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Effect of α-Tocopherol on Cytokines Production, Oxidative Stress, and Liver Injury Induced by Lipopolysaccharides in Male Albino Rats

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Abstract

Cytokines are secreted by specific cells of immune system and can mediate the balance between humoral and cell- mediated immune responses and they are involved in pathologic processes occurring in the liver. An imbalance of pro-inflammatory and anti-inflammatory cytokines production influences their immunopathogenesis. The current study aimed to detect the ameliorative effect of α-Tocopherol on cytokines production, oxidative stress biomarkers, and liver injury in Lipopolysaccharides (LPS) intoxicated male albino rats. Adult rats were classified as, (Group I): administered with saline solution (0.9%NaCl) as intraperitoneal injection (i.p), (Group II): administered orally with α-Tocopherol (40 mg /kg b. w) twice a week for one month, (Group III): injected (i.p) with a single dose of LPS (1mg/kg b. w), (Group IV): administered orally with α-Tocopherol for a month, then, intoxicated with a single dose of LPS. Rats were scarified at 4 and 24 hours post the last injected doses. The results of the present study revealed that intoxication with LPS induced imbalance in production of cytokines as it caused significant increases in the production of pro- inflammatory cytokines (tumor necrosis factor-α, interleukin-1β and interleukin-6) and anti-inflammatory cytokine (interleukin-10). Also, it increased liver lipid peroxide (MDA), hydrogen peroxide (H2O2), total cholesterol (TC) and triglycerides, liver enzymes as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) compared with the control group. Conversely, intoxication with LPS significantly decreased liver antioxidant enzymes as catalase, superoxide dismutase (SOD) and reduced glutathione (GSH) activity, and induced adverse histopathological changes in liver, including, increasing in number of active kuppfer cells and in leukocytes infiltration. Pre-treatment with α-Tocopherol significantly ameliorated the all previous studied parameters compared with LPS group, in particular, it decreased the levels of the pro-inflammatory cytokines (TNFα, IL-1β and IL-6) and increased the level of the anti-inflammatory cytokine (IL-10) and improved adverse histopathological changes in liver induced by LPS challenge, including, decrease in number of active kuppfer cells and reducing leukocytes infiltration in liver. Conclusion: we could conclude that α-Tocopherol has immunomodulatory effect demonstrated by ameliorating cytokines production and reducing active kuppfer cells and leukocytes infiltration in liver, anti-inflammatory effect as it increased the activity of the anti-inflammatory cytokine and decreased the level of the pro-inflammatory cytokines and its antioxidant effect was proven by alleviating lipid peroxidation through scavenging of free radicals, or by enhancing the activity of the antioxidants in liver. Moreover, improving the adverse histopathological changes in liver induced by LPS intoxication showed that it has hepato-protective effect and could attenuate liver injury.

Keywords: Pro-inflammatory cytokines, anti-inflammatory cytokines, kuppfer cells, Liver injury, α-Tocopherol, Lipopolysaccharides, oxidative stress.

1. Introduction

Cytokines are a large group of proteins, peptides or glycoproteins that are secreted by specific cells of immune system and can mediate the balance between humoral and cell- mediated immune

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responses. Cytokines are important in health and disease, specifically in host immune responses to infection, cancer, inflammation, sepsis and trauma. They act through cell surface receptors and consist of different types, including the interleukins, the chemokines, the tumor necrosis factor, the interferons and the transforming growth factor-b. Cytokines are produced by a wide variety of cells, mainly the Th1 and Th2 cells. Th1 cells secrete pro-inflammatory cytokines which are involved in cell-mediated immunity, and play a crucial role in protection from intracellular pathogens and are associated with recovery. The Th2 cells secrete anti-inflammatory cytokines which regulate humoral immune responses, and their rising levels are often associated with the development of persistent infections. Cytokines may be poor anti-inflammatory, suppress the activity and production of pro-inflammatory signals limiting inflammation and host damage; or pro-inflammatory, induce inflammation as a result of infection or injury. Different cytokine combinations give rise to distinct consequences, such as inflammation and insufficient production of cytokines may contribute significantly to the pathophysiology of a range of diseases including hepatic diseases (Allavena *et al.,* 2011, Huang *et al.,* 2020, Sultani *et al.,* 2012, Sun *et al.,* 2020, Chen *et al.,* 2012 and Guven, 2014).

Interleukin-10 (IL-10) is one of the most important anti-inflammatory cytokines, with important roles in modulating hyperactive immune responses to protect the body from excessive cell and organ damage. IL-10 can be produced by regulatory T cells, monocytes and B cells. In the liver, IL-10 can be produced by a variety of cell types, including Kupffer cells, sinusoidal endothelial cells and lymphocytes. Upon liver inflammation, it is upregulated under various conditions, and is always associated with protective impact during chronic liver disease. Interleukin-6 (IL)-6 is an interleukin that acts as both pro-inflammatory and anti-inflammatory cytokine. It is secreted from macrophages and T cells to stimulate immune response. Tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) are pleiotropic mediators of biologic responses related to infection, immunity, and inflammation. IL-1, TNFα and IL-6 are cytokines with overlapping biological properties which form a complex network of interactive signals (Fathia and Khaled, 2016).

The liver is a major organ in the production of cytokines and has a key role in metabolism of immune system. Moreover, cytokines are involved in pathologic processes occurring in the liver, liver growth and regeneration, as well as in inflammatory processes including liver fibrosis and cirrhosis (Perea *et al.,* 2017). An imbalance of pro-inflammatory and anti-inflammatory cytokines production influences their immunopathogenesis. In particular, alterations in serum and intrahepatic Th1 and Th2 cytokine patterns, including Il-6 and TNF-α, play a critical role in host immune response in liver. In the course of liver cirrhosis and failure, many pathological phenomena take place as abnormalities of the gut microflora and endotoxemia that leads to an increase in production of inflammatory cytokines, resulting in acute liver injury and failure (Michelena *et al.,* 2015, Fathia and Khaled, 2016).

Lipopolysaccharides (LPS), also known as endotoxins, are large molecules consisting of a lipid and a polysaccharide and are found in the outer membrane of Gram-negative bacteria and causes uncontrolled disease progression. Liver injury induced by LPS is associated with inflammatory mediators including nitric oxide, tumor necrosis factor, interleukin-1β, interleukin-6, and other cytokines. In vitro pretreatment of human monocytes with lipopolysaccharide (LPS) induces "endotoxin tolerance" with blunted TNF and IL-6 release to rechallenge with LPS. The pro-inflammatory cytokines TNF and IL-6 are important mediators in sepsis and the high IL-6 concentration has been used as a marker of infection severity. Also, in sepsis, the *in vivo* exposure to endotoxin is detectable by the *ex vivo* analysis of lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF) production. Moreover, several studies indicated that LPS induce increase in lipid peroxidation, which is an index of oxidative stress. This effect has been both time- and dose-dependent. Also, LPS administration resulted in liver injury in experimental animals. Septic liver dysfunction causes several alterations, including, release of reactive oxygen species (ROS) and imbalance of endogenous enzymatic activity, such as glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) (Kao *et al.,* 2009, Song *et al.,* 2017).

Vitamin E" is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities because it can act as a strong scavenger of free radicals, so prevent oxidative stress induction. α-Tocopherol is the only one used by the human body. In addition to its activities as an antioxidant, α-Tocopherol is involved in immune function, cell signaling and regulation of gene expression. Previous studies demonstrated that vitamin E could reduce oxidative stress and inflammation in experimental animals (Kanter *et al.,* 2005).

The present study aimed at investigating the ameliorating effect of α-Tocopherol on disturbed levels of pro-inflammatory and anti-inflammatory cytokines, oxidative stress, liver injury and histopathological alteration induced by LPS intoxication in male albino rats.

2. Material and Methods

2.1. Chemicals

Pure olive oil was used as a solvent for α-Tocopherol and was purchased from the popular market. α-Tocopherol was purchased from oxford laboratory, Mumbai, India. Lipopolysaccharides extracted from E. coli serotype O127:B8, were purchased from Sigma-Aldrich chemical St. Louis, MO, USA. All additional chemical reagents and kits included in the study were of standard analytical grades and were obtained from Sigma Chemical Co. (St. Louis, O, USA).

2.2. Experimental Animals

In the current study, forty rats were included. All rats were adult, male albino and Sprague Dawely (*Rattus norvegicus*) with average weight (130 - 150 g). The rats were obtained from Faculty of Medicine, Minia, Egypt and were examined well to avoid any signs of microbial or parasitic diseases. Rats were housed in cages in well ventilated room under controlled laboratory conditions of temperature (25°C), with constant humidity and 12h/12h light/dark cycle and were supplied with commercial rodent diet and drinking tap water *ad libitum* for one week before carrying out the experiment procedures for adaptation. The study protocol was approved by the Animal Ethics Committee of the Zoology Department at the Faculty of Science, Minia University according to Helsinki principles.

2.3. Study Design

After the adaptation period, the rats were divided into four groups (n=10 in each group) as following:

1. Group I: that was considered as normal control group in which rats were administered intraperitoneally with saline solution (0.9% NaCl) in a dose of (0.5ml / rat) daily for one month.

2. Group II: rats were administered with α-Tocopherol (40 mg / kg b w) in olive oil, orally, and twice a week for one month (Venkatanarayana *et al.,* 2012).

3. Group III: rats were administered intraperitoneally with saline (0.9% NaCl) (0.5ml / rat) daily, then were injected with LPS (1mg/kg body weight) on the 30th day (El-Feki *et al.,* 2016).

4. Group IV: rats were administered with α-Tocopherol (40 mg/kg body weight) in Olive oil (0.5ml / rat) orally and twice a week, then, injected with LPS on the 30th day.

After LPS injection, all rats were sacrificed under anesthesia with diethyl ether after four hours and twenty-four hours (five rats at each time), and blood samples from each rat were collected immediately, left to coagulate, then centrifuged at 3000 rpm for 15 minutes to obtain serum for estimation of some immunological and biochemical parameters. Liver samples were obtained and prepared for tissue homogenate as following: a part of liver tissue was perfused with a phosphate buffer saline (PBS) solution, with pH = 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. The tissue was homogenized in 10 ml cold buffer (50 mM potassium phosphates, pH 7.5) per gram tissue. Tissue homogenate was centrifuged at 4000 r.p.m for 15 minutes. The supernatant was removed and stored at - 80 ° for antioxidants assay. Also, other parts of liver were taken rapidly, washed with normal saline and fixed in 10% formal saline solution for histopathological studies. Fixed liver tissues were then embedded in paraffin and sectioned to 5 µm thick. Ordinary Haematoxylin and eosin staining were used according to standard protocols.

2.4. Determination of immunological parameters

From all control and treated groups, serum samples were used for detecting levels of pro-inflammatory and anti-inflammatory cytokines. Serum samples were used for the determination of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL-10) release by Enzyme-Linked Immune Sorbent Assay (ELISA) using Rat IL-1β PicoKine ™ ELISA Kit (EK0393), Rat TNF- alpha PicoKine ™ ELISA Kit (EK0526), Rat IL-6 PicoKine ™ ELISA Kit (EK0412), and Rat IL-10 PicoKine ™ ELISA Kit (EK0418) from Boster Biological

Technology Co., Ltd.

2.5. Biochemical analysis

Liver function parameters were determined in serum. The levels of alanine and aspartate aminotransferases (ALT&AST) were measured in serum according to the method of Tietz (1995). Levels of gamma-glutamyl transferase (GGT) in serum were determined by the method of Fiala *et al.* (1972). Serum alkaline phosphatase (ALP) concentrations were estimated using the method of King (1965). Triglycerides (TG) concentration was determined according to the method described by Fossati and Lorenzo (1982). Total cholesterol (TC) level was calculated in serum using the method described by Tietz (1995).

Liver lipid peroxidation level was measured in the liver tissue as the MDA level according to the method of Elguindy *et al.* (2016). Hydrogen peroxide (H2O2) level was measured according to the method of Aebi, (1984). Liver reduced glutathione activity (GSH) was evaluated using the method of Beutier *et al.* (1963). Liver catalase activity was assayed depending on the method of Aebi, (1984) and superoxide dismutase (SOD) activity was measured according to the method of Nishikimi, *et al.* (1972).

2.6. Statistical analysis

The collected data statistically analyzed using SPSS program software version 20. Analysis was done for parametric quantitative variables using one way ANOVA test. The mean± standard deviation (M± SD) was used to express the findings. The significance of the student "t" test was used and variations were deemed to be significant at ($P \leq 0.05$), extremely significant at ($P \leq 0.01$) and very extremely significant at ($P \leq 0.001$).

3. Results

3.1. Immunological studies (cytokines production)

3.1.1. Tumor necrosis factor alpha (TNF- α) levels

The results of the present study revealed that rat group orally treated with α-Tocopherol (40 mg /kg body weight), showed insignificant alterations in the levels of serum TNF-α ($p > 0.05$) as compared to the control group at 4 and 24 hours. On the other hand, rats intoxicated with LPS (1 mg / kg body weight) showed a significant increase in TNF- α level ($p<0.001$) at 4 and 24 hours compared with the control group, while, the level of this cytokine decreased significantly ($p<0.001$) in rats orally pretreated with α-Tocopherol twice a week for a month before LPS injection as compared to the LPS group at the two times (Table 1).

3.1.2. Interleukin- 6 (IL-6) levels

The current data demonstrated that α-Tocopherol administration alone for a month did not induce significant alterations ($p > 0.05$) in the level of serum IL- 6 as compared to the control group at 4 and 24 hours (Table 1). Conversely, rats challenged with LPS, showed a significant increase in serum IL- 6 level ($p <0.001$) at the two times, its peak time was 4 h (911 ± 12 pg/ml) compared with the control group (35.4 ± 1.81pg/ml), while, the level of this cytokine decreased significantly ($p <0.001$and $p <0.01$ respectively) on supplementation with α-Tocopherol before LPS injection at 4 and 24 hours (676 ± 15.21 and 50.6 ± 7.53 pg/ml, respectively) as compared to the LPS group at the two times (Table 1).

3.1.3. Interleukin-1β (IL-1β) levels

It was proven that animals orally administered with α-Tocopherol, had insignificant alterations ($p > 0.05$) in the level of serum IL-1β as compared to the control group at 4 and 24 hours. While, rats intoxicated with LPS, showed a significant increase in serum IL-1β level ($p <0.001$) at the two times compared with the control group, but, the level of this cytokine decreased significantly ($p <0.001$and $p <0.05$ respectively) on supplementation with α-Tocopherol before LPS injection at 4 and 24 hours as compared to the LPS group (Table 1).

3.1.4. Interleukin-10 (IL-10) levels

The current study revealed that animals administered orally with α-Tocopherol showed insignificant changes ($p > 0.05$) in the activity of serum IL-10 as compared to saline group at 4 and 24 hours, but, rats intoxicated with LPS showed a significant increase in serum level of this cytokine ($p < 0.001$) at 4 and 24 hours compared with the control group, while, the level of that cytokine was significantly more increased ($p < 0.001$) at 4 hour only on supplementation with α-Tocopherol for one month before LPS intoxication as compared to the LPS group (Table 1).

Table 1: Effect of α-Tocopherol, lipopolysaccharides (LPS) and (α-Tocopherol & LPS) on serum levels of pro-inflammatory and anti-inflammatory cytokines in male albino rats at four and twenty four hours after LPS challenge.

Parameter	Groups	Saline	α-Tocopherol	LPS	α-Tocopherol & LPS
TNF- α	**4 h**	65 ± 1.48	63.4 ± 2.03	166.8 ± 3.49###	71.6 ± 2.07 ***
(pg/ml)	**24 h**	65 ± 1.58	64.2 ± 1.30	71.6 ± 2.07###	64.6 ± 3.64 ***
IL-6	**4 h**	35.4 ± 1.81	32.2 ± 2.12	911 ± 12.75 ###	676 ± 1.41 ***
(pg/ml)	**24 h**	34.2 ± 1.3	32.8 ± 1.64	64.4 ± 2.51###	50.6 ± 7.53 **
IL- 1β	**4 h**	46.88 ± 2.03	45.9 ± 2.03	122.12 ± 4.09 ###	85.9 ± 2. 26 ***
(pg/ml)	**24 h**	46.01 ± 2.03	44.50 ± 1.09	64.3 ± 4.65 ###	55.05 ± 3.02 *
IL-10	**4 h**	22.4 ± 1.14	20.8 ± 2.31	141.8 ± 5.21###	176 ± 5.83 ***
(pg/ml)	**24 h**	22.8 ± 1.3	21.6 ± 2.3	25 ± 2.82###	25.8 ± 2.7

Values are expressed as means ± SD (n= 5).
 # p<0.05, ## p<0.05 and ### p <0.001 vs saline. **p <0.05, ** p <0.01, *** p <0.001* vs LPS

3.2. Biochemical analysis of liver function

3.2.1. Alanine aminotransferase (ALT) levels

The present data showed insignificant ($p > 0.05$) alterations in the activity of ALT that were observed in rat group administered orally with α-Tocopherol alone as compared to the control group at 4 and 24 hours. Conversely, rats challenged with LPS had a significant increase in serum ALT level ($p < 0.001$) at 4 and 24 hours (60.2 ± 2.38 and 87.2 ± 8.01 U/L, respectively) compared with the control group (37.6 ± 2.07 and 37.2 ± 2.58 U/L, respectively), while, the levels of this enzyme decreased significantly ($p < 0.001$) on supplementation with α-Tocopherol before LPS injection as compared to the LPS group at 24 hours only (Table 2).

3.2.2. Aspartate aminotransferase (AST) levels

The present data demonstrated that administration of α-Tocopherol alone for a month did not induce significant alterations ($p > 0.05$) in the activity of serum AST as compared to the control group at 4, and 24 hours. On the other hand, rats challenged with LPS showed a significant increase in its level ($p < 0.001$) at the two times and peaked at 24 hour (185.6 ± 7.82 and 254.6 ± 6.11 U/L, respectively) compared with the control group (132.8 ± 1.48 and 131.8 ± 1.48 U/L, respectively), while, the level of this enzyme decreased significantly ($p < 0.01$ and $p < 0.001$) at 4 and 24 hours on supplementation with α-Tocopherol for a month before LPS challenge (165.4 ± 8.9 and 144.8 ± 4.54 U/L, respectively) as compared to the LPS group (Table 2).

3.2.3. Alkaline phosphatase (ALP) levels

Insignificant alterations ($p > 0.05$) in the level of ALP were observed in the rats treated with α-Tocopherol at 4 and 24 hours. While, rats challenged with LPS showed a significant increase in serum ALP level ($p < 0.001$) at 4 and 24 hours and peaked at 24 h (247.4 ±10.81 and 377.2 ± 8.28 U/L, respectively) compared with the control group (189.2 ± 2.38 and 187 ± 2.91 U/L, respectively). The level of this enzyme decreased significantly ($p < 0.001$) at 4 and 24 hours on supplementation with α-Tocopherol before LPS injection (220.6 ± 7.02 and 311.8 ± 12.69 U/L, respectively) as compared to the LPS group (Table 2).

3.2.4. Gamma-Glutamyl transferase (GGT) levels

The current results revealed insignificant($p > 0.05$) alterations in the level of GGT in rat group treated with α-Tocopherol alone for one month as compared to the control group at 4 and 24 hours.

On the other hand, rats intoxicated with LPS showed significant increase in GGT levels ($p < 0.001$) at 4 and 24 hours and peaked at 4 hours (17.31 ± 2.75 and 11.8 ± 0.68 U/L, respectively) compared with the control group (3.4 ± 0.36 and 3.44 ± 0.32 U/L, respectively), while, the levels of this enzyme decreased significantly ($p < 0.001$) on supplementation with α-Tocopherol before LPS injection (8.78 ± 1.06, and 4.58 ±0.83 U/L, respectively) as compared to the LPS group at the two times (Table 2).

3.2.5. Total cholesterol levels

The present data demonstrated that administration of α-Tocopherol alone for a month did not induce significant alterations ($p > 0.05$) in the level of serum cholesterol as compared to saline group at 4 and 24 hours. Rats intraperitoneally injected with LPS showed a significant increase in serum cholesterol ($p < 0.001$) at the two times and peaked at 24 h (75.6 ± 2.7 and 109.2 ± 3.76 mg/dL respectively) compared with the control group (63.6 ± 3.57 and 63.8 ± 2.77 mg/dL, respectively), while, the level of cholesterol decreased significantly ($p < 0.001$) on treatment with α-Tocopherol for one month before LPS injection (64.4 ± 3.91 and 79.2 ± 7.69 mg/dL, respectively) as compared with the LPS group at the two times (Table 2).

3.2.6. Triglycerides levels

Administration orally with α-Tocopherol alone for one month showed non-significant ($p > 0.05$) alterations in the level of serum triglycerides as compared to control groups at 4 and 24 hours. Rats challenged with LPS showed a significant increase in serum triglycerides level ($p < 0.001$) at the two times and peaked at 4 h (141.2 ± 3.56 and 88 ± 2.12 mg/dL, respectively) compared with the control group (63.6 ± 3.57 and 63.8 ± 2.77 mg/dL, respectively), while, the level of triglycerides decreased significantly ($p < 0.001$) at 4 hour only on supplementation with α-Tocopherol for one month before LPS intoxication as compared to the LPS group.

Table 2: Effect of α-Tocopherol, lipopolysaccharides (LPS) and (α-Tocopherol & LPS) on serum levels of liver function parameters in male albino rats at four and twenty four hours after LPS challenge.

Parameter \ Groups		Saline	α-Tocopherol	LPS	α-Tocopherol & LPS
ALT	4 h	37.6 ± 2.07	33.4 ± 2.7	60.2 ± 2.38 ###	59.2 ± 5.93
	24 h	37.2 ± 2.58	34.2 ± 3.11	87.2 ± 8.01 ###	59.4 ± 4.27 ***
AST	4 h	132.8 ± 1.48	132.8 ± 1.64	185.6 ± 7.82 ###	165.4 ± 8.91 **
	24 h	131.8 ± 1.48	133 ± 2	254.6 ± 6.11 ###	144.8 ± 4.54 ***
ALP	4 h	189.2 ± 2.38	187 ± 2	247.4 ± 10.81 ###	220.6 ± 7.02 ***
	24 h	187 ± 2.91	187.6 ± 2.79	377.2 ± 8.28 ###	311.8 ± 12.69 ***
GGT	4 h	3.4 ± 0.36	3.77 ± 0.22	17.31 ± 2.75 ###	8.78 ± 1.06 ***
	24 h	3.44 ± 0.32	3.73 ± 0.19	11.8 ± 0.68 ###	4.58 ± 0.83 ***
TG	4 h	66.4 ± 3.04	66.8 ± 3.7	141.2± 3.56 ###	108 ± 5.29 ***
	24 h	63.8 ± 2.77	64.6 ± 3.64	88 ± 2.12 ###	80.6 ± 5.45
CL	4 h	63.6 ± 3.57	63.8 ± 3.11	75.6 ± 2.7 ###	64.4 ± 3.91 ***
	24 h	63.8 ± 2.77	64.6 ± 3.64	109.2 ± 3.76 ###	79.2 ± 7.69 ***

Values are expressed as means ± SD (n= 5).
 # p<0.05, ## p<0.05 and ### p <0.001 vs saline. **p <0.05, ** p <0.01, *** p <0.001* vs LPS

3.3. Lipid peroxidation and antioxidant activity

3.3.1. Liver lipid peroxidation (MDA).

Administration of α-Tocopherol alone for a month did not induce significant alterations ($p > 0.05$) in the level of MDA as compared to the control (saline group) at 4 and 24 hours (Table 3). Animals intoxicated with LPS showed a significant increase in MDA level ($p < 0.01$) at 4 and 24 hours (6.78 ± 0.16 and 5.88 ± 0.21 nmol/g.tissue, respectively) compared with the control groups (4.76 ± 0.23 and 4.87 ± 0.31 nmol/g.tissue, respectively), while, the level of this enzyme decreased significantly ($p < 0.05$) in rats pretreated with α-Tocopherol for one month before LPS challenge as compared to the LPS group at 4 hours only (Table 3).

3.3.2. Liver hydrogen peroxide (H_2O_2).

The present data revealed that administration of α-Tocopherol alone for a month did not cause significant alterations ($p > 0.05$) in the level of Liver hydrogen peroxide (H2O2) as compared to saline group at 4 and 24 hours. But, rats challenged with LPS showed a significant decrease in (H2O2) level ($p <0.001$) at 4 and 24 hours compared with the control group, while, its level increased significantly ($p <0.001$) on supplementation with α- before LPS intoxication as compared to the LPS group at 4 and 24 hours (Table 3).

3.3.3. Liver reduced glutathione (GSH) activity

The current data determined that administration of α-Tocopherol alone for a month did not induce significant alterations ($p > 0.05$) in the activity of reduced glutathione (GSH) as compared to saline group at 4 and 24 hours. Rats challenged with LPS showed a significant decrease in GSH level ($p <0.001$) at 4 and 24 hours (6.98 ± 0.35 and 5.1 ± 0.48 mg/g. tissue, respectively) compared with the control group (11.29 ± 0.75 and 11.22 ± 0.56 mg/g. tissue, respectively), while, the level of this enzyme increased significantly ($p <0.001$) on supplementation with α-Tocopherol before LPS intoxication (9.86 ± 0.54 and 10.2 ± 0.44 mg/g. tissue, respectively) as compared to the LPS group at 4 and 24 hours (Table 3).

3.3.4. Liver superoxide dismutase (SOD) activity

The current results revealed insignificant ($p > 0.05$) alterations in the activity of (SOD) in rat group treated with α-Tocopherol alone for one month as compared to the control group at 4 and 24 hours. While, rats intoxicated with LPS showed a significant increase in (SOD) ($p <0.001$) at 4 and 24 hours compared with the control group, while, the levels of this enzyme decreased significantly ($p <0.001$) on supplementation with α-Tocopherol before LPS injection as compared to the LPS group at the two times (Table 3).

3.3.5. Liver catalase activity

The group of rats treated orally with α-Tocopherol alone showed insignificant alterations ($p > 0.05$) in the activity of catalase as compared to the control group at 4 and 24 hours. Rats challenged with LPS showed significant decrease in the activity of catalase ($p <0.001$) at the two times (0.738 ± 0.017 and 1.283 ± 0.111 U/g, respectively) compared with the control group (1.821 ± 0.072 and 1.833 ± 0.058 U/g, respectively), while, the activity of this enzyme increased significantly ($p <0.001$) on supplementation with α-Tocopherol before LPS challenge (1.264 ± 0.131 and 1.156 ± 0.075 U/g, respectively) as compared to the LPS group at 4 and 24 hours (Table 3).

Table 3: Effect of α-Tocopherol, lipopolysaccharides (LPS) and (α-Tocopherol & LPS) on lipid peroxidation, oxidative stress and antioxidant activity in liver in male albino rats at four and twenty four hours after LPS challenge

Groups / parameter		Saline	α-Tocopherol	LPS	α-Tocopherol & LPS
MDA	4 h	4.76 ± 0.23	4.71 ± 0.28	6.78 ± 0.16 ##	6.04 ± 0.27 *
	24 h	4.87 ± 0.31	4.8 ± 0.34	5.88 ± 0.21 ##	5.82 ± 0.24
H_2O_2	4 h	0.49 ±0.1	0.53 ± 0.01	1.89 ± 0.21 ###	0.99 ± 0.07 ***
	24 h	0.49 ±0.3	0.55 ± 0.21	2.01 ± 0.5 ###	1.02 ± 0.3 ***
GSH	4 h	11.29 ± 0.75	11.46 ± 0.71	6.98 ± 0.35 ###	9.86 ± 0.54 ***
	24 h	11.22 ± 0.56	11.4 ± 0.79	5.1 ± 0.48 ###	10.2 ± 0.44 ***
SOD	4 h	2.36 ± 0.09	2.61 ± 0.5	0.72 ± 0.1 ###	1.5 ± 0.32 ***
	24 h	2.36 ± 0.21	2.49 ± 0.08	0.87 ± 0.2 ###	1.97 ± 0.34 ***
CAT	4 h	1.821 ± 0.07	1.803 ± 0.07	0.738 ± 0.01 ###	1.264 ± 0.13 ***
	24 h	1.833 ± 0.058	1.813 ± 0.077	1.283 ± 0.11###	1.156 ± 0.075 ***

Values are expressed as means ± SD (n= 5).
 # p <0.05, ## p<0.05 and ### p <0.001 vs saline. ** p <0.05, ** p <0.01, *** p <0.001* vs LPS

3.4. Histopathological examination

Liver sections of rats administrated orally with α-Tocopherol showed nearly normal liver morphology. While, liver sections of LPS group showed a highly dilated and ruptured central vein

congested with hemolyzed blood, infiltrated with leucocytes, and increased number of active kuppfer cells with shrinking in hepatocytes in some areas, Signs of histological improvement could be observed in (α-Tocopherol & LPS) group that showed a moderate regular central vein infiltrated with less inflammatory cells and less activated kupffer cells on dilated blood sinusoids (Fig. 1).

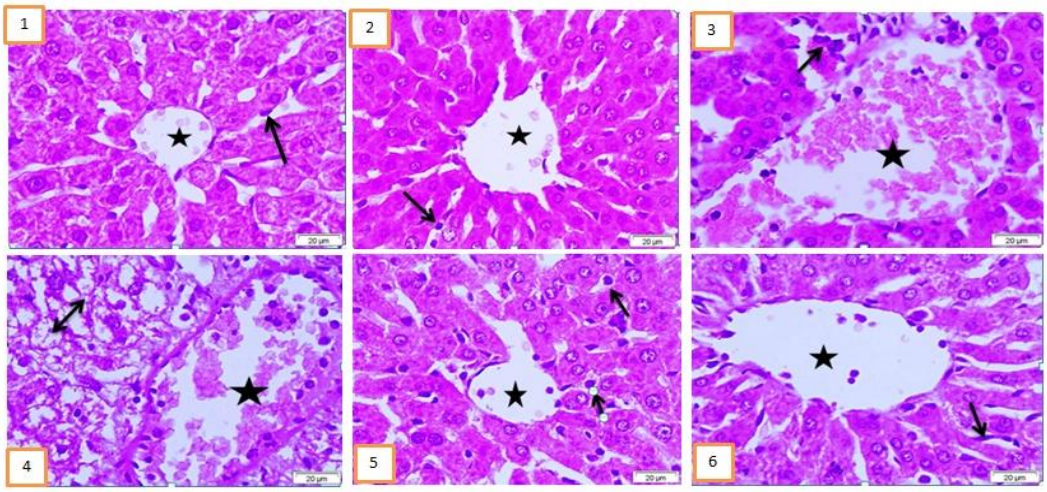

Fig.1: Liver sections of rats administrated with saline (1) and α-Tocopherol (2) showing nearly normal liver morphology, central vein (star), arranged strands of normal and polygonal hepatocytes, normal blood sinusoids and kupffer cells (thin arrow). While, liver sections of 4h LPS group (3) showed a highly dilated and ruptured central vein congested with hemolyzed blood, infiltrated with leucocytes, and increased number of active kuppfer cells with shrinking in hepatocytes in some areas, while, 24h LPS group (4) showed a highly congested central vein with hemolyzed blood, infiltrated with leucocytes, highly vacuolated hepatocytes associated with necrosis (douple arrow) and loss of typical hepatic cords organization and obliteration of blood sinusoid. Signs of histological improvement could be observed in group 4h α-Tocopherol & LPS (5) and 24h α-Tocopherol & LPS group (6) that showed a moderate regular central vein infiltrated with less inflammatory cells and less activated kupffer cells on dilated blood sinusoids.

4. Discussion

Liver consists of several cell types that under normal circumstances produce only minimal levels of cytokines. When liver cells, particularly immune cells called Kupffer cells (KC), become activated, cytokine production increases dramatically; therefore, if the liver has been damaged, cytokines mediate the regeneration of liver tissue. Also, KC can be activated by diseases caused by microorganisms. In this case, cytokines produced and released by the KC induce an inflammatory response in the liver (hepatitis), which is required to start the healing process. However, if the inflammation does not subside after a short time, persistent production of these same cytokines may lead to formation of fibrosis and cirrhosis. Thus, cytokine production can have both beneficial and harmful effects, depending on the amount and duration of cytokine release (Mohan *et al.,* 2019, Fathia and Khaled, 2016).

Toll-like receptors (TLR) are crucial factor in the pathophysiology of liver diseases. The most important one is TLR4 which mediates activation of hepatic stellate cells and Kupffer cells. Previous studies demonstrated that LPS endotoxin can bind to TLR4 and activate oxygen free radicals, NFκB, TNF-α overexpression, and induce inflammatory cytokines production to stimulate the liver cells for inflammation, injury and damage. The occurrence of these harmful mediators in circulation causes the adverse and toxic effects of LPS intoxication in liver and other organs (Görg *et al.,* 2005). The correlation between oxidative stress and TLR pathways is due to the imbalance of cellular pro-inflammatory/oxidative and cyto-protective mechanisms (Mohan *et al.,* 2019).

In the present study, LPS intoxication induced significant increases in the levels of Pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and the level of the anti-inflammatory cytokine (IL-10),

while, administration of α-Tocopherol significantly ameliorated these effects. Elevation of the inflammatory markers above normal levels is an independent predictor of several chronic diseases, including coronary heart disease, stroke, diabetes, atherosclerosis and liver injury. Previous studies indicated that pro-inflammatory cytokines contribute to liver injury by increasing the vascular permeability (Aspinall *et al.,* 2011). TNF- α and IL-6 could directly induce cytotoxic actions by activation of nuclear transcription factor kappa B of Kupffer cells and hepatocytes and, so, causing increase in expression of adhesion molecules, which further impel the inflammatory injury of hepatocytes (Yao & Yue, 2005). Previous studies demonstrated that IL-10 could protect from D-galactose amine and LPS-induced liver injury in experimental animals. IL-10 could exert its anti-inflammatory mechanism by inhibiting the activation of macrophages and monocytes by reducing the formation of pro-inflammatory cytokines (Uchida *et al.,* 2008). These results are in accordance with the results of the present study.

The results of the current study showed that α-Tocopherol could attenuate abnormal cytokines production induced by LPS intoxication and had anti-inflammatory effect as it increased the activity of the anti-inflammatory cytokine (IL-10) and decreased the activity of the pro-inflammatory cytokine. In addition to being strong antioxidant agent with an ability to reduce oxidative stress within hepatocytes, the significant ameliorating effect of α-Tocopherol on the levels of these cytokines (TNF-α, IL-1β and IL-6) after induction with LPS could be attributed to its ability to suppress Kupffer cell activation during liver injury (Godbout *et al.,* 2005). Also, the anti- inflammatory action of α-Tocopherol administration and its reverse influence on the increased levels of pro-inflammatory cytokines could be attributed to inhibition of chemotaxis of neutrophils by inhibition of cyclooxygenase, protein kinase C, tyrosine-kinase and 5-lipoxygenase as mentioned by previous studies (Singh *et al.,* 2005, Jaffer *et al.*, 2010).

Serum AST, ALT, ALP and GGT are biomarkers in the diagnosis of liver dysfunction as they are released into the blood circulation after hepatocellular damage. In the current study, intoxication with LPS to rats attributed to significant and noticed elevation in the serum levels of AST, ALT, ALP and GGT which is indicative of hepatocellular damage, as reported in different studies (Song *et al.,* 2017). The increase in the levels of these enzymes could be attributed to the release of these enzymes from hepatocellular cytoplasm to the blood circulation after rupture of the plasma membrane and occurrence of hepatocellular necrosis. These results agreed with the results reported by Heibashy *et al.* (2013) and agreed with the histopathological findings noticed in the present study. Victor *et al.* (2004) and Kao *et al.* (2009) suggested that administration of LPS leads to inflammatory reactions, hepatocyte injury and steatosis in the liver of experimental animals. Administration of α-Tocopherol before LPS intoxication in the present study ameliorated the elevated levels of ALT, AST, ALP and GGT that decreased towards the normal levels. These results are in a harmony with that of Hussein *et al.* (2012). This effect of α-Tocopherol may be due to its ability to reduce free radical-induced oxidative damage in the liver cells, so, could maintain the integrity of the plasma membrane, thereby suppressing the leakage of enzymes (Hobson, 2016).

Liver is considered as the most important site for metabolism and synthesis of phospholipids, cholesterol, bile acids and triglycerides, so, impaired liver function could lead to disturbances in the levels of the above mentioned parameters (Taniguchi *et al.,* 2002). Moreover, the inability of the diseased liver to remove cholesterol from circulation might lead to increased levels of cholesterol in the plasma (Tarascio, 2009). In the current study, LPS injection was sufficient to increase serum triglycerides and cholesterol significantly when compared with control group and these results came in accordance with a study carried out by Chung *et al.* (2017). Previous studies demonstrated that increase in serum total cholesterol may be due to the effect of LPS on hepatic HMG-CoA reductase activity, mass, and mRNA levels. HMG-CoA reductase is the rate controlling enzyme of mevalonate pathway that produces cholesterol, moreover, it was suggested that LPS induces hypertriglyceridemia by reducing lipoprotein catabolism, increasing hepatic lipoprotein secretion, reducing lipoprotein clearance and decreasing lipoprotein lipase activity (Feingold *et al.,* 1992). The current results showed that administration of α-Tocopherol before LPS intoxication significantly decreased concentration of serum cholesterol and triglycerides when compared with LPS intoxicated rats. This decrease in concentrations may be related to the increased rate of lipolysis by the increase of plasma lipase activity (Bennani *et al.,* 2000).

Several previous studies indicated that LPS induce lipid peroxidation in many cells and organs as liver, heart, and brain of rats which is considered as an index of oxidative stress (Kaur *et al.,* 2006). Under conditions of oxidative stress, reactive oxygen and nitrogen species (RONS) attack the polyunsaturated fatty acids (PUFAs) of cell membranes, causing destabilization, disintegration and alteration in membrane fluidity and permeability, all events which increase the rate of protein degradation and eventually leads to cell lysis. Decomposition products of lipid hydroperoxides such as MDA can interact with protein and nucleic acids, leading to oxidative protein and DNA damage (Pari and Shagirtha, 2012). Oxidative damage is a result of the imbalance between antioxidants and ROS or intensification of free radicals (Bharrhan *et al.,* 2010). In the present study, intoxication with LPS increased significantly the levels of MDA in liver, while, pre-treatment with α-Tocopherol before LPS intoxication significantly decreased concentration of MDA in liver which may be partly due to the ability of α-Tocopherol to scavenge free radicals**.** Moreover, increased plasma levels of the pro-inflammatory cytokines such as IL-6 and TNF-α influence nitric oxide that induces free radicals production and lipid peroxidation. In the current study, LPS increased levels of H2O2 and this result came in agreement with Lv *et al.,* (2017) who reported that LPS injection resulted in activation of peroxiredoxin gene, which is responsible for a significant increase in the ROS content in macrophage and reduced the antioxidant capacity of tissues. Also, α-Tocopherol supplementation showed an anti-peroxidative effect in the rat liver tissues by significantly decreasing the LPS-induced rise of H2O2 levels (Berg *et al.,* 2004).

Previous studies indicated that LPS intoxication can change tissue and circulating antioxidant enzymes levels, as well as, non-enzymatic antioxidants (Kaur *et al.,* 2006). Moreover, results of the current study showed a significant reduction in the activities of reduced GSH, SOD and catalase in the liver of rats intoxicated with LPS. This might end with a loaded anions of superoxide and wasteful decontamination of H2O2 which forms OH· ions, augmenting lipid membrane oxidation, thereby, leading to oxidative injury in various tissues (Tiwari *et al.,* 2018). The decrease in levels of these antioxidants in the liver of endotoxic rats constitutes evidence of severe oxidative stress and a breakdown of the redox balance in cells during endotoxemia (Kaur *et al.,* 2006). Naturally occurring antioxidants including vitamins A, C, and E, glutathione, SOD, catalase, and glutathione peroxidase can protect from oxygen-derived radical injury (Zaidi, 2004). Therefore, any increase in these antioxidant enzymes after α-Tocopherol administration was expected. Vitamin E supplementation was observed to increase the levels of GSH, SOD and catalase significantly in the LPS-challenged animals in previous investigation (Gulec *et al.,* 2006). In this study, α-Tocopherol -treated rats showed an enhancement in the activity of reduced GSH, SOD and catalase when compared to LPS-challenged group. Similarly, α-Tocopherol is considered as one of the most important antioxidant used in protecting cells, organs and tissues from oxidative stress because it is safe and available (Kanter *et al.,* 2005). It is a fat-soluble antioxidant that defends cell membranes against peroxidation and protects polyunsaturated lipid from ROS attack by inducing the activation of various signal transduction pathways (Hobson, 2016). Consequently, the sensitive antioxidant action of α-Tocopherol has ameliorating effect on oxidative stress induced by LPS in rat cells and organs (Aldana *et al.,* 2001, Bullón *et al.,* 2015). The free radical scavenging properties of α-Tocopherol and its ability to reduce tissues injury have been attributed to decreasing expression of cytochrome p450 (CYP450) which has an important role in the metabolism of toxins, steroids, drugs, and others (Butt *et al.,* 2017).

In the present study, liver sections of LPS group showed a highly dilated and ruptured central vein congested with hemolyzed blood, infiltrated with leucocytes, and increased number of active kuppfer cells with shrinking in hepatocytes in some areas. Also, highly vacuolated hepatocytes associated with necrosis, loss of typical hepatic cords organization and obliteration of blood sinusoid were noticed. However, pretreatment with α-Tocopherol showed improvement in most of the histopathological features, including, decreasing in number of active kuppfer cells and in leukocytes infiltration in liver.

The first manifestation of hepatic injury is the accumulation of fat within hepatocytes (steatosis), this is followed by the development of necro-inflammatory (steatohepatitis) activity that leads to cirrhosis. The pathogenesis of liver cell damage may be related to several immunologic mechanisms and may be due to selective immune responses deficiencies and the production of inappropriate cytokine patterns. In LPS intoxication, the production of abnormal cytokine levels, which was indicated in the present study, appears to contribute in the progression of liver injury.

Moreover, previous studies showed that cytokine genes polymorphisms located within the coding/regulatory regions have been shown to affect the overall expression and secretion of cytokines (Su GL, 2002 and Luster *et al.,* 1994).

The importance of cytokines as molecular effectors in liver damage has been particularly well demonstrated in patients and animals ranging from steatosis to cirrhosis. The involvement of macrophage derived cytokines such as TNF-α and IL1β in the production of inflammation has been described. TNF-α plays a critical role in epithelial cell injury and acts as important mediator in liver injury and generally associated with several known cirrhosis-related complications since it promotes activation of stellate cells, matrix-gene expression, and matrix remodeling. Moreover, TNF-α is positively related with the extent of liver necrosis. Recent studies have indicated that deficiency of interleukin-1 in KC reduces liver inflammation and expression of inflammatory cytokines. IL-6 is also released along with IL-10, TNF-α and other cytokines by KC after LPS intoxication. IL-6 and IL-10 are two cytokines that play roles in reducing liver injury and inflammation. On the other hand, IL-6 knockout mice fed chronic alcohol showed increased liver fat accumulation, lipid peroxidation, mitochondrial DNA damage, and sensitization of hepatocytes to TNF-α induced apoptosis, which was prevented by the administration of recombinant IL-6 (Fathia and Khaled, 2016, Chiao *et al.,* 2005 and Izeboud *et al.,* 2004).

5. Conclusion

We could conclude that α-Tocopherol has immunomodulatory effect demonstrated in ameliorating cytokines production and reducing active kuppfer cells and leukocytes infiltration in liver, anti-inflammatory effect as it increased the activity of the anti-inflammatory cytokine (IL-10) and decreased the level of the pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and its antioxidant effect was proven by reducing liver lipid peroxide (MDA), hydrogen peroxide (H2O2) and increasing the antioxidant agents as reduced glutathione, superoxide dismutase and catalase and has anti-hyperlipidemic effect. Moreover, improving the adverse histopathological changes in liver induced by LPS intoxication showed that it has hepato-protective effect and could attenuate liver injury.

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