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Research Article

Study of the toxic effect and safety of vitamin E supplement in male albino rats after 30 days of repeated treatment

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ABSTRACT

The aim of these investigations was to study vitamin E supplement effect in male albino rats after 30 days of repeated treatment. Four groups of six male rats were orally administered distilled water (control), 500, 1000 and 2000 mg/kg body weight vitamin E daily for 30 days. The impact of the treatment on percent body weight and mortality was determined and compared to the control group. Some hematological analysis, biochemical parameters and histological examination of different body organs were assessed. The rats treated with different doses of vitamin E supplement showed no deaths recorded in 30 days. The treatment with higher dose Vitamin E supplementation" caused significant alteration at the hematological, biochemical and histological level. Therefore, oral administration of vitamin E supplement in rats for 30 days was not safe for the liver and kidney and in the other hand, safe for the testes therefore that side effect on the liver and kidney should be considered when recommended vitamin E for therapeutic purpose. Care should be taken in taking high doses of vitamin E.

1. Introduction

The Greater part of the world's population relies on vitamin supplement for their health care needs (World Health Organization, 2005). Vitamin E is widely applied for the fields of medicine and livestock production (Betoret et al., 2011). And its safety deserves more attention. At the beginning of this century, the United States and the European Union carried out calculation of the maximum tolerable intake of vitamin E for adults by epidemiological investigation and animal toxicity tests which is 1000 mg/kg (Monsen, 2000).

Alpha (α)-tocopherol is one of eight types of vitamin E (Miller et al., 2005) which is a lipid-soluble molecule that functions as a chain-breaking antioxidant (Sies and Stahl, 1995). Vitamin E is essential for such body functions as growth, reproduction, prevention of various diseases, and protection of tissues (Hardie et al., 1990). Human daily recommendations for vitamin E are typically 1000 mg/day (Kappus and Diplock, 1992). Vitamin E supplements often taken at high dosages are regularly consumed in many countries all around the world (Miller et al., 2005), particularly by patients with cardiovascular diseases (Lonn et al., 2005), cancer (Lippman et al., 2009), disorders in the central nervous system (Vatassery et al., 1999) and male infertility with unjustified roles (Bassey et al., 2018).

Although vitamins E is considered relatively safe compared to other fat-soluble vitamins (Tappel, 1972), mortality progressively increased for approximately greater than 150 IU/d which lower than the tolerable upper intake level for vitamin E, which is currently designated at 1,000 mg of any form of supplementary α-tocopherol per day (corresponding to 1,100 IU of the synthetic vitamin E per day or 1,500 IU of natural vitamin E per day) (Lim et al., 2005). In general, Vitamin E are used as drug for treatment some clinical problems as muscular dystrophies and some nervous disease (Bicknell, 1940), male infertility (Keskes-Ammar et al., 2003), nervous disease (Muller et al., 1983), cardiovascular disease (Lee et al., 2005), lowering blood triglyceride (Engelhard et al., 2006), improvement in insulin action and in diabetic patients (El-Aal et al., 2018; Panda et al., 2018; Pavithra et al., 2018), inhibition of platelets adhesion (Steiner, 1991) and aging (Chen et al., 2005; Mocchegiani et al., 2014). Many physicians use vitamin E randomly for patients (Vivekananthan et al., 2003). Vitamin E is required in preventing or minimizing free-radical damage associated with specific diseases and lifestyle patterns and processes, including cancer, aging, circulatory conditions, arthritis, cataract, pollution, and strenuous exercise (Packer, 1991). Vitamin E consumption increased dramatically during the last decade,

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both through self-prescription and from the fortified vitamins supplemented foods. Therefore, it is necessary to assess the clinical safety and toxicity of vitamin E supplementation and a premise for pharmacodynamic studies". However, in recent years, there are few experimental researches or report on vitamin E safety evaluation. In this study, the toxic effects of vitamin E different doses on rats were observed. These studies are necessary to evaluate vitamin supplement possible undesirable side effects and to determine appropriate dosage levels and regimens to avoid overdosing or harming of patients (Hathcock, 1997).

2. Materials and methods

2.1. Chemical

Vitamins E (tocopherol acetate) clear, slightly yellow, viscous liquid miscibility with water was purchased from Sigma. 1 ml of the product equivalent to 500 mg DL-α-tocopherol acetate. The LD50 of tocopherols for Rats is $>$ 7000 mg/kg b.w orally according to (Additives and Food, 2015).

2.2. Test animal

Adult Male healthy albino rats with average age 12 weeks old and weighing 120–130 g were used. The animals were kept in plastic cages at animal house, in an air-conditioned environments with six rats in each cage and maintained at room temperature of (25 ± 2) °C with relative humidity (60% ± 10%) under 12 h night and light cycle. The animals were allowed free access to food and water. The rats were acclimatized for 14 days before the experiment. The protocol of the study was approved by an animal ethics committee and according to the National Institutes of Health guide to the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The reason for choosing male rats in this study is based on the popular use of vitamin E to increase fertility (Keskes-Ammar et al., 2003).

2.3. Experimental design

The animals were randomly and equally divided into four groups with six rats for each. Before dose administration, the body weight of each animal was determined and the dose was calculated according to the body weight. The doses administrated orally by a gavage tube not exceed 10 μl/g b.wt."The first group (control group) received orally distilled water, the second group (vitamins E 500 mg/kg) orally received vitamin E 500 mg/kg body weight daily for 30 days, the third group (vitamins E 1000 mg/kg) orally received vitamin E 1000 mg/kg body weight daily for 30 days. The fourth group (vitamin E 2000 mg/kg) orally received vitamin E 2000 mg/kg body weight daily for 30 days. The body weight of each rat was assessed using a sensitive balance once before beginning of treatment and on the day of sacrifice and then calculating the percentage of weight increase for each rat. The mortality observed daily during the experiment.

2.4. Laboratory procedures and sample collection

At the end of the four-weeks treatment and consequently after 24 h of the last oral administration, blood samples were collected morning via the orbital plexus into a standard test tube from all animals under ketamine anesthesia and the animals were sacrificed by cervical dislocation. The blood samples were collected in tube containing EDTA for determining hematological parameters, and the serum from "the blood samples without EDTA were" collected for determining clinical biochemical parameters. "the blood samples without EDTA were stood for half an hour and then centrifuge at 500x g for 15 min at 4 °C to separate serum and stored at −70 °C.

2.5. Histological examination

After sacrificing rats in each group, the liver, kidney, and testis were quickly removed and weighed. The relative organ weight was then calculated as follows: Relative organ weight = absolute organ weight/body weight of rats on sacrifice day×100. The tissue was fixed in "in 10% formalin in saline" for 24 h for tissue fixation. Afterwards, it was rinsed with distilled water, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. Finally, they were cut into 5 μm section with a rotary microtome and stained with haematoxylin and eosin and examined under a microscope (Fischer et al., 2008; El-Hak and Mobarak, 2018; El-Hak et al., 2018). A minimum of three fields for each slide were examined and scored semiquantitatively for severity of changes unaware of the type of treatment. The histological examination is described into a Numerical Score (McInnes and Scudamore, 2014).

Numerical	Description
0	Tissue considered to be within normal limits, under the conditions of the study (no change)
1	The amount of change present is minimal exceeds that which is considered to be within normal limits (mild)
2	The lesion is slightly easily identified, but of limited severity (moderate)
3	The degree of change is Severe occupies most of the organ (severe)

The hepatic tissue was evaluated for any alterations in the architecture, portal or lobular inflammation, sinusoidal dilation, degeneration, necrosis and fatty change used to identify the extent of injury associated with each group. Renal tissue was evaluated for any alteration in renal tubules and malpighian corpuscles and for the presence of necrosis and pyknotic nuclei. The data were compared to find the injury associated with each group."

2.6. Hematological parameters

After collecting blood from the cardiac puncture into EDTA containing tubes, hemoglobin (Hb), total white blood cells (WBCs) and platelet count were evaluated using a hematology automatic system.

2.7. Biochemical indices

The serum separated was used for various parameters such as creatinine, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipid peroxidation (Malondialdehyde) and reduced GSH by using commercial kits (El-Hak and Mobarak, 2018), Serum testosterone which was quantitatively measured by adopting enzyme-linked immunosorbent assay (ELISA) technique using kits purchased from Rapid Labs Ltd., (Essex, U.K). Malondialdehyde formed from the breakdown of polyunsaturated fatty acids that served as a convenient index for determining lipid peroxidation that reacts with thiobarbituric acid to give a red color absorbing at 535 nm (Draper and Hadley, 1990). Reduced glutathione determination is based on the formation of a yellow color after reacting with 5,5′dithiobis-2-nitrobenzoic acid (DTNB) which is then read at 412 nm (Jain and McVie, 1994).

2.8. Statistical analysis

Statistical analysis was performed as mean of variance ±SEM ($n = 6$) followed by an ANOVA test using statistical package for social science (SPSS) for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA) for multiple comparison test between the groups followed by followed by Dunnet's multiple comparison test. A probability level of $p < 0.05$ was considered to be statistically significant. Relative organ weight and percent weight were compared between groups using nonparametric analysis of variance (Kruskal-Wallis test) followed by Mann-Whitney U test comparing groups against each other.

3. Results

No mortality and behavior changes were observed in any group during the treatment period. The percentage of body weight increase of the the vitamin E treated groupswere not significantly different compared to the control groups ($p > 0.05$). Treatment the rats with vitamin E for 30 days showed a significant effect on the relative weight of the liver and testes and non significant difference in the relative weight of the kidney compared with rats in the control group (Table 1).

Microscopical and histological examinations showed remarked difference between the control group and the experimental treated groups. Histopathological changes were observed in all samples of the liver, kidneys and testes (Figs. 1, 2, and 3 and Table 2).

Kidney of control rats showed normal histological examination (Fig. 1A) with normal tubular brush borders and intact glomeruli. Numerous proximal convoluted tubules were noticed with small lumen composed of large cuboidal cells with granular cytoplasm; distal convoluted tubules were observed with larger and regular lumen surrounded by smaller and more distinctly cuboidal cells. The kidney of rats which received vitamins E with daily dose 500 mg/kg b.wt. for thirty days showing mild dilation of renal tubules (Fig. 1B). Histological examination of rat kidney which received the vitamins E with a daily dose of 1000 (Fig. 1C) and 2000 mg/kg b.wt (Fig. 1D) for thirty days showed from mild to moderate dose depend damages. Histological damage detected in kidney were degeneration and dilation of proximal and distal convoluted tubules, the nuclei of epithelial cells of some proximal tubules showed pyknotic nucleus with chromatin condensation and mild atrophy of glomeruli tuft.

Histological examination of the liver of control rats (Figs. 2A and 3A) showed normal histological structure and rats which received vitamins E with a daily dose (500, 1000 and 2000) mg/kg b.wt. for thirty days showed abnormal histological changes (Figs. 2 and 3). Liver tissues in groups treated with different doses of vitamin E supplement showed hydropic degeneration, necrosis and infiltration of inflammatory cells in the portal area.

The seminiferous tubules of control rat showed the normal arrangement of germinal epithelium spermatogonia and Sertoli cells resting on the basement membrane. The typical structure of primary spermatocytes was as round and elongated spermatids and late-stage sperms (Fig. 4A). Histological examination of rat testes which"received vitamin E with the daily doses (500, 1000 and 2000) mg/kg b.wt. for thirty days showed the absence of histological damage with normal testicular histology in all successive stages of spermatogenesis with lumen filled with sperm (Fig. 4 B, C and D, respectively).

The results of the hematological tests after subacute oral administration of the vitamin E to rats are'summarized in (Table 3). The tested hemoglobin parameters were within normal limits compared to the control group. A significant increase in total white blood cell was observed in all the treated group with vitamin E and a significant decrease in platelets was observed in rats treated with 1000 and 2000 mg/kg b.wt. Vitamin E compared to the control group.

The results of the serum biochemical parameter are summarized in Table 4. Significant variations in parameters were noted between groups. Oral administration of vitamin E supplementation with the doses of 500 and 1000 mg/kg showed non significant changes in creatinine, urea, ALT and AST levels when compared to control group. However, Oral administration of vitamin E supplementation with the dose of 2000 mg/kg showed significant increase of AST and ALT when compared to control group ($p < 0.005$). Lipid peroxidation content in" treated groups with the doses of 500, 1000 and 2000 mg/kg b.wt vitamin Efor 30 days was significantly decreased compared to control. The data showed that blood reduced glutathione (GSH) content showed a significant increase ($p \leq 0.05$) after treatment with with the doses of 500, 1000 and 2000 mg/kg b.wt vitamin E and a non-significant increase in treated groups with dose of 500 mg/kg b.wt vitamin E″ in comparison to the control group.

Table 1
Effect of oral administration of vitamin E on relative organ weight of rats and percentage of body weight increase.

Organ	Relative organ weight (%)			
	Control	Vitamin E 500 mg/kg	Vitamin E 1000 mg/kg	Vitamin E 2000 mg/kg
Liver	5.87 ± 0.9	6.52 ± 0.11*	7.11 ± 0.44 *	7.81 ± 0.27*
Kidney	1.61 ± 0.07	1.63 ± 0.02	1.54 ± 0.08	1.57 ± 0.054
Testes	3.21 ± 0.18	4.36 ± 0.15*	4.91 ± 0.20*	5.45 ± 0.18*
Percentage of body weight increase	16.78 ± 0.9	19.07 ± 1.4	15.73 ± 2.0	15.27 ± 1.5

Values are expressed as mean ± SEM. Kruskal–Wallis was used to determine any differences among groups followed by Mann–Whitney U test was used to compare groups against each other. *significant at $p \leq 0.05$.

4. Discussion

The present study is helpful in providing data on dosage regimens, target organ toxicity and find the observable adverse effect of a certain chemical. This study will provide useful information in the future for getting knowledge of the adverse reactions and side effects of vitamin E supplement.

The percent body weight changes serve as a sensitive indicator of general health status of animals (Ullman-Culleré and Foltz, 1999). The increases or decreases in the percentages are accompanied with the accumulation of fats and the physiological adaptation responses to the supplement not to the toxic effects that lead to decrease appetite and hence, lower the caloric intake by the animal (Jackman et al., 2008). In the present study, after 30 days of treatment of the vitamin E supplement, all the animals exhibited a normal change in body weight percentage, which indicated the absence of interference with the normal metabolism of animals. The protocol of weighing relative organs in toxicity studies includes their sensitivity to predict toxicity and it correlates well with histopathological changes (Morrissey et al., 1988) to confirm whether the organ weight was exposed to injury or not (Dudley et al., 1985). Non significant changes in the kidney weight were observed after administration of vitamin E supplement at subacute oral doses and that may indicate the absent of severe injury.

The hematological parameters can be used to find the blood relating functions of the supplement used (Davis et al., 2008). The hemopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological states in both humans and animals (Tchounwou et al., 2012). The present study showed a significant increase in total leukocyte count with the vitamin E treatment which may be responding to the liver and kidney tissue injury. The elevation of white blood cell count isnot in agreement to Ambali et al. (2011) found pretreatment with 75 mg/kg b.wt. vitamin E to albino mice showed decrease in white blood cells and explained that result due to leukocytosis and lymphocytosis with unknown reason.A marked decrease in the hemoglobin concentrationshemoglobin concentrations was observed in rats that received vitamin E supplement for 30 days that may be result from red blood cell reduction and that result is not in agreement to Jilani et al. (2008) that found Vitamin E supplementation enhanced Hemoglobin and erythropoietin levels in mildly anemic adults that difference may be due to species difference. A significant decrease in in the platelet countwas observed in rats that received vitamin E supplement for 30 days that result is in agreement to to Steiner (1991) study may be due to slight reduction of platelet cyclooxygenase activity and inhibition of lipid peroxide formation.

This study demonstrated minimal and moderate renal and hepatic toxicity of vitamin E supplement dependent on the increase of the dose. In the present study, sub-acute administration of 2000 mg/kg vitamin E caused liver toxicity by altering the levels of ALT, AST and the liver

Table 2
Results of histopathological examination of internal organs of control rats and treated rats with vitamin E daily for 30 days.

Organs	Histopathological changes	Categories	Number of animals with histopathological change/number of animals examined (%)			
			Control	Vitamin E 500 mg/kg	Vitamin E 1000 mg/kg	Vitamin E 2000 mg/kg
Kidney cortex	Dilation of renal tubules	2	0/6	5/6 (83%)	4/6 (66%)	4/6 (66%)
	Hydropic degeneration of proximal and distal tubules	2	0/6	0/6	5/6 (83%)	4/6 (66%)
	Atrophy glomerulus	2	6/6 (100%)	6/6 (100%)	5/6 (83%)	5/6 (83%)
	Pyknotic nucleus in the tubes	2	2	0/6	0/6	5/6 (83%)
Liver	Hydropic degeneration	1	0/6	4/6 (66%)	5/6 (83%)	4/6 (66%)
	Infiltration of inflammatory cells	1	0/6	3/6 (50%)	4/6 (66%)	4/6 (66%)
	Individual cell necrosis	2	0/6	0/6	1/6 (16%)	3/6 (50%)
	Focal necrosis	1	0/6	0/6	1/6 (16%)	3/6 (50%)
Testes	Spermatogenic stages	0	6/6 (100%)	5/6 (83%)	6/6 (100%)	6/6 (100%)

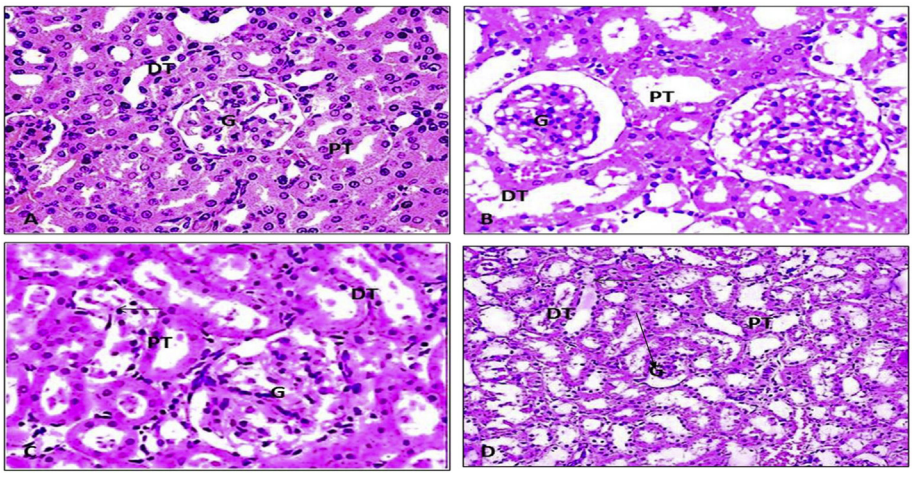

Fig. 1. (A): Photomicrograph of kidney of control rats showing normal glomerulus (G), proximal convoluted tubules (PT) and distal convoluted tubules (DT). (HX& E, 20X). (B): Photomicrograph of kidney of rat which received 500mg/kg b.wt. vitamin E daily for thirty days showing normal glomerulus (G) and mild dilatation of proximal convoluted tubules (PT) and distal convoluted tubules (DT). (HX& E, 20X). (C): Photomicrograph of kidney of rat which received 1000mg/kg b.wt vitamins E daily for thirty days showing normal glomerulus (G) and mild dilatation of proximal convoluted tubules (PT) and distal convoluted tubules (DT), degeneration of distal tubules with pyknotic nucleus arrow. (HX& E, 20X). (D): Photomicrograph of kidney of rat which received 2000mg/kg b.wt vitamins E daily for thirty days showing atrophy glomerulus (G) and mild dilatation of proximal convoluted tubules (PT) and distal convoluted tubules (DT), degeneration of convoluted and distal tubules with pyknotic nucleus arrow. (HX& E, 10X).

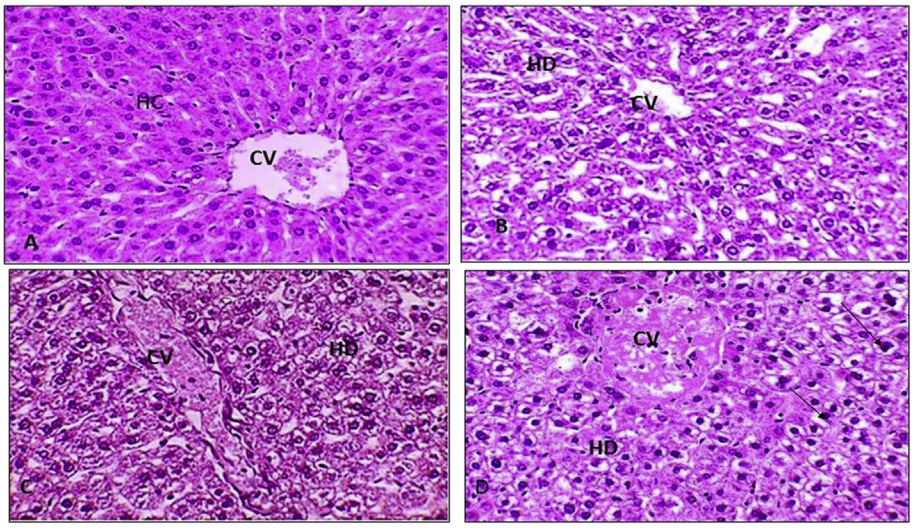

Fig. 2. (A): Liver section from a control rat showing normal hepatocytes (HC) radiating around the central vein (CV). (HX& E., 20X). (B): Liver section of treated rat, which received 500mg/kg b.wt. for 30 days with vitamin E showing hydropic degeneration (HD) of the hepatocyte surrounding the central vein (CV) (HX& E., 20X). (C): Liver section from a rat, which received 1000mg/kg b.wt for 30 days with vitamin E showing hydropic degeneration (HD) of the hepatocyte surrounding the central vein (CV). (D): Liver section from a rat, which received 2000mg/kg b.wt for 30 days vitamins E showing hydropic degeneration (HD) of the hepatocyte at the peripheral of the central vein with pyknotic nucleus ↓.(H.&E., 40X).

histological structure. The liver histological damage by the high dose of vitamin E treatment resulting from its role as the primary target stored organ for these vitamin (Zimmerman, 1999; Kayden and Traber, 1993). ALT and AST elevation may be due to hepatic necrosis which may change to diffuse necrosis to be replaced with new cells and normal hepatic architecture by the remarkable ability of the liver to regenerate itself (Bollard et al., 2009) or may be the degenerative changes in hepatocytes which affect the synthesis and the release of the enzyme (El-Daly, 1994). The kidney is considered the second route of excretion of vitamin E (Simon et al., 1956) Measurements of urea and creatinine levels in the blood are commonly performed to assess kidney function effects on the kidneys (Levey et al., 1999) which found to be nonsignificant difference in comparison to the control. Examination of the microscopic histopathology of the kidneys treated with vitamin E revealed histological damage which did not disturbs the kidney function as explained by Choudhury and Ahmed (2006) that found kidney injuries affect the renal function when more than two third of the nephron damage and in the present study most of the histological damage in the kidney appeared mild or moderate. these result is different from the finding of Soelaiman et al. (2011) that found the increase of kidney weight and raise in kidney

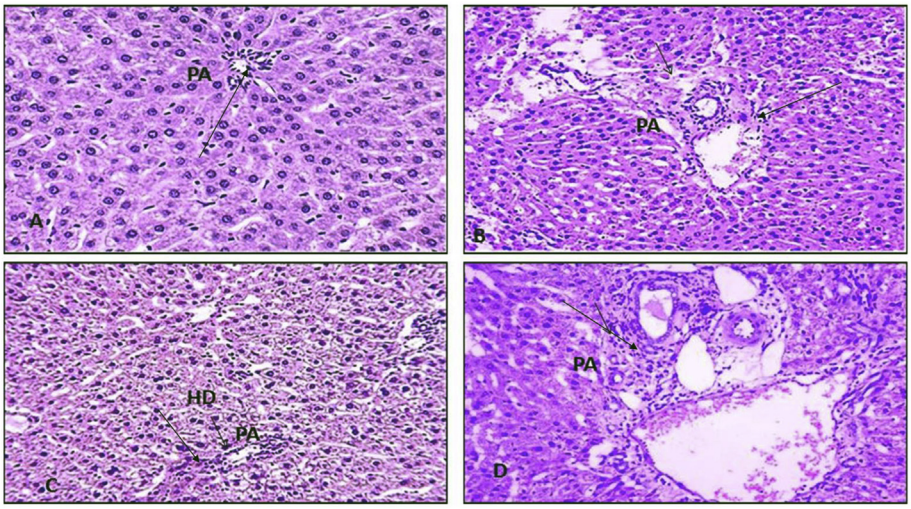

Fig. 3. (A): Liver section from a control rat showing normal hepatocytes and a portal area (PA) contain a branch of the portal vein, hepatic artery and bile duct (HX& E., 20X). (B): Liver section from a rat, which received 500 mg/kg b.wt. for 30 days with vitamin E showing mild infiltration cells (↓) in the portal area (PA) (HX& E., 20X). (C): Liver section from a rat, which received 1000 mg/kg b.wt for 30 days with vitamin E showing mild infiltration cells (↓) in the portal area (PA) (HX& E., 20X). (D): Liver section from a rat, which received 2000 mg/kg b.wt for 30 days vitamins E showing moderate infiltration cells (↓) in the portal area (PA) (HX& E., 20X).

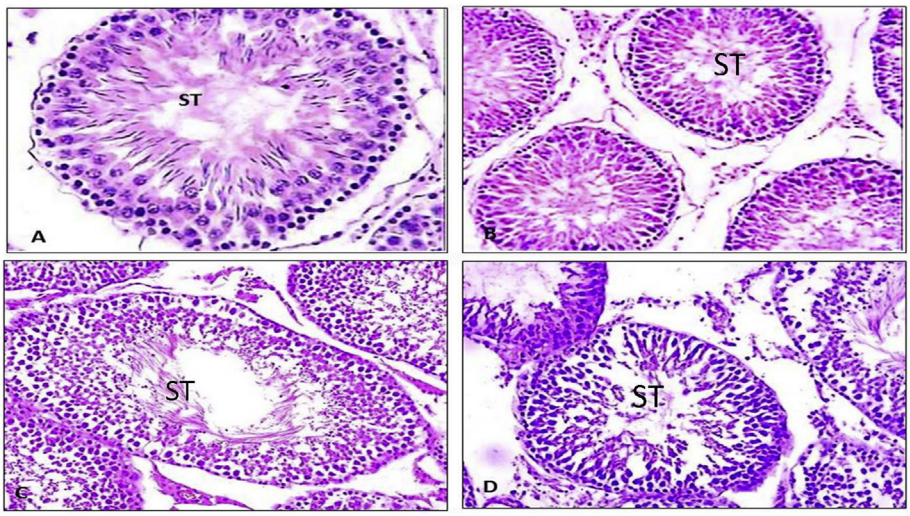

Fig. 4. (A): Testis section of a control rat showing the normal appearance of the seminiferous tubules (ST) with all the successive stage of spermatogenesis, lumen filled with spermatozoa (HX & E., 40 X). (B): Testis section of treated rat with 500 mg/kg, vitamin E for 30 days showing the normal appearance of the seminiferous tubules (HX & E., 20 X). (C): Testis section of treated rat with 1000 mg/kg, vitamin E for 30 days showing the normal appearance of the seminiferous tubules (ST) (HX & E., 20 X). (D): Testis section of treated rat with 2000 mg/kg, vitamin E for 30 days showing the normal appearance of the seminiferous tubules (ST) (HX & E., 20 X).

Table 3
Effect of vitamin E treatment on hematological parameters.

Parameters	Control	Vitamin E 500 mg/kg	Vitamin E 1000 mg/kg	Vitamin E 2000 mg/kg
Hemoglobin (g/L)	13.12 ± .33	13.86 ± .46	12.83 ± .18	12.53 ± .15
Platelet count (10^9/L)	712.83 ± 22.6	695.66 ± 35.35	632.66 ± 25.43	562.66 ± 10.68*
WBC (10^9/L)	7.38 ± .66	8.73 ± .16	8.74 ± .33	8.78 ± .23*

Values are expressed as mean ± SEM. * significant at $p \leq 0.05$ when compared to the control group. One-way ANOVA followed by Dunnet's post hoc test.

Table 4
Effect of vitamin E treatment on biochemical parameters.

Parameters	Control	Vitamin E 500 mg/kg	Vitamin E 1000 mg/kg	Vitamin E 2000 mg/kg
Urea (mg/dL)	22.83 ± .27	23.23 ± 0.17	23.27 ± 0.25	23.74 ± 0.72
Creatinine (mg/dL)	0.22 ± 0.015	0.23 ± 0.02	0.26 ± 0.23	0.29 ± 0.03
AST (IU/L)	86.5 ± 0.99	86.66 ± 1.25	89 ± 0.93	93.8 ± 1.24*
ALT (IU/L)	50 ± 3.07	51.66 ± 2.84	53.67 ± 3.16	60.33 ± 2.74*
Testosterone (U/L)	0.71.07±	1.3 ± 90*	1.8 ± 0 .19*	2.84 ± 0.44*
Lipid peroxidation (MDA nmol/ml)	2.69 ± 0.11	2.32 ± 0.12*	1.94 ± 0.05 *	1.53 ± 0.12*
Glutathione Reduced (μmol/l)	7.01 ± 0.62	8.23 ± .28*	11.98 ± .49*	14.86 ± 1.42*

Data are expressed as mean ± SEM. * significant at $p < 0.005$ when compared to control group. One-way ANOVA followed by Dunnet's post hoc test.

function and no change in the liver function and weight in studying vitamin E in palm oil that may be due to the presence of alpha tocopherol, alpha tocotrienol, gamma toctrienol and delta tocotrienol in palm (Aggarwal et al., 2010).

Testicular weight is an important parameter in the reproductive evaluation of males owing to its high and positive correlation to sperm production. In this work, there was a highly significant increase in treating group with 500, 1000 and 2000 mg/kg b.wt. vitamin E for 30 days in testicular weight in comparison to the control group. In this study showed an increase in serum levels of testosterone in rats treated with vitamin E important to the qualitative and the quantitative maintenance of spermatogenesis. Therefore the increase of testosterone led to increasing spermatogenesis that appeared in the treated testicular tissues so that present study is in agreement with Umeda et al. (1982) which observed elevation of testerosterone levels in both the rat testicular tissue

and plasma after treatment with vitamin E. The results of the present study also show that vitamin E did not affect the histological structure of the testes. Vitamins E is essential for normal spermatogenesis (Mason, 1933; Ichihara, 1967) and absence of such vitamins, the animals showed dysfunction of the testis germinal layers (Al-Attar, 2011). Supplementation of vitamins E has been established to reduce testicular ROS and restore normal testicular function in cadmium-exposed rats (Amara et al., 2008). Vitamin E, a strong lipid-soluble antioxidant present in the cell, naturally accumulates in the membranes of mitochondria and endoplasmic reticulum and protects cells from lipid peroxidation (Matés et al., 1999). These results suggest that vitamin E supplement exerts activator effects on testicular function and leads to increase in the fertility of rats and confirmed the important role of vitamin E in the biosynthesis of pituitary gonadotropins and testicular testosterone.

In addition, rats treated with 500, 1000 and 2000 mg/kg of vitamin E for 30 days compared with the control group showed an increase in the reduced glutathione (GSH) concentration and decrease in lipid peroxidation and our result is in agreement with Meagher et al. (2001) study on the impact of vitamin E on lipid peroxidation in healthy person and Wagner et al. (1996) found vitamin E slows the rate of free radical-mediated lipid peroxidation in cells. The present result in oxidative stress parameter confirming vitamin E role as an important antioxidant facing free radical damage (Devasagayam et al., 2004).

Based on these results administration of vitamin E with high doses for 30 days isnot safe by affecting hematological parameters and liver function. It also induce histological damage to the liver and the kidney and but the result ist not agreement with (Tsai et al. 1978) study in volunteer of male and female consumption of 600 IU vitamin E daily for 30 days did not change the state of the body in the measured parameter in addition to decreased leukocyte count.

5. Conclusions

Theses studies on vitamin E were obtained in order to define its safety to humans use. In the light of these findings, we may conclude that taking vitamins E for 30 days is not completely safe with the studied doses. Co-supplementation is needed to investigate in research with vitamin E that can modulate the biochemical and the histological damage obtained when using vitamins E. More investigations are needed to further confirm the possible recovery after stopping treatment with vitamin E supplement. The obtained result can provide references for long term toxicity experiment and clinical application about vitamin E.

Declarations

Author contribution statement

Heba N. Gad EL-Hak, Eman E. Elaraby, Ahmed K. Hassan, Osama A. Abbas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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