The potential curative and preventive effects of garlic on testosterone-induced benign prostatic hyperplasia in orchiectomized rats

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Abstract
Benign prostatic hyperplasia (BPH) is a common aging disease in men. Garlic is known to have anti-proliferative effects. Therefore, this study was designed to investigate the curative and preventive effects of garlic on BPH in rats. Rats were divided into five groups: control group, orchiectomized group (where rats were subjected to bilateral orchiectomies operation), BPH group [BPH was induced by intramuscular injection of testosterone (TE) enanthate once weekly for five weeks after orchiectomy], curative group (where rats were injected with TE for five weeks followed by daily administration of garlic powder for other five weeks), and preventive group (where rats were given garlic powder simultaneously with TE injections for five weeks). Serum levels of TE and prostate-specific antigen (PSA) were measured, and prostate weighed and processed for light microscopic, immunohistochemical and transmission electron microscopy (TEM) examination. Serum levels of TE and PSA, and prostate weight (PW) were significantly increased in BPH group and significantly decreased in curative and preventive ones. Histologically and morphometrically, BPH group showed epithelial hyperplasia, stromal expansion and reduced acinar lumens that were significantly improved in both curative and preventive groups. Proliferating cell nuclear antigen (PCNA) expression was decreased in BPH group. These results were reversed in both curative and preventive groups. TEM showed nuclear irregularities, dilated endoplasmic reticulum (ER) cisterns, and lost cell boundaries, secretory vesicles and apical microvilli. Most of the previous changes were minimized in preventive group more than in curative one.

Keywords: benign prostatic hyperplasia, orchiectomy, testosterone, garlic.

Introduction
Benign prostatic hyperplasia (BPH) is one of the commonest diseases in males that is characterized by unregulated but non-malignant proliferation of prostatic epithelial and stromal cells [1]. At birth, the weight of prostate is only few grams; however, it continues to increase to be 20±6 g in young adults. This increase in size is via androgen-dependent process [2, 3]. After age of 60, more than 50% of males will have classical symptoms of BPH as a result of considerable both stromal and epithelial proliferation in the transitional zone compressing the urethra [4, 5].

Although some aspects of etiology of BPH are not understood yet, several mechanisms were believed to trigger this process [6]. Normally androgen levels during aging is declining and at the same time estradiol levels remain as it is, so there is an increase in estradiol to testosterone (TE) ratio [7]. This imbalance between androgens and estrogen and the shift towards estrogen dominance may be implicated in prostate growth. Nevertheless, it is thought that high androgen levels are pre-requisites for BPH to develop [8].

In the prostate, TE is converted into its active metabolite, dihydrotestosterone (DHT), by 5α-reductase enzyme, which is an important mediator for prostate tissue growth [9]. As a result, the traditional medical treatment of BPH is directed toward inhibition of this enzyme using Finasteride or Dutasteride [10]. However, these drugs are not devoid of adverse effects including prostate cancer, risk of osteoporosis, erectile dysfunction, decreased libido, acne and nasal congestion [11–13].

Herbal medicines are gaining popularity that increases by the time due to their benefits [14]. Garlic (*Allium sativum*) is one of these herbs that was used for years to alter risk factors of various diseases [15]. The main compositions of garlic are: water (65% of fresh weight), proteins, carbohydrates, lipids, sulfur compounds (1.1–3.5% of fresh weight), fibers, minerals and vitamins [16].

Allicin is one of sulfur compounds of garlic that causes the characteristic odor of garlic and also acquires garlic its healing properties [15]. It is a volatile and highly unstable ingredient because it immediately gives diallyl monosulfide and polysulfides, mainly diallyl disulfide (DADS) and diallyl trisulfide (DATS), when decomposed [17]. Garlic is known to have anti-proliferative, anti-mutagenic, anticarcinogenic, antioxidant, antimicrobial, hypolipidemic, antihypertensive, antiatherosclerotic, anti-thrombotic, hepatoprotective, heavy metal poisoning antidote, antidiabetic and immunomodulatory effects [18–21]. However, these desired effects depend on the method used for extraction of its ingredients [22].

Therefore, this study was aimed to compare between the curative and protective effects of garlic on TE-induced BPH in rats.
Materials and Methods

Animals

A total 50 Sprague–Dawley male rats (three months old, weighing 220±30 g) were purchased from Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. Rats were housed in separate standard plastic cages and maintained in an air-conditioned room with a 12 hours light/dark cycle. They were provided tap water and standard rodent pellets ad libitum.

Experimental design

All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care. After the acclimatization period of two weeks, rats were randomly divided into five equal groups, according to the performed procedures regarding the first day of TE injection as day 1 of the experiment. Each group contained 10 rats and divided randomly as following:

Group I (control group) was subdivided into group IA (negative control subgroup), where the animals received 100 μL of olive oil, as a vehicle, once weekly for successive five weeks [23], and group IB (sham control subgroup), in which the scrotum was incised, manipulated and then sutured without resection of the testes [24].

Group II (orchiectomized group), where rats were subjected to bilateral orchiectomies operation [25]. The operation was performed seven days before induction of BPH, to prevent the influence of intrinsic TE [23].

Group III (BPH group), where BPH was induced by intramuscular injection of TE enanthate starting three days after orchiectomy and given once weekly for five weeks [23].

Group IV (curative group), where rats were subjected to BPH induced by TE enanthate for five weeks, followed by daily administration of garlic powder for other five weeks.

Group V (preventive group), in which rats were subjected to BPH induced by TE enanthate, simultaneously with daily administration of garlic powder for other five weeks.

By the end of the experiment, all rats were sacrificed and the prostate was extracted, weighed and processed for light microscopy, immunohistochemical (IHC) and transmission electron microscopy (TEM) examination.

Orchiectomy procedures

Anesthesia was induced by intraperitoneal injection of Ketamine (90 mg/kg) and Xylazine (12 mg/kg) [27]. After confirmation of complete anesthesia, 1.5 cm incision was performed in the ventral aspect of the scrotum, through which the testis and epididymal fat were gently pulled, then the spermatic cord and testicular vessels were ligated and cut. After resection of both testes, incisions were sutured via 4-0 silk sutures (Figure 1) [25]. The animals were given prophylactic Ampicillin (4000 IU/kg) by intraperitoneal injection for three days and Coloplast paste (Humlebaek, Denmark) was applied locally.

The experiment was maintained and managed in accordance with the guidelines of the National Institutes of Health (NIH) for the care and use of laboratory animals (NIH Publication No. 85-23, Rev. 1985).

Blood collection and biochemical analysis

At the end of the experiment (after 10 weeks), all rats were fasted overnight and then sacrificed by an over dose of Ketamine and Xylazine. Immediately, the blood samples were collected from the abdominal aorta and centrifuged at 3000×g for 15 minutes, in order to obtain the serum. Serum levels of TE and prostate-specific antigen (PSA) were measured by the enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer’s instructions [30, 31].

Prostate weight index

After sacrifice, prostate glands were collected, carefully cleared from the extraneous tissues and weighed. The prostate weight (PW) index was calculated according to the following equation: PW index = (PW/Body weight) × 100 [32].

Histopathological study

Tissue samples from ventral lobes of the prostate were fixed by submersing in 10% neutral buffered formalin solution, for at least 24 hours prior to preparation of the paraffin blocks, then 4 μm thick paraffin sections were prepared. Finally, sections were mounted on glass slides.
for staining with Hematoxylin and Eosin (HE) [33] and Masson’s trichrome [34].

IHC study

For proliferating cell nuclear antigen (PCNA) immunostaining [35], paraffin-embedded tissue sections were deparaffinized. Sections were hydrated using ethanol-graded concentrations, and then heated in citrate buffer (pH 6) for 20 minutes. After that, the sections were blocked for two hours using 5% bovine serum albumin in Tris-buffered saline (TBS). The sections were then incubated for two hours with mouse monoclonal anti-PCNA antibody, as a primary antibody (Bohai Biotechnology Development Co., Ltd., Hebei, China). Sections were washed with TBS and incubated for one hour with the secondary antibody, at room temperature. Visualization of PCNA expression was done by 0.06% 3,3’-diaminobenzidine (DAB), giving a brown color, then sections were counterstained by Hematoxylin [35].

For caspase-3 immunohistochemistry, tissue sections were deparaffinized, rinsed with phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked by 0.1% H2O2 for 30 minutes. After rinsing in PBS, sections were incubated in 10% normal goat serum for 60 minutes, at room temperature. The sections were incubated with the primary antibody (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) [36], rinsed with PBS and incubated with secondary biotinylated antibody for 20 minutes, at room temperature. Visualization of caspase-3 expression was achieved by staining with 0.06% DAB and then Hematoxylin counterstaining was done. Finally, sections were dehydrated by ethanol-graded concentrations [36].

Morphometric study

By using four non-overlapping fields, histomorphometric analysis was performed to measure the epithelial height, acinar luminal area and stromal area. Measurement of the epithelial height was performed by drawing 30 lines per field through the whole length of the acinar epithelial layer. On the other hand, the measurement of acinar luminal area was performed by drawing a line around the perimeter of the acini in four fields per prostate and then the acinar areas were calculated. Furthermore, the stromal area was calculated by subtracting the total acinar luminal area from the total field area. The epithelial height measurements were performed under 400× magnification, while that of acinar luminal and stromal areas were performed at 100× magnification [37].

Under 400× magnification, the percentage of epithelial cells that expressed immunopositive PCNA reaction to the total epithelial cell number was calculated according to the following equation: (Positive epithelial cell number/Total epithelial cell number) × 100 [38].

All histomorphometric data were collected and analyzed using Image J software by only a single-blinded investigator, to reduce the inter-observer bias.

TEM study

Specimens of 1 mm³ size were cut and fixed in 2.5% glutaraldehyde for two hours. Post-fixation was done using 1% osmium tetroxide (pH 7.4) for one hour. Specimens were dehydrated in ethanol-graded concentrations and then embedded in resin. Semithin sections were stained with 1% Toluidine Blue, to be examined by light microscopy, to determine the areas of interest. Then, ultrathin sections were double stained with uranyl acetate and lead citrate [39, 40]. Finally, sections were examined using a JOEL TEM (Columbia, South Carolina, USA), at the Electron Microscope Unit, Al-Azhar University, Cairo, Egypt.

Statistical analysis

The statistical analysis was done by using the Statistical Package for the Social Sciences (SPSS) software (16.0; SPSS, Inc., Chicago, IL, USA). All values were expressed as mean±standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test. Significance of the data was determined by p values, where a p value ≤0.05 was considered to be significant [41].

Results

Biochemical analysis

A statistical significant decrease in the serum levels of TE and PSA were found in the orchietomized group, when compared to the control one, and showed a statistical significant increase in BPH group, when compared to control and orchietomized groups. Levels of both TE and PSA in the curative and preventive groups showed a significant decrease, when compared to BPH group. Moreover, the preventive group showed a significant decreased TE level, when compared to that of curative one (Figure 2).

Body and prostate weight changes

No statistical significant difference was found regarding the body weight gain among the different groups (Table 1). Regarding the PW and PW index, the BPH group showed a statistical significant increase, when compared with
control one. Administration of garlic, either in curative or preventive groups, resulted in a significant reduction of the PW and PW index, with no significant difference between both groups. Moreover, orchiectomized group showed a statistical significant decrease of PW and PW index, when compared to all other study groups (Table 1).

Table 1 – Body weight, PW and PW index changes

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Initial body weight [g]</th>
<th>Final body weight [g]</th>
<th>Body weight gain [g]</th>
<th>PW [mg]</th>
<th>PW index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Initial body weight</td>
<td>205.1±1.7</td>
<td>294.67±2.64</td>
<td>89.57±3.79</td>
<td>799±40.6</td>
<td>0.271±0.013</td>
</tr>
<tr>
<td></td>
<td>Final body weight</td>
<td>305.65±6.67</td>
<td>111±18.5±3.3</td>
<td>935±37.7</td>
<td>0.036±0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final body weight</td>
<td>238.75±14.09</td>
<td>80.15±15.7</td>
<td>888±109.8</td>
<td>0.289±0.047</td>
<td></td>
</tr>
</tbody>
</table>

PW: Prostate weight; BPH: Benign prostatic hyperplasia. Values are mean±SD. *p<0.05 vs. control group, †p<0.05 vs. orchiectomized group, ‡p<0.05 vs. curative group, and ††p<0.05 vs. preventive group. Statistical analysis was performed by ANOVA, followed by Tukey’s post-hoc test. SD: Standard deviation; ANOVA: Analysis of variance.

Gross morphology of the prostate

The prostate glands of BPH group appeared to be larger than that of control group, with hyperemia and associated with large congested seminal vesicles. Few urinary bladders of BPH rats were occupied by spherical stones, with a spike extending into the urethra. The prostate glands of curative and preventive groups were smaller in size and with no urinary stones. The prostate glands of orchiectomized group were markedly atrophic and pale in color, with atrophied seminal vesicles (Figure 3, A–F).

Histopathological results

HE- and Masson’s trichrome-stained sections of the control group showed normal prostatic tissue, with acini lined with a single layer of low columnar epithelial cells, with an unremarkable stroma (Figure 4A). Fine collagen fibers scattered in the stroma between the acini (Figure 5A). Orchiectomized group showed marked expansion of the stroma, with inflammatory infiltration. Furthermore, there was reduced thickness of acinar wall, with flattening or loss of the acinar epithelial cells (Figure 4B). The stroma was expanded, with abundant collagen fiber deposits (Figure 5B).

BPH group revealed remarkable histological alterations. The acinar lumens were markedly narrowed by the epithelial hyperplasia, which project into large involutions. However, there were some areas of epithelial cell loss and some epithelial cells showed intracellular vacuolations (Figure 4C). Excessive collagen fiber deposition appeared in the stroma (Figure 5C).

Curative and preventive groups showed marked improvement and restoration of many of the normal prostatic characteristics, in the form of reduced acinar epithelium height, with absent involutions and decreased stromal thickness (Figure 4, D and E). Moderate amount of collagen fibers was seen in the curative group (Figure 5D), while minimal collagen deposits in the stroma appeared in the preventive one (Figure 5E). Thus, the preventive group showed more histological improvement, when compared to the curative group.

IHC results

IHC staining of the PCNA, as a marker for proliferation, showed minimal immunoreexpression, however IHC staining of caspase-3, as an apoptotic marker, was mostly negative in the control group (Figures 6A and 7A). Furthermore, orchiectomized group showed minimal nuclear PCNA immunoreexpression, and massive nuclear caspase-3 immunoreexpression in the epithelial cells and cytoplasmic caspase-3 expression in the stromal cells (Figures 6B and 7B). On the other hand, BPH group showed dramatically increased nuclear PCNA immunoreexpression in both epithelial and stromal cells, but was mostly negative for caspase-3 immunostaining (Figures 6C and 7C). Curative group showed minimal nuclear PCNA immunoreexpression and mild nuclear caspase-3 immunoreexpression of the epithelial cells (Figures 6D and 7D). Preventive group shows minimal nuclear PCNA immunoreexpression, and moderate nuclear and cytoplasmic caspase-3 immunoreexpression of epithelial cells (Figures 6E and 7E).
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Figure 4 – Photomicrographs of HE-stained (×400) sections of prostate: (A) Control group showing normal prostatic tissue, with acini lined by a single layer of low columnar epithelial cells [E] and a scanty stroma [S] in between; (B) Orchiectomized group showing massive expansion of the stroma [S], with inflammatory infiltration [I] and reduced thickness of acinar wall, with flattening [F] or loss [L] of the acinar epithelial cells; (C) BPH group showing that the acinar lumen is narrowed by the epithelial hyperplasia projecting into large involutions [H], areas of epithelial cell loss [L], detached epithelial cells inside the lumen [D], appearance of many vacuolated epithelial cells [V], with abundant stroma in between the acini [S]; (D) Curative group showing absence of involutions, flattened epithelial cells [F], areas of epithelial cell loss [L], and moderate amount of stroma [S]; (E) Preventive group showing that the acini are lined by flattened epithelium [F], with markedly reduced stroma [S]. Note absence of the involutions. Scale bar = 25 μm. HE: Hematoxylin–Eosin; BPH: Benign prostatic hyperplasia.

Figure 5 – Photomicrographs of Masson’s trichrome-stained (×400) sections of prostate: (A) Control group showing fine collagen fibers [C] scattered in the stroma between the acini; (B) Orchiectomized group showing marked expansion of the prostatic stroma, with abundant collagen fiber deposits [C]; (C) BPH group showing excessive collagen fiber deposits [C] in the stroma; (D) Curative group shows moderate increase in the amount of stromal collagen [C]; (E) Preventive group showing minimal collagen deposits [C]. Scale bar = 25 μm. BPH: Benign prostatic hyperplasia.
Figure 6 – Photomicrographs of prostate sections immunohistochemically stained with anti-PCNA antibody (×400): (A) Control group showing one epithelial cell with nuclear immunoexpression (arrow); (B) Orchiectomized group showing minimal nuclear immunoeexpression of the epithelial and stromal cells (arrows); (C) BPH group showing dramatically increased nuclear immunoeexpression of the epithelial and stromal cells (arrows); (D) Curative group showing minimal nuclear immunoeexpression of the epithelial cells (arrows); (E) Preventive group showing minimal nuclear immunoeexpression of the epithelial cells (arrows). Scale bar = 25 μm. PCNA: Proliferating cell nuclear antigen; BPH: Benign prostatic hyperplasia.

Figure 7 – Photomicrographs of prostate sections immunohistochemically stained with anti-caspase-3 antibody (×400): (A) Control group showing negative nuclear immunoeexpression; (B) Orchiectomized group showing massive increase in positive nuclear immunoeexpression of the epithelial cells (arrows) and massive increase in positive cytoplasmic immunoeexpression of the stromal cells (arrow heads); (C) BPH group showing negative immunoeexpression; (D) Curative group showing mild nuclear immunoeexpression of the epithelial cells (arrows); (E) Preventive group showing moderate nuclear and cytoplasmic immunoeexpression of epithelial cells (arrows). Scale bar = 25 μm. BPH: Benign prostatic hyperplasia.
Morphometric study

A statistical significant decrease of the epithelial height in the orchiectomized group was observed when compared to the control one. In contrary, it showed a significant increase in BPH group, when compared to control and orchiectomized groups. On the other hand, administration of garlic in the curative and preventive groups showed a statistical significant decrease in epithelial height, when compared to BPH group. However, the epithelial height was significantly lower in preventive group, when compared to curative one reaching near to control values (Table 2).

A statistical significant decrease of the luminal area in both orchiectomized and BPH groups was noticed when compared to that of control group. There was increase of the luminal area in both curative and preventive groups. This increase was statistically non-significant in the curative group and significant in the preventive group, when compared to BPH one (Table 2).

Table 2 – Epithelial height, acinar luminal area, stromal area and PCNA percentage changes

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Epithelial height [μm]</th>
<th>Acinar luminal area [μm²] ×10⁶</th>
<th>Stromal area [μm²] ×10⁶</th>
<th>PCNA percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td>15.86±2.78</td>
<td>442.49±37.74</td>
<td>267.95±37.74</td>
<td>1.25±0.46</td>
</tr>
<tr>
<td>Orchiectomized</td>
<td></td>
<td>6.01±3.25</td>
<td>119.91±17.55</td>
<td>590.52±17.55</td>
<td>0.74±0.31</td>
</tr>
<tr>
<td>BPH group</td>
<td></td>
<td>25.75±7.91</td>
<td>271.92±145.52</td>
<td>438.52±145.52</td>
<td>35.6±7.61</td>
</tr>
<tr>
<td>Curative group</td>
<td></td>
<td>19.84±5.3</td>
<td>381.79±139.19</td>
<td>328.65±139.19</td>
<td>16.97±6.78</td>
</tr>
<tr>
<td>Preventive group</td>
<td></td>
<td>16.69±4.11</td>
<td>442.67±143.27</td>
<td>267.77±143.27</td>
<td>5.63±1.98</td>
</tr>
</tbody>
</table>

PCNA: Proliferating cell nuclear antigen; BPH: Benign prostatic hyperplasia. Values are mean±SD. *p<0.05 vs. control group, *p<0.05 vs. orchiectomized group, *p<0.05 vs. BPH group, *p<0.05 vs. curative group, and *p<0.05 vs. preventive group. Statistical analysis was performed by ANOVA, followed by Tukey’s post-hoc test. SD: Standard deviation; ANOVA: Analysis of variance.

TEM results

Ultrathin sections of the ventral lobe of prostate of the control group showed normal secretory epithelial cells, with oval euchromatic nuclei with prominent nucleolus. Its cytoplasm showed parallel non-dilated endoplasmic reticulum (ER) cisterns and intracellular dense secretory vesicles. Distinct cell membrane appeared separating the acinar cells. The apical region was rich in dense secretory vesicles and showed well-developed apical microvilli. Furthermore, smooth muscle cells were occasionally present surrounded by delicate elastic fibers (Figure 8).

Orchiectomized group showed secretory cells containing nuclei with slightly irregular nuclear envelope, and hypodense nucleolus and chromatin. The cytoplasm showed intracellular vacuolations and many large dense phagosomes. Secretory vesicles were mostly absent, but only few of them were hypodense and found near the apex of the secretory cells. Apical microvilli were mostly lost. Fibroblasts were flat structures with long irregular nucleus and collagen fibers were densely deposited in the stroma between the acini. However, the cell boundaries were well distinct. The blood capillaries were lined by abnormal variable sized endothelial cells, with irregular nuclei and surrounded by fibroblast and extensively collagen fibers deposits (Figure 9).

BPH group showed many secretory cells arranged into multiple layers with an inflammatory cells in-between. Their nuclear envelopes were highly infolded and the ER was dilated. The boundaries between the cells were mostly lost. The secretory cell appeared with multiple variable sized intracellular vacuolations. The secretory vesicles were rare to be demonstrated. Apical microvilli were mostly lost. The stroma showed deposition of fibroblast and extensive collagen fibers (Figure 10).

Curative group showed that the nuclei of the secretory cells were still irregular, with condensed chromat and their envelopes showed invaginations. However, ER had slightly dilated cisterns. Moreover, the boundaries between the cells were distinct. Some of the apical microvilli were lost and others were short. Few well-developed apical secretory vesicles were also seen (Figures 11).

Preventive group showed that secretory cells had regular nuclei, with slight invagination of their nuclear envelope. Secretory vesicles and slightly dilated ER was seen in the cytoplasm. Moreover, the boundaries between the cells were distinct. Most of the apical microvilli were regained and appeared normal, in addition to increased number of the apical secretory vesicles (Figure 12).

The previous findings indicated more improvement in the preventive group, in comparison to the curative one. The sections of the preventive group appeared near the normal features of the control one.

Discussions

Clinically, BPH is characterized by voiding and storage lower urinary tract symptoms affecting the life quality of the patients. Moreover, BPH has many dangerous complications including urinary tract infection, acute urinary retention, gross hematuria, renal insufficiency, urinary stones in addition to renal failure [42].
Figure 8 – Electron micrographs of ventral lobe of a prostate gland of control group: (A) A secretory epithelial cell appears normal with oval euchromatic nucleus [N], prominent nucleolus [n], flat parallel endoplasmic reticulum [ER], well distinct cell boundaries [B] and many cytoplasmic electron dense secretory vesicles [S]; (B) The apical microvilli [M] are well-developed and the apical region is rich in dense secretory vesicles [S]; (C) A smooth muscle cell [SMC] appeared normal, with flat nucleus [N] and thin elastic fibers (arrows). Transmission electron microscopy (TEM): (A) ×8000; (B and C) ×10 000. Scale bar = 2 μm.

Figure 9 – Electron micrographs of ventral lobe of a prostate gland of orchiectomized group: (A) A secretory cell nucleus with slightly irregular nuclear envelope [N] and hypodense nucleolus [n] and chromatin (arrows). The cytoplasm is rarefied [R] and contains many large phagosomes [P] and cytoplasmic vacuolations [V]. Secretory vesicles are mostly absent. The cell membranes are highly distinct [M]; (B) Two flat fibroblasts [F] contain long irregular nuclei [N] with thick collagen fiber deposits (C) are seen in the stroma between the acini. Transmission electron microscopy (TEM): (A) ×8000; (B) ×10 000. Scale bar = 2 μm.
Figure 9 (continued) – Electron micrographs of ventral lobe of a prostate gland of orchiectomized group: (C) A blood capillary (B) is lined by abnormal variable sized endothelial cells with irregular nuclei (E) and surrounded by fibroblast (F) and extensively collagen fibers deposits (C); (D) The apical microvilli (M) are mostly lost and the apical secretory vesicles (S) are fewer in number and less in density than that of control group. Transmission electron microscopy (TEM): (C and D) ×10,000. Scale bar = 2 μm.

Figure 10 – Electron micrographs of ventral lobe of a prostate gland of BPH group: (A) Many secretory cells are arranged into multiple layers with an inflammatory cell (I) in-between. Their nuclear envelopes (N) are highly infolded and the ER is massively dilated. The boundaries between the cells are mostly lost; (B) A secretory cell appears with multiple variable sized intracellular vacuolations (V), with mostly absent secretory vesicles. Transmission electron microscopy (TEM): (A) ×6000; (B) ×10,000. Scale bar = 2 μm. BPH: Benign prostatic hyperplasia; ER: Endoplasmic reticulum.
Figure 10 (continued) – Electron micrographs of ventral lobe of a prostate gland of BPH group: (C) Fibroblasts are present in the stroma {F}, with extensive collagen fiber deposits {C}; (D) Secretory cells with loss of most of apical microvilli. Transmission electron microscopy (TEM): (C) ×15 000; (D) ×10 000. Scale bar: (C) 0.5 μm; (D) 2 μm. BPH: Benign prostatic hyperplasia.

Figure 11 – Electron micrographs of ventral lobe of a prostate gland from curative group: (A) Nuclei {N} of secretory cells are still irregular with condensed chromatin (arrows) and their envelopes show invaginations (arrowheads). However, ER has slightly dilated cisterns. Moreover, the boundaries between the cells are distinct {B}; (B) Some of the apical microvilli are lost and others are short {M}. Few well-developed apical secretory vesicles {S} are also seen. Transmission electron microscopy (TEM): (A) ×8000; (B) ×10 000. Scale bar = 2 μm. ER: Endoplasmic reticulum.
To date, the etiology of BPH is highly debating [43]. However, BPH pathogenesis is not well understood, several etiological factors, including hormone alteration, prostatic inflammation, growth factors and metabolism syndromes seem to be implicated in its development [44].

To our knowledge, the present study is the first one demonstrating a comparison between protective and curative effects of garlic on TE-induced BPH in rats based on laboratory, histological, morphometric, IHC and TEM findings.

Previous studies demonstrated that garlic or its components have antiproliferative effect on prostatic tissue [26, 45–47]. For example, Chung et al. [26] induced BPH in rats by daily TE propionate injections for four weeks after castration. They used garlic daily treatment along TE administration that reduced PW by 1.16 times in comparison with BPH group. In the current study, garlic reduced PW by 1.52 times and 1.6 times in curative and preventive groups, respectively, in comparison to BPH group. This better improvement in PW in our study than the results of Chung et al. [26] may be due to longer use of garlic or due the different type of TE we used.

In the present study, prostate glands of rats that had orchiectomy without TE administration showed increase in the stromal mass concomitant with epithelial atrophy. On the other hand, orchiectomized rats developed histopathological features of BPH after five weeks of TE injections, in form of massive epithelial hyperplasia, reduction of the acinar lumens and stromal expansion, with marked collagen deposition that were more improved in preventive group than in curative one. In accordance with our results, Lee et al. [25] found that castration in rats creates glandular atrophy and reduction in epithelial thickness. Furthermore, Jang et al. [23] reported that intramuscular injection of TE for five weeks developed epithelial cells hyperplasia and the prostate tissue was excessively developed.

Moreover, Chung et al. [26] showed that TE propionate injection for four weeks caused epithelial hyperplasia, with epithelial cytoplasmic vacuolations, in addition to decrease in the acinar luminal area. These pathological features were mostly improved with garlic treatment for four weeks, simultaneous with TE injection and this matches our results in the preventive group. Arunkumar
et al. [46] evaluated the effect of DADS, one of the components of garlic, on rat prostate carcinoma induced by subsequent injections of Cyproterone acetate, then TE propionate, then propylene glycol. They reported that DADS produced reduction of the hyperplastic and dysplastic histological changes in the prostate.

PCNA is a positive marker indicating cellular proliferation as it has a nuclear expression during the deoxyribonucleic acid (DNA) synthesis phase of the cell division process [48]. At the same time, eukaryotic nuclear factor-κB (NF-κB) signaling pathway is known to stimulate cellular proliferation by producing the transcription of target genes [49]. In harmony of our data, Sarbishegi et al. [50] demonstrated that TE increased the number of PCNA-positive cells in prostate tissue. In addition, in the present study, garlic reduced the number of PCNA-positive cells. However, in preventive group, garlic was more efficient in decreasing PCNA expression than in curative one.

In addition to its role in cellular proliferation, NF-κB pathway activation could reduce apoptosis, as well as produce cell survival [51]. At the same time, caspase-3 is a major executioner protein in proteolytic degradation during the process of apoptosis [52]. Zheng et al. [53] showed the effects of Qianliening capsule in treatment of TE-induced BPH. Caspase-3 expression was low in BPH group, which was increased with high dose Qianliening capsule treatment. This was concomitant with our results according to BPH group. Furthermore, garlic administration increased caspase-3 IHC expression that was more in preventive group than in curative one.

The morphometric evaluation, in the current study, showed that the epithelial height and the stromal area of the prostate tissue were significantly increased in BPH group but the acinar luminal area was decreased. Also, according to Bharali & Chetry [54], daily subcutaneous TE injections for 21 days produced significant increase in the epithelial height, when compared with the control rats. Gonzales et al. [37] also reported that TE administration in mice increased the value of stromal area that is in accordance of our results. However, according to Gonzales et al. [37], TE did not produce significant changes in the epithelial height, compared to control group, and significantly increased the acinar area, which was in contrary to our results.

In the current study, ultrathin prostate sections revealed that five weeks injections of TE after bilateral orchietomy produces irregularity of the secretory cell nuclei, dilatation of ER, loss of most of cell boundaries, cytoplasmic vacuolations, mostly absent secretory vesicles, loss of apical microvilli and stromal collagen fiber depositions. Azmy & Abdallah [55] evaluated the ultrastructure of epithelial prostatic hyperplasia by Metoclopramide-induced hyperprolactinemia. They found that the nuclei of the secretory cells were pleomorphic in shape with excessively infolded nuclear envelope, the cytoplasm contains vacuolations, dilated ER and the apical microvilli were discon-tinuous. However, with hyperprolactinemia, the secretory vesicle were present but mostly with central flocculent material surrounded by electron-lucent zones, which is different compared to TE-induced BPH where secretory vesicles were mostly absent. Furthermore, these TEM findings were mostly improved by more extent in preventive group than in curative one.

A series of events can be caused by acute and chronic inflammation, which could lead to proliferative events in the prostate tissues [56]. For example, chronic intra-prostatic inflammation provokes chronic tissue remodeling and subsequent prostatic diseases progression, including BPH [57]. In case of exogenous TE injections, this could initially lead to increase the intra-prostatic TE level that produces a higher activity of the 5α-reductase enzyme, leading to an accumulation of DHT. Accumulation of DHT in prostatic tissue is sufficient to produce proliferation/apoptosis imbalance, by increasing the androgen-dependent growth factors expression, as well as by genomic and non-genomic stimulation of NF-κB/p65 signaling pathways, creating inflammatory cytokines expression such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, IL-6, IL-8 [58, 59]. NF-κB transcription factor family controls the expression of genes that are involved in inflammatory and immune responses in addition to cellular development and growth [60, 61].

When IL-17 is binding to its receptor, multiple signaling intermediates are contributed to transduce downstream signaling. One of the early events is activation of the Act1 and TNF receptor-associated factor 6 (TRAF6) E3 ubiquitin ligases. These can provoke degradation of the IκB inhibitor as well as the NF-κB transcription factor translocation into the nucleus [62]. Previous studies showed that IL-17 is not expressed in healthy prostates, in contrast to BPH, where IL-17 expression is present [59]. The interesting fact is that androgens seem to have critical anti-inflammatory effects on prostatic tissue. This was shown in men having anti-androgen therapy develop prostatic inflammatory reactions; also, this was shown previously in mouse models [63]. In the current study, this may explain that decreased TE serum levels in orchietomized group might lead to the inflammatory reaction that appeared in the prostate tissue in this group.

Indeed, stromal BPH cell proliferation can be promoted by IL-6 and IL-8 by the fibroblast-to-myofibroblast trans-differentiation and also by indirect stimulation of basic fibroblast growth factor secretion. This can have a potent stimulating effect in prostatic growth [45]. In addition, TNF-α exerts a variety of important functions in inflammation, cell differentiation, cell proliferation and cell death [64].

Recently, DATS has been demonstrated to exert suppressive effects on NF-κB pathway [65, 66]. Despite of that, the detailed mechanisms of NF-κB pathway suppressive effects of DATS are still unknown [65]. However, it is well recognized that cellular proliferation can be suppressed by allyl sulfides by blocking cells in the G2/M phase and also by the induction of apoptosis [67]. This can explain the ameliorative effect of garlic on TE-induced BPH in the current study. Garlic administration in preventive group showed better effects than in curative group this may be due to the earlier administration of garlic that led to early suppression of NF-κB pathway.

**Conclusions**

The present study showed that garlic has both preventive and curative effects on TE-induced BPH in rats; however, its preventive effect is more obvious than the curative effect.
Conflict of interests

The authors declare that they have no conflict of interests.

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Revised: November 8, 2018

Accepted: April 23, 2019

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