.10 ^a	123.67±4.07 ^{bc}	108.00±2.45 ^{cd}	103.50±5.36 ^d
Uric Acid(mg/dl)	1.33±0.18 ^b	1.54±0.18 ^{ab}	2.04±0.27 ^a	1.44±0.16 ^b	1.16±0.12 ^b	1.50±0.14 ^{ab}
T. Cholesterol. (mg/dl)	56.63±2.81 ^{ab}	62.74±2.37 ^a	59.49±3.45 ^{ab}	54.97±1.95 ^{ab}	51.83±4.67 ^b	53.63±2.40 ^{ab}
Triacylglycerol (mg/dl)	70.04±4.0 ^{bc}	104.13±10.87 ^a	103.0±15.63 ^a	80.74±6.35 ^{ab}	44.73±5.47 ^c	66.59±1.84 ^{bc}
Phospholipids (mg/dl)	40.14±14.18 ^{bc}	75.46±15.87 ^{ab}	95.02±20.96 ^a	43.90±8.54 ^{bc}	15.08±2.60 ^c	20.86±2.86 ^c
Free FA (ng/dl)	12.33±1.03 ^{cd}	21.50±1.32 ^b	27.0±3.34 ^a	17.25±1.80 ^{bc}	16.50±1.32 ^{bc}	9.25±1.25 ^d
ALT (U/L)	71.89±3.87 ^c	81.24±3.02 ^b	93.09±4.08 ^a	67.99±2.55 ^{cd}	75.31±2.29 ^{bc}	60.0±1.83 ^d
AST (U/L)	236.44±17.39 ^c	326.38±2.09 ^a	301.05±17.53 ^{ab}	289.10±8.95 ^{ab}	281.84±17.35 ^b	260.06±9.31 ^{bc}
GGT(U/L)	1.79±0.225 ^b	1.61±0.282 ^b	5.37±1.32 ^a	1.46±0.131 ^b	2.79±0.529 ^b	1.14±0.170 ^b
Haptoglobin (g/l)	20.33±3.03 ^b	34.13±2.70 ^{ab}	48.73±9.39 ^a	27.70±4.93 ^b	24.93±4.72 ^b	21.63±5.48 ^b
α-1-acid Glycoprotein (ng/ml)	39.93±2.51 ^d	85.13±2.54 ^b	103.53±3.80 ^a	77.45±4.59 ^{bc}	69.93±5.86 ^c	7.38±1.50 ^e
Vitamin E (µmol/ml)	4.75±0.104 ^c	4.48±0.206 ^c	4.68±0.125 ^c	5.40±0.268 ^b	5.05±0.194 ^{bc}	6.43±0.155 ^a
Vitamin C (mg/L)	3.08±0.124 ^c	3.08±0.124 ^c	3.20±0.167 ^c	3.50±0.134 ^c	3.50±0.134 ^c	3.12±0.097 ^c
L-MDA (nmol/ml)	34.25±4.49 ^{ab}	35.40±3.01 ^{ab}	42.00±4.01 ^{ab}	47.80±7.49 ^a	31.80±2.71 ^b	30.50±3.14 ^b
NO (µmol/l)	2.30±0.178 ^a	2.60±0.570 ^a	2.40±0.324 ^a	2.25±0.096 ^a	1.44±0.561 ^a	1.30±0.367 ^a
GSH (mg/dl)	19.50±0.817 ^{bc}	16.02±0.715 ^{de}	14.34±1.10 ^e	18.66±0.690 ^{cd}	21.66±1.23 ^{ab}	22.94±0.755 ^a

Mean (± S.E.) values with different superscript letters in the same column are significantly different at (P<0.05).

Table 2 Effect of pretreatment with vitamin E on liver SOD, GPX, CAT and GR activities, vitamin E, vitamin C, GSH and L-MDA concentrations at 2 and 5 hours after endotoxin injection in rats

Parameter	Animal group					
	Control group	Endotoxin group		Endotoxin + vitamin E group		Vit. E group
		2 hrs	5 hrs	2 hrs	5 hrs	
SOD (U/g.tissue)	383.60±34.73 ^a	194.61±18.18 ^b	234.42±10.47 ^b	391.41±39.63 ^a	437.24±73.39 ^a	482.34±49.26 ^a
GPX (nmol NADPH/min./g. tissue)	0.024±0.005 ^{ba}	0.036±0.013 ^a	0.037±0.014 ^a	0.032±0.002 ^a	0.032±0.002 ^a	0.041±0.004 ^a
CAT (U/g. tissue)	1.80±0.354 ^a	0.57±0.099 ^b	18.45±1.96 ^b	1.53±0.085 ^a	1.40±0.158 ^a	1.65±0.132 ^a
GR (U/g.tissue)	79.07±2.78 ^a	74.94±4.51 ^a	74.47±2.82 ^a	71.30±2.33 ^a	74.15±1.83 ^a	71.30±1.72 ^a
Vit. E (µmol/g.tissue)	0.170±0.011 ^c	0.148±0.021 ^c	0.135±0.018 ^c	0.240±0.027 ^b	0.180±0.009 ^c	0.340±0.015 ^a
Vit. C (mg/g.tissue)	0.244±0.017 ^b	0.216±0.025 ^b	0.240±0.033 ^b	0.332±0.027 ^a	0.224±0.019 ^b	0.300±0.035 ^{ab}
GSH (mg/g.tissue)	9.92±0.56 ^b	6.90±0.44 ^c	7.08±0.46 ^c	9.05±0.67 ^b	13.49±0.30 ^a	12.28±0.71 ^a
L-MDA(nmol/g.tissue)	15.92±1.04 ^b	0.36±0.104 ^b	31.36±3.05 ^a	15.13±2.19 ^b	14.00±1.58 ^b	15.21±0.83 ^b

Mean (± S.E.) values with different superscript letters in the same column are significantly different at (P<0.05).

These results came in accordance with the recorded data of Campbell *et al.*, [15] who provided that, decrease phospholipids concentration after 5 hr. from LPS injection and treatment with oral administration of vitamin E. This is because vitamin E is an endogenous lipid soluble chain-breaking antioxidant that is known to protect cells from the diverse actions of free oxygen radicals by donating its hydrogen atom leading to decreasing of phospholipids concentration [13].

The mean value of serum FFA concentration increased significantly in rats injected LPS when compared with control group as showed in (Table 1). These results agreed with the recorded data of Chunga *et al.*, [18] who recorded that, free fatty acid increased by 6 h. post-LPS, compared to 0 and 1.5 h, regardless of tocopherol supplementation. This may be related to that LPS-triggered oxidative stress and inflammatory responses induced serum dyslipidemia and tocopherol

supplementation decreased or maintained these responses. Moreover, the LPS-mediated increases in α -TNF likely free fatty acid release from adipocytes by stimulating lipolysis. Likewise, hepatic free fatty acid accumulation induces oxidative stress through several of intracellular pathways [27]. The presented data demonstrated that, the mean value of serum FFA was significantly decreased in vitamin E pretreatment group when compared with endotoxin treated rats only (5hr.) and these results came in accordance with the recorded data of Pascoe and Reed, [67] who demonstrated that, vitamin E protects fatty acids and protein thiol groups against oxidation. Furthermore, the amount of vitamin E present in tissues is related to fatty acid concentration. In addition, the majority of vitamin E in tissues is present in those membranes with the highest fatty acid composition. It is a lipid-soluble vitamin and its main function is to protect polyunsaturated fatty acids (PUFA) against oxidative stress. It can break the chain, prevent lipid peroxidation, trap peroxy free radicals and prevent disruption of the membrane integrity [59]. Vitamin E plays a role in protecting FFA in cell membranes and LDL from oxidation by donating the hydrogen of the hydroxyl group, therefore, blocking the initiation and propagation of lipid peroxidation [20].

The mean value of serum ALT and AST activities significantly increased in rats injected with LPS when compared with control group (Table 1). These results came in accordance with the recorded data of Hanan and Hagar, [35] who found that, induction of endotoxemia with LPS resulted in increased serum activities of ALT and AST as a measure of hepatic damage. Injection of LPS for 5 hr. before the onset of endotoxin intoxication markedly attenuated LPS-induced hepatic dysfunction and accompanying oxidative stress in liver. In addition, high concentrations of AST and ALT were measured in the systemic circulation as a

result of acute destruction of hepatocytes and treatment by vitamin E restored the levels of these indicators of liver damage indicating that the reactive oxygen intermediates (ROIs) are also involved [22]. Therefore, the increase in the serum ALT and AST activities might perhaps be an indication of liver damage. This increase could be explained that, free radical production which reacts with polyunsaturated fatty acids of cell membrane leading to impairment of mitochondrial and plasma membrane results in enzyme leakage [69]. The obtained data showed that, the mean value of serum ALT and AST activities were significantly decreased in pretreatment vitamin E group when compared with endotoxin injected rats (Table 1). These results are in a harmony with the data of Sushma *et al.*, [78] who showed that, LPS caused a marked rise in serum level of ALT and AST where the activities of liver enzymes were decreased significantly on supplementation with tocopherol. Furthermore, administration of tocopherol before LPS challenge resulted in a significant reduction in the serum levels of these enzymes as a result of vitamin E has been reported to confer protection against such changes in formaldehyde and monosodium glutamate induced-hepatotoxicity and oxidative stress in rats [34].

The presented data showed in Table 1 revealed that, mean value of serum γ -glutamyltransferase (GGT) activity increased significantly in endotoxin injected rats (5hr.) when compared with control group. These results agreed with the recorded data of Hanan and Hagar, [35] who investigated that, induction of endotoxemia with LPS resulted in increased serum levels of GGT, as a measure of hepatic damage. LPS induced significant increase in the activity of GGT during the whole time course of the experiment (8 days) [70]. This could be attributed to increase in GGT occurred to restore glutathione used in the metabolism

of drugs which accounts for the elevated activity assayed [44].

The mean value of serum haptoglobin concentration increased significantly in endotoxin injected rats (5hr.) when compared with control group as displayed in Table 1. These results are similar with Nielsen *et al.*, [61] who recorded that, the serum concentration of haptoglobin was increased by LPS and caused high mortality compared with the control group. Likewise, plasma haptoglobin was increased by LPS treatment at 24 h. post injection in rats [26]. The increase in plasma haptoglobin after the initiation of the experiment indicates the presence of an inflammatory condition in rats. Nevertheless, during the acute phase of inflammatory process there is a characteristic increase in some plasma proteins called collectively acute phase reactant (APR), LPS that induces a strong acute phase response, indicated by high level of haptoglobin as confirmed by Silveira and Limaos, [76]. Even though, the mean value of serum haptoglobin was significantly decreased in pretreatment vitamin E group (5hr.) when compared with endotoxin injected rats group only. These results are in a harmony with the data of Carter *et al.*, [16] who reported that, serum haptoglobin concentrations were significantly lower on days 0 and 7 than concentrations for rat that required > 1day treatment with vitamin E administration. Serum haptoglobin concentration on day 0 significantly correlated with the number of antimicrobial treatments required.

The data showed in Table 1 illustrated that, intraperitoneal injection of endotoxin to normal rats significantly increased the value of serum α -1-acid glycoprotein concentration after 2 and 5 hours in comparison with control group. These results are similar with the recorded data of Nielsen *et al.*, [61] who recorded that, the serum concentration of α -1-AGP was increased by LPS and induced high mortality compared with the control group. α -1-AGP has also been reported to be

involved in nonspecific resistance to infection by Gram-negative bacteria as well as in protection against LPS [39]. The increase of α -1-AGP could be related to that, α -1-AGP interacts directly with LPS and this α 1-AGP-LPS complex is removed by macrophages, enhancing clearance of LPS from the body [24]. Furthermore, α -1-AGP an acute phase reactant produced by the liver and elevated during inflammation to control inappropriate or extended activation of the immune system [29]. However, serum α -1-AGP was significantly decreased in pretreatment vitamin E group (5hr.) when compared with LPS injected rats. These results are in harmony with the data of Carter *et al.*, [16] who found that, serum α -1-AGP concentrations were significantly lower on days 0 and 7 than concentrations for rat that required > 1day treatment with vitamin E administration. Serum α -1-AGP concentration on day 0 significantly correlated with the number of antimicrobial treatments required.

On the other hand, there was no significant change in the serum and liver vitamin E concentration in rats injected LPS (2hr. and 5hr.) group when compared with control group and significant increase in pretreatment vitamin E (2 hr.) group in comparison with rats injected LPS (2 hr.) (Table1). These results are similar with the data of Rojas *et al.*, [71] who reported that, LPS did not affect vitamin E levels either in animals with low or with high liver vitamin E levels. Moreover, vitamin E, the main lipid-soluble antioxidant, was not modified by the LPS treatment in rats when compared with control groups [65]. Furthermore, tissue vitamin E level did not affect by LPS in animals either with low or with high LPS concentrations and increase tissue vitamin E in pretreatment vitamin E group [40].

No significant change in the serum and liver vitamin C concentration in rats injected LPS (2hr. and 5hr.) group when compared with control group and other groups as illustrated in Table 1 & 2. These

results are in a harmony with the recorded data of Alessio *et al.*, [5] who discussed that, vitamin C has little or no effect on oxidative stress, as measured by plasma thiobarbituric acid reactive substances (TBARS), at levels 1.0 gm and less. The differences in the reported results might be due to dose and time of 5 treatments. Likewise, LPS did not change the concentration of tissue vitamin C when compared with all groups and significant increase in pretreatment with vitamin E after 6 hr. from LPS [63]. This may be attributed to that, vitamin C is a potent scavenger of free oxygen radicals and it has been shown that marginal LPS results in intracellular oxidative damage in the rats could not be make any change in vitamin C [81].

Intraperitoneal injection of endotoxin to normal rats caused non-significant increase in the value of serum L-MDA concentration after 2 and 5 hours in comparison with control group as found in (Table 1). These results agreed with the data of Ouchi *et al.*, [65] who stated that, lipid peroxidation in the guinea pig was not significantly modified by endotoxin (LPS). Hence, Taillé *et al.*, [14] showed that, L-MDA had insignificantly changed in LPS treated animals at 6, 12, and 24 h from injection. The obtained data showed that, a non-significant change in serum L-MDA concentration in pretreatment vitamin E group (2 and 5hr.) when compared with LPS injected rats and other groups. These results are similar with the recorded data of Ross and Moldeus, [72] who proved that, after 5-week dietary supplementation of α -tocopherol don't change LPS-triggered lipid peroxidation, inflammation, and hepatic damage. This is resulted in Intracellular vitamin E is associated with lipid rich membranes such as mitochondria and endoplasmic reticulum but vitamin E here hasn't effect role in protecting against membrane lipid peroxidation by reactive lipid peroxy and alkoxyl radicals.

The results displayed in Table 1 proved that, no significant change in the serum nitric oxide (NO) concentration in rats injected LPS (2hr. and 5hr.) group when compared with control group. These results agreed with the data of Taylor *et al.*, [82] who mentioned that, NO production by hepatocytes is not significantly enhanced by LPS, but was markedly enhanced by cytokines and even LPS-activated Kupffer cells conditioned medium. Therefore, sub-stimulatory doses of LPS can effectively reprogram macrophages for altered responses to subsequent activation by LPS and other microbial stimuli and no enhancement is observed with NO production following LPS-dependent re-programming. The observed enhancement of the NO response by LPS-dependent reprogramming is not strictly dependent upon enhanced macrophage responsiveness for NO production, since neutralizing antibody to mouse NO only partially no change for LPS response. Collectively, these data support the conclusion that the LPS-dependent reprogramming event involved a more generalized intracellular regulatory event cannot control NO production [38].

In the same way, there was no significant change in the liver GPx activity in rats injected LPS (2hr. and 5hr.) group when compared with control group (Table 2). These results came in accordance with the recorded data of Gilad *et al.*, [31] who indicated that, no changes were observed in the activity of glutathione peroxidase in the brain of rats exposed to LPS. Moreover, Aydin *et al.*, [6] reported that, no significant change GPx enzyme activity in LPS rats determined compared to control group. Decrease GPx activities or its hidden may indicate that high amount of hydrogen peroxide which may have accumulated in the cells. Increased hydrogen peroxide may be transform to hydroxyl radicals and increase oxidative stress.

Concerning to liver GR, also there was no significant change in the liver GR activity in endotoxin injected rats (2hr. and 5hr.) group when compared with control group (Table 2). These results are in a harmony with the data of Rojas *et al.*, [71] who recorded that, a dose of LPS which induced endotoxic shock did not alter any antioxidant enzyme or GR in the guinea pig myocardium, making the interpretation of the changes in the rest of the parameters studied more easy. GR is a key enzyme of the antioxidative system that protects cells against free radicals. The enzyme catalyzes the reduction of GSSG to GSH by the NADPH-dependent mechanism. No change of GSH/GSSG ratio may be contributes to decrease oxidative stress and there is increasing evidence indicating that oxidative stress plays an important role in the pathogenesis of many diseases [79]. The obtained data in Table 1 & 2 demonstrated that, the mean value of serum and tissue GSH concentration decreased significantly in endotoxin injected rats (2hr. and 5hr.) group when compared with control group. These results are in a harmony with the data of Ballatori *et al.*, [7] and Lee *et al.*, [50] who observed that, increased oxidative stress following endotoxin administration is supported by decreased levels of reduced glutathione (GSH) in experimental animals. However, GSH is an important regulator of cellular redox balance and plays a major role in protecting against oxidative stress by reacting directly with ROS. The impact of GSH on immune function has been investigated as confirmed by Adler *et al.*, [2] who provided that, GSH depletion in rats impairs T-cell and macrophage immune function and the level of GSH is depressed in the serum [55]. Moreover, serum and liver GSH was significantly increased in pretreatment vitamin E group (5hr.) when compared with endotoxin injected rats only. These results are similar with the data of Gilad *et al.*, [31] and Nadia *et al.*,

[58] who observed that, treatment with α -Tocopherol increased GSH by approximately 15.4% compared with control. This is because of glutathione in the reduced state (GSH) is present in human plasma intracellular, it has antioxidant properties to inhibit free radical formation and functions more generally as a redox buffer [23].

The data showed in Table 2 stated that, intraperitoneal injection of endotoxin to normal rats significantly increased liver L-MDA concentration after 5 hours in comparison with control group. These results are in a harmony with the recorded data of Freeman and Crapo [30] who reported that, L-MDA is a major oxidation product of peroxidized poly-unsaturated fatty acids and increased L-MDA content is an important indicator of lipid peroxidation and his study has shown a significant elevation in L-MDA levels with significant decline in rat liver homogenates after LPS administration. Formation of lipid peroxides in the crude homogenates resulted in response to the administration of LPS. This may be due to an enhanced generation of O_2^- and H_2O_2 that accelerated peroxidation of native membrane lipids. Peroxidation of mitochondrial membrane led to loss of cell integrity, increase in membrane permeability and alteration of Ca^{2+} homeostasis that contribute to cell death due to alteration in the inner membrane potential [41]. Nevertheless, L-MDA an end product of lipid peroxidation was shown to increase in the mitochondria from rat liver in response to exhaustive treadmill running in rats. Rise in L-MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these rats [53]. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. The lipid peroxides and free radicals may be important in pathogenesis of sepsis [75].

The mean value of liver L-MDA concentration decreased significantly in vitamin E pretreatment group (5hr.) when compared with endotoxin injected rats. These results agreed with the data of McDowell, [56] who indicated that, positive effect of vitamins E is evidence for liver MDA level decreased and it is known that vitamin E is the first line of defense against lipid peroxidation. Vitamin E and other hydrophobic antioxidants function mainly in and around the membrane/lipid bilayers, which halt lipid peroxidation by acting as peroxy radical trapping, chain breaking antioxidants [42]. Liver SOD activity was decreased significantly in rats injected LPS (2hr. and 5hr.) group when compared with control group (Table 2). These results are in a harmony with the recorded data of Pilkhwal *et al.*, [68] who reviewed that, LPS markedly decreased liver SOD levels indicating oxidative stress in rats as compared with control group after injection 4 hours and this decrease persist after 8 hours. Decrease of SOD activities may indicate that high amount of hydrogen peroxide which may have accumulated in the cells. Increased hydrogen peroxide may be transform to hydroxyl radicals and increase oxidative stress [6]. While, Pretreatment with vitamin E significantly increased liver SOD activity at 2 and 5 hours after endotoxin injection when compared with endotoxin treated rats only. These results came in accordance with the recorded data of Sushma *et al.*, [78] who investigated that, tocopherol supplementation in LPS-challenged rats increased the SOD level in both pre and post-LPS challenged groups which were supplemented with tocopherol for 15 days, possibly because α -Tocopherol decreased lipid peroxidation, leading to decreased levels of $O\bullet_2$ and endotoxemia is also accompanied by significant changes in the reductive oxidative balance of critical target organs [86].

Liver catalase activity decreased significantly in rats injected LPS (2hr. and 5hr.) group when compared with control group as showed in Table 2. These results are in a harmony with the recorded data of Sushma *et al.*, [78] who recorded that, LPS significantly reduced the levels of liver catalase as compared to the control group. It may be suggested that after LPS administration in the mice there may be decreased H_2O_2 in the liver, and not scavenge enough those increased oxidant burden liver tissue so not more catalase expression [26]. Meanwhile, liver catalase activity was significantly increased in vitamin E pretreatment group (2 and 5hr.) when compared with endotoxin injected rats. These results came in accordance with the recorded data of Sushma *et al.*, [78] who investigated that, catalase activity was also significantly increased at only in tocopherol supplemented and LPS challenged group. Moreover, Gilad *et al.*, [31] provided that, vitamin E treatment (pre or post) significantly reversed depleted catalase activity in liver and brain while no protective effect was observed in kidney tissue. It had been thought that intracellular superoxide amount is increased as a result of increased NADPH oxidase enzyme of stimulated phagocytic leucocytes. At the same time, increased SOD activity protects the oxygen metabolizing cells against the harmful effects of free radicals such as lipid peroxidation and take place in the intracellular termination of phagocytic bacteria. It can be thought that based on the increase of SOD enzyme activity, hydrogen peroxide can increase as well. It could be concluded that, endotoxemia could potentially influence serum and liver tissues biochemical parameters. So that, LPS has been reported to disrupt the cell membrane. Indeed, this work demonstrated the action of LPS is mainly due to the formation of free radicals that attack the cell membranes. This study provides novel evidence that, α - tocopherol (Vitamin E) decreased LPS-triggered pathogenic

responses by mitigating liver damage, being counteracted the oxidative stress and prevented the increase of lipid peroxidation (MDA). Moreover, vitamin E protects against lipid peroxidation, oxidative stress and decrease the inflammatory response to endotoxin injection.

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دراسة التأثير الوقائي لفيتامين هاء على الدلالات البيوكيميائية للاجهاد التأكسدي ودلالات الالتهابات في نموذج التسمم البكتيري للفئران

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قسم الكيمياء الحيوية- كلية الطب البيطري بمشهر - جامعة بنها

الملخص العربي

في هذه الدراسة تم تقييم التأثير الوقائي لفيتامين هاء على التغيرات في مستوى جلوكوز ودهون الدم ، حمض البوليك ، الأكسدة الكلية للدهون، بعض دلالات الالتهاب في مصل الدم والإنزيمات المضادة للأكسدة في دم وأنسجة الفئران المحقونة بالاندوتوكسين. هذا وقد استخدم لاجراء هذه الدراسة عدد 80 من ذكور الفئران البيضاء أعمارهم تتراوح من 8-10 أسابيع و أوزانها من 150-200 جرام وقسمت إلي أربعة مجموعات اشتملت كل مجموعة على عدد عشرون فأر وتم توزيعها كالاتي: المجموعة الأولى: (المجموعة الضابطة): تكونت من 20 فأر لم تعطى أي أدوية سوى زيت الذرة . المجموعة الثانية: تكونت من 20 فأر تم جرعههم لفيتامين هاء فقط لمدة 35 يوم بجرعة 100 ملليجرام لكل كيلوجرام من الوزن. المجموعة الثالثة:تكونت من 20 فأر تم حقنهم بيكتريا مسممة(الايشريشياكولاي)(الاندوتكسين) 20 ملليجرام لكل كيلوجرام من الوزن. المجموعة الرابعة: اشتملت على 20 فأر تم جرعههم لفيتامين هاء لمدة 35 يوم ثم حقنهم بالاندوتوكسين. وقد تم تجميع عينات الدم بعد خمس اسابيع على فترات: بعد ساعتين وبعد خمس ساعات من حقن الاندوتوكسين في أنابيب نظيفة ومعقمة وقد تم فصل مصل الدم واستخدامه مباشرة لقياس سكر الدم ثم تم حفظ المصل المتبقى في الفريزر لقياس:حمض البوليك - دهون كاملة (كوليسترول كلى - دهون ثلاثية -الدهون الفوسفورية-احماض دهنية حرة) - اوكسيد النيتريك - اسبرتات امينو ترانس فيراز - الانين امينو ترانس اميناز - جاما جلوتاميل ترانس فيراز - هابتوجلوبين - الفا-1- جليكوبروتين - فيتامين سى - فيتامين هاء - جلوتاثيون مختزل وال-مالون داي الدهيد ثم تم ذبح الفئران واخذ الكبد وذلك لتقدير سوبراكسيد ديسميوتيز- جلوتاثيون مختزل - كتالاز - فيتامين سى - فيتامين هاء ,ال-مالون داي الدهيد, جلوتاسيون ريديكتازوجلوتاثيون بيروكسداز .وقد أسفرت نتائج التحليل البيوكيميائي عن وجود زيادة في كلا من تركيز سكر الدم-حمض البوليك - دهون ثلاثية -الدهون الفوسفورية- احماض دهنية حرة - اسبرتات امينو ترانس فيراز - الانين امينو ترانس اميناز - جاما جلوتاميل ترانس فيراز - هابتوجلوبين - الفا-1-جليكوبروتين -جلوتاثيون مختزل بالمصل بالإضافة إلى زيادة في معدل سوبراكسيد ديسميوتيز- جلوتاثيون مختزل - كتالاز - وال-مالون داي الدهيد في نسيج الكبد. وخلصت الدراسة على أن التسمم البكتيري له تأثير ضار على المكونات الكيميائية الحيوية للمصل وأنسجة الكبد وذلك من خلال إضراب وخلل في تركيب غشاء الخلية الذي يرجع أساسا إلى تكوين الجذور الحرة التي تهاجم غشاء الخلايا. وان حقن ألفا-توكوفيرول (فيتامين هاء) أدى إلى الحد من الآثار الضارة والناجمة للتسمم البكتيري المسببة لتلف وتدمير خلايا الكبد وذلك من خلال منع الأكسدة الفوقية للدهون والإجهاد التأكسدي والتقليل من استجابةالالتهابات نتيجة حقن الأندوتوكسين. لذلك ينصح بتناول فيتامين هاء في حدود الجرعة الطبية الموصى بها وذلك للوقاية من تلف الخلايا التي تتجم عما يسمى الجذور الحرة أو الذرات الطليقة، وتشمل تلك الأمراض السرطان والتهاب المفاصل وأمراض الحساسية والقلب وغيرها من الأمراض الضارة .

(مجلة بنها للعلوم الطبية البيطرية: عدد 23(2)، ديسمبر 2012:121-136)