Role of Alpha-lipoic acid and Naringin in Modulating Bisphenol-A Induced Perturbations of Prostate in Adult Rats (Histological and Immunohistochemical Study)

Original Article

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ABSTRACT

Introduction: Bisphenol-A is categorized as an endocrine disruptor. A biological thiol antioxidant that lowers oxidative damage is alpha-lipoic acid. Flavone naringin has antioxidant properties.

Aim of the Work: To evaluate the ability of α-Lipoic Acid and Naringin to ameliorate disruptive effects of BPA on the prostate gland in adult rats.

Materials and Methods: Seventy-two adult rats were divided randomly into nine groups. Group I: (IA, IB and group IC). Group II: BPA, group III: BPA+α-LA, group IV: BPA+N, group V: BPA+α-LA+N, group VI: α-LA group, and group VII: N. After 30 days, the rats were sacrificed and evaluation of blood T, E2, weight gain, prostate volume, weight, index, and ventral lobe weight were done. The ventral lobes of Prostate were handled for the histological, immunohistochemical and morphometric analyses.

Results: Bisphenol-A significantly reduced serum T and E2 levels, decreased the weight gain, increased the prostatic volume, prostatic weight, and the prostatic index. It induced histopathological alterations of the ventral lobe of the prostate by histological and immunohistochemical studies. These changes were also confirmed by the morphometric assessment of the prostate tissue. Alpha-lipoic acid exhibited mild improvement in all parameters. Naringin had a moderate improvement in the parameters disrupted by the BPA. The combination of the α-LA and the N showed synergistic effects compared to the BPA+α-LA and the BPA+N groups. It showed marked improvement in all parameters to be near to normal.

Conclusion: Both Naringin and Alpha-lipoic acid have the potential to be effective protective agents against reproductive toxicity caused by endocrine disruptors.

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Key Words: Alpha-lipoic acid, bisphenol-A, naringin, rat prostate.

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INTRODUCTION

Worldwide, infertility is a problem for public health. Around 8–12% of couples are affected, with roughly 50% of those having a male role as the main contributor[1]. Delivery of hormone-disrupting substances (EDCs) to environment has been connected to decrease male reproductive health. EDCs disrupt homeostasis, reproduction, and developmental processes[2].

Bisphenol-A (BPA) is synthetic white crystalline organic compound, classified as a one of the endocrine disrupting chemicals. BPA is utilized in various fields as production of materials used in dentistry and optics in medicine, in the field of food industry as reusable dishes, bottles, internal coating of food containers, and microwavable utensils and in the process of production of water pipes[3,4]. BPA has an intracellular anti-androgenic action which is attributed to the formation of Androgen receptor Bisphenol-A complex AR/BPA complex[5]. BPA produces cellular injury to both protein and lipid components by liberation of reactive oxygen species (ROSs) in the tissues where it concentrates[6]. The major endocrine disruption caused by BPA exposure was due to estrogen and androgenic action[7].

Exposure to BPA has been linked to numerous male reproductive disorders and prostate diseases[8]. Low-dose BPA administration to adult rats increased weight of the ventral prostate, compromise spermatozoa, disrupt hypothalamic–pituitary–gonadal axis, causing hypogonadotropic hypogonadism and prostatic epithelium squamous metaplasia[9,10].

Alpha-lipoic acid (α-LA) is antioxidant thiol that decreases oxidative harm and boosts the antioxidants. It has high reactivity to free radicals, increases glutathione,

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reduces lipid peroxides, renovating antioxidant enzyme and alleviate damage of testes a result of harmful effects of BPA as it is potent antioxidant and cell-protecting compound[11,12].

Naringin (N) is flavone existing in citrus fruits, cherries and cocoa[13]. It had nephroprotective, anti-inflammatory, anti-oxidative, hepatoprotective, and anticancer properties. Both N and Naringenin (the active form of naringin) are powerful free radical scavengers that prevent lipid peroxidation[14]. Naringin restore normal testicular and epididymal structures in mice with testicular and epididymal disrupted structures by cyanide toxicity[15]. Naringin also found to reduce toxic effects of BPA on reproductive organs by adjusting level of lipid peroxidation, reducing ROS, and preserving homeostasis[16].

Therefore, current work directed to assess the role of α-LA and N in mitigating BPA produced perturbations in mature rat prostate.

MATERIAL AND METHODS

The current research was conducted in Department of Human Anatomy, Faculty of Medicine, Suez Canal University.

Animals

Animals were procured from Veterinary Medicine Faculty, Suez Canal University. Young adult male albino rats, weighing between 210 and 240 grammes, with ages ranging from two to three months. Animals housed in wire cages with good ventilation, provided food and water, and adapted for one week before experimental use. Researchers handled animals exclusively during the experiment. Suez Canal University's Faculty of Medicine approves study protocol.

Chemicals

Bisphenol-A (BPA): Bisphenol-A 99% in the form of powder (cat.no.239658: purity of 99%; Sigma-Aldrich Co, St Louis, Missouri, USA) was given orally for 30 consecutive days in a dose of 50 mg/kg[16,17] (10 mg BPA: 1ml Corn oil)[18].

Alpha-lipoic acid (α-LA): Alpha-lipoic acid (Eva Pharma Cairo, Egypt) in the form of powder suspended in distilled water was given orally 100 mg/kg/day[19] during 30 consecutive days[20].

Naringin (N): Rats received N (95% pure: Sigma-Aldrich Co, USA) 160 mg/kg/day[16,17] orally during 30 days[21] powder dissolved in distilled water[22].

Experimental designs

Seventy-two male rats randomly divided to nine groups and subgroups as follows:

I: Control group: consisted of three equal subgroups (eight rats per each).

- IA: rats received nothing.
- IB: rats administered orally corn oil 5 ml/kg[18].
- IC: rats received distilled water 3ml/kg orally (positive control II)[22].

II: Bisphenol-A (BPA) group: Bisphenol-A was given orally for 30 consecutive days.

III: (BPA+α-LA) group: Rats received α-LA concomitantly with BPA orally for 30 consecutive days.

IV: Bisphenol-A plus Naringin (BPA+N) group: Rats received N concomitantly with BPA orally for 30 consecutive days.

V: Bisphenol-A plus Alpha-lipoic acid and Naringin (BPA+α-LA+N) group: Rats received BPA concomitantly with α-LA plus N orally for 30 successive days.

VI: (α-LA) group: the rats received α-LA orally for 30 successive days.

VII: Naringin (N) group: Rats received N only orally for 30 successive days.

Every rat from all groups weighed daily then doses of the drugs were adjusted accordingly (Figure 1).

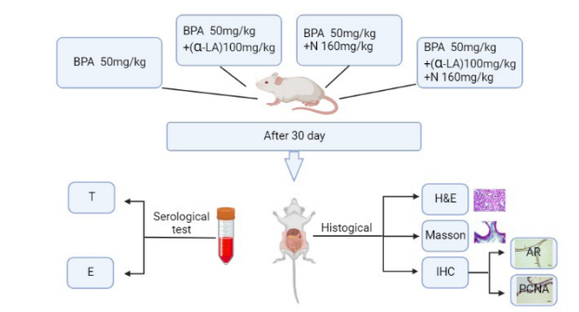

Fig. 1: Graphical abstract: Summary for the study design

Blood samples collection: 24 hours after the last dosage, samples of blood from retro-orbital plexus under light anesthesia were obtained from animals using capillary glass tubes. Every rat's blood was obtained on a cleanse tube at 37˚C until coagulation of blood. After that It underwent centrifugation to separate the serum. then serum E2 and T concentrations were estimated using enzyme-linked immunosorbent test (ELISA) kits, as directed by the manufacturer's guidelines[23,24].

At experiment end (30 days), 24 hours following final doses, the weight of the rats were measured then anesthetized by ketamine (70 mg/kg) + xyaline (7mg/kg), then sacrificed by cervical dislocation[25].

The prostate parameters

After sacrifice, the prostate gland separated, gathered, and trimmed away from the additional fascia. Volume of prostate (PV) calculated using water displacement procedure[26]. The prostate weight (PW) was measured. The prostate index (PI) determined depending on the subsequent equation:

$$PI = \frac{\text{(prostate weight (PW))}}{\text{(body weight (BW))}} x100$$ [27,28].

The two ventral lobes were excised, separated, and then weighed.

Histological Assessment

The prostate ventral lobes were fixed by being immersed in 10% formalin solution 24 hours period prior to making paraffin blocks, then, paraffin slices 4 m thick were made.

Paraffin-embedded Prostate ventral lobe slices stained by H & E[29] and Masson trichrome staining[30]. Sections were examined by a light microscope (Olympus, Japan) under x100, x400, and x1000 magnifications.

Immunohistochemistry

The paraffin embedded sections were immunohistochemically analyzed using:

a. **PCNA stain:** degrees of proliferation were measured by PCNA monoclonal antibodies antigen according to the method described by[28,31].

b. **Androgen receptor (AR):** To assess degree of AR expression in the prostatic acini, according to the method described by[32].

Morphometric study

By using FIJI program software, histomorphometry analysis of the ventral lobe of the prostatic sections was performed[33]. Four non-overlapping fields per prostate were randomly chosen to be studied and analyzed[34].

The height of the epithelial cells, acinar luminal, and areas of stroma were measured in four non-overlapping fields per prostate per rat[34].

The epithelium height was estimated by drawing a line through the whole length of acinar epithelium (a field with 30 measurements) under 400x magnification[34].

The area of the acinar lumen was measured. by tracing a line around the acini's edge under 100x magnification[34].

The area of the stroma was measured by deducting the total area of acinar luminal from the total area of the field under 100x magnification[34].

The collagen area percentage was measured under 400x magnification[9,35].

The epithelial cells proportion expressed by immunopositively PCNA reaction to the total epithelium cell number was determined[36,37].

The epithelial cells proportion expressed immunopositively AR outcome to the total epithelial cell number was determined[38].

Statistical analysis

The mean, and standard deviation for each parameter was calculated and significance of the variance in the values among various groupings were performed using one-way ANOVA test then the post-hoc test (Tukey). The statistical program performed statistical analysis for social sciences (IBM SPSS Statistics Version 22.0). A statistical significance threshold is reached when the results of *p-value* > 0.05.

RESULTS

The present work demonstrated that There was no discernible difference between different controls subgroups regarding the biochemical, gross, histological, and morphometric assessment and therefore, they are presented as one control group. Additionally, not statistically significant existed among control and either naringin (N) or alpha-lipoic acid (-LA) groups (so data not shown for simplicity)

Testosterone (T) and Estradiol (E2) levels

According to current work, there were highly statistically significant decreases in both T and E2 levels of BPA, BPA+α-LA, and BPA+N groups in comparison with C group. But on the other side, there was no statistically significant alteration among group BPA+α-LA+N in comparing with the control.

Both T and E2 levels showed a statistically significant increase between BPA+α-LA, BPA+N and BPA+α-LA+N groups in comparison with BPA group.

Moreover, T and E2 levels exhibited a statistically significant increase of BPA+α-LA+N group compared to the BPA+α-LA.

T levels showed statistically significant increase compared to the BPA+N groups. But the E2 levels compared to the BPA+N group exhibited insignificant difference.

Additionally, T and E2 levels in the BPA+N group was a statistically highly significantly increased compared to those of the BPA+α-LA group (Figure 2)

Body weight assessment

The first rat weight wasn't statistically significant between the various study groups.

The final weight and weight gain was statistically highly significantly decreased in BPA, BPA+α-LA, and BPA+N groups in comparison with the control group.

Compared to BPA group, final weight and weight gain were statistically highly significantly increased in BPA+α-LA, BPA+N and BPA+α-LA+N groups (Figure 3A).

The prostatic parameter

Compared to the C group, the prostate volume (PV), weight (PW), ventral lobe weight (VLW) and the prostatic index (PI) were statistically significantly increased in BPA, BPA+α-LA, and BPA+N groups. There were no statistically significant differences regarding BPA+α-LA+N group in comparison with the C group.

In contrast, the prostate volume (PV), weight (PW), ventral lobe weight (VLW) and the prostatic index (PI) were statistically significantly declined in BPA+α- LA, BPA+N and BPA+α-LA+N groups compared to the BPA group.

Regarding the BPA+α-LA+N group, the prostate volume (PV) weight (PW), ventral lobe weight (VLW) and prostatic index (PI) statistically significantly decreased in comparison with the BPA+α-LA and the BPA+N groups.

In addition, prostate volume (PV), weight (PW), ventral lobe weight (VLW) and the prostatic index (PI) of BPA+N group were statistically decreased in comparison with BPA+α-LA group (Figure 3B).

Histopathological results of the prostatic tissues

The H & E-stained sections of ventral lobe prostatic gland of the adult rat control group showed normal histological architecture. The ventral lobe consisted of closely packed acini with variable shapes and sizes and surrounded by a fibromuscular stroma. The acini showed very scarce infoldings. The epithelial linings of the acini ranged from cubical to low-columnar cells with basophilic cytoplasm and rounded to oval basal nuclei which rested on a regular intact basement membrane. Fibromuscular stroma in between was thin and unremarkable consisting of loose connective tissue and blood vessels (Figures 4A,5A,6A).

The histological architecture of prostate's ventral lobe of BPA group was disrupted. The ventral lobe showed irregularity of the acini shape and size containing few eosinophilic secretions and surrounded by a wide fibromuscular stroma. It showed presence of inflammatory cell infiltration, dilated blood vessels with appearance of acidophilic homogenous masses. Some acini showed thickening of the epithelial layers and many infoldings were visible in the acini in addition to presence of areas of interrupted and disorganized epithelium. Moreover, the basement membrane and epithelium separated from one another with the presence of vacuolated epithelium. Some epithelial cells were found to have irregular hyperchromatic nuclei (pyknotic nuclei) (Figures 4(B1, B2), 5(B1, B2), 6 (B1, B2))

Most of the acini of ventral lobe prostate gland of the BPA+α-LA group, were irregular and the fibromuscular stroma was wide and thickened. The epithelium lining was thickened showing multiple rows of flat nuclei with few infoldings (Figures 4C, 5C,6C).

The prostatic ventral lobe of BPA+N group exhibited irregular acini with various sizes and shapes having some infoldings. The acini lumen contains few vacuolations. The fibromuscular stroma was thin. The epithelial lining of most acini was nearly normal ranging from cubical to low columnar cells (Figures 4D,5D,6D).

In the BPA+α-LA+N group, the ventral lobe prostate showed that the ventral lobe consisted of closely packed acini of variable shapes and sizes having moderate number of eosinophilic secretions. The fibromuscular stroma in between was scanty with infiltration of few inflammatory cells and dilatation of the blood vessels. The epithelial cells which line the acini ranged from cubical to low columnar cells with basally situated nuclei. Little infoldings were observed (Figures 4E,5E,6E).

Masson trichrome stained sections

Masson trichrome-stained slices of the adult rat prostatic ventral lobe gland in control group presented typical thin and scanty positively bluish to greenish fine stained scattered stroma collagen fibers and thin fine collagen fibers at the acini basement membrane of the prostate.

The collagen fibers area percentage was statistically highly significantly increased in the BPA, BPA+ α-LA, BPA+N and BPA+α-LA+N groups compared to the C group.

In contrast, the area percentage showed highly significantly statistically decreased in the BPA+α-LA, BPA+N and BPA+α-LA+N groups compared to the BPA group.

The area percentage was statistically significantly reduced in BPA+α-LA+N group compared to the BPA+α-LA and BPA+N groups. There was also a high statistically significant reduction in area percentage in BPA+N group compared to BPA+α-LA group (Figure 7).

PCNA and (AR) immuno-stained sections

Both PCNA and AR -immuno-stained sections of the control group of adult rat prostate's ventral lobe showed numerous positive nuclear immunoreactions in the acinar cells represented by a single layer of brown nuclei lying on the basement membrane.

The percentage of both PCNA and AR positive nuclei was highly statistically significantly decreased in the BPA, BPA+α-LA, BPA+N, and BPA+α-LA+N groups compared to the C group.

The percentage of both PCNA and AR positive nuclei was statistically highly significantly increased in BPA+α-LA, BPA+N and BPA+α-LA+N groups in comparison with BPA group.

In addition, percentage of both PCNA and AR positive nuclei was statistically significantly increased in BPA+α-LA+N group when compared with BPA+α-LA and BPA+N groups.

The percentage of both PCNA and AR positive nuclei was highly statistically significantly increased in BPA+N group in comparison with BPA+α-LA group (Figures 8,9).

Morphometric results

When compared to the C group, the epithelium height and stromal area were statistically significantly increased with significant decrease of acinar luminal area in BPA,

BPA+α-LA, and BPA+N groups. On the other hand, there was no statistically significant difference among BPA+α-LA+N in comparison with the C group.

When compared to BPA group, the epithelium height and stromal area demonstrated highly statistically significantly decreased with significant increase of acinar luminal area in the BPA+α-LA, BPA+N and BPA+α-LA+N.

According to the BPA+α-LA+N group, the epithelium height and stromal area were statistically significantly decreased with significant increase of acinar luminal area in comparison with BPA+α-LA and BPA+N group.

Also high statistically significant reduction of epithelium height with stromal area with a significant increase of acinar luminal area in BPA+N in comparison with BPA+α-LA group. (Table 1)

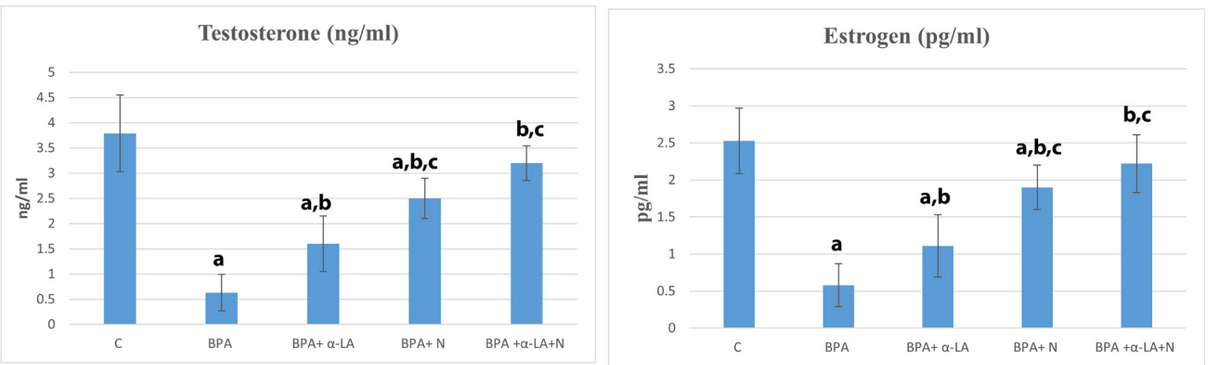

Fig. 2: The Mean ± Standard deviation (SD) of T and E2 levels among the different study groups One-way ANOVA, Post hoc test (Tukey):

a compared to control group b compared to BPA group C compared to BPA+ α-LA group

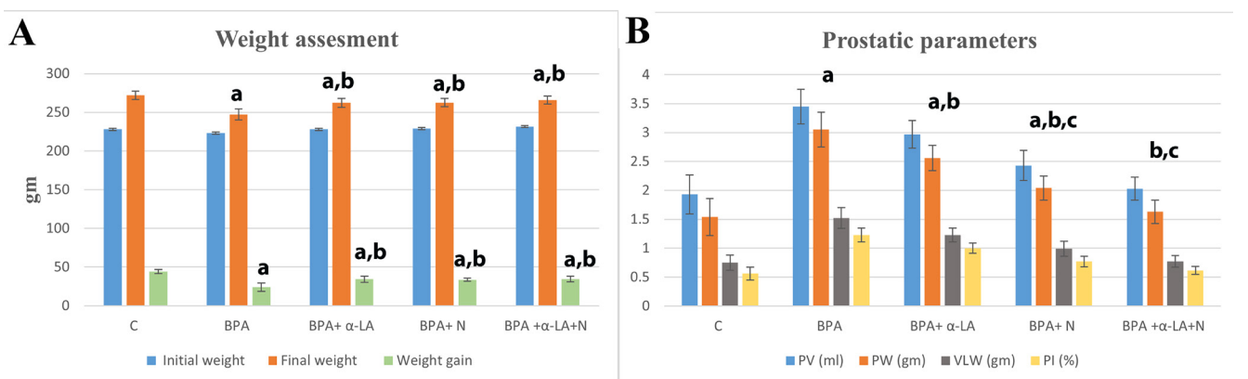

Fig. 3: (A) The Mean ± SD of rat weight (initial and final) and weight gain among the different study groups. (B) The Mean ± SD of the prostate weight, the prostatic index, the prostatic volume, and ventral lobe weight among the different studied groups. One-way ANOVA, Post hoc test (Tukey):

a compared to control group b compared to BPA group C compared to BPA+ α-LA group.

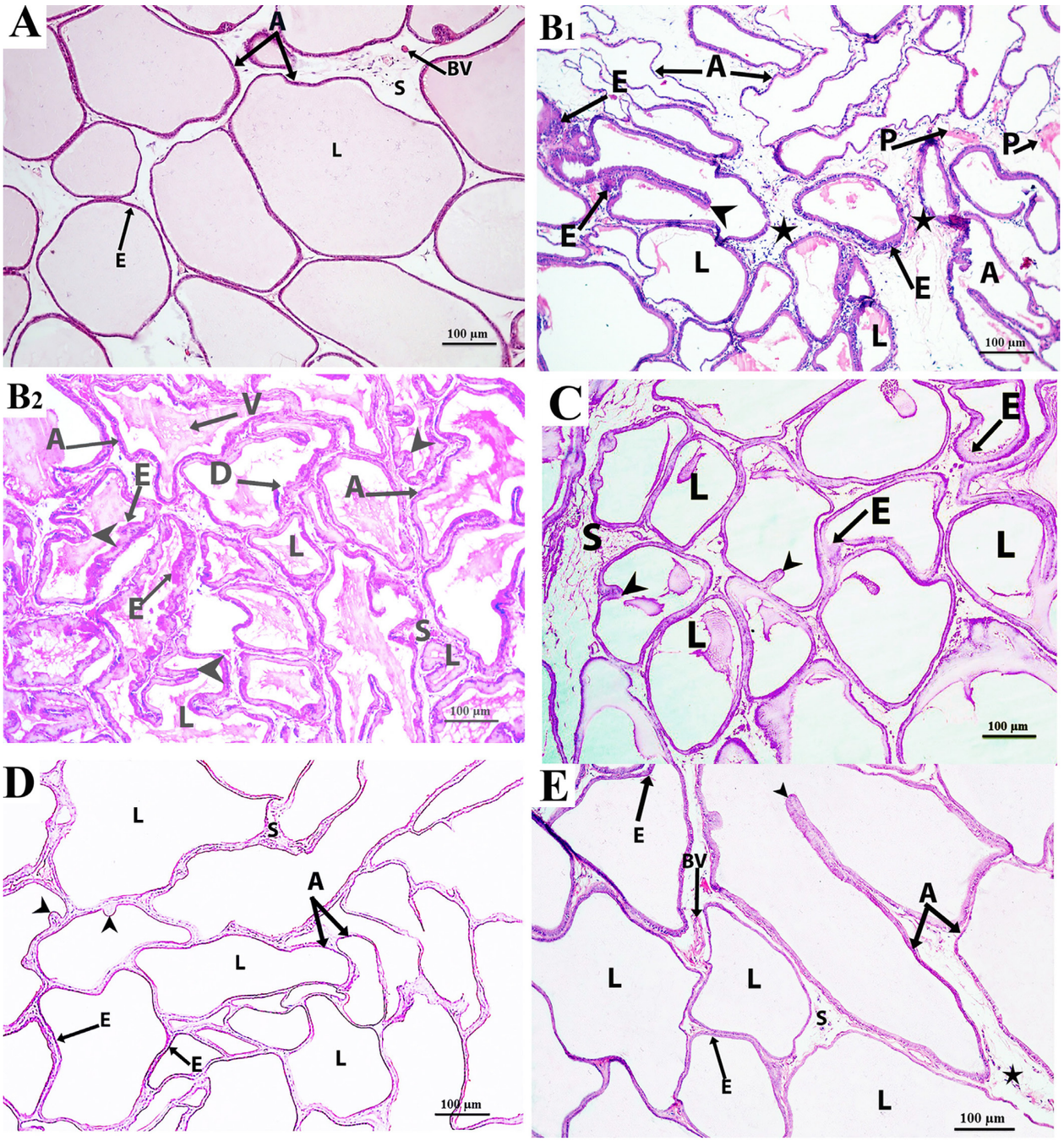

Fig. 4: A photomicrograph of a section of ventral lobe of rat prostate A: Control group showing closely packed acini with variable sizes, shapes, and intact normal epithelium. The acini are surrounded by normal thin fibromuscular stroma containing normal blood vessels.

B1 and B2: BPA group showing irregularly shaped acini surrounded by thickened fibromuscular stroma contains acidophilic homogenous masses with inflammatory cells infiltration. Thickening, infoldings and discontinuation of epithelium of some acini.

C: BPA+ α-LA group showing irregularly shaped acini with narrow lumen and little to absent eosinophilic secretions. Thick fibromuscular stroma and epithelium with few infoldings.

D: BPA+N group showing irregularly shaped acini with narrow lumen and scanty fibromuscular stroma. Normal lining epithelium with few infoldings.

E: BPA+α-LA+N showing adjacent acini with variable shapes and sizes and surrounded by scanty fibromuscular stroma with mild infiltration of inflammatory cells and dilated blood vessel. The epithelial lining of the acini is nearly normal with minimal infoldings (H&E x100). acini (A), epithelium (E), lumen (L), stroma (S), blood vessels (BV), vacuolations (V), plaques (P), inflammatory cells infiltration (asterisk), infoldings (arrowhead), discontinuation of epithelium (D).

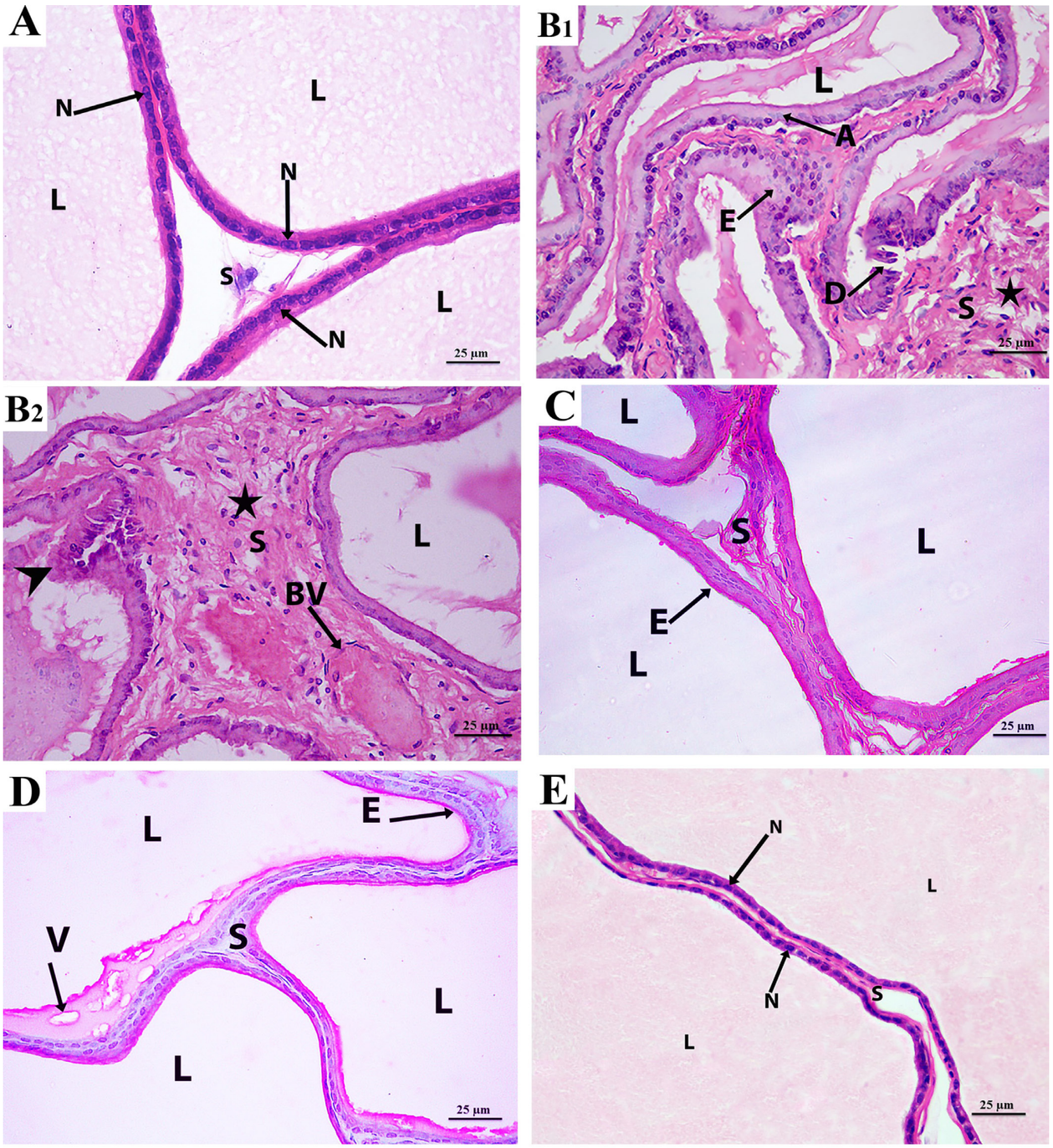

Fig. 5: A photomicrograph of a section of the ventral lobe of rat prostate A: Control group showing parts of three adjacent acini filled with eosinophilic secretions. The epithelium lining is low columnar to cubical cells with basal nuclei surrounded by normal thin fibromuscular stroma.

B1 and B2: the BPA group showing parts of numerous irregular acini. Thickened fibromuscular stroma is infiltrated by inflammatory cells. Some acini show thickened epithelium with some infoldings while others show discontinued epithelium and dilated blood vessels.

C: BPA+α-LA showing parts of four acini surrounded by thickened fibromuscular stroma and epithelium with multiple rows of flat nuclei.

D: BPA+ N group showing parts of three prostatic acini, their lumen contains few vacuolations. The fibromuscular stroma is thin. Epithelium consists of cubical cells.

E: BPA+α-LA+N group showing parts of two adjacent. The acini are surrounded by thin fibromuscular stroma with nearly normal epithelial lining with rounded to oval nuclei situated on the basement membrane (H&E x400). acini (A), epithelium (E), lumen (L), stroma (S), blood vessels (BV), vacuolations (V), plaques (P), inflammatory cells infiltration (asterisk), infoldings (arrowhead), discontinuation of epithelium (D), nuclei (N).

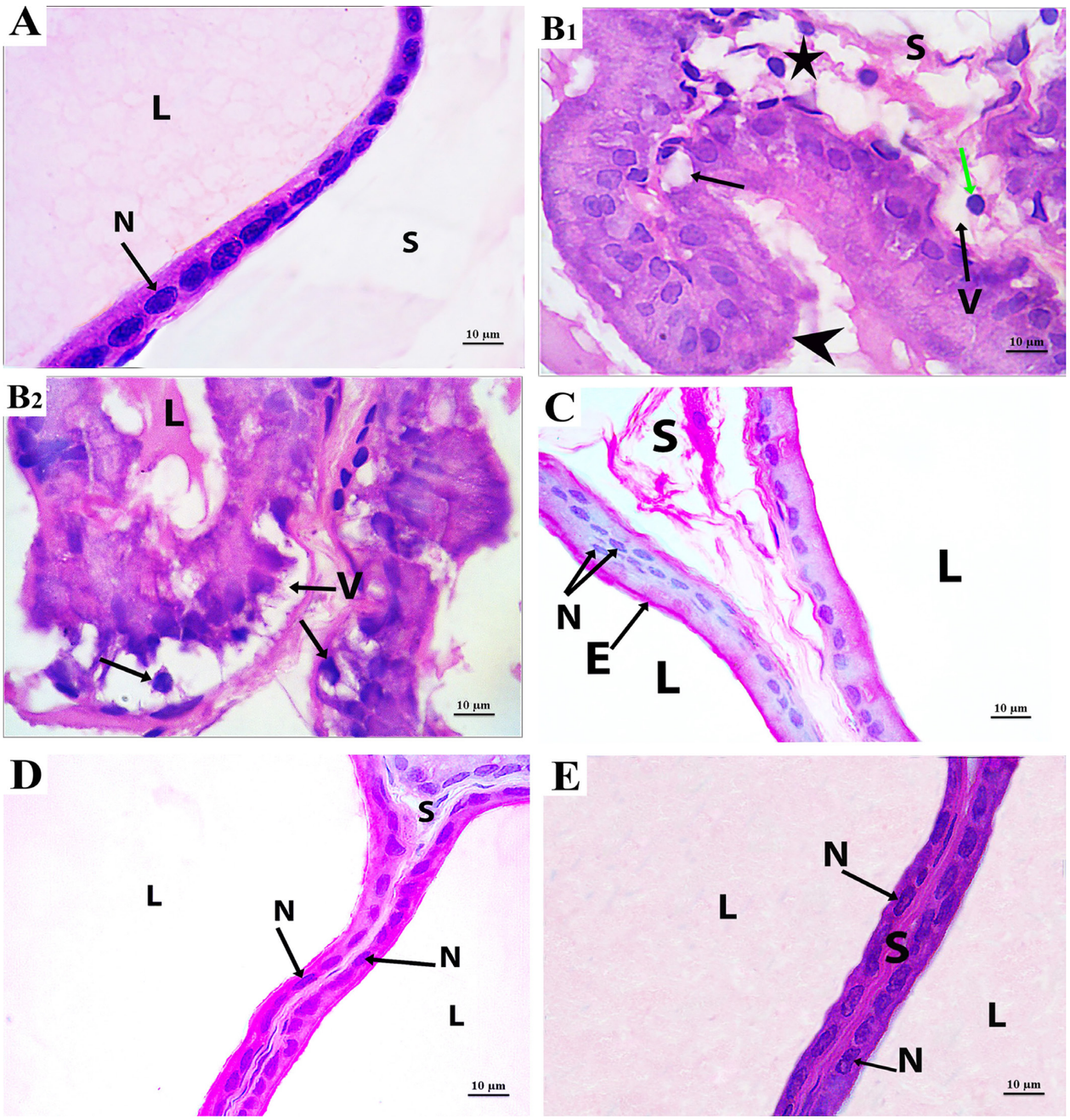

Fig. 6: A photomicrograph of a section of the ventral lobe of rat prostate. A: Control group showing part of one acinus with low columnar to cubical epithelial with basal nuclei. The acinus is surrounded by normal thin fibromuscular stroma.

B1& B2: the BPA group showing a prostatic acinus surrounded by fibromuscular stroma (S) infiltrated with inflammatory cells (asterisk). The inflammatory cell (green arrow) is surrounded by vacuolations (V). The epithelium shows infoldings (arrowhead) having cells dark small nucleus surrounded by halo (pyknotic nuclei) (arrow).

C: BPA+α-LA showing parts of two adjacent acini are surrounded by wide and thickened fibromuscular stroma. The epithelium is thickened with multiple rows of flat nuclei in one acinus.

D: BPA+ N showing two adjacent acini. The acini are surrounded by thin fibromuscular stroma. The epithelium ranged from low columnar to cubical cells with basal nucleus.

E: BPA+ α-LA+N showing parts of two adjacent acini filled with eosinophilic secretions surrounded by thin and narrow fibromuscular stroma, having nearly low columnar to cubical epithelial lining with nuclei situated basally on the basement membrane (H&E x1000).

acini (A), epithelium (E), lumen (L), stroma (S), blood vessels (BV), vacuolations (V), plaques (P), inflammatory cells infiltration (asterisk), infoldings (arrowhead), discontinuation of epithelium (D), nuclei (N)

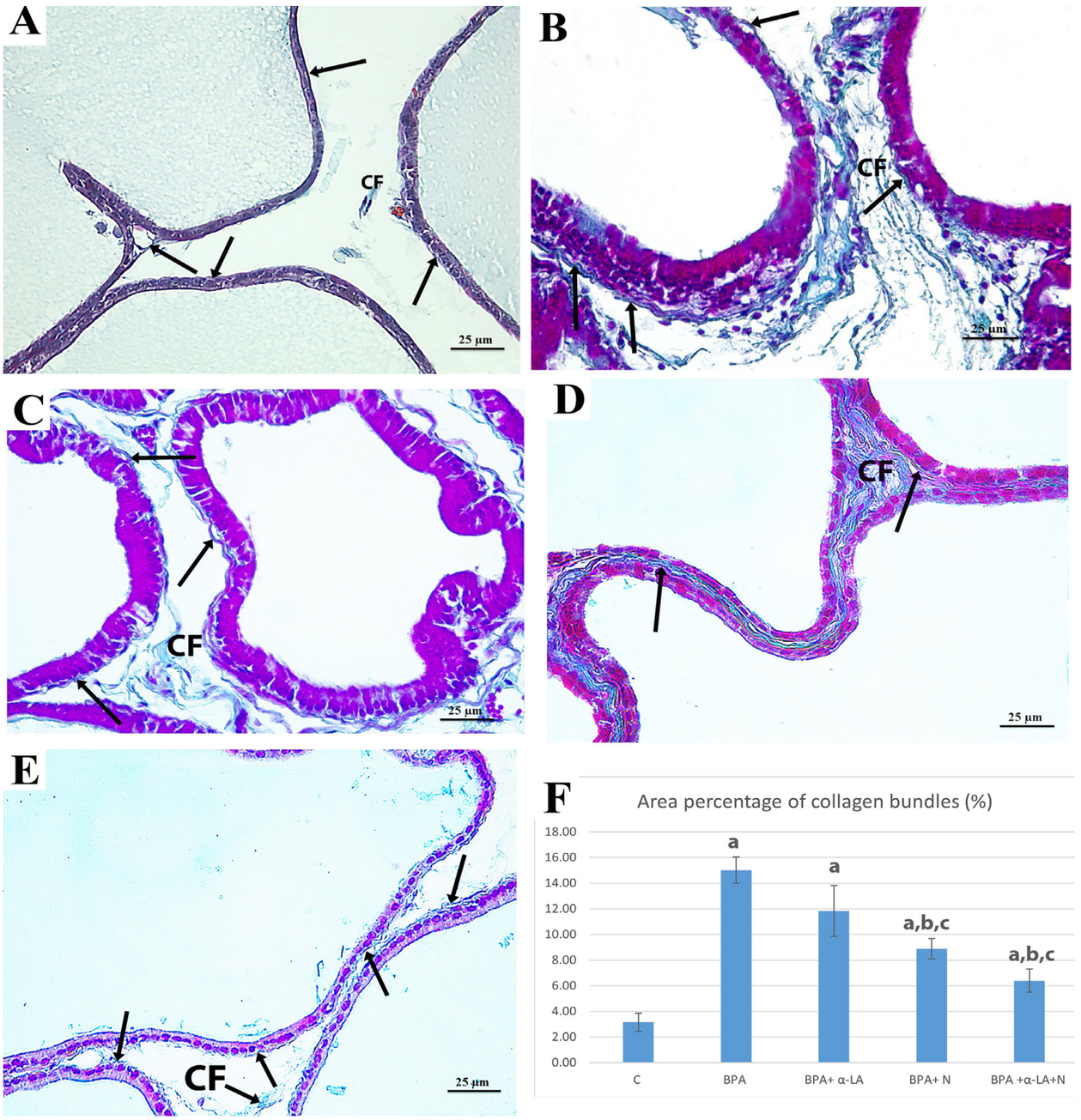

Fig. 7: A photomicrograph of a section of the ventral lobe of rat prostate A: Control group showing a normal thin and scanty amount of bluish to greenish collagen fibers in the stroma between the prostatic acini and fine thin thread like collagen fibers related to the basement membrane.

B: the BPA group showing abundant excessive amount of collagen fibers bluish to greenish

C: BPA+α-LA showing moderate amount of bluish to greenish collagen fibers.

D: the BPA+N group showing moderate amount of bluish to greenish collagen fibers.

E: the BPA+α-LA+N group showing scanty to minimal amount of bluish to greenish collagen fibers. (Masson Trichrome x400).

F: The Mean ± SD of area percentage of collagen fibers among the different studied groups.

collagen fibers (CF), basement membrane (arrows)

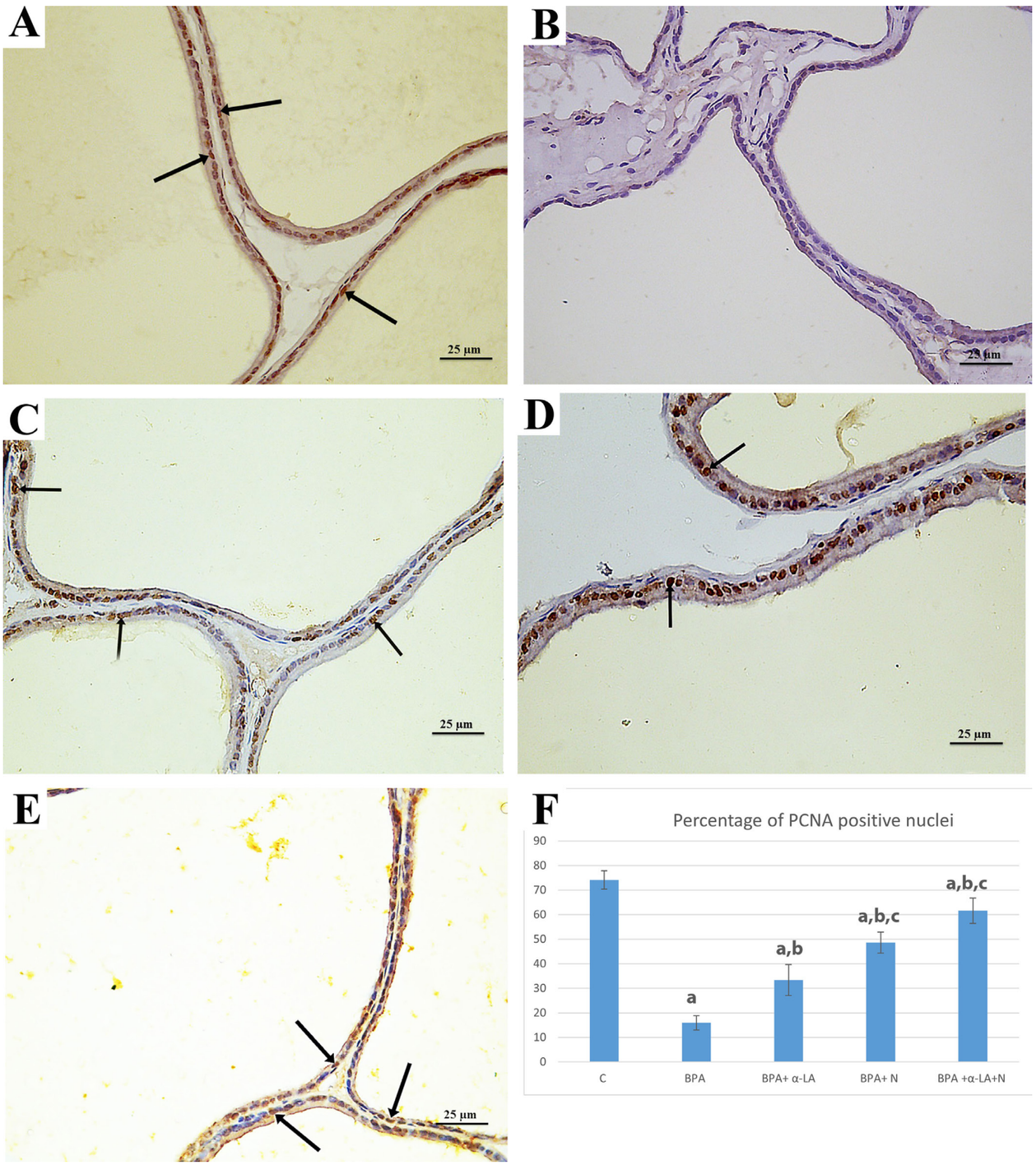

Fig. 8: A photomicrograph of a section of the ventral lobe of rat prostate A: Control group showing three adjacent prostatic acini with numerous brown PCNA-positive nuclei of acinar cells lying basally on the basement membrane (arrows).

B: the BPA group showing a marked decrease to absent expression of the brown PCNA-positive nuclei of acinar cells.

C: the BPA+α-LA group showing mild to moderate expression of brown PCNA-positive nuclei.

D: the BPA+N group showing moderate expression of the brown PCNA-positive nuclei.

E: the BPA+α-LA+N group showing increased expression of the brown PCNA-positive nuclei (PCNA immunostaining x400).

F: The Mean ± SD of the percentage of immunopositively PCNA nuclei among the different studied groups

PCNA-positive nuclei (arrows).

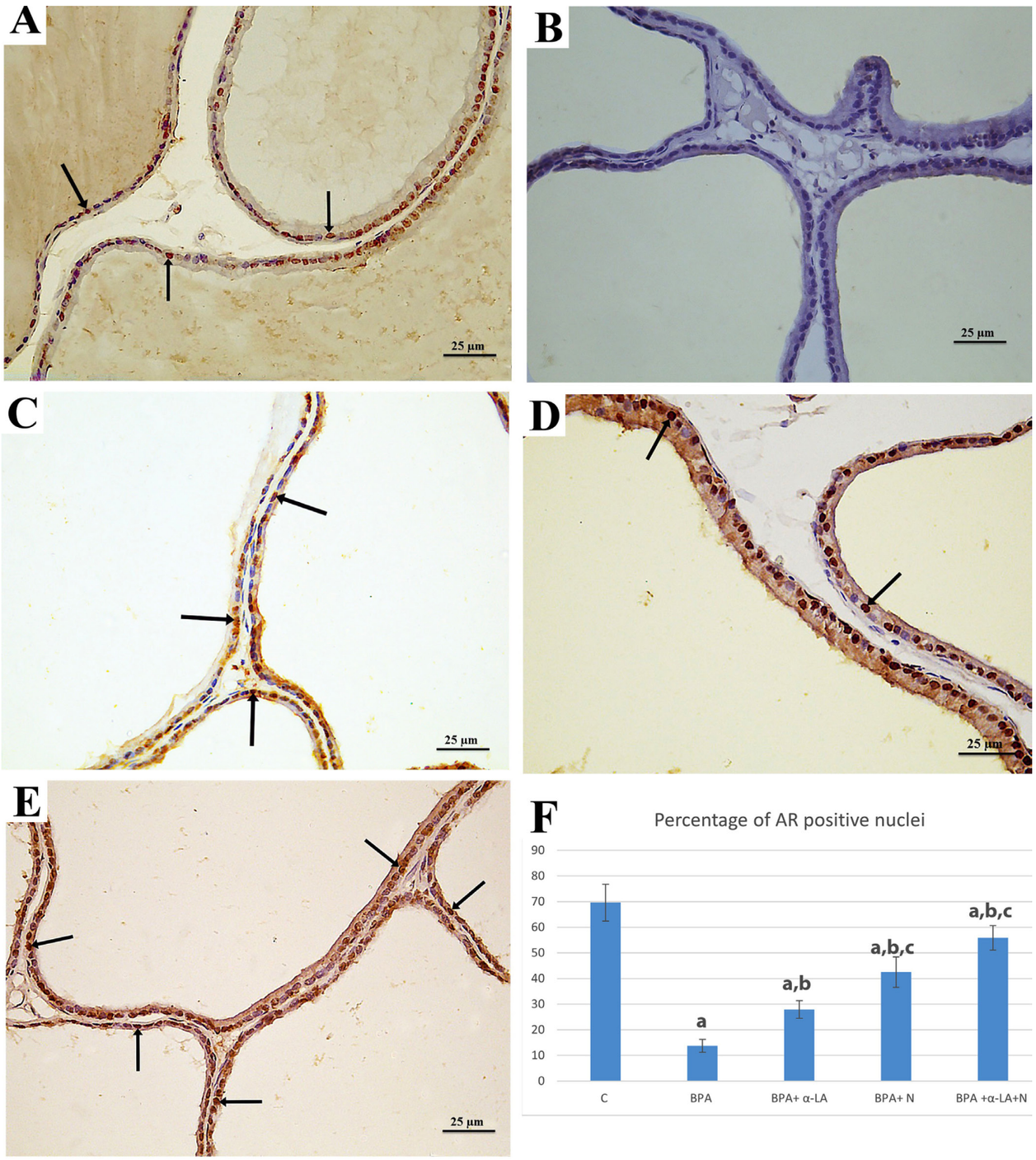

Fig. 9: A photomicrograph of a section of the ventral lobe of rat prostate A: Control group showing three acini with numerous brown AR-positive nuclei lying basally on the basement membrane.

B: the BPA group showing a marked decrease to absent expression of the brown AR-positive nuclei.

C: the BPA+α-LA group showing mild expression of brown AR-positive nuclei.

D: the BPA+N group showing moderate expression of the brown AR-positive nuclei.

E: the BPA+α-LA+N group showing increased expression of the brown AR-positive nuclei (AR immunostaining x400).

F: The Mean ± SD of the percentage of immunopositively AR nuclei among the different studied groups

AR-positive nuclei (arrows)

Table 1: The Mean ± SD of the morphometric parameters of the ventral lobe of prostate glands in the different study groups.

	Epithelium height (µm)	Acinar luminal area (µm^2) x10^3	Stromal area (µm^2) x10^3
C	8.01 ± 1.04	1130.06 ± 22.97	166.26 ± 22.97
BPA	20.61 ± 1.09 a**	756.59 ± 53.87 a**	539.73± 53.86 a**
BPA+α-LA	16.81 ± 0.99 a**,b**	864.24 ± 29.93 a**, b**	432.08 ± 29.92 a**, b**
BPA+N	13.53 ± 1.03 a**,b**,c**	962.26 ± 59.25 a**, b**, c**	334.06 ± 59.25 a**, b**, c**
BPA+α-LA+N	10.02 ± 1.59 b**, c**, d**	1060.09 ± 49.98 b**, c**, d**	236.23 ± 49.98 b**, c**, d**

One-way ANOVA, Post hoc test (Tukey):
* P<0.05
** P<0.01

DISCUSSION

BPA as a common endocrine disrupting agent disturbs the endocrine and reproductive system functions by stimulating or inhibiting the action of endogenous hormones so modifying the synthesis of hormones. BPA administration to male rats affects androgen receptors, T levels, testis and prostate and sexual behavior[39].

The goal of the current investigation was to ascertain the impact of α-LA versus naringin on alleviation of the disruptive BPA's effects on mature albino rat's prostates.

The current study showed significant weight loss of BPA group when compared with another groups and weight increased significantly after administration of α-LA and N. This outcome is compatible with Miao, *et al*.[40] who claimed that BPA significantly decrease rats weight related to a loss in appetite.

The present analysis revealed a considerable decline in serum level of both T and E2 in BPA group which increased significantly after administration of α-LA and N. This outcome was in line with other research that found that administering BPA altered spermatogenesis, inhibited Leydig cell proliferation and development, and decreased LH production from the pituitary gland, which drastically decreased T and E2 levels[41,42]. BPA dramatically increased lipid peroxidation, which resulted in DNA fragmentation, and decreased the of enzymatic and non-enzymatic antioxidants activity like CAT, SOD, in addition lowered GSH in rat testis, because the membrane of sperm contained abundant amount of unsaturated fatty acids and deficiencies the ability to repair the DNA, it might cause oxidative stress in spermatozoa, which lowers the T level[43,44]. Prathima, *et al*.[45] They showed that the administration of -LA reduces the antiandrogenic effect of linuron by significantly normalizing the T level, restoring the activity of testicular STAR mRNA expression, and lowering cholesterol levels. These may be related to α-LA steroidogenic effects and its capacity to reduce oxidative stress by reducing lipid peroxidation. Alboghobeish, *et al*.[16] They showed that injection of N improves spermatogenesis and restores the normal level of LH and FSH hormones, significantly normalizing T and E2 levels, and attenuating the antiandrogenic effect of BPA. Naringin also restores lactate dehydrogenase enzyme which is responsible for development of seminiferous tubules germinal epithelial layer and protects against oxidative damage markers by reduction of lipid peroxidation and ROS production, which normalizes E2 level[46,47].

The current study demonstrated a considerable rise in prostatic indices with ventral lobe weight of BPA group in comparison to every other group. This outcome is compatible with other authors, they reported that Prolactin and dihydrotestosterone levels were raised by BPA, which promote prostate epithelial growth and permanently increase prostatic size[48,49]. Hyperplasia, an increase in prostatic weight, and lymphocyte infiltration were all brought on by prolonged BPA exposure[50]. The rats treated with BPA had a higher prostatic index corroborated by significant increases in the PSA that indicates prostate problems, specifically BPH which is crucial in the identification of prostatic disorders. Chronic inflammation induced proliferation in the stroma of rat prostate characterized by increase in the prostatic weight[42,51].

In the present study, the prostatic parameters reduced BPA+α-LA and BPA+N groups in comparison with BPA group. This is compatible with Giammusso, *et al*.[52] who claimed that the treatment of -LA reduced prostatitis and prostate edema via blocking the vascular cell adhesion molecule and endothelial adhesion of human monocytes. Also, α-LA enhanced testicular inflammation through a reduction in (NF-κB) expression, decrease in (IL)-6, cyclooxygenase-2 expression, and enhanced appearance of anti-inflammatory cytokine (IL-10)[53]. Jeon, *et al*.[54] reported that N could restore the BPH by reducing the prostatic weight and prostatic index. This is explained by anti-inflammatory and antioxidant effect of N. Flavonoids reduce the hyperplastic effect on prostatic tissue by reducing oxidative stress and inflammation[55].

The present research revealed that BPA group showed histopathological alterations in the form of irregularity of acini. The acini showed many infoldings and the lining was thickened (hyperplasia) with disorganization and separation of epithelium from the basement membrane. Few vacuolations of the epithelium were observed. Some epithelial cells had pyknotic nuclei. The fibromuscular stroma became wide with infiltration with inflammatory cells accompanied with congested blood vessels. Moreover, there was accumulation of excessive collagen bundles between the prostatic acini. These histological findings agree many authors[41,42]. These negative consequences of

BPA on the ventral lobe structure were explained in previous studies by the oxidative stress induced by endocrine disrupting agent. BPA impairs the natural oxidative balance by increasing oxidative mediators and decreasing antioxidant enzymes (SOD, CAT, GRx, and GPx) and increasing hydrogen peroxide[56,57]. BPA increases the cytokines expression that cause inflammation, like IL-1, IL-6, IL-8, and TNF-α. BPA stimulated inflammatory activity pathway of NF-KB by producing hydrogen peroxide and ROSs. Moreover, this NF-κB pathway was responsible for fibrosis as bundles of collagen accumulation between acini induced by BPA[6].

The current investigation demonstrated that stroma of BPA expanded with abundant excessive collagen fibers in between the prostatic acini compared to other groups. These results are accordant with Tolba and Mandour[41]. They explained this excessive collagen fibers by the fibroblast hyperplasia, oxidative stress inducing fibrosis and excessive production of collagen in the prostate gland. This mechanism occurred through the nuclear transcription factor κB. BPA elevates pro-inflammatory cytokines (IL-1β) and lowering the cytokine that fights inflammation and fibrosis (IL-10) Additionally, it increases oxidative stress by inhibiting the action of the antioxidant enzyme CAT and decreases GSH levels, which causes fibrosis[58].

The present study showed mild improvement in prostate histopathological alteration and collagen fibers deposition in BPA+α-LA and BPA+N groups in comparison with BPA group. This agrees with Avci, *et al*.[20] who explained the ameliorative effect of α-LA on BPA induced oxidative damage through the decrease of Malondialdehyde MDA levels. Deore, *et al*.[59] demonstrated that α-LA protect the testes from the harmful effects of nanoparticles of zinc oxide by ameliorating DNA damage, inflammation, and metabolic imbalance. Also, the α-LA may significantly decrease fibrosis by inhibiting pro-fibrotic genes' expression through inhibition of p65 acetylation and inhibiting TGF-β1 expression in fibroblasts[53,60]. Naringin may defend the rat brain from oxidative stress brought on by BPA by lowering lipid peroxidation and nitrite levels. Moreover, N could restore the decline in GSH, SOD, CAT and GPx, inhibit nitric oxide synthase enzyme and eliminating nitric oxide free radicals[61]. Naringenin acts as anti-inflammatory and antioxidant by inhibiting leukocyte recruitment, increasing the GSH capability for antioxidants. Macrophages are impacted by naringenin to activate Nrf2, which triggers anti-inflammatory and antioxidant reactions. Naringenin inhibit the production of IL-33, TNFα, IL-1β, IL-6 and expression of cyclooxygenase-2 mRNA[62]. N decreased fibrosis by downregulating expression of mRNA genes in the liver[63].

In the present study, PCNA-positive nuclei in the rat prostatic tissues of the BPA group were very few, α-LA administration could to some extent increase PCNA expression, N administration could restore expression of PCNA positive nuclei. This supports the conclusions of Sahu, *et al*.[64] who claimed that PCNA expression was diminished of BPA administered rats. Cell proliferation and repair of DNA damage were both impaired through BPA. They explained that the production of steroid hormones is hampered by BPA, and it also causes embryonic lethality and germ line apoptosis. BPA caused apoptosis in Leydig, germ cells and disrupted spermatogenesis in mouse testis by upregulating active caspase-3 protein expression. The α-LA increased the expression of PCNA and decreased apoptosis of cells, α-LA increased expression of the protective protein PCNA, along with offering neuroprotection by regulating p53 protein, responsible to produce PCNA by means of the promoter's attachment. When cells experience oxidative stress and apoptosis, which is mediated by DNA damage, the p53 gene is activated[65]. Naringin reduce apoptosis via lowering the expression of p53 and caspase-3, which attenuates apoptosis and the disruption of renal tubular cells caused by cisplatin[66].

The current study showed few complete absences of AR-positive nuclei in the BPA group, α-LA showed mild improvement in the decrease of AR positive nuclei and N. improved the decreased expression of AR-positive nuclei. This is to the extent of Teng, *et al*.[67] who affirmed that the BPA significantly inhibited the transcriptional activity of the AR that was stimulated by DHT through exerting inhibitory reaction the AR activation cascade at any stage. A connection exists between reduced AR expression and oxidative stress, explained by a decrease in poly ADP-ribose polymerase (PARP), a protein family involved in a variety of cellular processes including DNA repair, genomic stability, and programmed cell death[68]. Ibrahim, *et al*.[69] who reported that α-LA could preserve the testis architecture while restoring AR expression to near-normal levels. They explained that α-LA has two functions: intracellular and extracellular. Thus, the regular expression of several immunological markers provides double protection and improves the pathology of testicular tissue. The ability of α-LA to protect the cells from DNA harm and oxidative stress brought by exercise in male patients[70]. The protective effects of N against testicular and reproductive disturbances on male reproductive architecture and function[71]. In rats treated with cisplatin and doxorubicin, naringenin avoided reductions in serum T and inhibin B levels[72]. This effect is explained by the antioxidant ability of N on the DNA repair and reduction of the oxidative stress[73].

The current study showed that the combined treated group (BPA+α-LA+N) showed a highly significant restoration in all parameters. This is consistent with Saleh, *et al*.[74] whoever said that Naringenin conjugate co-drug with α-LA mediate the neuroprotection in a rat model of oxidative stress. That research demonstrated that the combination of these two drugs is more potent than a single antioxidant on brain tissue. This explained that the co-drug enhances the endogenous cellular antioxidant systems and considered direct scavenging agent, powerful antioxidant, anti-inflammatory, and anti-apoptotic agent than each alone.

The histological findings of the current study were confirmed by the morphometric ones as the BPA group showed a very big rise in epithelium height, decrease of acinar luminal area, and increase of stromal luminal area. These changes agree with Olukole, *et al*.[42], and Tolba and Mandour[41]. The increased height of the epithelium was explained by increased hyperplasia correlates with elevated PSA; chronic inflammation increased prostate epithelial cell proliferation. BPA significantly increased the prostate's smooth muscle layer and accumulation of collagen fibers between acini contribution to increase of the prostatic stromal area[41,42,75].

CONCLUSION

Bisphenol-A decreased the levels of T and E2, decreased weight gain of rats, increased all measured parameters of the prostate of the mature young rats, and disrupted histological architecture of the prostatic ventral lobe. Alpha-lipoic acid showed mild improvement in the measured parameters. Naringin showed moderate amelioration in all estimated parameters. The combination of both α-LA and N exhibit beneficial synergistic effects, and so it showed great improvement in the all the measured parameters.

ABBREVIATIONS

AR: Androgen Receptor, **AR/BPA complex:** Androgen Bisphenol-A complex, **BPA:** Bisphenol-A, **BPH:** Benign prostatic hyperplasia, **CAT:** Catalase, **E2:** Estradiol, **EDCs:** Endocrine-disrupting chemicals, **FSH:** Follicular stimulating hormones, **GPx:** Glutathione peroxidase, **GRx:** Glutathione Reductase, **GSH:** Glutathione, **IL-β:** Interleukin-beta, **IL-1:** Interleukin-one, **IL-1 β:** Interleukin-one beta, **LH:** Luteinizing hormone, **MDA:** Malondialdehyde, **mRNA:** Messenger ribonucleic acid, **Na+, K+ ATPase:** Sodium, potassium adenosine triphosphate, **NF-KB:** Nuclear Factor-Kappa B, **P53:** Tumor protein 53, **PARP:** Poly ADP ribose polymerase, **PCNA:** Proliferating cell nuclear antigen, **PI:** Prostate index, **PSA:** Prostatic specific antigen, **PV:** Prostate volume, **PW:** Prostate weight, **ROSs:** Reactive oxygen species, **SD:** Standard deviation, **SOD:** Superoxide dismutase, **T:** Testosterone, **TGF-β1:** Transforming growth factor beta, **TNF-α:** Tumor necrosis factor alpha, **VLW:** Ventral lobe weight, **VP:** Ventral prostate, **α-LA:** Alpha-lipoic acid

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دور حمض الألفا ليبويك و النارينجين في تعديل اضطرابات البروستاتا الناجمة عن بيسفينول-أ فى الجرذان البالغة (دراسة نسيجية ، نسيجية مناعية)

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المقدمة: تعتبر مشكله العقم من المشاكل التى تواجه الأزواج فى جميع انحاء العالم وارجع الانخفاض التدريجى فى الصحه الانجابيه للذكور الى المواد الكميائيه المسببه لاختلال عمل الغدد الصماء. ومن هذه المواد الكميائيه ماده البسفينول-أ حيث تعمل هذه الماده على اعاقه عمل الهرمونات الطبيعيه داخل الجسم بتثبيطها او تحفيزها او تعديلها. حيث تعتبرماده بيسفينول-أ أن لها خصائص مضاده لعمل هرمون الاندروجين الداخلى للجسم كما يمكنها أيضا ان تحدث خلل فى عمل هرمون الاستروجين الداخلى.

اثبتت الدراسات ان تعاطى جرعه عاليه من البيسفينول-أ للجرذان البالغه قد يؤدى الى حدوث ضرر بالحيوانات المنويه لديها كما يعطل عمل هرمونات تحت المهاد والغده النخاميه. ويمكنه كذلك ان يسبب قصور فى عمل الغدد التناسليه و كذلك قد يسبب تحولا حرشفيا للنسيج الظاهرى لغده البروستاتا.

تسبب ماده البيسفينول-أ تلف خلوي لهياكل البروتين والدهون عن طريق إطلاق و تراكم جزيئات الأكسجين النشط فى الأنسجة. حمض الألفا ليبويك مركب بيولوجى يقلل من الضرر التأكسدى ويعزز مستويات مضادات الأكسده الأخرى. وقد اثبت أنه يمكنه أن يخفف من الإجهاد التأكسدي للخصية, و يقلل من بيروكسيدات الدهون الناجمة عن ماده بسفينول-أ. ماده النارينجين من الفلافونات الموجوده فى الحمضيات والطماطم والكريز والجريب فروت والكاكاو. وقد ثبت ان النارينجين يمكنه ان يستعيد التركيب الطبيعى الخصية والبربخ في الفئران. كما أنها قللت من السمية الإنجابية الناجمة عن بيسفينول-أ عن طريق ضبط مستوى بيروكسيد الدهون.

.لذلك هدفت هذه الدراسه الى تقييم دور كل من حمض الالفا ليبويك وماده النارينجين فى تحسين اضطرابات البروستاتا الناجمة عن التعرض لمادة بيسفينول-أ فى الجرذان البيضاء البالغة.

طريقه البحث: تم استخدام عدد ٧٢ من الجرذان البيضاء البالغه في التجربة وتم تقسيمهم بالتساوي وبشكل عشوائى إلى تسع مجموعات رئيسية وفرعية.

المجموعة الظابطة: وتتكون هذه المجموعه من ثلاث مجموعات فرعيه. كل مجموعه بها ثمانيه جرذان لكل مجموعة فرعية.

وكانت باقى المجموعات كالتالى:

مجموعة البيسفينول-أ و مجموعة البسفينول-أ+حمض الألفا ليبويك و مجموعة البيسفينول-أ+النارينجين و مجموعه البيسفينول-أ+حمض الالفا ليبويك+النارينجين و مجموعة حمض الألفا ليبويك و مجموعة النارينجين.

في نهاية التجربة، تم التضحية بالجرذان والتعامل معها لتقييمها :

حيث تم قياس هرمون التستوستيرون وهرمون الإستراديول فى الدم و كذلك التغييرفى الوزن و قياس حجم البروستاتا ووزنها ومؤشرها و ايضا قياس وزن الفصوص الباطنية وعمل تحليل الانسجة والقياسات المرفوميترية لها.

النتائج: أظهرت النتائج ان ماده البيسفينول-أ خفضت بشكل كبير هرمون التستوستيرون و الاستراديول فى الدم . الى جانب ذلك ، فإنها خفضت وزن الجرذان. كما انها عملت على زياده حجم البروستاتا ، ووزنها. وزادت من وزن الفص الباطني للجرذان. وكذلك تسببت في تغيرات نسيجية للفصوص الباطنيه لغدد البروستاتا وتم تاكيد ذلك بمجموعه من التغيرات فى القياسات المرفوميتريه بالمقارنة مع المجموعة الظابطه.

وباستخدام حمض الألفا ليبويك أظهر تحسنا طفيفا في النتائج التي اختلت بسبب ماده بيسفينول-أ.

الخلاصة: اثبت استخدام ماده النارينجين فى هذه الدراسه أن لها تأثيرأفضل بالمقارنة مع حمض الألفا ليبويك فقد أظهرت تحسنا متوسطا فى النتائج التى اختلت بسبب مادة بيسفينول-أ.

أوضحت النتائج أيضا ان الجمع بين حمض الألفا ليبويك والنارينجين فى مجموعه واحده كان له تأثيرات تآزريه كبيرة بالمقارنة بين مجموعة بسفينول-أ وحمض الالفا ليبويك و بين مجموعة البيسفينول-أ والنارينجين. فهناك استعادة لجميع القياسات المختلفه لتشبه المجموعة الظابطه إلى حد كبير.