Original Article

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The Effects of Vitamin E on Liver and Kidney Damage Induced by Dianabol in Small Laboratory Mice

H. Morovvati (PhD)*[1], M. Babaei (PhD)[1], Z. Tootian (PhD)[1], S. Fazelipour (PhD)[2], H. Anbara (MSc)[1], A. Akbarzadeh (MSc)[1]

1.Department of Basic Siences, Faculty of Veterinary Medicine, University of Tehran, Tehran, I.R.Iran

2.Department of Anatomy, Faculty of Tehran Medical Sciences, Islamic Azad University, Tehran, I.R.Iran

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ABSTRACT

BACKGROUND AND OBJECTIVE: Anabolic steroids, especially dianabol, are used by athletes as a performance-enhancing drugs that damage the liver and cause structural changes. The aim of this study was to evaluate the effects of vitamin E on liver and kidney toxicity caused by dianabol.

METHODS: In this experimental study, 72 adult male mice were randomly divided into 8 groups of 9. Four groups of mice received 100 IU/kg vitamin E orally for 42 days through gavage. Three groups of the above groups received 5, 10 and 20 mg/kg oral dianabol four hours after receiving vitamin E, respectively. The control group and the groups receiving only 5, 10 and 20 mg/kg oral dianabol were also considered. 24 hours after the final treatment, serum samples were collected for biochemical evaluations and tissue samples were collected for histological, histomorphometric and histochemical evaluations.

FINDINGS: The results showed that dianabol significantly increased the level of AST (158.52±9.76), ALT (113.70±11.02), and ALP (141.30±5.94), and significantly decreased albumin (1.04±0.47) compared to the control group (72.61±7.54, 41.47±7.03, 112.80±4.30, 3.14±0.25, respectively) ($p<0.05$). Administration of vitamin E significantly increased the level of AST (110.56±9.86), ALT (80.19±4.02) and ALP (120.52±4.94) and improved albumin (2.1±0.28) ($p<0.05$).

CONCLUSION: The results of the study showed that vitamin E can reduce the oxidative damage caused by dianabol in the liver and kidney of the mouse.

KEY WORDS: *Vitamin E, Dianabol, Liver, Kidney, Mouse.*

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***Corresponding Author: H. Morovvati (PhD)**

Address: Section of Tissue and Embryology, Department of Basic Sciences, Faculty of Veterinary Medicine, Tehran University of Medical Sciences, Tehran, I.R.Iran

Tel: +98 21 61117117

E-mail: hmorovvati@ut.ac.ir

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Introduction

The use of androgenic – anabolic steroids by athletes started about 50 years ago and its use is significantly increasing (1, 2). Androgenic – anabolic steroids are referred to a group of synthetic hormones that are mainly derived from testosterone (3). Many athletes and adolescents use androgenic–anabolic steroids for fitness and appearance. It is noteworthy that many athletes who use anabolic steroids believe that the side effects of these drugs are not serious and permanent, while some doctors reported changes in the kidney and liver along with the progression of liver tumors in those taking these drugs (4, 5).

Studies have shown that the use of anabolic steroids in laboratory animals causes damage to the liver and structural changes and leads to liver tumors in small laboratory mice (6). On the other hand, it has been shown that anabolic steroids cause inflammation in rabbit's liver, sinusoidal congestion and the formation of fat vacuoles in the rabbit's liver tissue (7). In the analysis of liver changes in rats receiving anabolic steroids, the researchers also found that these drugs can change the liver metabolism capacity abnormally, and cause abnormal proliferation of liver cells (8). Other studies also found that the use of anabolic steroids in the female rats caused liver damage (9).

One of the most widely used androgenic – anabolic steroids among athletes is methandrostenolone ($C_{20}H_{28}O_2$), which is found in the market under the brand names Dianabol, Averbol, and Metandienone (3). Dianabol is an androgenic – anabolic steroid that is used by athletes, especially bodybuilders, for fitness, and since this drug is metabolized in the liver, it may impair liver function (8).

Dianabol causes muscle mass growth and strengthens the muscles of the body. It also delays cell death and degradation, but causes complications such as liver cancer, liver and kidney failure, hepatic necrosis, hepatocyte apoptosis, excess urination, hepatic peliosis, hepatic adenoma, hepatocarcinoma, and kidney and heart disorders (10–12). The oxidation of anabolic steroids, especially Dianabol in the body, leads to the production of active oxygen species, oxidative stress formation and lipid peroxidation, and causes cellular damage and increased apoptosis (13).

The physiological functions of vitamin E as a fat-soluble vitamin are mainly related to the degradation properties of oxidation reactions (14). Vitamin E has different biological effects and its antioxidant property is one of its most important characteristics (14, 15). This vitamin has the potential to neutralize free radicals and, as a potent antioxidant, prevents the harm caused by free radicals and can play a key role in delaying the pathogenesis of various degenerative diseases, such as liver and kidney disease (14, 16). Since some anabolic steroids have been able to cause changes in the liver and kidneys by increasing free radicals, Dianabol may also cause changes in their function and cause liver and kidney toxicity. Considering the widespread use of these drugs, especially by athletes, and the lack of any research on the effects of this drug on the liver and kidney, the researchers have sought to study the effects of Dianabol on these organs in mice and to find whether vitamin E can act as an strong antioxidant through the glutathione peroxidase pathway and whether it has positive effects on reducing the possible damage caused by Dianabol, and get the young and athletes acquainted with the complications of Dianabol. Therefore, the present study was conducted to evaluate the protective effect of vitamin E on histology, histomorphometric parameters, renal and hepatic enzymes, and finally the histochemistry of kidney and liver tissue in small white laboratory mice treated with Dianabol.

Methods

After approval at the Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran (code of ethics 7506001/6/14), this randomized controlled trial was carried out on 72 adult male NMRI rats weighing 20 to 25 grams prepared from Animal Breeding Center, Faculty of Science, University of Tehran. All the stages of this study were performed according to Directive 86/609/EEC of European Commission for experimenting on animals.

Animals were kept in 12 hr light/12 hr dark cycle at a temperature of 25±2 °C and relative humidity of 50±10% in polyethylene cages with free access to tap water. All animals were equally fed from mouse pellets. All criteria and conditions for keeping the animals and experimenting on them were carried out in accordance with the instructions of the Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran. Before starting the treatment period, animals were kept for two weeks to adapt to the new environment, and after marking them, male rats were randomly divided into eight groups of nine and received oral Dianabol(17) and vitamin E (18) by gavage for 42 consecutive days:

1. Control group (Con): Animals of this group received 0.3 ml physiological serum by gavage.

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2. Second group (D5): Animals of this group received Dianabol alone at a dose of 5 mg/kg body weight by gavage.
3. Third group (D10): Animals of this group received Dianabol alone at a dose of 10 mg/kg body weight by gavage.
4. Fourth group (D20): Animals of this group received Dianabol alone at a dose of 20 mg/kg body weight by gavage.
5. Fifth group (E): Animals of this group only received vitamin E at 100 IU/kg body weight by gavage.
6. Sixth group (D5E): Animals of this group received 5 mg/kg body weight Dianabol with 100 IU vitamin E by gavage.
7. Seventh group (D10E): Animals of this group received 10 mg/kg body weight of Dianabol with 100 IU vitamin E by gavage.
8. Eighth group (D20E): Animals of this group received 20 mg/kg body weight of Dianabol with 100 IU vitamin E by gavage.

All animals of the eight groups were sacrificed one day after the 42-day treatment period and blood samples were collected directly from the heart by sterile syringes. Blood samples were then poured into 2 cc microtubes and after blood clotting, samples were centrifuged at 3000 rpm for five minutes for serum separation. The separated serum samples were stored and kept at a temperature of -20 °C until the time of experiment. After blood collection, the mice were necropsied and ultimately the liver and kidney tissue was removed and placed in Bouin solution (equal volumes of 0.2% picric acid in phosphate buffer and 2% formaldehyde in phosphate buffer).

Then, the samples were tested to determine serum Total Antioxidant Capacity (TAC) (based on regenerative capacity of trivalent iron) and malondialdehyde (MDA) of lipid peroxidation (by measuring malondialdehyde based on the reaction with thiobarbituric acid, extraction with normal butanol, absorbance measurement by spectrophotometric method and comparison of absorption by standard curve) (19).

The serum activities of aspartate aminostransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine, urea and albumin were measured using biochemical kits (Commercial kits, Pars Azmoon Inc., Tehran, Iran) by spectrophotometry (20, 21). After getting fixed, the liver and kidney tissues were placed in special containers along with the specifications of each sample and molded using molten paraffin wax through passage of tissue. After molding and preparation of paraffin molds, 6 µm sections were prepared from paraffin molds using a microtome and finally hematoxylin-eosin staining was used for staining the samples. Dino-Lite digital microscope (AnMo Electronics Corporation, Taiwan) (21) was used to study the diameter of hepatocytes, the diameter of the hepatocyte nuclei, and the number of Kupffer cells per square millimeter in liver (20). Masson's trichrome stain kit was used for histochemical evaluation of the liver and kidney tissue in accordance with the manufacturer's instruction (Shimi Pajouhesh Asia) (22). In the Masson's trichrome staining method, the collagen fiber dispersion in the liver and kidney tissues was investigated. The results of this study were evaluated using SPSS software version 19. Significant differences among groups was performed by ANOVA followed by Tukey test. $P<0.05$ was considered significant.

Results

Results of the histomorphometric studies of the liver: Histomorphometric results of liver tissue revealed that Dianabol consumption increased the diameter of hepatocytes, the diameter of the hepatocyte nuclei, and the number of Kupffer cells per square millimeter in liver compared to the control and vitamin E groups ($p<0.05$). The diameter of hepatocytes ($p<0.05$) in the D10 (13.0±86.87) and D20 (16.1±65.37) groups was significantly higher than the control group (11.03±1.03) and vitamin E group (11.1±11.13), whereas the D5 group (12.1±09.25) had no significant difference with control group and vitamin E group. Moreover, the increase in the diameter of hepatocytes in the D20E group (16.1±1.41) was significant ($p<0.05$) compared to the control and vitamin E groups, but had no significant difference with their corresponding groups (Fig. 1).

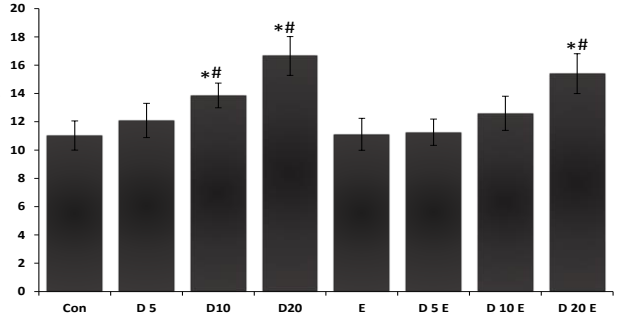

Figure 1. Comparison of mean diameter of hepatocytes (µm) in different experimental groups

Regarding the morphometric indices of the diameter of the hepatocyte nuclei, these indices were significantly higher ($p<0.05$) in the D10 (6.0±78.67) and D20 (8.0±35.66) groups compared with control group (5.04±0.42) and vitamin E group (5.0±09.36),

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while the D5 group (5.0±12.37) had no significant difference with control and vitamin E groups. Furthermore, there was a significant difference between D10E (5.0±98.39) and D20E (7.1±9.62) groups and the control group and vitamin E group ($p<0.05$), but had no significant difference with their corresponding groups (Fig. 2). The number of Kupffer cells per square millimeter in liver tissue in the D10 (8.1±79.77) and D20 (13.2±35.16) groups was significantly higher than the control (5.0±83.92) and vitamin E (5.1±41.16) groups ($p<0.05$). Moreover, the increase in number of Kupffer cells per square millimeter in the D20E group (12.1±6.61) had a significant difference ($p<0.05$) with control and vitamin E groups, but had no significant difference with their corresponding groups (Fig. 3).

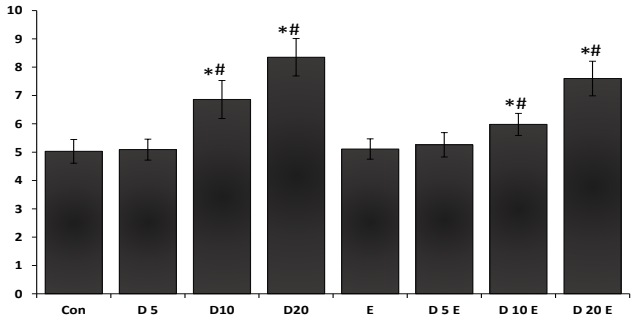

Figure 2. Comparison of mean diameter of hepatocyte nuclei (µm) in different experimental groups

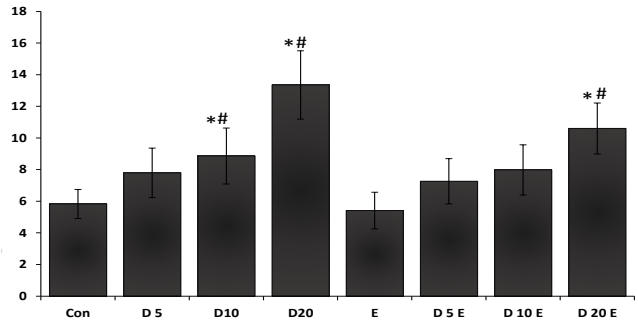

Figure 3. Comparison of the mean number of Kupffer cells per square millimeter in liver tissue in different experimental groups

Con: Control, D5: 5 mg Dianabol, D10: 10 mg Dianabol, D20: 20 mg Dianabol, E: Vitamin E, D5E: 5 mg Dianabol with vitamin E, D10E: 10 mg Dianabol with Vitamin E, D20E: 20 mg Dianabol with vitamin E. * Significant difference compared with the control group ($p<0.05$), # significant difference compared with the vitamin E group ($p<0.05$), + the existence of a significant difference in compared with the corresponding group ($p<0.05$).

Results of serum biochemical analysis of liver: Compared to the control group and vitamin E group, administration of Dianabol significantly increased serum levels of AST, ALT, ALP and LDH in D5, D10 and D20 groups ($p<0.05$). Two groups of D5E and D10E had a significant difference ($p<0.05$) with control group and vitamin E group and their corresponding groups in serum levels of AST and ALT, but had no significant difference with control group and vitamin E group regarding serum levels of ALP and LDH. Moreover, the D20E group had a significant difference ($p<0.05$) with control group and vitamin E group in all serum indices except for ALP, and had significant difference ($p<0.05$) with the corresponding groups in all serum indices (Table 1).

Results of serum and biochemical tests of kidney: Administration of Dianabol in D5, D10, and D20 groups significantly increased ($p<0.05$) serum levels of creatinine, and urea and decreased serum levels of albumin in mice compared to control group and vitamin E group. Among the groups receiving Dianabol with vitamin E, only D20E group had a significant difference ($p<0.05$) with control group and vitamin E group regarding serum levels of creatinine and albumin. Moreover, this group had a significant difference ($p<0.05$) with the corresponding group. D5E and D10E groups only had significant difference ($p<0.05$) with their corresponding group in serum levels of creatinine, urea and albumin (Table 2).

Results of Serum Total Antioxidant Capacity (TAC) measurement: Serum total antioxidant capacity measurement in different groups showed that serum total antioxidant capacity levels in D5 (0.0±341.008), D10 (0.0±301.015), and D20 (0.0±238.007) groups decreased significantly compared with control (0.0±567.007) and vitamin E (0.0±578.008) ($p<0.05$). The serum total antioxidant capacity levels in D5E (0.0±401.001), D10E (0.0±339.011), and D20E (0.0±305.011) groups showed significant difference with control group and vitamin E group ($p<0.05$). Moreover, the aforementioned groups showed a significant increase ($p<0.05$) in serum total antioxidant capacity levels compared to their corresponding group (Table 3).

The results of malondialdehyde (MDA) measurement: Measuring the levels of lipid peroxidation in animals showed that administration of Dianabol in D5 (0.0±306.007), D10 (0.0±366.013), and D20 (0.0±405.011) groups caused significant increase ($p<0.05$) compared with the control group (0.0±220.005) and vitamin E group (0.0±221.008), and the levels of malondialdehyde in D5E (0.0±266.014), D10E (0.0±313.009) and D20E (0.0±327.112) groups showed significant difference compared with the

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control and vitamin E groups (P<0.05). D5E, D10E, and D20E groups showed significant decrease (p<0.05) in malondialdehyde levels compared to their corresponding group (Table 3).

Table 1. The results of mean serum parameters of liver in different experimental groups

Groups	AST (U/l) Mean± SD	ALT (U/l) Mean±SD	ALP (U/l) Mean±SD	LDH (U/l) Mean±SD
Con	72.61±7.54	41.7±47.03	112.80±4.30	617.71±32.54
D 5	130.4±02.78 *#	94.6±08.07 *#	129.6±65.58 *#	951.93±92.12 *#
D 10	139.9±47.13 *#	105.9±20.89 *#	134.7±77.13 *#	1084.75±51.57 *#
D 20	158.9±52.76 *#	113.11±70.02 *#	141.5±30.94 *#	1261.69±43.43 *#
E	70.40±6.65	40.8±91.59	110.26±6.30	632.64±81.70
D 5 E	103.7±92.88 *#+	64.5±51.74 *#+	113.7±93.42 +	798.93±06.32
D 10 E	108.4±64.73 *#+	73.4±13.89 *#+	116.7±16.12 +	790.65±12.14 +
D 20 E	110.9±56.86 *#+	80.4±19.02 *#+	120.4±52.94 +	841.33±82.67 *#+

U/l: international unit per liter, Con: Control, D5: 5 mg Dianabol, D10: 10 mg Dianabol, D20: 20 mg Dianabol, E: Vitamin E, D5E: 5 mg Dianabol with vitamin E, D10E: 10 mg Dianabol with Vitamin E, D20E: 20 mg Dianabol with vitamin E. * Significant difference compared with the control group (p<0.05), # significant difference compared with the vitamin E group (p<0.05), + the existence of a significant difference in compared with the corresponding group (p<0.05).

Table 2. The results of biochemical tests of renal enzymes in different experimental groups

Groups	Creatinine (mg/dl) Mean±SD	Albumin (mg/dl) Mean±SD	Urea (mg/dl) Mean±SD
Con	0.0±53.071	3.0±14.25	42.7±61.05
D 5	0.0±73.069 *#	1.80±0.31 *#	64.7±74.09 *#
D 10	0.0±82.061 *#	1.41±0.50 *#	68.7±36.15 *#
D 20	0.0±84.064 *#	1.04±0.47 *#	71.8±73.46 *#
E	0.0±52.86	3.0±22.35	43.8±17.4
D 5 E	0.0±61.068	2.97±0.43 +	49.8±33.87 +
D 10 E	0.0±63.071 +	2.59±0.32 +	54.6±27.93 +
D 20 E	0.0±69.072 *#+	2.0±10.28 *#+	59.8±49.10

Mg/dl: milligrams per deciliter, Con: Control, D5: 5 mg Dianabol, D10: 10 mg Dianabol, D20: 20 mg Dianabol, E: Vitamin E, D5E: 5 mg Dianabol with vitamin E, D10E: 10 mg Dianabol with Vitamin E, D20E: 20 mg Dianabol with vitamin E. * Significant difference compared with the control group (p<0.05), # significant difference compared with the vitamin E group (p<0.05), + the existence of a significant difference in compared with the corresponding group (p<0.05).

Table 3. The results of the mean Serum Total Antioxidant Capacity and Malondialdehyde in different experimental groups

Groups	TAC(mMol/mg) Mean±SD	MDA(µmol/ml) Mean±SD
Con	0.567±0.007	0.0±220.005
D 5	0.341±0.008 *#	0.306±0.007 *#
D 10	0.301±0.015 *#	0.366±0.013 *#
D 20	0.238±0.007 *#	0.405±0.011 *#
E	0.578±0.008	0.221±0.008
D 5 E	0.401±0.001 *#+	0.266±0.014 *#+
D 10 E	0.339±0.011 *#+	0.313±0.009 *#+
D 20 E	0.305±0.011 *#+	0.327±0.112 *#+

µmol/ml: micromole per milliliter, mMol/mg: millimol per milligram,, Con: Control, D5: 5 mg Dianabol, D10: 10 mg Dianabol, D20: 20 mg Dianabol, E: Vitamin E, D5E: 5 mg Dianabol with vitamin E, D10E: 10 mg Dianabol with Vitamin E, D20E: 20 mg Dianabol with vitamin E. * Significant difference compared with the control group (p<0.05), # significant difference compared with the vitamin E group (p<0.05), + the existence of a significant difference in compared with the corresponding group (p<0.05).

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Results of histological and histochemical analysis of liver tissue: In histological analysis during hematoxylin-eosin staining, it was found that liver tissue sinusoids were normal and the arrangement of hepatocyte cells and hepatic lobules were also in normal shape in control group and vitamin E group. However, among the groups receiving Dianabol, depending on the amount Dianabol received, the dilatation of liver sinusoids was abnormally increased in D5, D10 and D20 groups.

In addition, the hemorrhagic and inflammatory spots in these groups were observed in different parts of the liver, especially around the lobular central vein. In addition to the hepatic lesions in these groups, spots with hepatic necrosis were frequently observed in hepatic parenchyma of these groups, especially the D20 group. Co-administration of vitamin E with Dianabol in D5E, D10E and D20E groups has also been able to reduce these hepatic lesions and somewhat modify the hemorrhagic and inflammatory spots present in hepatic parenchyma. In histochemical studies and for Masson's trichrome staining, the distribution of collagen fibers in the liver tissue sections and in the control and vitamin E groups was normal.

However, increased fibrotic tissue was observed in the tissue sections of the groups receiving Dianabol, i.e. D5, D10, and D20 groups, compared to the control group and vitamin E group. Increased fibrotic tissue was also observed in groups receiving vitamin E with Dianabol, i.e. D5E, D10E, and D20E groups (Fig 4).

Results of histological and histochemical analysis of the kidney tissue: Results of histological analysis of the kidney tissue showed that the use of Dianabol in groups D5, D10 and D20 led to glomerular structure degradation and increased urinary space. The cellular arrangement in the proximal tubule had more basophil nuclei than the control group and vitamin E group, and the cytoplasm in these cells was pale acidophilus. The use of Dianabol in D5, D10 and D20 groups caused hyperemia and necrosis of the kidney tissue, and the group receiving high levels of Dianabol (the D20 group) had more severe damage to the kidney, such as hyaline exudates.

Co-administration of vitamin E in the D5E, D10E, and D20E groups improved the glomerular structure and proximal tubule, and improved the tissue lesions and somewhat brought them back to normal condition. The histological study and Masson's trichrome staining showed that tissue fibrosis increased in the kidney in groups receiving Dianabol, i.e. D5, D10 and D20 groups, compared to the control group and vitamin E group. However, in the groups receiving vitamin E with Dianabol, i.e. D5E, D10E and D20E groups, the distribution of collagen fibers was lower than the corresponding groups and tissue fibrosis decreased in these groups (Fig 5).

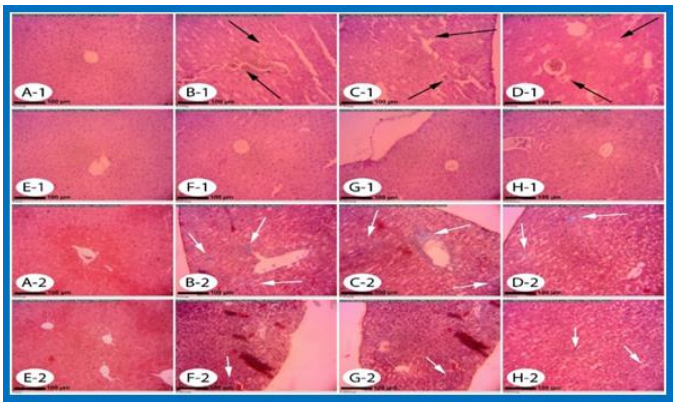

Figure 4. Microscopic view of the liver tissue in different experimental groups related to hematoxylin-eosin staining (images series 1, 100× magnification) and Masson's trichrome staining (images series 2, 100× magnification).

A-1: Control group. The natural structure of the liver tissue is observed.

B-1, C-1, D-1: Groups receiving 5, 10 and 20 mg Dianabol. Indicating Dianabol-induced liver tissue damage, dilatation of hepatic sinusoids, hemorrhagic and inflammatory spots, and hepatic necrosis (black arrows).

E-1: Vitamin E group. The natural structure of the liver tissue is observed. There was no significant difference in liver tissue structure compared with control group.

F-1, G-1, H-1: Groups receiving 5, 10 and 20 mg Dianabol along with vitamin E. Vitamin E has largely compensated for histological damage caused by Dianabol. Inflammatory tissue injuries and necrotic spots were less observed.

A-2: Control group. Masson's trichrome staining with uniform distribution of collagen fibers along with the natural structure of the liver tissue.

B-2, C-2, D-2: Groups receiving 5, 10 and 20 mg Dianabol. Indicating Dianabol-induced liver tissue damage. Increased tissue fibrosis is evident in the Dianabol group compared to the control group and Vitamin E group (white arrows).

E-2: Vitamin E group. Uniform distribution of collagen fibers is observed. There is no significant difference in the structure of the liver tissue compared with the control group.

F-2, G-2, H-2: Groups receiving 5, 10 and 20 mg Dianabol along with vitamin E. Vitamin E have been moderately able to modify the collagen fibers in the liver tissue, and reduce the level of tissue fibrosis in the liver.

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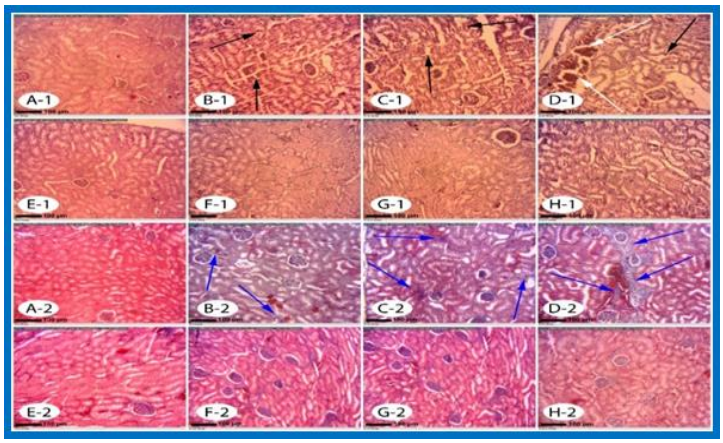

Figure 5. Microscopic view of the kidney tissue in different experimental groups related to hematoxylin-eosin staining (images series 1, 100× magnification) and Masson's trichrome staining (images series 2, 100× magnification).

A-1: Control group. The natural structure of the kidney tissue is observed.
B-1, C-1, D-1: Groups receiving 5, 10 and 20 mg Dianabol. Indicating Dianabol-induced kidney tissue damage, degradation of glomerular and nephron structure, hyperemia and necrosis of the kidney (black arrows). In the D-1 group, hyaline spots can also be observed in the kidney (white arrows).
E-1: Vitamin E group. The natural structure of the kidney tissue is observed. There was no significant difference in kidney tissue structure compared with control group.
F-1, G-1, H-1: Groups receiving 5, 10 and 20 mg Dianabol along with vitamin E. Vitamin E has largely compensated for histological damage caused by Dianabol. Necrotic injuries were less observed in kidney tissue.
A-2: Control group. Masson's trichrome staining with uniform distribution of collagen fibers along with the natural structure of the kidney tissue.
B-2, C-2, D-2: Groups receiving 5, 10 and 20 mg Dianabol. Indicating Dianabol-induced kidney tissue damage. Increased tissue fibrosis is evident in the Dianabol group compared to the control group and Vitamin E group (blue arrows).
E-2: Vitamin E group. Uniform distribution of collagen fibers is observed. There is no significant difference in the structure of the kidney tissue compared with the control group.
F-2, G-2, H-2: Groups receiving 5, 10 and 20 mg Dianabol along with vitamin E. Vitamin E have been moderately able to modify the collagen fibers in the kidney tissue, and reduce the level of tissue fibrosis in the kidney.

Discussion

In this study, the administration of vitamin E in animals treated with Dianabol improved tissue damage and serum parameters in all the three doses compared with those receiving Dianabol alone. The results of this study showed that there was a significant increase in the diameter of the hepatocytes, the diameter of the hepatocytes nuclei and the number of Kupffer cells in the mice receiving Dianabol, which is consistent with previous animal studies regarding the role of Dianabol in morphological changes of hepatocytes, such as hypertrophy and increase in the diameter of their nuclei (23). Moreover, the results of this study showed that administration of Dianabol significantly increased the levels of AST, ALT, ALP and LDH enzymes in liver, which are important indices for assessing liver toxicity, and these results are consistent with previous studies (24). Administration of Dianabol increased the serum levels of creatinine, urea and albumin in all of the mice receiving Dianabol, which is similar to previous studies, indicating that anabolic steroid drugs have been responsible for changes in these biochemical parameters in the kidney of rats (25).

In addition, the administration of Dianabol in mice in this study caused tissue damage in the kidney and liver and reduced the levels of serum total antioxidant capacity and increased malondialdehyde in the serum, which is in line with previous studies, indicating that anabolic steroid drugs decrease total antioxidant capacity and increase the levels of malondialdehyde in the kidney and liver of male rats (26).

The liver and kidney are one of the most important organs in the body, whose role in detoxification and metabolism of materials has been proven (27, 28). Anabolic steroid drugs are mainly metabolized in the liver and excreted through the kidneys (27, 28). Considering the special role of the liver as a vital organ that plays an important role in the detoxification and metabolism of toxic compounds in addition to the storage and production of nutrients, evaluation of functional indices and cellular structures of these organs, especially in animal models, has attracted much attention (29). Previous studies have suggested that anabolic steroids, as oxidant compounds, have the potential to cause oxidative damage to hepatocytes by increasing the production of free radicals during cellular oxidation in the liver tissue (30–32).

On the other hand, the unique position of kidney as an important organ for purification and disposal of xenobiotic compounds, such as anabolic steroids, and regulating the balance between water and electrolytes, has made histological evaluation as an indicator for

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observing the health of this organ (27). Studies have shown that anabolic steroids such as Dianabol cause oxidative damage in kidney tissue through the formation of catabolic products, which are potential catalysts for the damage caused by free radicals, along with oxidative metabolites of anabolic steroids (33). According to the results of the present study, which is also consistent with the results of numerous studies in this regard, it seems that the formation of lipid peroxidation and the weakening of the antioxidant defense system following the administration of Dianabol are the main mechanisms involved in liver and kidney toxicity caused by Dianabol.

Many studies indicate the role of liver damage in the incidence of morphological and morphometric changes in hepatocytes, such as hypertrophy and the increase in the diameter of the nucleus, which is consistent with the results of the histological evaluations of the present study, and can reflect the increased activity of hepatocytes in response to degenerative injuries (34, 35). One study found that dose-dependent Dianabol could change the liver morphology and liver enzymes activity, leading to hepatocyte hypertrophy, increase in the diameter of the hepatocytes, and change in the size of the reticuloendothelial cells of the sinusoid wall (23). Kupffer cells play a key role in the pathogenesis of several deficiencies and disorders of the liver as the most frequent intrinsic immune cells in the liver tissue (36). These cells are activated after liver damage and lead to the release of inflammatory mediators as well as active oxygen species, which in turn increase the liver damage (37).

Therefore, according to the histological findings of the present study, it seems that increase in the proliferation and activation of Kupffer cells can also play a significant role in liver damage caused by Dianabol. The results of this study indicate the significant protective effect of vitamin E on histological parameters of liver tissue in Dianabol-induced hepatotoxicity. The results of previous studies in this field also confirmed the efficacy of compounds with anti-oxidant and anti-inflammatory properties in reducing the toxicity caused by phenylhydrazine on histometric parameters of liver tissue (21). In order to detect liver function, measuring the serum activity of liver enzymes is a good indicator of liver damage. That’s because any damage to hepatocytes is followed by the release of these enzymes into the blood flow and increase in their serum levels (38). It seems that disorder in the structure and function of the hepatocyte membrane due to the invasion of active oxygen species, which is caused by the interactions between the compounds derived from the metabolism of Dianabol and the antioxidant defense system, leads to the release of liver enzymes into the blood flow of mice treated with this compound in this study. Considering the key role of the kidney in performing filtration and as one of the detoxification places in the body, it is directly affected by various drugs such as anabolic steroids in a way that the metabolites resulted from these drugs cause damage to kidney cells. Damage to kidney parenchyma increases the levels of urea and creatinine, which are the ultimate protein metabolism products, and decreases albumin (20).

In the present study, the levels of urea and creatinine increased in the groups receiving Dianabol while the levels of albumin decreased. These results are fully consistent with previous studies who reported that biochemical parameters of kidney are destructively changed in athletes who use Dianabol (27). The results of this study show that the activity of liver enzymes are associated with liver tissue damage caused by anabolic steroids. The use of anabolic steroid drugs leads to the formation of free radicals that are made inside the body cells, and since they have unpaired electrons, they seek to absorb the electrons of the healthy cells of the body to balance themselves, and they bind to proteins and lipoproteins of the cell membrane to fulfil their outer ring electrons, and this way, they apply their cytotoxic effect (33).

In the histological analysis of the present study, dilation of hepatic fibrosis, hepatocyte necrosis, and hemorrhage and local inflammation were induced by Dianabol in the liver tissue. Research has shown that anabolic steroidal drugs lead to the production of radical lipids with oxygen molecules by creating free radicals in cells and combining them with cell membranes and unsaturated fatty acids, and as a results, the phospholipids in the endoplasmic reticulum network are decomposed and enzymes are released, and ultimately these reactions lead to necrosis and cell death. As previous studies have shown, anabolic steroid drugs may cause degenerative changes, inflammation, and necrosis around the central veins of liver (39). On one hand, it has been shown that the anabolic steroid drugs cause hypertrophy and fibrosis of the liver cells, which is consistent with the findings of Masson's trichrome staining in this study, which shows that Dianabol may cause hypertrophy and subsequently, fibrosis of the liver tissue in mice (40). Anabolic steroid drugs cause

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oxidative damage in the kidney through formation of free radicals (41). Considering the findings of this study, which is similar to the results of numerous studies in this area, it seems that lipid peroxidation and weakening of the antioxidant defense system of the body after the administration of Dianabol are the main mechanisms involved in Dianabol-induced renal toxicity. On the other hand, several reports confirm the role of oxidative stress in the incidence of cellular damage and structural changes in the tissues of all animals treated with other oxidative and anabolic steroids (20, 42).

Moreover, the increase in the production of free radicals is one of the main causes of renal tubular degeneration, especially in proximal tubules (20). Therefore, based on the results of histological analysis of the present study, the formation of oxidative stress after Dianabol administration seems to cause glomerular structure degeneration and damage to renal proximal tubular epithelial cells and thus lead to hyperemia and necrosis of the kidney tissue (20, 42).

In addition, the increase in the distribution of collagen fibers in the renal tissue, or in other words, the increase in fibrotic tissue is consistent with the results of previous studies that show an increase in fibrotic tissue in the kidney due to oxidative factors (40, 43). Administration of vitamin E in this study resulted in a significant reduction in liver and kidney damage in Dianabol-treated mice. The results of previous studies in this field also confirmed the efficacy of compounds with antioxidant properties in reducing tissue damage induced by anabolic steroid drugs (40).

Vitamin E seems to be responsible for the improvement of oxidative damage in the liver and kidney of the mice through inhibition of lipid peroxidation and inflammatory reactions, as well as the improvement of the activity of the antioxidant defense system due to its antioxidant and anti-inflammatory functions (44, 45). Previous studies have shown that vitamin E has protective effects on renal toxicity caused by para-nonylphenol (46) as well as cadmium-induced renal damage in rats (47), and other recent studies suggest that vitamin E can improve the inflammatory reactions and carbon tetrachloride-induced liver damage in the fatty liver of rat (48).

The results of this study showed that Dianabol causes cell damage and structural impairment in the liver and kidney of the mice, causes serum and biochemical changes in the liver and kidney, decreases body antioxidant capacity and increases oxidative damage in these organs by increasing the production of free radicals, formation of oxidative stress and weakening of the antioxidant defense system. Vitamin E, on the other hand, reduces the tissue and biochemical complications of Dianabol administration in the liver and kidney of the mice through its antioxidant and anti-inflammatory properties. However, confirmation of the efficacy of vitamin E in clinical cases of Dianabol-induced liver and kidney toxicity require further extensive experimental studies and clinical trials.

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