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Possible protective and curative effects of selenium nanoparticles on testosterone-induced benign prostatic hyperplasia rat model

W.A. Elfakharany[1], M.M. Safwat[1], A.S. Essawy[2, 3]

[1]*Human Anatomy and Embryology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt*
[2]*Human Anatomy and Embryology Department, Faculty of Medicine, Menoufia University, Menoufia, Egypt*
[3]*Anatomy Department, Ibn Sina National College for Medical Studies, Jeddah, Saudi Arabia*

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Background: *Men over the age of 40 are more likely to develop benign prostatic hyperplasia (BPH). BPH is characterised by proliferation of the prostatic epithelium and stroma. Selenium in the form of nanoparticles is an essential metalloid mineral and antioxidant. In this study, selenium nanoparticles (SeNPs) were tested for their potential protective and curative impacts on BPH in rats.*

Materials and methods: *Fifty male Sprague-Dawley rats were randomly divided into five groups: Group I (Control group); Group II (Orchiectomised group): bilateral orchiectomy was conducted on rats; Group III (BPH group): testosterone (TE) enanthate injection was used to induce BPH; Group IV (Protective group): rats were given SeNPs before subjecting rats to BPH; Group V (Curative group): rats were succumbed to BPH, followed by administration of SeNPs. Measurement of prostate specific antigen (PSA) and TE in serum was performed and prostates were weighed and prepared for histological, immunohistochemical and ultrastructural examination.*

Results: *In the BPH group, serum TE and PSA levels, as well as prostate weight, increased significantly and significant decreases were observed in the protective and curative groups. Reduced acinar lumen, expansion of stroma and epithelial hyperplasia were noticed in the BPH group, which were ameliorated significantly in both the protective and curative groups. There was an increase in proliferating cell nuclear antigen immunoreaction in the BPH group and a decrease in both the protective and curative groups. On transmission electron microscopy of BPH group, the nuclei appeared irregular with dilated endoplasmic reticulum, loss of cell boundaries and apical microvilli. The protective group showed more improvement than the curative group.*

Conclusions: *The effects of SeNPs on BPH induced by TE in rats, were both protective and curative, although the protective effects were more pronounced.*
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Key words: selenium nanoparticles, testosterone, benign prostatic hyperplasia, rat

Address for correspondence: Lecturer A.S. Essawy, Faculty of Medicine, Menoufia University, Menoufia, Egypt and Ibn Sina National College for Medical Studies, Almahjar Street, Jeddah, Saudi Arabia, tel: 00966546637821, e-mail: doc_asmaa@hotmail.com

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is characterised by proliferation of the prostatic epithelium and the stroma, distinguished by an unregulated growth of the prostate [59]. The condition is common among men over 40 and 68% of males over the age of 50 have symptoms associated with the disease [18]. In more than 50% of males over the age of 60, classical BPH symptoms will manifest when stromal and epithelial cells proliferate in the transitional zone, which compresses the urethra [15, 16].

Benign prostatic hyperplasia is distinguished by an increase in volume of prostate caused by hyperplasia of stromal and epithelial cells impairing urine flow. Ultimately, this leads to polyuria, urinary retention, nocturia, urinary hesitancy, and increased risks for urinary tract infections [20]. While the pathogenesis of BPH remains an enigma, several lines of evidence have suggested that hormonal and vascular changes trigger prostatic cell proliferation and accelerate BPH development [13].

With age, the normal decline of androgen accompanied by a constant rise in oestradiol results in an increase of the ratio of oestradiol to testosterone (TE) [33]. This androgen-oestrogen disparity, as well as the shift toward oestrogen dominance, may be linked to growth of prostate. Furthermore, it is assumed that elevated androgen levels are essential for the development of BPH [49].

The 5-alpha-reductase enzyme in the prostate converts TE to its active metabolite, dihydrotestosterone (DHT), which is a key mediator for growth of the prostate [45]. The pathophysiology of BPH has been linked to differentiation, apoptosis, and epithelial/ /stromal interaction. Transforming growth factor (TNF)-beta1 is an imperative pro-inflammatory cytokine that regulates cellular proliferation and apoptosis [19]. An increased expression of the angiogenic factor vascular endothelial growth factor was also observed in patients with BPH [50].

The field of nanotechnology presents a host of potential applications in biology and biomedicine. Because of nanomaterials' unique properties, such as they are smaller, biocompatible, and able to penetrate cellular membranes to carry drugs, they are used in many biomedical applications [58].

Among the most important micronutrients for our health are selenium nanoparticles (SeNPs), an essential metalloid mineral and antioxidant. Selenium also has cancer chemo-preventive properties [4]. There is a strong association between selenium deficiency and many physiological disorders, such as higher cancer risks. Due to their low therapeutic index, different selenium supplements fail to restore selenium levels. In this regard, it would be advantageous to have a form of selenium that is less toxic with potential anti-cancer properties [53].

Considering the high health concerns associated with benign prostatic hyperplasia, the aim of this work was to illustrate, for the first time to our knowledge, the possible ameliorative effects of selenium nanoparticles on benign prostatic hyperplasia induced by TE and to elucidate the underlying mechanisms through which selenium nanoparticles act based on biochemical, histological, immunohistochemical and ultrastructural studies.

MATERIALS AND METHODS

Chemicals

Induction of benign prostatic hyperplasia. To induce BPH in rats, TE enanthate intramuscular injections (Cidoteston 250 mg ampoule, CID Company, Giza, Egypt) were used once weekly, for 5 consecutive weeks, at a dose of 25 mg/rat [29]. To dilute the dose of TE, 100 μL of olive oil were used as the vehicle [12].

Selenium nanoparticles (Nano Se, SeNPs). SeNPs were purchased from the Nano Tech Egypt for Photo-Electronics Communication Centre, City of 6 October, Al-Giza, Egypt, in the form of orange suspension (concentration 7.8 ppm and average size 20 ± 25 nm) [37]. A daily dose of 0.1 mg/kg of SeNPs was administered orally by gavage for 14 days [2].

Animals

Through the entire study, 50 three-month-old male Sprague–Dawley rats weighing 200–250 g were utilised. Rats were bought from Animal House of Faculty of Medicine, Ain Shams University, Cairo, Egypt. They were acclimatised for two weeks in stainless steel cages prior to starting the experiment. They were kept in a climate-controlled room where they had 12 hours of light/dark cycles. The rats received access to tap water and rodent pellets as needed.

Experimental design

All experimental procedures and animal handling were carried out according to accepted animal care standards and approved by Ain Shams University's ethical committee. Following acclimatisation, we divided the rats randomly into five groups (10 rats/group):

— **Group I (Control group)**: subdivided into group IA (negative control subgroup), where 100 μL of olive oil were administered each week for successive 5 weeks as a vehicle [29] and group IB (sham control subgroup), where incision, manipulation and stitching of the scrotum were done without resecting the testes [63];
— **Group II (Orchiectomised group)**: bilateral orchiectomy was performed on rats [35]. In order to prevent the influence of intrinsic TE, the operation took place seven days before BPH induction [29];
— **Group III (BPH group)**: in which TE enanthate was injected intramuscularly 3 days after orchiectomy once a week for 5 successive weeks to induce BPH [29];
— **Group IV (Protective group)**: in which rats were given SeNPs (0.1 mg/kg) daily by oral gavage for 14 days [2] before subjecting rats to benign prostatic hyperplasia through injection of TE enanthate intramuscularly for successive 5 weeks;
— **Group V (Curative group)**: in which BPH was induced by injection of TE enanthate intramuscularly for successive 5 weeks followed by SeNP treatment (0.1 mg/kg) by oral gavage for 14 days.

Rats were sacrificed at the end of the experiment and their prostates were instantly extracted, weighed and processed for histological, immunohistochemical and ultrastructural examination.

Orchiectomy procedures

Ketamine (90 mg/kg) and Xylazine (12 mg/kg) were intraperitoneally injected to induce anaesthesia [52]. After confirming complete anaesthesia, the testis and epididymal fat were gently removed through a ventral 1.5 cm incision on the scrotum, followed by ligation and cutting of the spermatic cord and testicular vessels. Incisions have been sutured using 4-0 silk sutures after both testes were removed [35]. An intraperitoneal dose of Ampicillin (4000 IU/kg) was given for 3 days as a prophylactic measure, and local application of Coloplast paste (Humlebaek, Denmark) was also administered.

Biochemical analysis

After the 5 weeks of the experiment, the rats were fasted overnight and then overdosed with Ketamine and Xylazine before they were sacrificed. To obtain the serum, we took blood samples directly from the abdominal aorta and centrifuged them for 15 minutes at 3000 g. A testosterone ELISA kit (Biocheck, USA) and DHT ELISA kit (Abnova, USA) were used to measure TE and prostate specific antigen (PSA) in serum using an enzyme-linked immunosorbent assay (ELISA) [34, 46].

Prostate weight index

Prostate glands were carefully removed after sacrification and weighed. Calculation of prostate weight (PW) index was done using this formula: PW index = = (PW/body weight) $\times$ 100 [56].

Evaluation of markers of prostate oxidative stress

To homogenize prostate tissues, ice-cold phosphate-buffered saline (PBS; 50 mM potassium phosphate, pH 7.5) was used. The level of reduced glutathione (GSH) in tissues homogenates was measured using a commercially available kit (Biodiagnostic, Cairo, Egypt) [54]. Furthermore, a commercial kit (Biodiagnostic, Cairo, Egypt) was used to determine the catalase (CAT) and superoxide dismutase (SOD) activities. Lipid peroxidation has been measured spectrophotometrically by measuring malondialdehyde (MDA) level using thiobarbituric acid reactive substance (TBARS) method, following the description of Mihara and Uchiyama [41].

Histological study

Tissue samples from the prostate's ventral lobes were preserved for at least 24 hours in a 10% neutral buffered formalin solution prior to the fabrication of the paraffin blocks, the paraffin sections were then made at a thickness of 4 cm. A final step was to mount sections on glass slides and stained by haematoxylin and eosin (H&E) [9] and Masson's trichrome [7].

Immunohistochemistry

The paraffin sections on poly-L-lysin coated slides were deparaffinised and rehydrated. In order to inhibit endogenous peroxidase, the sections were immersed in 3% hydrogen peroxide (H_2O_2). Microwave antigen retrieval procedure was used. The sections were incubated with primary anti-proliferating cell nuclear antigen (PCNA) antibody, cellular regeneration marker (Sigma-Aldrich, St. Louis, Missouri, USA). Afterwards, the biotinylated polyvalent secondary antibody was applied. Next the sections were incubated in preformed streptavidin peroxidase. In the end, the DAB substrate chromogen (3,3'-diaminobenzidine tetrahydrochloride) was applied and the slides were counterstained with haematoxylin [8].

Morphometric study

A histomorphometric analysis was conducted for measurement of the epithelial height, the acinar luminal area, and the stromal area. To determine epithelial height, 30 lines per field were drawn through the acinar epithelium. To estimate the luminal area of an acinus, a line was drawn around its perimeter and then calculating the acinar area. In addition, the stromal area was calculated through subtraction of the acinar luminal area from the total area of the field. Luminal and stromal areas were measured using 100× magnification, whereas epithelium height was measured using 400× magnification [28].

Using a magnification of ×400, the mean area percentage of PCNA was measured. To reduce inter-observer bias, a single blinded examiner conducted all histomorphometric analyses using Image J software. Each parameter was explored in ten non-overlapping fields for five different rats/experimental group.

Transmission electron microscopy (TEM)

Small prostate specimens were immersed in 2.5% buffered glutaraldehyde in 0.1 PBS at pH 7.4 and 4°C for 2 hours, then replaced with 1% osmium tetroxide in 0.1 mol/L for 1 hour at 4°C to fix the specimens. Thereafter, specimens were dehydrated in ascending serial dilutions of ethanol (50, 70, 90, 95 and four times 100%, each for 30 minutes) followed by acetone dehydration for 30 minutes. Lastly, the fixed specimens were embedded in epoxy resin (Epoxy Embedding Medium Kit; Sigma). Using an ultramicrotome (RMC PT-XL PowerTome Ultramicrotome), semi- and ultra-thin sections were cut. Toluidine blue staining of semithin slices (1 μm thick) was performed and the sections were examined using an Olympus BX61 light microscope. Ultrathin sections (70–90 nm) were cut by ultramicrotome then stained by 2% uranyl acetate as a principal stain and lead citrate as counter stain to be examined using JEM-1400 Plus (JEOL, Japan) transmission electron microscope at the Electron Microscope Unit, Faculty of Science, Alexandria University, Alexandria, Egypt [60].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software (16.0; SPSS, Inc., Chicago, IL, USA). The data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), followed by the Tukey's post-hoc test was used to analyse the data. P values ≤ 0.05 were significant.

Table 1. Testosterone (TE) and prostate-specific antigen (PSA) serum levels changes

Group	TE [ng/mL]	PSA [ng/mL]
Control group	4.04 ± 0.5^{c}	4.65 ± 0.24^{c}
Orchiectomised group	0.05 ± 0.02^{d}	3.29 ± 0.15^{d}
BPH group	12.67 ± 0.7^{a}	10.25 ± 0.40^{a}
Protective group	4.74 ± 1.44^{c}	4.97 ± 0.25^{c}
Curative group	8.15 ± 1.06^{b}	5.65 ± 0.54^{b}

Values are mean ± standard deviation. Statistical analysis was performed by analysis of variance (ANOVA), followed by Tukey's post-hoc test; a, b, c, d: There is significant difference ($p > 0.05$) between any two groups, within the same column not having the same superscript letter; BPH — benign prostatic hyperplasia

RESULTS

Clinical observations

No mortality or behavioural changes were observed during the treatment period in any group. Also, no differences were observed in the general health status of the control and treated groups.

Biochemical analysis

The orchiectomised group showed a statistically significant decrease in TE and PSA serum levels in comparison with the control group. In comparison with the control and orchiectomised groups, the BPH group showed a statistically significant increase. The protective and curative groups showed significant decrease in TE and PSA levels, compared to the BPH group. Furthermore, the protective group revealed a significant decrease in TE and PSA levels when compared with the curative group (Table 1).

Body and prostate weight changes

The difference in body weight gain between the groups was not statistically significant. The BPH group showed statistically significant increases in PW and PW index when compared to the control group. In both protective and curative groups, SeNP administration significantly reduced the PW and PW index compared with the BPH group. Furthermore, a statistically significant decrease in the PW and the PW index was observed in the orchiectomised group when compared with all other groups (Table 2).

Oxidative stress markers

In the BPH group, TE treatment increased MDA levels significantly compared to the control group. SeNPs administration, either in a protective or curative group, significantly alleviated testosterone's effect

Table 2. Body weight, prostate weight (PW) and PW index changes

Group	Initial body weight [g]	Final body weight [g]	Body weight gain [g]	PW [mg]	PW index
Control group	207.22 ± 2.8	290.53 ± 4.71	82.7 ± 5.48^{a}	510 ± 30.5^{d}	0.175 ± 0.011^{d}
Orchiectomised group	218.22 ± 3.85	306.35 ± 7.65	88.10 ± 7.43^{a}	100 ± 19.6^{e}	0.326 ± 0.006^{e}
BPH group	230.89 ± 15.3	312.00 ± 4.12	82.0 ± 18.14^{a}	1365 ± 215.4^{a}	0.439 ± 0.067^{a}
Protective group	225.54 ± 10.55	315.47 ± 2.83	90 ± 12.02^{a}	690 ± 64.9^{c}	0.219 ± 0.021^{c}
Curative group	235.22 ± 6.91	321.5 ± 13.34	86.30 ± 7.54^{a}	840 ± 28.9^{b}	0.261 ± 0.077^{b}

Values are mean ± standard deviation. Statistical analysis was performed by analysis of variance (ANOVA), followed by Tukey's post-hoc test; a, b, c, d, e: There is significant difference ($p > 0.05$) between any two groups, within the same column not having the same superscript letter; BPH — benign prostatic hyperplasia

Table 3. Markers of oxidative stress in the prostate

Group	Malondialdehyde [nmol/mg protein]	Glutathione [mmol/mg protein]	Superoxide dismutase [U/mg protein]	Catalase [U/mg protein]
Control group	0.235 ± 0.063^{d}	0.135 ± 0.010^{b}	40.5 ± 2.45^{a}	0.56 ± 0.045^{a}
Orchiectomised group	0.252 ± 0.075^{d}	0.152 ± 0.008^{a}	41.6 ± 1.76^{a,b}	0.58 ± 0.020^{a}
BPH group	1.423 ± 0.146^{a}	0.058 ± 0.007^{d}	27.4 ± 2.43^{d}	0.40 ± 0.022^{c}
Protective group	0.352 ± 0.043^{c}	0.103 ± 0.012^{c}	38.7 ± 1.33^{b}	0.50 ± 0.035^{b}
Curative group	0.783 ± 0.112^{b}	0.08 ± 0.002^{e}	33.8 ± 1.56^{c}	0.49 ± 0.051^{b}

Values are mean ± standard deviation. Statistical analysis was performed by analysis of variance (ANOVA), followed by Tukey's post-hoc test; a, b, c, d, e: There is significant difference ($p > 0.05$) between any two groups, within the same column not having the same superscript letter; BPH — benign prostatic hyperplasia

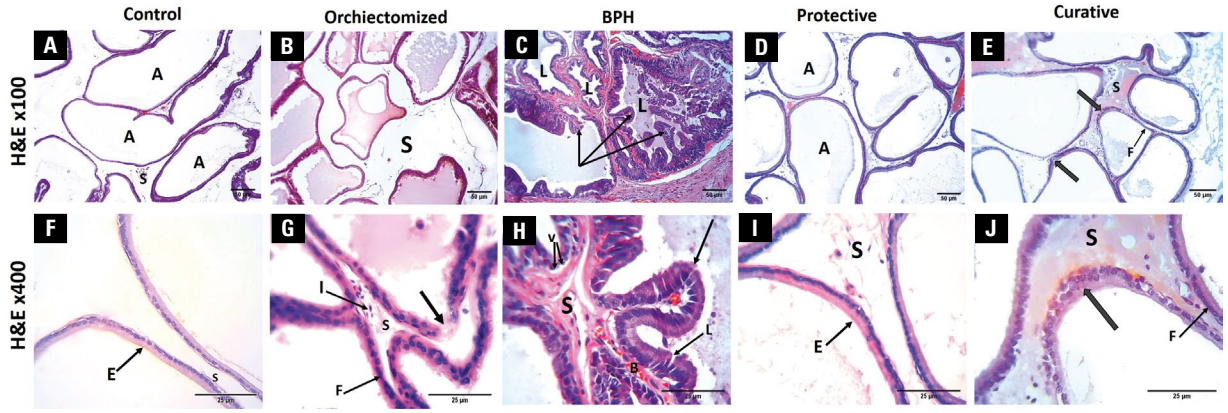

Figure 1. Photomicrographs of sections of prostate; **A, F.** Control group showing normal prostatic tissue, formed of numerous acini (A) lined with a single layer of low columnar epithelium (E) and sparse stroma (s) in between; **B, G.** Orchiectomised group showing expanded stroma (s), with inflammatory infiltrate (I) and flattened (F) or loss (arrow) of epithelial cells in some acini; **C, H.** Benign prostatic hyperplasia group showing that epithelial hyperplasia markedly narrowing the acinar lumens (L) and produce large involutions (arrows), cell loss in some epithelial areas (L), congested blood vessels (B), other epithelial cells display vacuolations (v), with plentiful stroma in between the acini (s); **D, I.** Protective group showing that the acini (A) lined with single layer of low columnar epithelium (E) with no involutions and reduced stromal thickness (s); **E, J.** Curative group showing no involutions, some acini are lined by flat epithelium (F), others have multiple layers of epithelium (thick arrows), and moderate amount of stroma (s). H&E, A, B, C, D, E ×100, scale bar = 50 μm; F, G, H, I, J ×400, scale bar = 25 μm.

on lipid peroxidation. Furthermore, the BPH group had significant decrease in GSH, SOD and CAT levels compared with the control group. Nonetheless, both protective and curative groups that received nano-selenium had significant increases in GSH, SOD and CAT levels in comparison with the BPH group (Table 3).

Histopathological results

Sections of the control group stained with H&E and Masson's trichrome revealed normal prostate tissue, formed of numerous acini that appeared lined with a single layer of low columnar epithelium, and sparse stroma (Fig. 1A, F). In between the acini, fine collagen

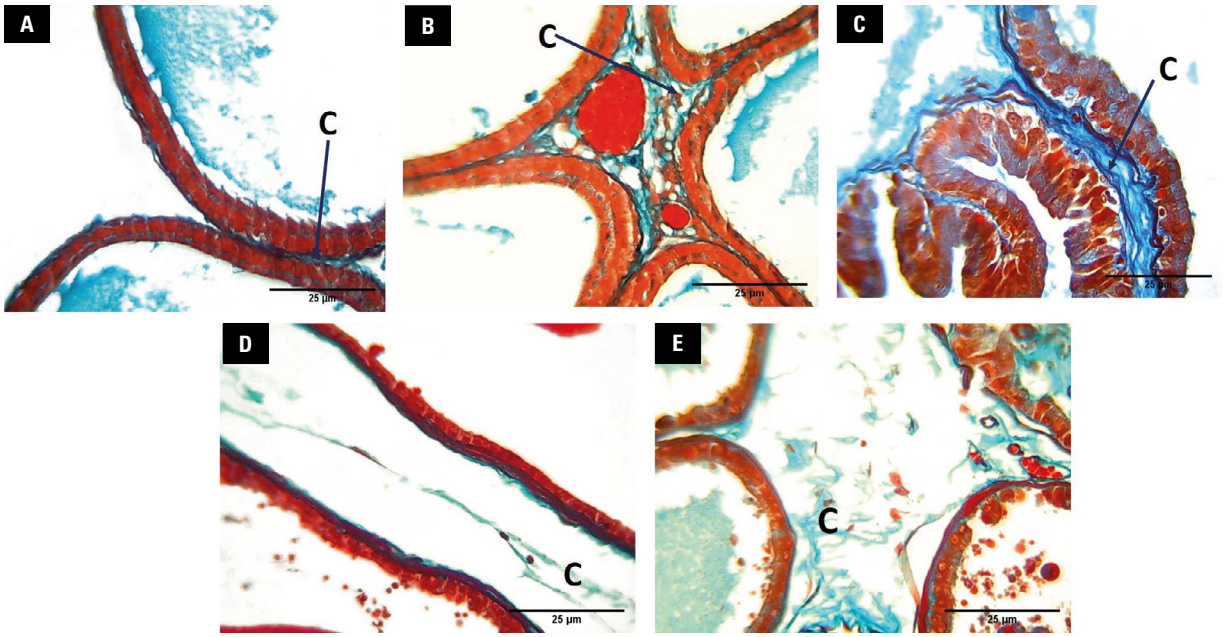

Figure 2. Photomicrographs of sections of prostate; **A.** Control group showing fine collagen fibres (C) dispersed in the stroma between the acini; **B.** Orchiectomised group showing expanded stroma, with plentiful collagen fibre deposits (C); **C.** Benign prostatic hyperplasia group showing abundance of collagen fibres (C) in the stroma; **D.** Protective group showing minimal increase in the amount of collagen in the stroma (C); **E.** Curative group showing moderate collagen deposits (C). Masson trichrome ×400, scale bar = 25 μm.

fibres were visible dispersed in the stroma (Fig. 2A). The orchiectomised group exhibited an inflammatory infiltrate and expansion of stroma. In some acini, the epithelial cells were flattened or lost (Fig. 1B, G). Plentiful collagen fibre deposits could be seen in the stroma (Fig. 2B). The BPH group showed striking histological changes. Epithelial hyperplasia markedly narrowed the acinar lumens and produced large involutions. There was cell loss in some epithelial areas and other epithelial cells displayed vacuolations. Congested blood vessels were noticed in the stroma between acini as well (Fig. 1C, H). There was an abundance of collagen fibres in the stroma (Fig. 2C). Both protective and curative groups displayed obvious improvement and restored many normal characteristics of the prostate. In the protective group, the acini appeared lined by low columnar epithelium with no involutions and reduced stromal thickness (Fig. 1D, I). There were also no involutions in the curative group, though some acini were lined by flat epithelium and others had multiple layers of epithelium accompanied by moderate amount of stroma (Fig. 1E, J). Minimal amount of collagen was found in the stroma of the protective group (Fig. 2D), while the curative group showed moderate levels of collagen fibres (Fig. 2E). Accordingly, the protective group showed more improvement in histological characteristics than the curative group.

Immunohistochemical results

As a proliferation marker, immunohistochemical staining of PCNA in control tissue demonstrated minimal immunoexpression (Fig. 3A). Moreover, PCNA expression in epithelial cells of the orchiectomised group was minimal (Fig. 3B). In contrast, the BPH group showed pronounced increase in nuclear PCNA immunoexpression in epithelial cells (Fig. 3C). Both protective and curative groups displayed minimal and moderate nuclear PCNA immunoexpression in the epithelial cells respectively (Fig. 3D, E).

Morphometric results

A statistically significant reduction in epithelial height was seen in the orchiectomised group compared to the control group. In contrast, in the BPH group, there was a significant increase when compared to the control group along with the orchiectomised group. Conversely, when compared to the BPH group, treatment of SeNPs in the protective and curative groups led to a statistically significant decrease in epithelial height. Despite this, the epithelial height was clearly lower in the protective group, in comparison with the curative one, and was nearer the control values (Table 4).

A statistically significant reduction in luminal area was observed in both orchiectomised and BPH groups

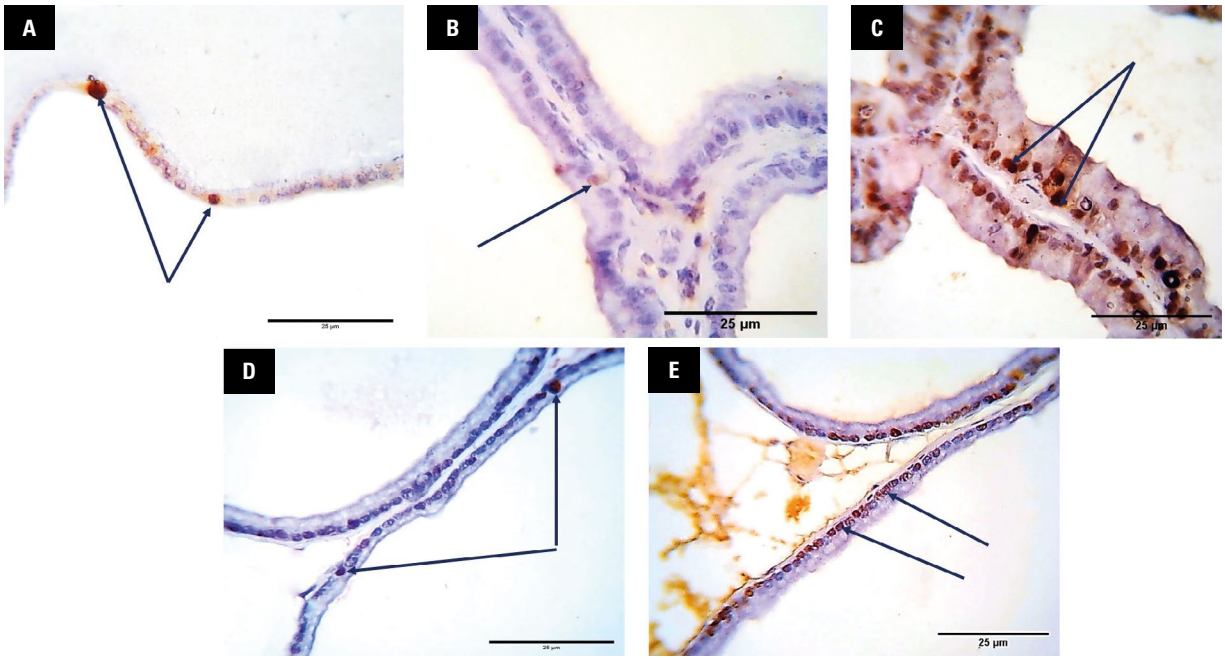

Figure 3. Photomicrographs of sections of prostate; **A.** Control group showing minimal nuclear immunoexpression (arrows); **B.** Orchiectomised group showing minimal nuclear immunoexpression in the epithelial cells (arrow); **C.** Benign prostatic hyperplasia group showing pronounced increase in nuclear immunoexpression in the epithelial cells (arrows); **D.** Protective group showing minimal nuclear immunoexpression in the epithelial cells (arrows); **E.** Curative group showing moderate nuclear immunoexpression in the epithelial cells (arrows). PCNA ×400, scale bar = 25 μm.

Table 4. Epithelial height, acinar luminal area, stromal area and proliferating cell nuclear antigen (PCNA) percentage changes

Group	Epithelial height [μm]	Acinar luminal area [μm^2] × 10^3	Stromal area [μm^2] × 10^3	PCNA [%]
Control group	15.55 ± 1.34^c	441.89 ± 1.90^a	267.40 ± 1.65^c	1.25 ± 0.11^d
Orchiectomised group	6.23 ± 0.99^d	120.00 ± 1.56^c	589.74 ± 2.09^a	2.28 ± 0.31^d
BPH group	23.74 ± 2.96^a	330.38 ± 1.63^b	380.49 ± 3.90^b	32.51 ± 1.70^a
Protective group	15.60 ± 1.94^c	442.05 ± 1.00^a	268.92 ± 3.39^c	5.01 ± 1.14^c
Curative group	19.51 ± 1.25^b	331.11 ± 1.68^b	379.28 ± 2.29^b	16.17 ± 0.92^b

Values are mean ± standard deviation. Statistical analysis was performed by analysis of variance (ANOVA), followed by Tukey's post-hoc test. a, b, c, d: There is significant difference ($p > 0.05$) between any two groups, within the same column not having the same superscript letter; BPH — benign prostatic hyperplasia

compared to the control group. Protective and curative groups showed significant increases in luminal area. This increase was not statistically significant in the curative group, but it was statistically significant in the protective group when compared to the BPH one (Table 4).

The stromal area of the orchiectomised as well as the BPH groups was significantly increased when compared with the control group. The stromal area was statistically decreased when SeNPs were added to both the protective and curative groups. However, when compared to BPH, this decrease in stromal area was only significant in the protective group and non-significant in the curative group (Table 4).

In comparison with the control group, a significant increase in PCNA expression in the BPH group was found. On contrast, PCNA percentages decreased significantly in protective and curative groups, compared to the BPH group. However, in the protective group, PCNA percentage decreased significantly less than that in the curative group (Table 4).

Transmission electron microscope results

In the control group, ultrathin sections of the ventral lobe of the prostate revealed normal epithelial cells with oval euchromatic nuclei and prominent nucleoli. A parallel flat endoplasmic reticulum (ER) and dense intracellular secretory vesicles can be seen in

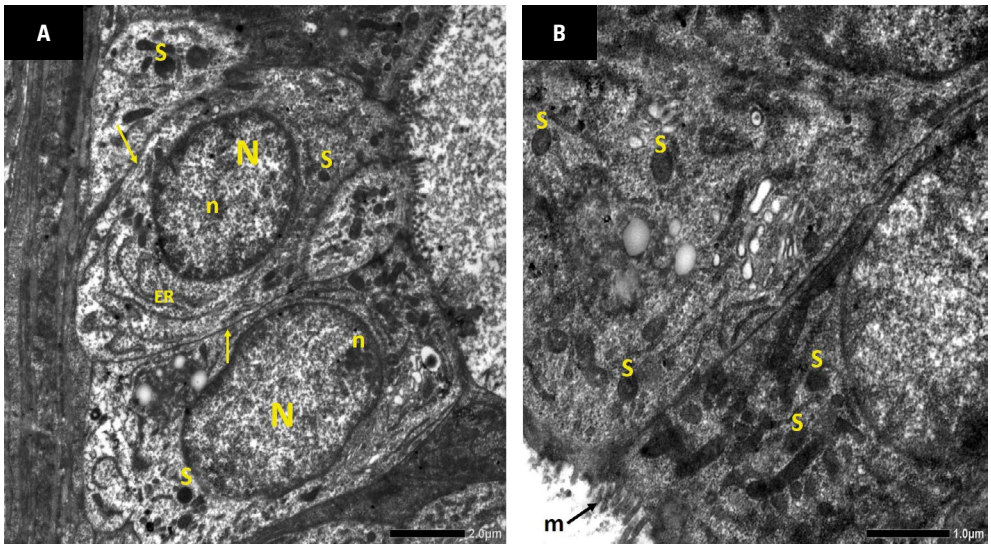

Figure 4. Electron micrographs of the ventral lobe of prostate gland of control group showing; **A.** Normal secretory epithelial cells with oval euchromatic nuclei (N), prominent nucleoli (n), parallel flat endoplasmic reticulum (ER), distinctive cell membranes (arrow) and numerous cytoplasmic electron dense secretory vesicles (S); **B.** Well-developed apical microvilli (m) and the apical region contains many dense secretory vesicles (S). TEM, A ×3000, scale bar = 2 μm; B ×6000, scale bar = 1 μm.

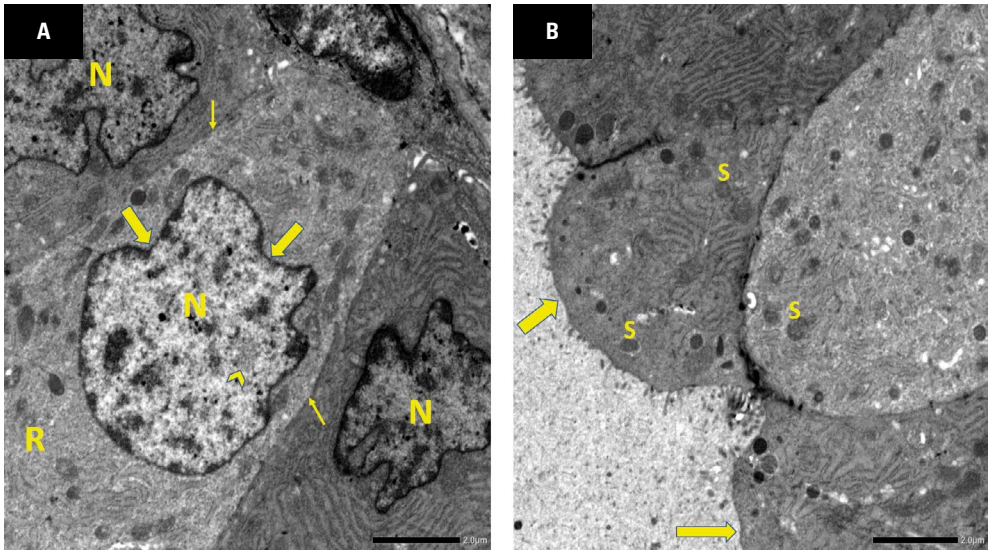

Figure 5. Electron micrographs of ventral lobe of prostate gland of orchiectomised group showing: **A.** Secretory cells' nuclei (N) with irregular nuclear envelopes (thick arrows) and hypodense chromatin (arrowhead). The cytoplasm is rarefied (R). Most of secretory vesicles are absent. Cells' boundaries are clearly defined (arrows); **B.** Most of apical microvilli are lost (thick arrows) and few hypodense apical secretory vesicles (S) are noticed. TEM ×3000, scale bar = 2 μm.

the cell cytoplasm. The acinar cells were separated by obvious cell membranes. As well as dense secretory vesicles, there were well-developed apical microvilli in the apical region of the cells (Fig. 4).

Secretory cells of the orchiectomised group are characterised by nuclei with irregular nuclear envelopes and hypodense chromatin. Rarified cytoplasm could be noticed. The boundaries of the cells were clearly defined. There was an absence of most secretory vesicles, but a few were hypodense and located at the apex of the cell. Most of the apical microvilli have been lost (Fig. 5).

In the BPH group, multiple layers of secretory cells were seen. The nuclear envelopes of these cells were folded, with their ER was extremely dilated. Most of the cell boundaries were lost. Several variable sized intracellular vacuoles could be seen in the secretory cells. Secretory vesicles were rarely

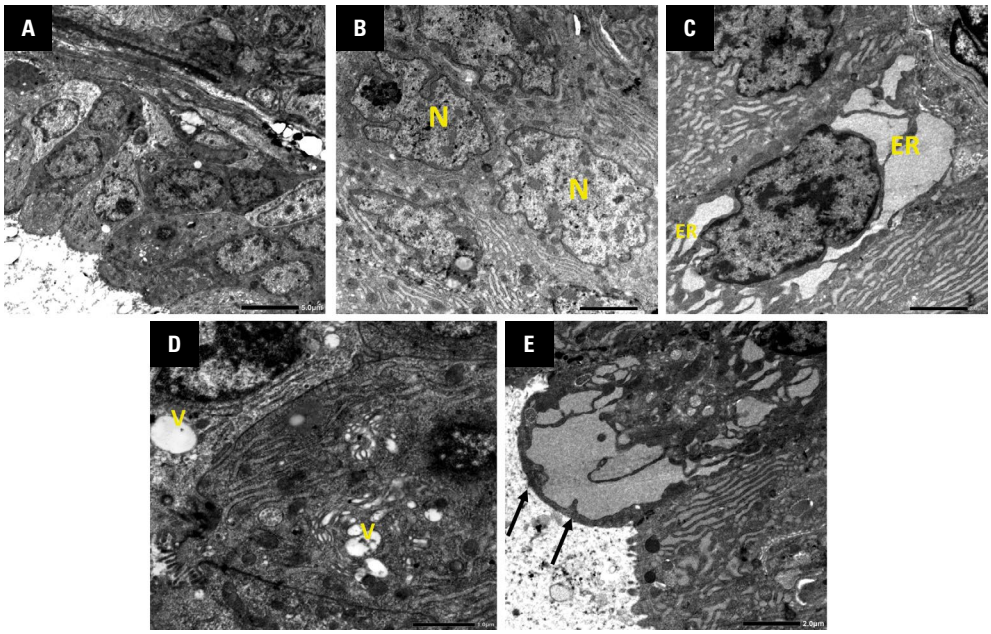

Figure 6. Electron micrographs of ventral lobe of prostate gland of benign prostatic hyperplasia group showing: **A.** Several secretory cells can be seen in multiple layers; **B.** The nuclear envelopes (N) of these cells are highly folded and most of the cell boundaries are lost; **C.** The endoplasmic reticulum (ER) is extremely dilated with absence of most secretory vesicles; **D.** Variable sized intracellular vacuoles can be seen in the secretory cells (V); **E.** Most of the apical microvilli are lost (arrows). TEM, A ×1000, scale bar = 5 μm; B, C, E ×3000, scale bar = 2 μm; D ×6000, scale bar = 1 μm.

observed. Most of the apical microvilli were missing (Fig. 6).

The protective group revealed secretory cells arranged in one layer with normal nuclei, but their nuclear envelopes had slight invagination. The cytoplasm contained secretory vesicles and had slightly dilated ER. Moreover, the cells had distinct cell boundaries. Microvilli were largely restored to normal and secretory vesicles were also increased apically (Fig. 7).

In the curative group, secretory cells' nuclei appeared irregular, having condensed chromatin and invaginations on their nuclear envelopes. Though, ER cisterns were somewhat dilated. Additionally, the cells' boundaries were still indistinct. There were some losses in the apical microvilli and some short microvilli. Apical secretory vesicles were also evident in some cells (Fig. 8).

DISCUSSION

Benign prostatic hyperplasia is the most common benign tumour in men, which increases with aging [22]. BPH presents with symptoms of urinary retention and voiding difficulties, which negatively affect patients' quality of life. Furthermore, there are several dangerous complications associated with BPH, including urinary tract infections, gross haematuria, acute urinary retention, along with renal failure [36].

Benign prostatic hyperplasia's aetiology is very arguing [61]. The pathogenesis of BPH is still unclear, but numerous factors, such as hormones, inflammation, growth factors, and metabolism disorders, appear to contribute to its progress [57].

According to our best knowledge, this is the first work to demonstrate protective and curative effects of selenium nanoparticles on BPH induced by TE in rats using biochemical, histological, immunohistochemical, morphometric and transmission electron microscopic analysis.

In the current work, prostate glands of orchiectomised group revealed atrophy of the epithelium associated with an increase in the stromal mass. Furthermore, following 5 weeks of TE injections, orchiectomised rats developed histopathological signs of BPH, taking the form of massive epithelial hyperplasia, acinar lumens reduction and stromal expansion, as well as marked deposition of collagen, that were more ameliorated in the protective group than in the curative one. In agreement with these findings, Nasr El-Din and Abdel Fattah [44] reported that castration in rats resulted in a decrease in thickness of the epithelium. In addition, Jang et al. [29] reported that 5 weeks of intramuscular TE injection resulted in hyperplasia of epithelial cells and excessive development of prostate tissue.

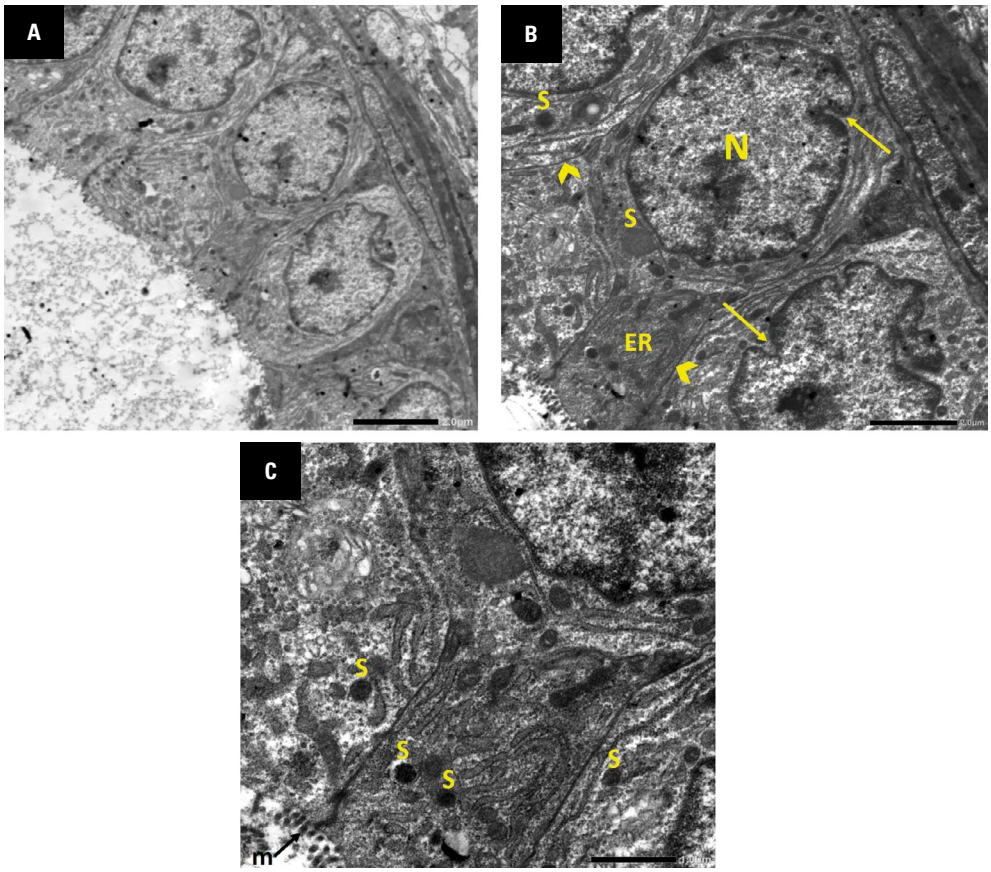

Figure 7. Electron micrographs of ventral lobe of prostate gland of protective group showing: **A.** Secretory cells appear arranged in one layer; **B.** Nuclei (N) are normal, but their nuclear envelopes have slight invagination (arrow). The cytoplasm contains secretory vesicles (S) and has slightly dilated endoplasmic reticulum (ER). The cells have distinct boundaries (arrowheads); **C.** Microvilli (m) are largely restored to normal and secretory vesicles (S) are also increased apically. TEM, A, B ×3000, scale bar = 2 μm; C ×6000, scale bar = 1 μm.

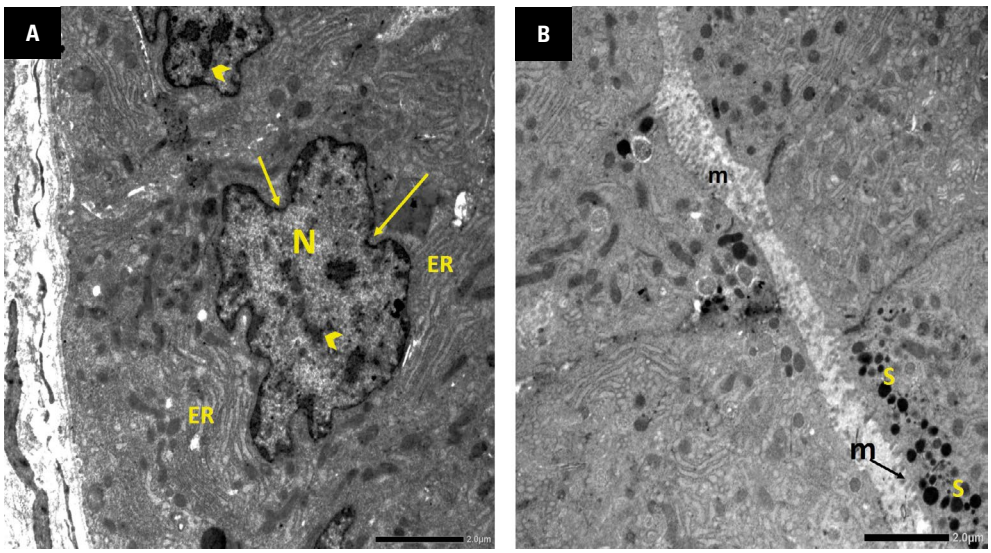

Figure 8. Electron micrographs of ventral lobe of prostate gland of curative group showing: **A.** Nuclei (N) of the secretory cells appear irregular, with condensed chromatin (arrowhead) and invaginations on their nuclear envelopes (arrows). The endoplasmic reticulum (ER) has some what dilated cisterns. The cells' boundaries were still indistinct; **B.** There are some losses in the apical microvilli and some short microvilli (m). Apical secretory vesicles (S) were also evident in some cells. TEM ×3000, scale bar = 2 μm.

Moreover, Minutoli et al. [42] showed that TE propionate injection completely deranged the prostate tissue and caused severe hyperplasia compared with control animals. Most of these pathological features improved with selenium treatment coinciding with TE injection. They added that selenium (Se) and lycopene combining with serenoa repens reduces prostate hyperplasia most effectively. In addition, Lindshield et al. [38] reported that selenium decreased prostate adenocarcinoma growth in male rats. Also, lentinan-functionalised selenium nanoparticles combined with zoledronic acid have a good antitumor effect on prostate cancer, according to An and Zhao [3]. Furthermore, Barbanente et al. [10] showed that platinum-loaded, selenium-doped hydroxyapatite nanoparticles selectively reduce proliferation of prostate and breast cancer cells without reducing proliferation of bone marrow stem cells.

Nanominerals are minerals that have been made into nanoparticles using nanotechnology. A few studies have been done to check the effectiveness of nanominerals [1]. It is demonstrated that nanoparticles have unique properties including large surface areas, high surface activity, many surface-active centres, high catalytic efficiency, low toxicity, and high absorbed ability [62]. Due to their high biological efficacy, elemental Se nanoparticles (NSe) have recently drawn considerable interest. When compared to routine Se, its efficacy is primarily attributed to its high bioavailability, high surface activity, and low toxicity [24]. NSe has been used as a nutritional supplement and for medical therapy because of its high stability and nano-defined size in the redox state of zero [26]. In comparison to inorganic or organic Se, NSe would be more effective as a dietary supplement [1].

In the current study, the MDA levels were higher in rats treated with TE while the levels of SOD, catalase and GSH were lower in comparison with the control group. These results are in harmony with Kim et al. [31] who found significant increase of MDA and significant decrease of SOD, catalase and GSH levels in the prostate of rats administered TE. The authors attributed these findings to oxidative damage within the tissue of prostate induced by TE administration. In the present study, nano-selenium treatment of rats before and after TE exposure appeared to restore prostate antioxidant activity by reverting the GSH, MDA concentration, catalase and SOD activities to nearly normal levels. The same findings were reported by Dkhil et al. [21] who found that SeNPs restored antioxidant enzyme activity and reduced lipid peroxidation and nitric oxide levels in the testis of streptozotocin-induced diabetic rats.

Proliferating cell nuclear antigen is a cell proliferation marker, as well as a cofactor of DNA polymerase delta, which is involved in replicating, excising, and repairing DNA [30]. Oxidative stress is believed to be mediated by the mechanisms that are involved in prostate proliferation [6]. By regulating transcription of target genes, eukaryotic nuclear factor kappa B (NF-κB) encourages cell proliferation [51]. In accordance with our results, Nasr El-Din and Abdel Fattah [44] showed that PCNA-positive cells were significantly increased in prostate tissue following TE. Additionally, in the current work, selenium nanoparticles decreased the percentage of PCNA-positive cells. However, selenium nanoparticles were more effective in decreasing PCNA expression in the protective group than in the curative group. Our results were harmonic with Dkhil et al. [21] who found that streptozotocin-induced diabetic rats treated with SeNPs showed abundant PCNA-positive cells in their testes, indicating strong PCNA expression.

Regarding the morphometric measurements, the present study revealed decreased acinar luminal area, but increased prostate epithelial height and stromal area in BPH group. Similarly, Bharali and Chetry [11] demonstrated that 21 days of subcutaneous TE injections significantly increased epithelial height compared to control group. Likewise, Gonzales et al. [28] reported that TE administration increased the stromal area in mice, which agrees with our results. On the contrary, Gonzales et al. [28] showed that epithelium height did not change significantly after treatment with TE and significantly increased the acinar area compared to control group which was opposing to our findings.

In the present work, ultrathin examination of prostate specimens exhibited dilatation of ER, irregular nuclei, loss of cell boundaries, loss of apical microvilli, and mostly absent secretory vesicles after 5 weeks of TE injection after bilateral orchiectomy. Similar findings have been reported by Nasr El-Din and Abdel Fattah [44] who examined epithelial prostatic hyperplasia ultrastructure induced by TE. They reported that the nuclear envelopes of the secretory cells were infolded and the ER was dilated. Multiple intracellular vacuolations of various sizes appeared in the secretory cell. Most apical microvilli were lost.

An important contributor to BPH is prostate inflammation [27]. High-grade prostatic inflammation

is significantly associated with larger prostate volumes and worse symptom scores in BPH patients [47]. In many experimental studies, TE-induced BPH was shown to be mediated by inflammation, and protective agents have been found to reduce inflammation [48]. In prostatic tissue, DHT accumulation is adequate to cause imbalance between proliferation and apoptosis as it increases expression of androgen-dependent growth factors, along with, by genomic and nongenomic stimulation of the NF-κB/p65 signalling pathways, producing inflammatory cytokines expression [32, 39]. The family of NF-κB transcription factors controls gene expression in inflammatory and immune responses, also in the development and growth of cells [5, 39].

Several signalling intermediates participate in the transduction of downstream signals by interleukin (IL)-17 as it binds to its receptor. Activation of Act1 and TNF receptor-associated factor 6 (TRAF6), E3 ubiquitin ligases, is an early event. As a result, they can stimulate degradation of the IκB inhibitor and also promote nuclear translocation of the transcription factor NF-κB [17]. Studies have shown that IL-17 is not expressed in healthy prostates but is found in BPH [32]. Androgens have been shown to reduce inflammation of the prostate. Anti-androgens result in inflammatory reactions of the prostate; mouse experiments have demonstrated this as well [14]. Possibly, this may explain why inflammatory responses in prostate tissue in the orchiectomised group were caused by decreased TE serum levels.

In fact, both IL-6 and IL-8 have been shown to promote proliferation of stromal BPH cells through trans differentiation of fibroblasts to myofibroblasts as well as motivation of basic fibroblast growth factor secretion indirectly that can stimulate prostatic growth effectively [23]. Furthermore, TNF-alpha plays a serious role in inflammation, cellular differentiation, proliferation and death [40].

Selenium nanoparticles have been shown to inhibit MAP kinase, NF-κB and decrease TNF-alpha levels [43]. According to another study, SeNPs showed anti-inflammatory effects in multiple animal models [25]. According to Vunta et al. [55], Se increases an endogenous inhibitor of IKK, which resulted in inhibition of NF-κB. Selenium may also inhibit NF-κB activation through increasing the expression of GPx genes and inhibiting NF-κB phosphorylation through the redox pathway [55]. This could explain SeNPs' beneficial effects on BPH induced by TE in the existing work. Treatment with SeNPs to the protective group gave better results than to the curative group, likely due to the fact that early treatment inhibited NF-κB pathway activation.

Limitations and clinical implications

Other studies of longer duration and multiple dosages of selenium nanoparticles are needed to uncover its potential therapeutic benefits for benign prostatic hyperplasia. Clinical trials on humans should also be undertaken.

CONCLUSIONS

It was shown in the present study that SeNPs had both curative and protective actions on BPH in rats induced by TE, though it was more apparent that the protective effect outweighed the curative effect.

Conflict of interest: None declared

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