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RESEARCH ARTICLE

Lycopene protects against renal cortical damage induced by nandrolone decanoate in adult male rats

Amany Mohamed Shalaby*, Dina Fouad El Shaer

Histology and Cell Biology Department, Faculty of Medicine, Tanta University, Tanta 31527, Egypt

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ABSTRACT

Nandrolone decanoate is an anabolic androgenic steroid that is abused worldwide by young athletes and bodybuilders to enhance their physical performance. Many clinical reports among those abusers demonstrated a variety of renal disorders. Lycopene is one of the dietary carotenoids found in fruits like tomato, watermelon, and grapefruit and has attracted considerable attention as an antioxidant. Therefore, the present study was designed to evaluate the protective effect of lycopene against nandrolone decanoate induced renal cortical damage. Forty adult male rats were equally divided into four main groups: group I served as the control, group II received lycopene 4 mg/kg/day, group III received nandrolone 10 mg/kg/week, and group IV received nandrolone and lycopene at a dose similar to the previous groups. At the end of the experiment, urea, creatinine and oxidative stress indicators were measured, then the kidneys were sampled for histopathological and immunohistochemical studies. Sections of the group (ΠI) showed variable histopathological alterations in the form of distorted shrunken glomeruli and almost complete loss of the glomerular capillaries, in addition to vacuolation and shedding of the tubular epithelium. In conclusion, these results showed that nandrolone decanoate induced toxic effects in the kidney of rats and lycopene had protective effects versus such evoked renal damage.

1. Introduction

Anabolic androgenic steroids (AAS) are defined as synthetic derivatives of the endogenous sex hormone testosterone. These compounds have been clinically used to treat many diseases such as hypogonadism, anemia, and protein deficiency as well as severe weight loss associated with chronic diseases (Kurling-Kailanto et al., 2010). AAS are often abused by competing athletes desiring a rapid increase in muscle mass and non-athletes aiming to improve their physical appearance (Angell et al., 2012). According to the National Institute on Drug Abuse, nandrolone is one of the most used anabolic steroids, because of its moderate androgenic potential associated with the good anabolic properties (Andreato et al., 2013).

Previous studies reported many serious side effects resulted from abusing these anabolic drugs which include; a cardiovascular disorder that can lead to sudden death, acute hepatitis, jaundice, testicular dysfunction with subsequent infertility, hypertension, and behavioral disorders (Al-Kennany and Al-Hamdany, 2014). Some studies have demonstrated that exposure to nandrolone induced some functional and structural abnormalities in the renal system. As regards the kidney function, there were an elevation of serum creatinine, blood urine nitrogen, and uric acid (Juhn, 2003). Moreover, acute renal failure might occur as a consequence of rhabdomyolysis (Hageloch et al., 1988).

Other studies have revealed structural changes after nandrolone exposure such as membranoproliferative glomerulonephritis, increased volume of the renal cortex, and decreased density of α_{1B}-adrenoceptors in the kidney (Hoseini et al., 2009; Lindblom et al., 2005; Revai et al., 2003). However, numerous studies showed that anabolic androgens can exert a direct toxic effect on podocytes causing their depletion, glomerular cell damage, and accumulation of mesangial matrix (Herlitz et al., 2010). The mediating steps between exposure of the kidney to nandrolone and initiation of the cascade of responses leading to kidney abnormality have not yet been completely clarified. Studies have often postulated that oxidative stress or inflammation mechanisms are involved (Riezzo et al., 2014; Summers et al., 2012). Others have shown that alterations in the expression of nephrin and podocin genes led to worsening of proteinuria, glomerulosclerosis, and renal function in diabetic nephropathy (Wang et al., 2007)

Lycopene is considered the most prevalent antioxidant carotenoid in the Western diet. It is present in tomatoes, watermelon, pink grapefruit, and several other red fruits (Story et al.,

* Corresponding author.
E-mail address: dr_amany_shalaby@yahoo.com (A.M. Shalaby).

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2010). Consumption of tomatoes or tomato products is usually associated with increased lycopene blood levels and reduced oxidative damage of lipids, proteins, and DNA (Palabiyik et al., 2017). Recent studies have reported that the supplementation of lycopene-rich diets is associated with reduced risk of many chronic diseases, cancer, heart diseases, diabetes, and other degenerative diseases in humans (El-Gerbed, 2014). Hence, in the current study, we evaluated the role of lycopene against the harmful effects of nandrolone decanoate on kidney tissue of male rats, as regards both the histological and functional abnormalities.

2. Materials and methods

2.1. Experimental animals

This work was carried out on 40 adult (non-breeding) male Wistar rats. Their weight ranged between 180 and 200 g. All animals were housed in suitable, clean, properly ventilated cages under controlled conditions of temperature (22 ± 2 °C), humidity (50 ± 10%) and a 12-h light/dark cycle, and were fed on a similar commercial laboratory diet and water. The animals were acclimatized to their environment at least one week before starting the experiment.

The animal procedures were performed according to the national guidelines for animal care and were approved by the local Institutional Animal Ethical Committee of Faculty of Medicine, Tanta University, Egypt.

2.2. Chemicals

Nandrolone decanoate (Deca-Durabolin) was in the form of ampoules (25 mg/ ampoule). It was produced by the Nile Company pharmaceuticals — Cairo, under license of N.V. Organon–oss–Holland. While, Lycopene was in the form of 20 mg capsules manufactured by Puritan's Pride, INC, USA.

2.3. Experimental design

The rats were randomly divided into four main groups (10 rats per each):

Group I (control group): was further subdivided into two equal subgroups:

Subgroup (i): kept without any treatment throughout the experiment.

Subgroup (ii): received 0.5 ml of corn oil, the diluting vehicle for lycopene via oral gavage for eight weeks.

Group II (lycopene group): received lycopene at a dose 4 mg/kg body weight/day dissolved in corn oil by oral gavage for eight weeks. This dose was selected based on previous studies (Pandir et al., 2016).

Group III (nandrolone group): was given nandrolone by intramuscular injection in the femoral muscle at a dose of 10 mg/kg body weight/week (supraphysiological dose) for eight weeks according to previous studies done by Abdelhafez (2014), Riezzo et al. (2014) and Marqueti et al. (2015). This dose is comparable to the dosage frequently used by athletes.

Group IV (Nandrolone–lycopene group): was given nandrolone and lycopene at a dose similar to the previous groups.

- The mean body weight which was measured weekly from the beginning to the end of the study and was used to recalculate the dosage of nandrolone decanoate.

At the appropriate time, one week after the last injection, all rats were fasted overnight, then anesthetized with an intraperitoneal sodium pentobarbital injection (30 mg/kg) according to Karthikeyan et al. (2009). Blood samples from each rat were collected from puncture of the retro-orbital plexus using capillary tubes without heparin (Barakat et al., 2015). Blood samples were incubated at room temperature for 10 min and left to clot. Then they were centrifuged at 3000 rpm for 10 min. Clear serum samples were carefully separated and frozen at −80 °C until biochemical analysis of urea and creatinine. Under the anesthesia, the thoracic and abdominal walls were opened exposing the viscera. The abdominal wall was retracted and both kidneys were removed, washed with cold saline, blotted on filter paper then weighed. The left kidneys were frozen in liquid nitrogen then divided into pieces and stored at −80 °C until the preparation of tissue homogenates. While the right kidneys were put into 10% buffered formalin to perform a histopathological evaluation.

2.4. Biochemical analysis

Urea and creatinine serum level were measured by using commercial kits from the Biolabo French Company.

2.5. Preparation of kidney homogenates and assessment of oxidative stress

Kidney homogenates were prepared as reported by El-Moghazy et al. (2012). Briefly, each piece of the left kidney was weighed and homogenized separately with a Potter Elvenhjem tissue homogenizer. The crude tissue homogenate was centrifuged at 3000 rpm for 10 min at 4 °C and the resultant supernatant was used for estimation of malondialdehyde (MDA) which acts as an indicator of lipid peroxidation, reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) to assess the activity of antioxidant enzymes.

2.6. Light microscopic studies

The right kidney from each animal was cut longitudinally into two halves and was fixed in 10% buffered formalin solution for 24 h, dehydrated in ascending grades of ethanol and embedded in paraffin. Serial sections of 5 μm thickness were cut and subjected to the following techniques:

- Hematoxylin and Eosin (H&E) stain: to reveal the general histological features (Gamble, 2008).
- Periodic acid–Schiff (PAS) stain: for a demonstration of the parietal layer of Bowman's capsule, basal laminae and brush border of the proximal and distal convoluted tubules (Layton and Bancroft, 2013).
- Immunohistochemical stains using streptavidin-biotin-peroxidase technique according to Van Noorden (1990).

Sections were deparaffinized, rehydrated, and rinsed in tap water, embedded in 3% hydrogen peroxide for 10 min then immersed in antigen retrieval solution. Nonspecific protein binding was blocked by incubating the sections in 10% normal goat serum in phosphate buffer solution (PBS). Sections were then incubated for two hours with the diluted primary antibody against desmin (desmin mouse monoclonal antibody) as a marker for podocyte damage (Phillips et al., 2001), that was purchased from Dako, North America, Inc., cat number (M 0760), and a monoclonal antibody against Bcl2 (antiapoptotic protein) (Dako, Carpentaria California, USA). Drops of streptavidin-peroxidase were added for 20 min then washed with PBS for five minutes. Diaminobenzidine (DAB) was added to as chromogen and Mayer's hematoxylin was used as a counterstain. Lastly, the slides were dehydrated, cleared and mounted with DPX. All slides were examined and photographed using a Leica light microscope with a built-in camera.

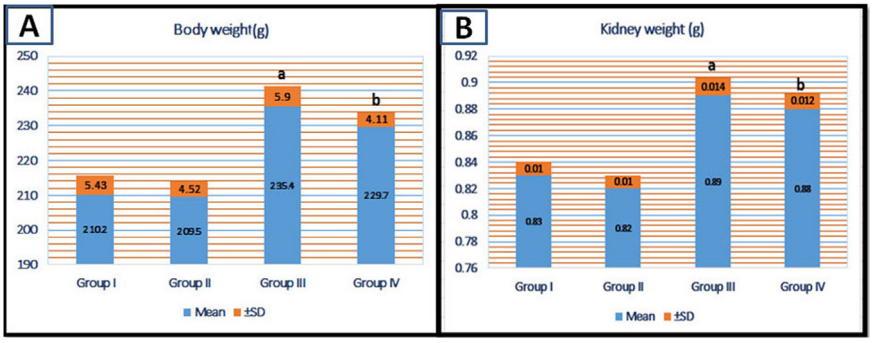

Fig. 1. A significant increase in the rat body weight and kidney weight. Graphs showing; body weight [A] and kidney weight [B] in the examined groups. Two-way ANOVA followed by Tukey's post-hoc test was used. [a] $P < 0.05$ vs. control group (group I); [b] $P > 0.05$ vs. nandrolone group (group III).

2.7. Morphometric study

- A Leica light microscope (DM500, Switzerland) coupled to a Leica digital camera (ICC50, Switzerland) was used for image acquisition at the histology department, Faculty of Medicine, Tanta University. The software "Image J version 1.47 software" (National Institute of Health Bethesda, Maryland, USA) was used for image analysis.
- The measurements were done in 10 non-overlapping randomly chosen fields in slides of each animal in each group by an independently blinded histologist.
- Mean area percentage and mean optical density of PAS-positive reaction of brush borders and basal laminae of proximal and distal convoluted tubules, in PAS-stained sections at a magnification of ×400.
- Mean area percentage of desmin-positive immunoreaction, in desmin-stained sections at a magnification of ×400.
- Mean area percentage and mean optical density of Bcl2 expression, in Bcl2 stained sections at a magnification of ×400.

2.8. Statistical analysis

- Obtained data of the biochemical lab and morphometric results were tabulated and analyzed. Data were summarized as means and standard deviations.
- Data were analyzed by two-way ANOVA followed by Post Hoc Tukey's test. The differences were considered statistically significant when the probability of chance (P value) <0.05.
- Analyses of all data were performed using the software Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, Illinois, USA) (Dawson-Saunders and Trapp, 2001).

3. Results

During the study period (eight weeks), no signs of morbidity or mortality were recorded in the experimental animals. In addition, no significant statistical differences in the biochemical, histological and immunohistochemical results were noticed between the subgroups of the control group (I and II). Thus, they were represented as the control group (I) in figures to simplify the presentation of the results. Moreover, no significant difference in the biochemical, histological and immunohistochemical results was noticed between group I and group II.

3.1. Body and kidney weights results

There was a significant ($P < 0.05$) increase of the body and kidney weights in nandrolone group (group III) (235.4 ± 5.9 g, 0.89 ± 0.014 g, respectively) as compared to the control group (group I) (210.2 ± 5.43 g, 0.83 ± 0.01 g, respectively). However, there was a non-significant difference ($P > 0.05$) in body and kidney weights in the nandrolone–lycopene group (group IV) (229.7 ± 4.11 g, 0.88 ± 0.012 g, respectively) as compared to the nandrolone group (group III) (Fig. 1).

3.2. Biochemical results

The influence of nandrolone and lycopene either individually or combined on the serum levels of urea and creatinine. The nandrolone group (group III) showed a significant increase in the level of serum urea and creatinine (94.05 ± 5.3 mg/dL, 5.41 ± 0.49 mg/dL, respectively) as compared to the control group (group I) (24.9 ± 3.68 mg/dL, 0.71 ± 0.03 mg/dL, respectively). Co-administration of lycopene with nandrolone (group IV) significantly decreases the level of serum urea and creatinine ($P < 0.05$) (31.5 ± 3.11 mg/dL, 0.96 ± 0.08 mg/dL, respectively) as compared to the nandrolone group (group III) (Fig. 2A and B).

3.3. Oxidative stress assessment

Nandrolone group (group III) showed a significant increase in MDA level (99.72 ± 6.8 nmol/g) and a significant decrease in the levels of GSH (5.31 ± 0.52 μmol/g), GSH-Px (6.59 ± 1.67 units/g), and SOD (4.82 ± 0.74 units/mg) as compared to the control (group I) (64.38 ± 2.96 nmol/g, 10.11 ± 0.85 μmol/g, 17.77 ± 1.31 units/g, 9.35 ± 1.02 units/mg respectively). On the other hand, co-administration of lycopene with nandrolone (group IV) significantly ($P < 0.05$) ameliorated the increase in MDA level (79.66 ± 4.15 nmol/g) and the decrease in the levels of GSH (8.46 ± 0.48 μmol/g), GSH-Px (10.76 ± 1.32 units/g) and SOD (7.25 ± 1.36 units/mg) (Fig. 3A–D).

3.4. Histological results

Histological examination of H&E-stained sections of both control and lycopene groups was similar and showed the normal histological architecture of the renal cortex with well-defined renal corpuscles and many convoluted tubules. Renal corpuscles were formed of a tuft of capillaries (glomerulus) enveloped by Bowman's capsule. The proximal convoluted tubules (PCT) were rounded with a narrow lumen and lined with pyramidal cells with rounded nuclei and deeply acidophilic cytoplasm. However, distal convoluted tubules (DCT) appeared with a wider lumen, less acidophilic cytoplasm and lined with cuboidal cells (Fig. 4A and B).

In the nandrolone group, glomerular changes were detected in the form of distorted, shrunken glomeruli with dilatation of the capsular space. Some glomeruli appeared congested while others showed complete loss of their capillaries. The tubules showed dilatation, vacuolation, shedding of their lining epithelium

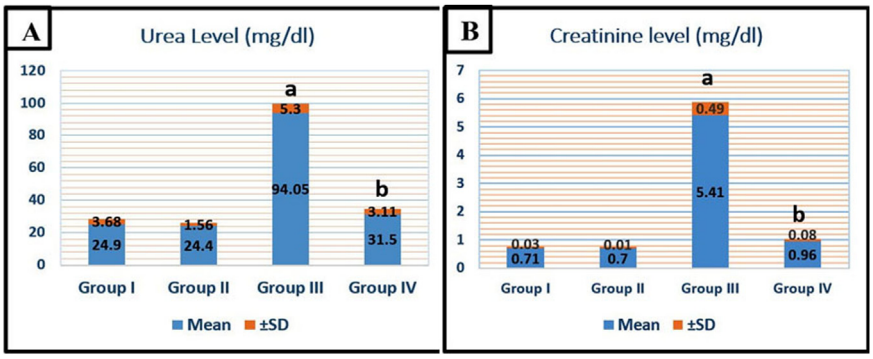

Fig. 2. A significant increase in the serum levels of urea and creatinine. Graphs showing; urea [A] and creatinine [B] levels in the examined groups. Two-way ANOVA followed by Tukey's post-hoc test was used. $^{a}P < 0.05$ vs. control group (group I); $^{b}P < 0.05$ vs. nandrolone group (group III).

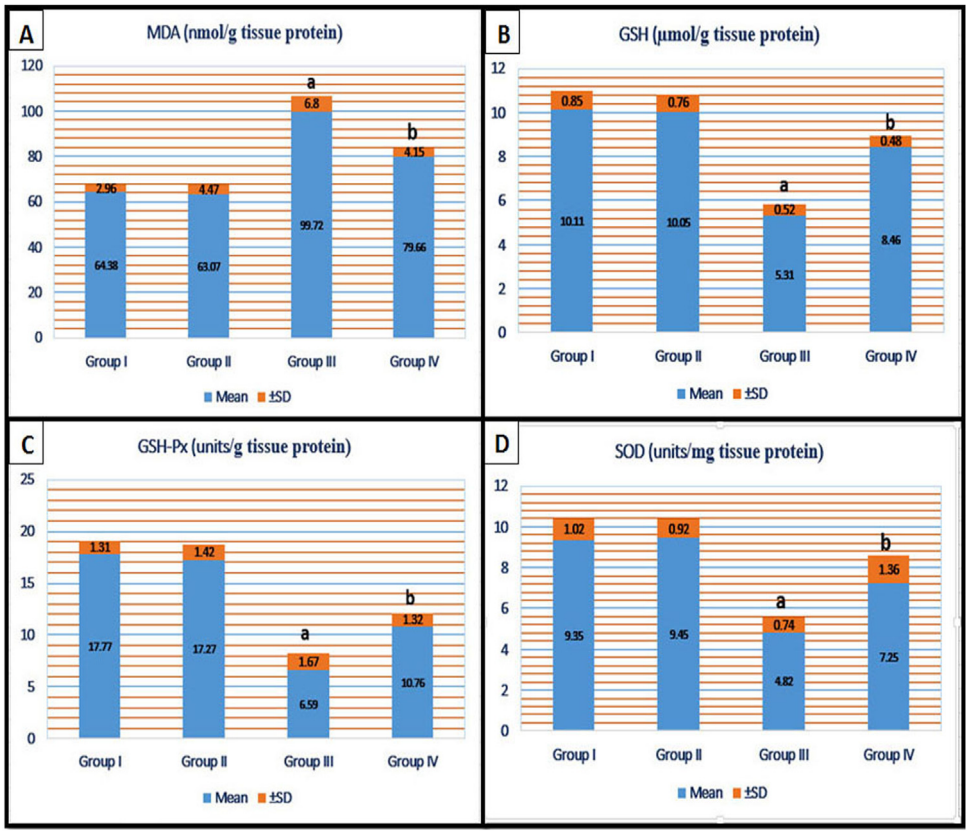

Fig. 3. A significant increase in MDA level and a significant decrease in the levels of reduced GSH, GSH-Px, and SOD. Graphs showing; MDA [A], reduced GSH [B], GSH-Px [C] and SOD [D] values in the examined groups. Two-way ANOVA followed by Tukey's post-hoc test was used. $^{a}P < 0.05$ vs. control group (group I); $^{b}P < 0.05$ vs. nandrolone group (group III).

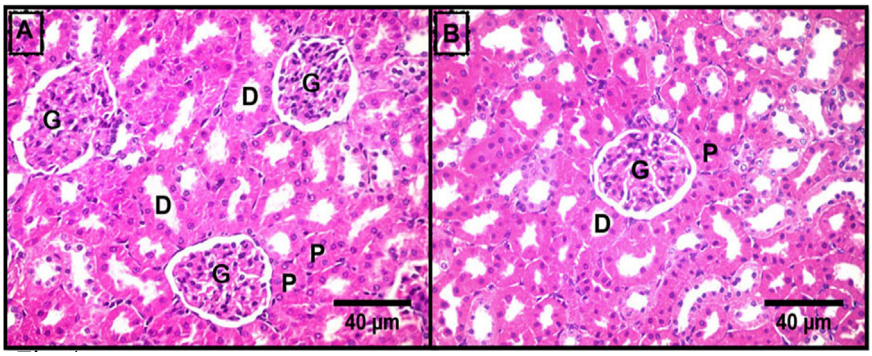

Fig. 4. Normal histological structure of the renal cortex. Photomicrographs of hematoxylin and eosin-stained sections; [A] group I (control group) and [B] group II (lycopene group) showing multiple normal glomeruli surrounded by Bowman's capsule (G). PCTs (P) are lined with pyramidal cells with deeply acidophilic cytoplasm and have a narrow lumen. DCTs (D) are lined by acidophilic low cuboidal cells with a wider lumen.

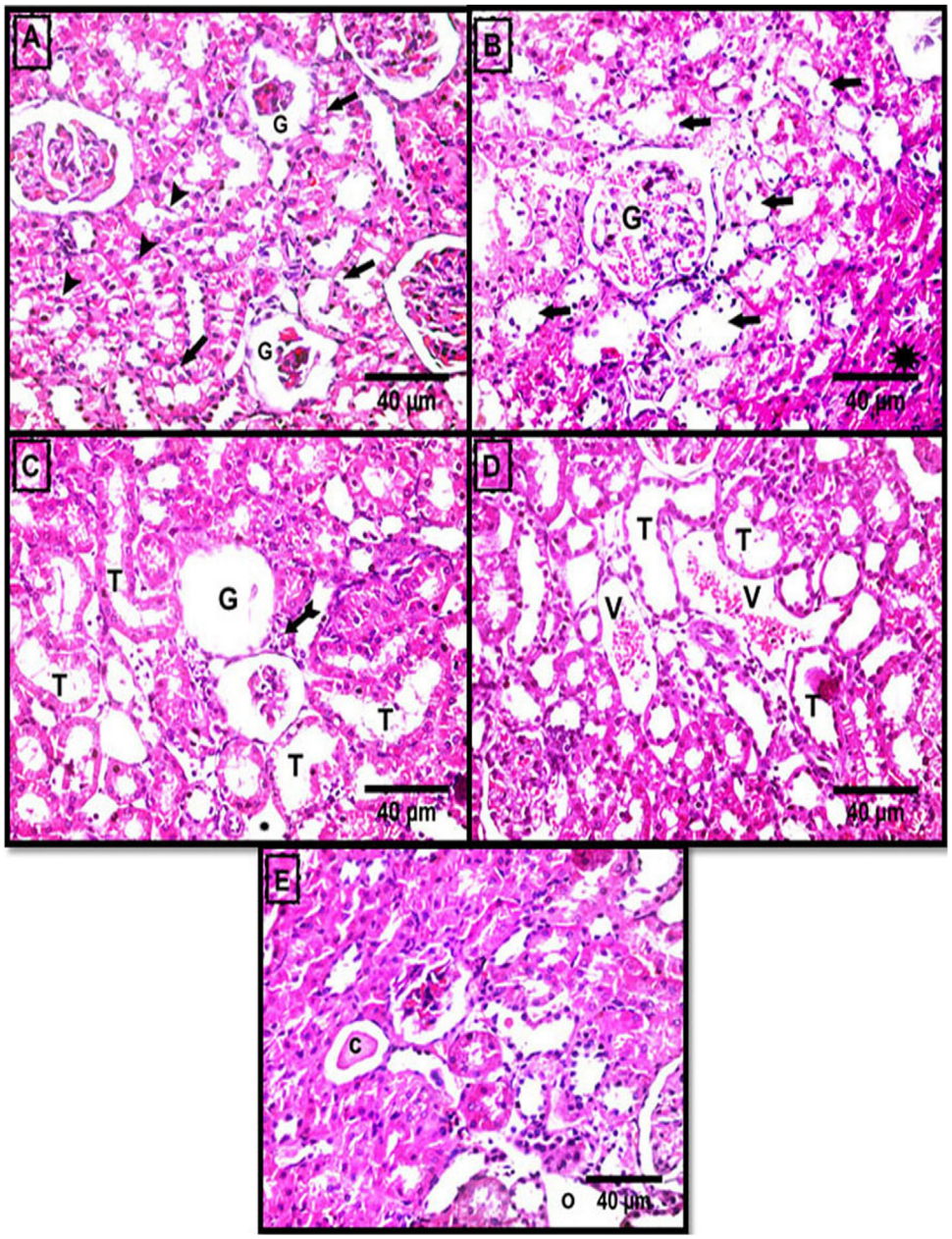

Fig. 5. Nandrolone induced histopathological alterations in the renal cortex. Photomicrographs of hematoxylin and eosin-stained renal cortical sections of group III (nandrolone group) showing [A] distorted shrunken glomeruli with widened Bowman's space (G), vacuolation (arrow) and shedding (arrowhead) of the lining epithelium in the surrounding tubules. [B] Dilated congested glomerular capillaries (G), degenerated tubules with pyknotic nuclei (arrow) and distortion of renal architecture (star). [C] Malpighian corpuscle with almost complete loss of glomerular capillaries and widening of Bowman's space (G), dilatation of the tubules (T) and cellular infiltrations (bifid arrow). [D] Marked dilatation and congestion in the inter-tubular blood vessels (V) and dilatation of some tubules (T). [E] Acidophilic hyalinized material (cast) (C) in the tubular lumen and edema (O) in between the tubules.

and occasional cast formation (acidophilic proteinous material). Moreover, degenerated tubules with pyknotic nuclei were also detected. In addition, loss of normal architecture of the renal cortex, peritubular congestion and edema were obviously appeared together with infiltrations by mononuclear inflammatory cells (Fig. 5A–E).

Administration of lycopene with nandrolone ameliorated to a great extent the histological changes induced by nandrolone. The renal cortex appeared with histological architecture nearly similar to the control group. There were few areas of focal inflammatory cells infiltration as well as mild congestion and hemorrhage in the interstitium (Fig. 6A and B).

3.5. PAS-stained results

Histological examination of PAS-stained sections of both control and lycopene groups was similar and showed the normal histological architecture of renal cortex with prominent PAS + ve reaction in the basement membrane of the parietal layer of Bowman's capsule as well as in the basement membrane and brush border of renal tubules (Fig. 7A and B). On the other hand, renal cortical sections of the nandrolone group revealed apparent weak PAS + ve reaction and disruption of the basement membrane of the parietal layer of Bowman's capsule and of some surrounding renal tubules as well as focal or complete loss of the brush border in most of the cortical tubules (Fig. 7C). This atypical reaction

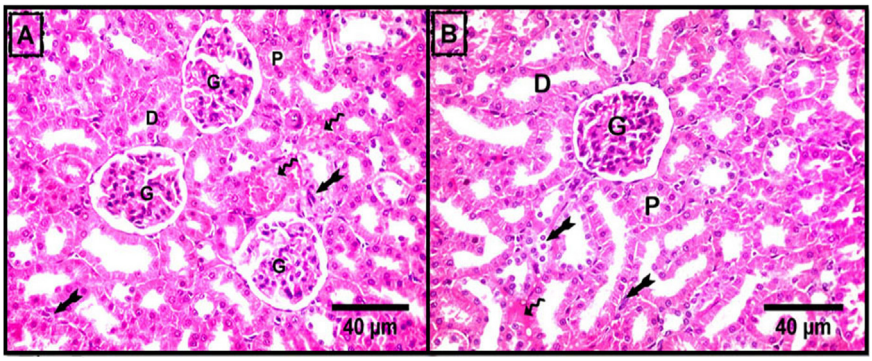

Fig. 6. Lycopene protects against histopathological changes in the renal cortex. [A & B] Photomicrographs of hematoxylin and eosin-stained renal cortical sections of the nandrolone–lycopene group (group VI) showing to a great extent normal histological structure with normal glomeruli (G) PCTs (P) and DCTs (D). Notice, focal inflammatory cell infiltration (bifid arrow) with mild congestion and hemorrhage (wavy arrow) in the interstitium.

returned back to almost normal one when lycopene was administered with nandrolone in group IV (Fig. 7D). Regarding the mean area percentage and optical density of PAS + ve material, there was a significant decrease ($P < 0.05$) in the nandrolone group (group III) (18.06 ± 0.88, 0.83 ± 0.01, respectively) as compared to the control group (33.03 ± 2.18, 1.33 ± 0.05, respectively). On the other hand, there was a significant increase ($P < 0.05$) in the mean area percentage and optical density of PAS + ve material in group IV (27.51 ± 0.89, 1.29 ± 0.07, respectively) as compared to group III (Fig. 7E and F).

3.6. Results of immunohistochemistry

3.6.1. The immunohistochemical reaction of desmin

Renal cortical sections of control and lycopene groups were similar and revealed minor localized desmin immunoreactivity (Fig. 8A and B). This immunoreaction became abundant in sections of nandrolone group (Fig. 8C). On the other hand, nandrolone–lycopene group manifested localized desmin immunoreactivity (Fig. 8D). Statistical analysis of morphometric data revealed a significant increase in the area percentage of desmin-positive cells in cortical tissue of nandrolone group (III) (6.04 ± 0.21) as compared to that of the control group (I) (0.32 ± 0.04). While, nandrolone–lycopene group (group IV) manifested a significant ($P < 0.05$) decrease in the area percentage of desmin-positive cells (0.62 ± 0.06) in comparison with nandrolone group (group III) (Fig. 8E).

3.6.2. The immunohistochemical reaction of Bcl2

Examination of cortical sections from rats in both control and lycopene groups manifested moderate to a marked reactivity of Bcl2 in the cytoplasm of the cortical cells (Fig. 9A and B). Bcl2 immunoexpression in the cortical tissue of the nandrolone treated group appeared with weak reaction as compared to that of the control group (Fig. 9C). Sections from nandrolone–lycopene treated rats in group IV manifested a strong reaction of Bcl2 as compared to rats received nandrolone alone (Fig. 9D). Statistical analysis of morphometric data revealed a significant decrease ($P < 0.05$) in the area percentage and optical density of Bcl2 positive cells in the cortical tissue of nandrolone group (21.19 ± 1.31, 0.6 ± 0.014 respectively) as compared to that of the control group (52.12 ± 0.68, 0.75 ± 0.009 respectively). While, the nandrolone–lycopene group manifested a significant ($P < 0.05$) increase in the area percentage and optical density of Bcl2 (49.96 ± 1.08, 0.73 ± 0.016, respectively) in comparison with the nandrolone group (Fig. 9E and F).

4. Discussion

In this research we studied the protective effect of lycopene, which is considered the most effective antioxidant among the carotenoids (Cohen, 2002) against nandrolone-evoked renal insult.

A previous study performed by Mulligan et al. (2005) revealed that women who received nandrolone as a treatment for human immunodeficiency virus, showed a significant increase in body weight and body mass. This agrees with the significant increase in body weight reported in this study. This increase is attributed to the anabolic effect of nandrolone and its ability to increase the fat-free muscle mass (Gold et al., 2006). Another finding in the present study was the significant increase in kidney weight of animals which received nandrolone. The same finding was noticed by Hasso (2009) in the kidneys of nandrolone treated rabbits. The effect of nandrolone on the kidney weight is due to tubular hypertrophy and an increase in glomerular size and cellularity (Hoseini et al., 2009). Lycopene administration along with the nandrolone did not show any significant alteration in body and kidney weight as compared to the nandrolone group. This agrees with Palabiyik et al. (2013) who revealed that lycopene administration with ochratoxin A changed neither body weights nor absolute kidney weights as compared to the ochratoxin A group.

In this study, we observed a significant increase in the level of serum urea and creatinine in the nandrolone treated group. This finding agrees with the previous studies of Hoseini et al. (2009) and Mwaheb et al. (2017). This elevation occurred as a result of the reduction in the glomerular filtration rate (Jwad and Mohammed, 2017). Their increases are indicators of renal damage as reported by Li et al. (2015). On the other hand, the level of serum urea and creatinine showed normal ranges in rats concomitantly treated with nandrolone and lycopene, which advocate the role of lycopene in the protection of the kidney from nandrolone evoked renal damage. These results are in agreement with the previous study performed by Karahan et al. (2005) who demonstrated normalization of serum urea and creatinine in drug-induced nephrotoxicity when treated with lycopene.

Results of the present study revealed a significant increase in MDA level and a significant decrease in the levels of GSH, GSH-Px, and SOD in nandrolone received group. These findings are in agreement with those obtained by Riezzo et al. (2014) who indicated that the long term administration of nandrolone, promoted oxidative injury in the kidneys of mice with a subsequent decrease in the ability of the kidney to scavenge toxic hydrogen peroxide and lipid peroxides. On the other hand, co-administration of lycopene with nandrolone significantly decreased MDA level and increased the levels of GSH, GSH-Px, and SOD. This coincided with the previous results of Ali and Agha (2009) who revealed that the administra-

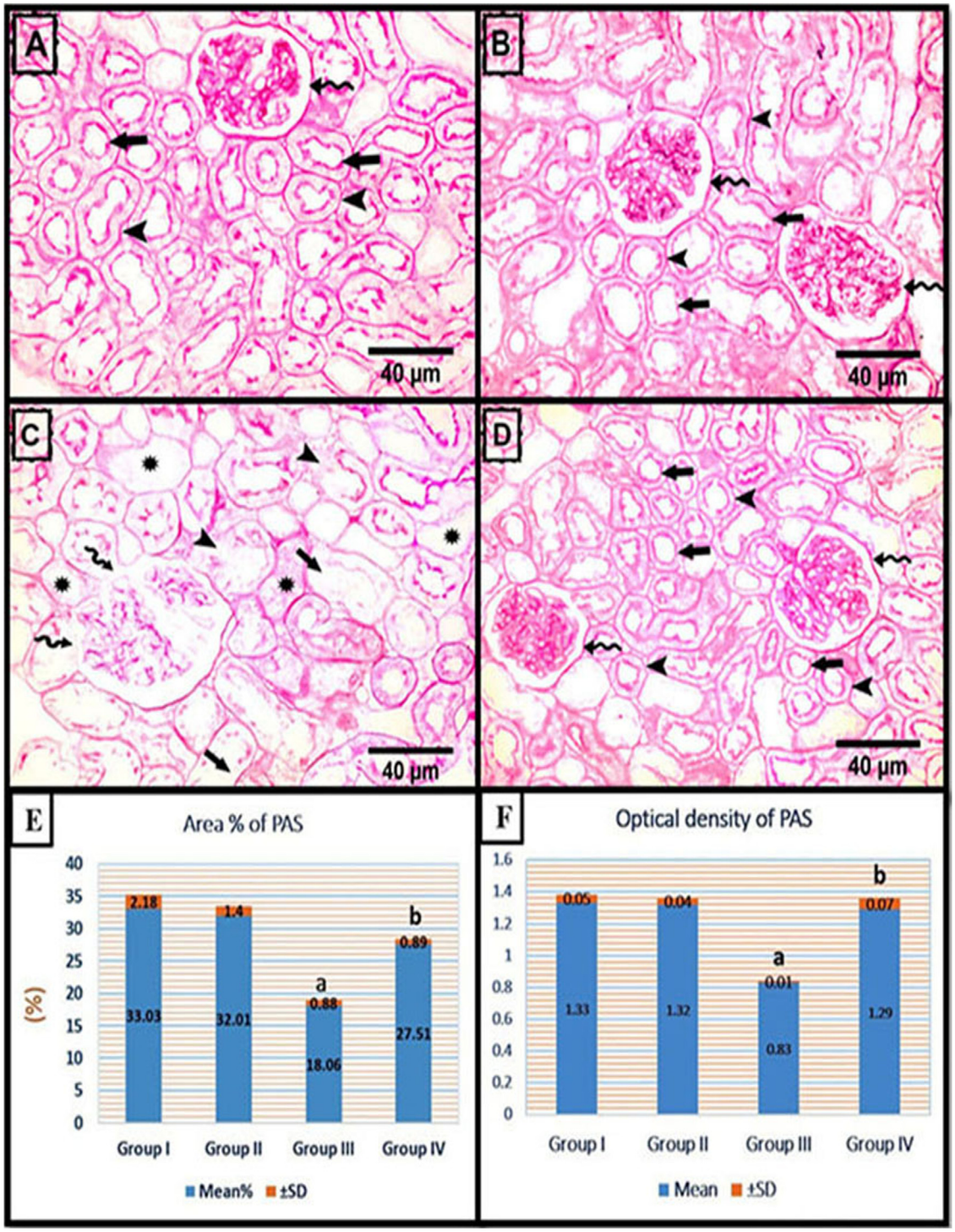

Fig. 7. Nandrolone induced weak PAS + ve reaction in the renal cortex. Photomicrographs of PAS-stained sections in the renal cortex of: [A] group I (control) & [B] group II, respectively, showing prominent PAS + ve reaction in the basement membrane of the parietal layer of Bowman's capsule (wavy arrow) as well as in the basement membrane (arrowhead) and brush border (arrow) of multiple cortical tubules. [C] Group III (nandrolone group) showing apparent weak PAS + ve reaction in the interrupted basement membrane of the parietal layer of Bowman's capsule (wavy arrow) and of some surrounding tubules (arrowhead) as well as focal (arrow) or complete loss (star) of the brush border in most of the cortical tubules. [D] Group IV (nandrolone–lycopene treated group) showing apparent moderate PAS + ve reaction in the basement membrane of the parietal layer of Bowman's capsule (wavy arrow) as well as in the basement membrane (arrowhead) and brush border (arrow) of the renal tubules. [E & F] Quantitative photomicrographs showing respectively the measurements of area% and optical density of PAS + ve material in renal cortex in the examined groups (two-way ANOVA followed by Tukey's post-hoc test was used. $^{a}P < 0.05$ vs. control group I and $^{b}P < 0.05$ vs. group III).

tion of lycopene to hyperglycaemic rats causes a decrease in the level lipid peroxidation as well as an increase in the activity of the antioxidant enzyme. The mechanism by which lycopene could affect total antioxidant, SOD and GPx activities, can be explained by its highly efficient antioxidant activity with a singlet-oxygen and free radical scavenging capacity (Stahl and Sies, 2003; Wertz et al., 2004).

In addition, nandrolone-treated rats revealed variable histopathological alterations in the renal glomeruli, tubules, and interstitium. These pathologic alterations are coordinated

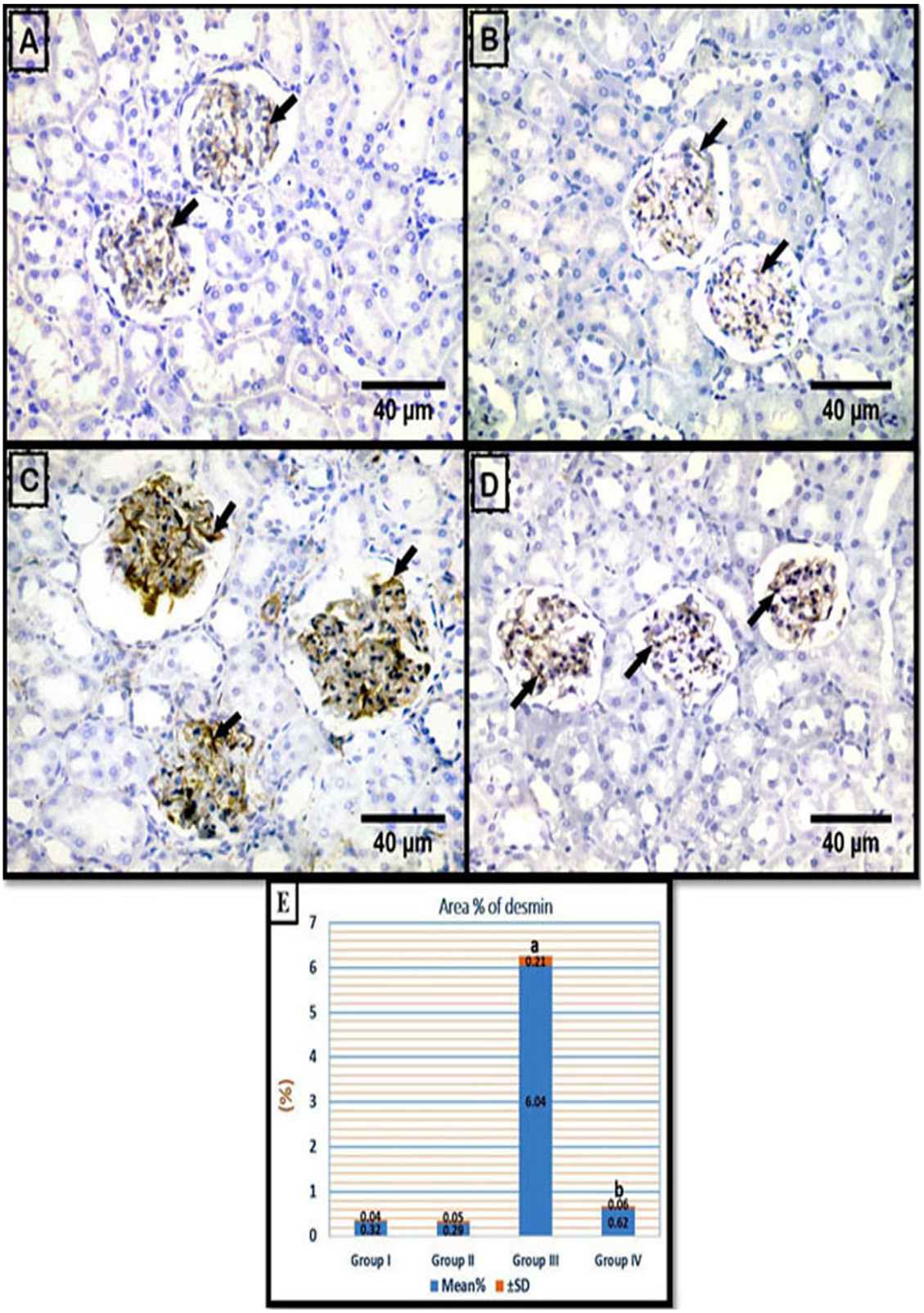

Fig. 8. Nandrolone induced widespread positive desmin immuno-reaction. Photomicrographs of desmin immunostained sections in the renal cortex showing [A] group I (control) & [B] group II (lycopene group), respectively, showing minimal glomerular positive immunoreaction in the podocytes (arrow). [C] Group III (nandrolone group) showing widespread positive desmin immuno-reaction in the podocytes (arrow). [D] Group IV (nandrolone–lycopene group) showing apparent diminution in the positive desmin immuno-reaction in the podocytes. [E] Quantitative photomicrograph showing the measurement of area% of desmin-positive cells in renal cortex in the examined groups (two-way ANOVA followed by Tukey's post-hoc test was used. $^{a}P < 0.05$ vs. control group I and $^{b}P < 0.05$ vs. group III).

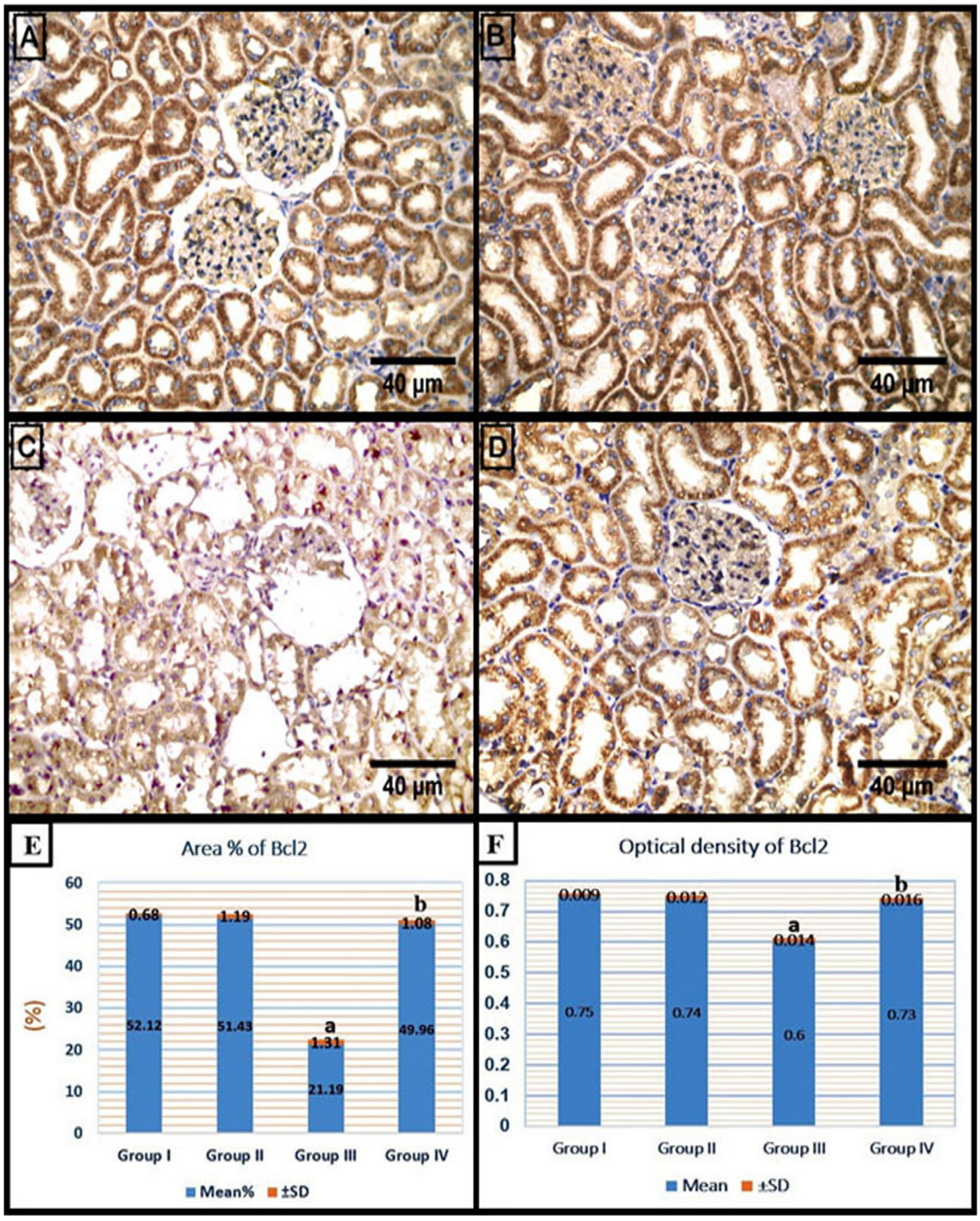

Fig. 9. Nandrolone induced weak immunoexpression of Bcl2. Photomicrographs of Bcl2 immunostained sections in the renal cortex showing [A] group I (control) & [B] group II (lycopene group) respectively showing marked Immunoexpression of Bcl2 activity. [C] Weak immunoexpression of Bcl2 activity in both cortical glomeruli and tubules of nandrolone group. [D] Moderate immunoexpression of Bcl2 activity in group administrated lycopene with nandrolone. [E & F] Quantitative photomicrographs showing respectively the measurements of area% and optical density of Bcl2 positive cells in renal cortex in the examined groups (two-way ANOVA followed by Tukey's post-hoc test was used. $^{a}P < 0.05$ vs. control group I and $^{b}P < 0.05$ vs. group III).

with other earlier researches of Takahashi et al. (2004) and Ebeye et al. (2016). Such pathological alterations cause an abnormal production of cytokines and growth factors which consequently promote the synthesis of extracellular matrix proteins and their deposition in the glomerulus that eventually lead to mesangial expansion, glomerular basement thickening and glomerular shrinkage (Hartung et al., 2001; D'Errico et al., 2011). These modifications increase hydrogen peroxide production in the mesangial cells and lipid peroxidation of the glomerulus. The products of cellular damage and lipid peroxidation lead to oxidative stress (Torres-Bugarin et al., 2007; Ali et al., 2017).

Renal tubules in the nandrolone group were obviously affected. Previous studies suggested that androgenic anabolic steroid affected the epithelial cells of urinary tubules via an androgen-dependent mechanism either directly or indirectly (Takahashi et al., 2004; Hasso, 2009). McWilliam (2007) reported that toxic agents such as drugs, chemical and environmental agents can cause tubular and interstitial disorders. The cytoplasmic vacuolations in

the lining epithelium of the renal tubules could be attributed to increased fluid uptake as a result of altered permeability of the cell membrane. The cell membrane damage could be referred to oxidative stress causing lipid peroxidation (Emanuel, 2001). The appearance of cells with pyknotic nuclei in the lining of the cortical tubules in our study can be considered as a pattern of nuclear changes caused by a nonspecific breakdown of DNA, leading to irreversible condensation of chromatin into a solid basophilic mass, in a cell undergoing necrosis or apoptosis (Kumar et al., 2015).

Another study performed by Riezzo et al. (2014) suggested that nandrolone increased the level of inflammatory cytokines TNF-α, which promotes apoptosis through the interaction with its membrane-bound receptor, TNFR1 or taking part in the mitochondrial membrane disturbances with subsequent release of cytochrome C and cell death.

Previous studies by Deshmukh et al. (2010) and Jashni et al. (2011) reported that anabolic-androgenic steroids are excreted as glucuronide compounds which may create casts in the urinary tubules. Some investigators explained that sloughing of the epithelial lining of the tubules results in cellular casts which in-turn obliterates the lumen of the tubules (Kumar et al., 2003; Rubin and Strayer, 2008).

Administration of lycopene with nandrolone, ameliorated to a great extent most of the previously mentioned histological changes. This is in agreement with Pandir et al. (2016), who reported that lycopene could protect the rat kidney against furan-induced oxidative damage by improving renal function, attenuating histopathologic changes, reducing MDA production and renewing the activities of antioxidant enzymes. Moreover, an earlier study done by Yilmaz et al. (2006) revealed that lycopene decreased renal changes induced by adriamycin and attributed this effect to its antioxidant properties that prevent lipid peroxidation and protect cellular GSH. Among the carotenoids, lycopene has been considered the most effective in protecting the cortical tubular cells from oxygen radical-induced injury and an efficient scavenger of free radicals which can prevent lipid peroxidation and generation of the oxidized products (Palabiyik et al., 2013; El-Gerbed, 2014).

In addition, lycopene was detected in the serum of rats fed with tomato lycopene complex and this confirms that consumption of lycopene improves the status of antioxidant agents in the serum and tissue. These data are in agreement with previous studies done by various antioxidant supplementations (Dogukan et al., 2011).

Furthermore, Velmurugan et al. (2002) observed that supplementation of lycopene significantly enhances the antioxidant levels, such as glutathione peroxidase, glutathione, vitamins C, and E. In fact, the favorable effect of lycopene is being as an anti-autophagic and antiapoptotic substance as well as its ability to attenuate oxidative stress in metabolic diseases and to promote a protection against oxidation of lipids, proteins, and DNA (Taheri et al., 2015).

Furthermore, the PAS-stained sections of the nandrolone group in the current study revealed weak PAS + ve reaction. These findings were supported by a significant decrease in the mean area percentage and optical density of PAS-positive material in this group. Moreover, complete or partial loss of the brush borders of most of the tubules could be explained based on that the brush border membranes are the first targets of ROS (Yousry et al., 2016). This atypical reaction returned back to almost normal one when lycopene was administered with nandrolone. This result coincides with the results of Nasr and Saleh (2014) who revealed preserved strong PAS reaction in the brush border of PCT, the basement membrane of PCT and DCT and the parietal layer of Bowman's capsule and attributed this effect to the antioxidant effect of aged garlic extract.

Another evidence of renal function deterioration in our study is the abundant desmin positive immunoreactivity within the cytoplasm of the podocytes of the nandrolone group. This was formerly supported by the significant increase in its mean area percentage versus the control group. This finding could be explained based on that podocytes are essential components of the renal glomerular filtration barrier that are injured at the early stages of glomerular damage and react to injury by altered expression of intermediate filaments and strong expression of desmin (Kakimoto et al., 2015).

On the other hand, diminished desmin positive immunoreactivity with a significant decrease in its mean area percentage in nandrolone–lycopene group was detected in our study. This finding supports the results of the previous study done by El-Gerbed (2014) who revealed that lycopene administration modulated podocyte foot process changes and had a protective effect on podocytes.

The present study revealed weak reactivity of Bcl2 in the nandrolone group. On the other hand, expression of Bcl2 significantly increased in the nandrolone–lycopene group which coincides with earlier published works concluding that lycopene inhibits apoptosis (Buyuklu et al., 2014; Bayomy et al., 2017). Morphometric results of the area percentage and optical density confirmed these results which suggest that lycopene has a potent antiapoptotic effect as it increases the expression of the antiapoptotic gene Bcl-2 and protects renal cells from undergoing apoptosis.

5. Conclusion

The biochemical, histological, and immunohistochemical results obtained in the current study support the view that a supraphysiological dose of nandrolone decanoate has harmful effects on the renal tissue and causes a variety of the histopathological changes in the renal cortex. Importantly, lycopene was shown to have beneficial effects versus renal damage. It is advisable to employ the lycopene supplement as a natural nephroprotective factor.

Ethical statement

The animal procedures were performed according to the national guidelines for animal care and were approved by the local Institutional Animal Ethical Committee of Faculty of Medicine, Tanta University, Egypt.

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