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Pumpkin-Seed Ethanolic Extract Has Protective and Therapeutic effect On Benign Prostatic Hyperplasia in Male Albino Rats

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ABSTRACT

This study aimed to determine the prophylactic and curative effects of pumpkin seed ethanolic extract (PSEE) in adult male rats with testosterone-induced BPH. Two study group were designed for this study; the first study examined the protective effect of (PSEE) against testosterone-induced BPH in five rats divided into three equal groups (control negative group (C1) received only drinking water; protective group (P) received testosterone enanthate concurrently with regular oral administration of (PSEE) for 30 days, and control positive group (CP) received only testosterone enanthate; although the second study examined the extract's therapeutic effect on testosterone-induced BPH, it used two equal groups (control negative group (C2) (injected testosterone enanthate only for 30 days) and therapeutic group (T) (injected testosterone enanthate for 30 days, then the injection stopped, and the rats received 100mg of pumpkin seed ethanolic extract orally for 27 days). Serum dihydrotestosterone(DHT) levels, 5α-reductase, and PSA were estimated, and prostates were histologically studied. Results showed a significant reduction in all examined parameters and histopathological improvement in protective and therapeutic groups compared to control groups. In conclusion, the pumpkin seed ethanolic extract has curative and protective activity on testosterone-induced BPH in rats, with a potential protective effect compared to curative activity.

Keywords: Pumpkin-seed, benign prostatic hyperplasia, albino rats, PSA; 5α-reductase.

INTRODUCTION

Benign prostatic hyperplasia (BPH}) is an abnormally non-cancerous increase in the prostate gland (Doku, 2006; Dordevic*et al.*, 2016; Perez Gutierrez, 2016; Jeon *et al.*, 2017;

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Omotosho*et al.*, 2018). It is also termed nodular hyperplasia of the Prostate (Doku, 2006). It is one of the common diseases in males that is provoked by androgen and dihydrotestosterone signals (Kim*et al.*, 2015). The testosterone is catalyzed and converted into dihydrotestosterone by an enzyme called 5α-reductase present in prostatic cells (el-mehi*et al.*, 2015).

The definite etiology of prostatic hyperplasia is not well resolved, but the aging males are affected by the androgen hormone (Ejike and Ezeanyika, 2011; Wang*et al.*, 2014; Kim*et al.*, 2015; Dwarakanath*et al.*, 2017). 3α-androstandiol and dihydrotestosterone seem the central hormonal stimuli for glandular and stromal proliferation in men with prostatic hyperplasia Perez Gutierrez, 2016; Mbaka*et al.*, 2017). Dihydrotestosterone results via testosterone conversion by 5α-reductase (Wang*et al.*, 2014; Jeon *et al.*, 2017). The main cures used to manage prostatic hyperplasia include 5α-reductase inhibitors and α-blockers (Wang*et al.*, 2014; el-mehi*et al.*, 2015). Treatment options of prostatic hyperplasia also target either relaxing prostatic muscle tone or reducing the prostate gland (Omotosho*et al.*,2018).

Recently there has been a global turnout to use herbal medicine to manage and treat different medical conditions (Mbondo, 2013; Ayaz*et al.*, 2015). Herbal medicines are an alternative, cheaper medicine than chemical types (Doku, 2006; Mohammadi*et al.*, 2014), and safe even when used for long periods (Karawya and Zahran, 2015). Pumpkin (*Cucurbita moschata*) is a fruit or vegetable as many commonly regarded in consumer terms present in Africa, Asia, Europe, and America and is widely used for medicinal purposes (Azizah*et al.*, 2009; Mbondo, 2013; Ayaz*et al.*, 2015; Perez Gutierrez, 2016) such as treatment or prevention of urinary disorders (Nishimura*et al.*, 2014; Medjkovic*et al.*, 2016; Perez Gutierrez, 2016) especially nocturia, healing efficiency of burn wounds (Bardda et al., 2016), antioxidant and antiulcer (Perez Gutierrez, 2016), anthelmintic (Rahman*et al.*, 2014; Ayaz*et al.*, 2015), treating cramps and fever (Rahman*et al.*, 2014), prevention of diabetic nephropathy, cardiac protection, antifungal, antibacterial, and anti-inflammation (Mbondo, 2013; Perez Gutierrez, 2016), Pumpkin seeds are rich in β-Sitosterol, γ and α-tocopherol, β-Carotene, amino acids, and fatty acids mainly palmitic, linoleic, stearic and oleic acids (Nishimura*et al.*, 2014; Kim*et al.*, 2015). The prostate-specific antigen (PSA) is synthesized only by the prostate gland (Rahman*et al.*, 2014). The PSA level elevation is considered a specific and sensitive marker for detecting prostatic diseases, especially prostatic cancer (Nogueira et al., 2009). There is evidence that PSA is possibly a marker to see BPH (Nogueira et al., 2009; Rahman*et al.*, 2014).

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The present study aimed to investigate the protective and curative effects of pumpkin seed ethanolic extract (PSEE) on testosterone-induced BPH in male rats.

MATERIALS AND METHODS

Animals

A 25 mature albino male rats weighing 230 ±20 g were used in this study. All animals were housed separately in a standard cage and provided with drinking water and a standard nutrition *ad libitum*.

Experimental design

All animals were performed under protocols approved by the Animal Care Committee of the college of veterinary medicine, university of Al-Qadisiyah, Iraq.

The study was designed in two separated experiments:

The first experiment was designed to investigate the protective effect of the PSEE on testosterone-induced BPH, which included three equal groups; each group involve five rats and divided as follows: Control negative group (C1) (received drinking water only), protective group (P) (injected testosterone enanthate 5mg /kg BW/ Sc for 30days to induce BPH, simultaneously with daily oral administration of 100mg PSEE), and Control positive group (CP) (injected testosterone enanthate only with the same protective group dose to induce BPH).

The second experiment was designed to investigate the therapeutic effect of the PSEE on testosterone-induced BPH, which included two equal groups; each group involve five rats and divided as follows: Control negative group (C2) (injected testosterone enanthate only with the same dose of the protective group to induce BPH), and the injection is then stopped and the rats left with no treatment as a negative control group, and therapeutic group (T) (injected testosterone enanthate, with the same dose of the protective group to induce BPH), and the injection is then stopped, and the rats received 100mg of PSEE orally for 27 days.

When both experiments of study ended, all animals were sacrificed, and the prostate glands were extracted for histopathology, and blood samples were collected for biochemical analysis.

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Preparation of 70% PSEE.

The dried Seeds of *Cucurbitamoschata* were obtained from a local market. Dried seeds were then crushed into powder using clean mortar and pestle. The dried seeds were homogenized to a fine powder, and 100 g of this powder was extracted by macerating with 500 ml of 70% ethanol alcohol at room temperature for seven days with frequent agitation for several minutes. The resulting solution was vacuum-filtration through Whatman filter paper No.2, and the resultant yield was concentrated to near dryness in a rotary evaporator (Labtech, Korea) under reduced pressure at a temperature of 40 ° C to remove the organic solvent. The solvent traces were further removed by incubated at 37° C for three days until the ethanol was evaporated entirely, then stored at 4°C until use, according to the method described by [23] with some modification.

Induction of BPH

BPH was induced by subcutaneous injection of testosterone enanthate (E-JECT 300. 10 ml vial, Novector Labs. Com., India) at a daily dose of 5mg / Kg BW for 30 days. The testosterone doses were diluted in olive oil as a vehicle.

Biochemical Analysis

ELISA kits estimated serum level of dihydrotestosterone, 5α-reductase, and PSA (Rat dihydrotestosterone, DHT ELISA kit, Sunlong Biotech Co. Ltd; Rat steroid 5 Alpha Reductase 2 (SRD5a2) ELISA Kit, Sunlong Biotech Co.Ltdand Prostate Specific Antigen ELISA kit, Elabscience, U.S.A. respectively).

Histopathological Examination

Histopathological examination of prostatic tissues was done according to the routine histological examination.

Statistical Analysis

The results of the standard concentrations of rat PSA, DHT, and 5α-reductase and their concentrations in the samples were read and analyzed based on the instructions in the booklet on each specific kit by using excel to plot the standard curve and then applied in the linear regression equation (y= mX+C) to calculate the values.

All data values are displayed as a mean ± standard average error (SEM).Excel and the IBM Statistical Package for the Social Sciences (SPSS.) Statistics 22 programs (International

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Business Machines Corp., Armonk, NY, USA) were used to analyze statistical significance. Results $p < 0.05$ and $p < 0.01$ were known as the statistical significance criterion.

RESULTS

In this study, no histopathological changes in the prostatic tissue of both control negative (C1) and protective (P) groups have been identified in the first experiment (Figure 1) and (Figure 5) compare the control-positive group (CP), unlike in the normal prostatic tissue formed an epithelial lining devoid of convolutions with enormously dilated hyperplastic glandular acini containing small laminated concrete known *corpora amylaceae* (Fig4). The induction of BPH by testosterone causing significant histopathological changes in the control groups' prostatic tissue that received testosterone, namely C2 (Figure 3) and CP (Figure 4).

On the other hand, the therapeutic group (T) in the second experiment showed a significant histological improvement compared to CP, which showed benign hyperplasia of the prostate with predominant glandular proliferation and reduced stroma, which contain small laminated concretion known as *corpora amylase* (Figure 2). (Figure 6, Figure 7, and Figure 8), all of which related to the positive behavior of PSEE on BPH in the control groups. There was a decrease in DHT and 5α- reductase levels (graph 6, graph 7, and graph 8). The PSA level in the protective(P) and therapeutic(T) groups showed a dramatic depression to be less than the grey zoon area, namely (4-10ng/ ml) in comparison to the groups that underwent to induction of BPH, i.e., CP and C2 groups (Figure 6), This result indicates a great and promised therapeutic and protective activity for PSEE on BPH.

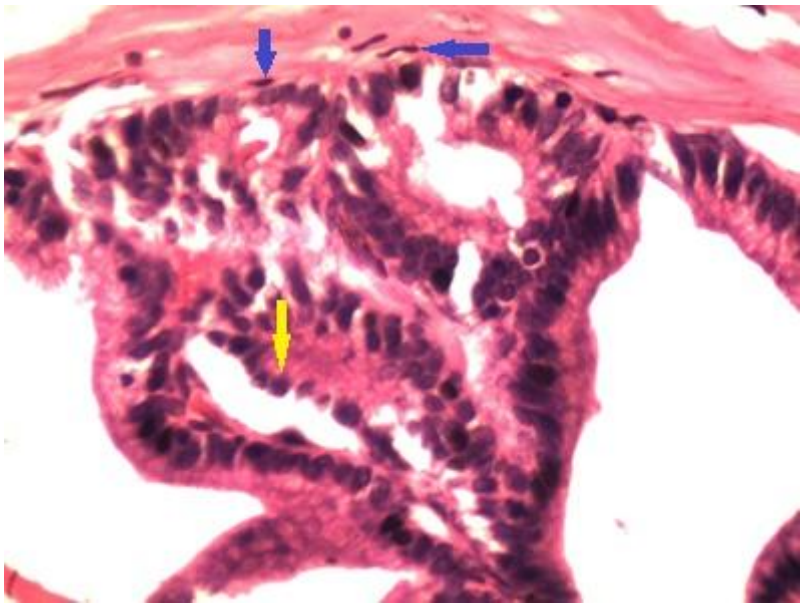

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Fig. 1-Control negative group in the first experiment (C1) shows normal prostatic tissue shows acinar cells (yellow arrow) and basal cells (blue arrows). H & E, X400

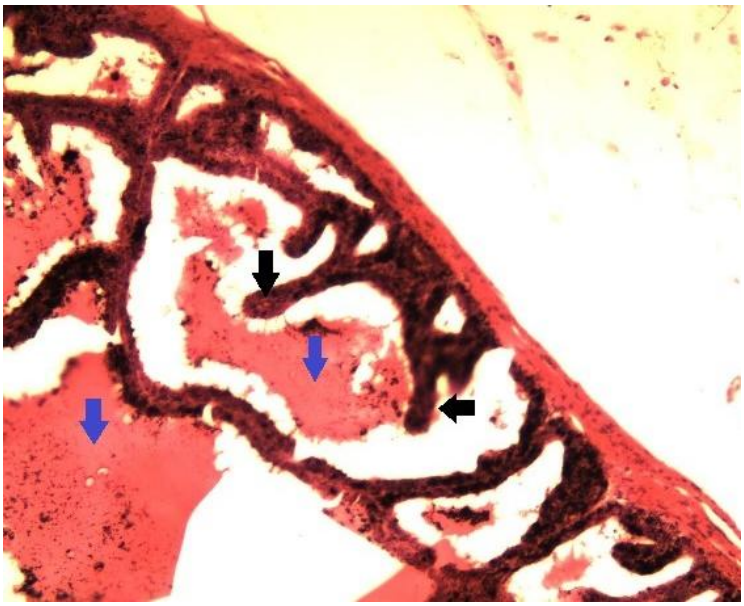

Fig. 2-Control negative group in the second experiment (C2) shows benign hyperplasia of the Prostate with predominant glandular proliferation (black arrows) and reduced stroma contains small laminated concretion known as *corpora amylaceous*(blue arrows). H & E, X100 and 400.

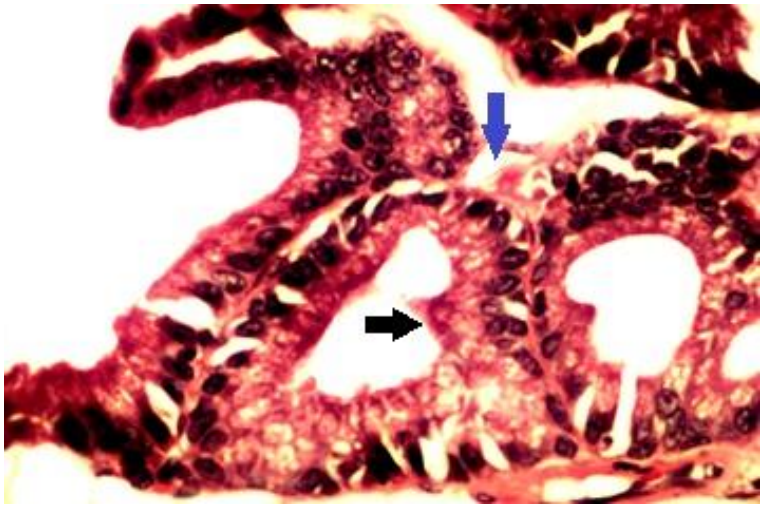

Fig. 3-Therapeutic group (T) in the second experimentshowsnormal thick intraglandular epithelia convolution (Black arrow) and scanty interglandular smooth muscle fibers (blue arrow). H&E stain, X400.

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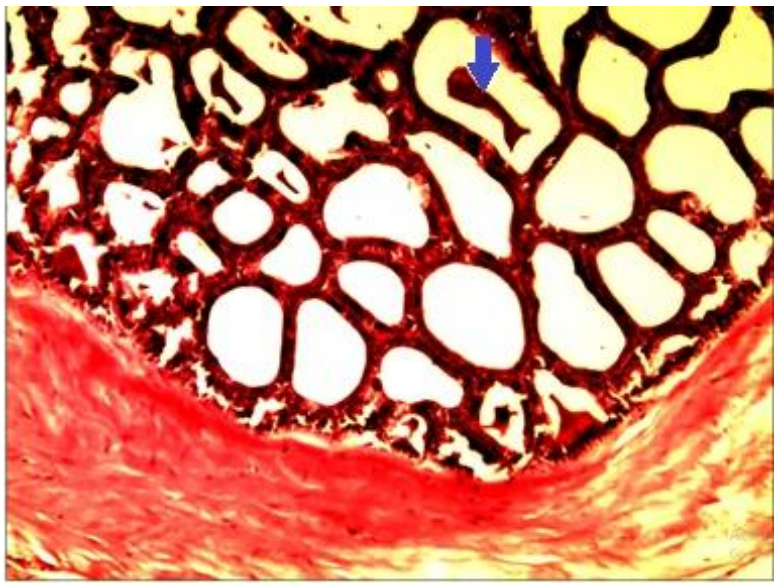

Fig. 4-Control positive group (CP) in the first experiment,the glands, unlike in the normal prostatic tissue, formed an epithelial lining devoid of convolutions and contained small laminated concretion *corpora amylaceae* (blue arrow). H&E, X100.

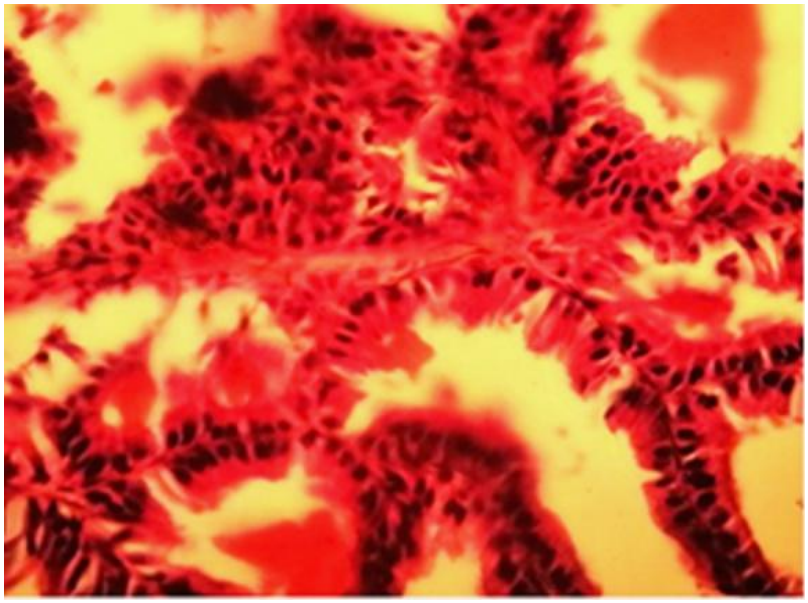

Fig.5- Protective group in the first experiment (P) **s**howed almost normal prostatic tissue, but the deposition of *corpora amylaceae* also has been noticed (black arrow). H & E, X100.

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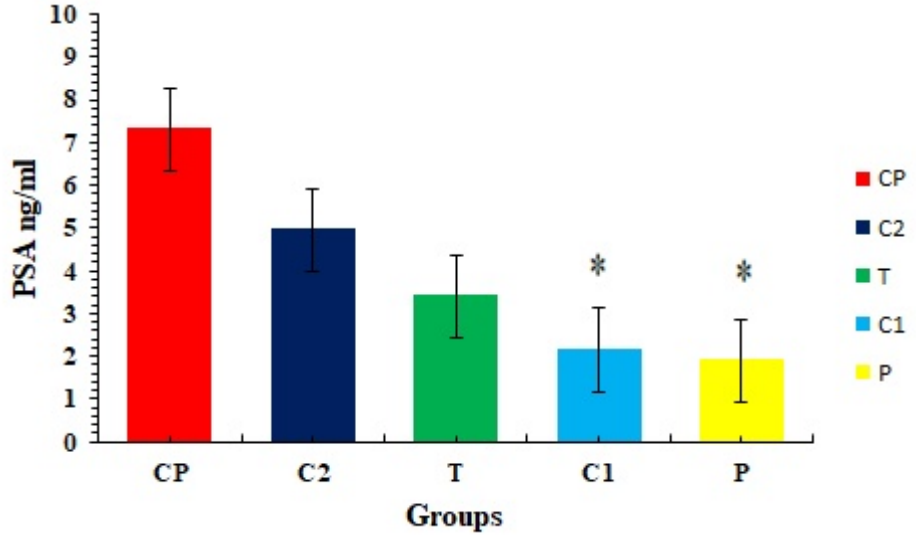
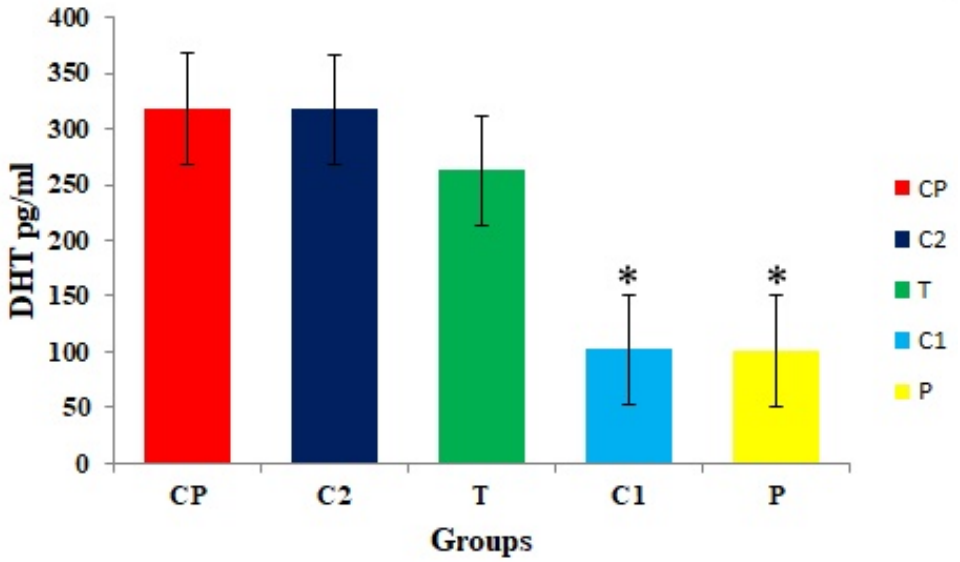


Graph 6. PSA value of different groups. *The *F*-ratio value is 10.69802. The *p*-value is 0.000084. The result is significant at $p < .05$.CP=control positive in the first experiment; C2=control negative group in the second experiment; T=therapeutic group in the second experiment; P =protective group in the first experiment; C1=control negative in the first experiment.



Graph 7. DHT values of different groups. *The *F*-ratio value is 4.96062. The *p*-value is 0.006075. The result is significant at $p < 0.05$.CP=control positive in the first experiment; C2=control negative group in the second experiment; T=therapeutic group in the second experiment; P =protective group in the first experiment; C1=control negative in the first experiment.

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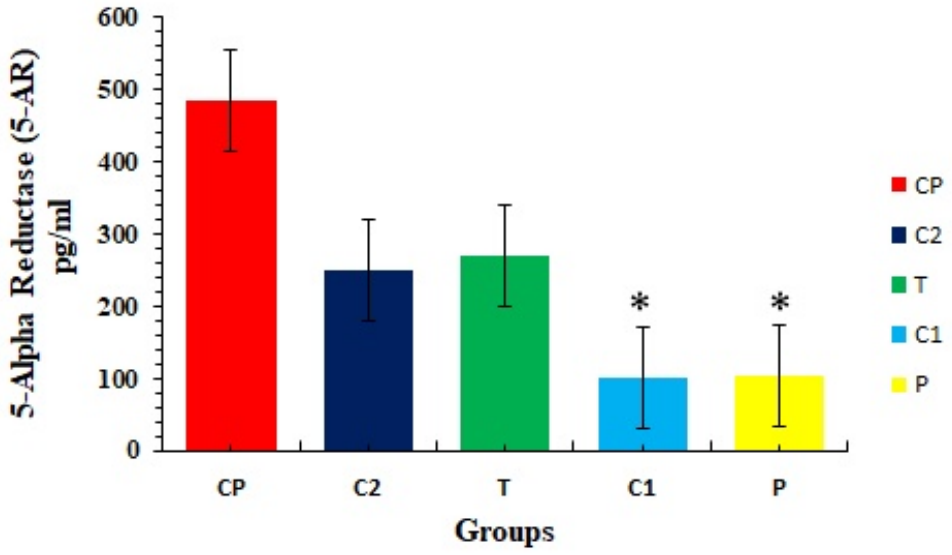

Graph 8.5α-reductase value of different groups. * The *F*-ratio value is 12.31714. The *p*-value is 0.000033. The result is significant at $p < 0.05$.CP=control positive in the first experiment; C2=control negative group in the second experiment; T=therapeutic group in the second experiment; P =protective group in the first experiment; C1=control negative in the first experiment.

DISCUSSION

Our results revealed a dramatic effect for the PSEE on the testosterone-induced BPH in rats on different lines, i.e., histological and biochemical improvement and protective and therapeutic levels. The histological results reflect the positive effects of the PSEE on BPH in rats at both the protective and curative levels. Besides, the findings of our biochemical study (Dihydrotestosterone, PSA, and 5-α-reductase) suggest the foreword of histological changes as all of the chemical mentioned above parameters showed depression in the protective (P) and therapeutic (T) groups close to the expected value in the control groups. Histologically, the use of PSEE prevents any hyperplastic changes from taking place in the defensive group (P) and also causing a significant improvement in the prostatic tissue of the therapeutic group (T) (Figure 1). These results indicate the positive protective and therapeutic activities of PSEE on the BPH. Our results agree with multiple previous studies in the same line (Xanthopoulou*et al.*, 2009; Vahlensieck*et al.*, 2014; Karawya and Zahran, 2015; Dordevic*et al.*, 2016; Shaban, 2017; Alhakamy*et al.*, 2019; Leibbrand*et al.*, 2019). A biochemical assay confirmed the histologically indicated findings for DHT, PSA, and 5-alpha reductase when all parameters in the protective (P) and therapeutic (T) groups were reduced close to their expected values, reflecting the positive effects of PSEE on BPH and suggesting its inhibitory

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activity on the 5α-reductase enzyme responsible for converting testosterone hormone to dihydrotestosterone. This outcome is concurrence with a few past investigations in a similar line (Perez Gutierrez, 2016; Alhakamy*et al.*, 2019), but contradicting the consequence (Shirvani et al., 2014), who recorded no pumpkin impact on PSA value. The PSA value in the protective(P) and therapeutic (T) groups was reduced to be within the average level, i.e., less than the grey zone area (4-10ng/ml) (Malati*et al.*, 2006). Reducing the PSA level less than the grey zone area reflects the Prostate's regular activity (Xanthopoulou*et al.*, 2009), which indicates the positive effect of PSEE on protecting and treating BPH. The positive activity of PSEE on BPH may be due to the phytosterols content of PSEE, which is well known to interfere with dihydrotestosterone biological actions (Perez Gutierrez, 2016). On the other hand, the specific effect of 5α-reductase inhibitors may be through elaborating the epithelial tissue of the Prostate, which is the primary source of PSA; the reduction of dihydrotestosterone by 5α-reductase inhibitors indirectly lead to PSA reduction (Kim*et al.*, 2015).

CONCLUSIONS

Our results revealed that the pumpkin seed ethanolic extract has curative and prophylactic activities on testosterone-induced BPH in rats, with a potential preventive effect comparable to corrective activity.

ANIMAL RIGHTS STATEMENT

The experiments on animals were conducted following the local Ethical Committee laws and regulations regarding the care and use of experimental animals.

All procedures performed in studies involving animals were under the institution's ethical standards or practice at which the studies were conducted. The ethical status of animals' use in our research was clarified in detail in the materials and process section, including their references.

ETHICAL APPROVAL

The manuscript was not previously published in any language and is not considered in any other peer-reviewed media in the same or substantially similar way. This study was conducted under the ethical committee's permission in the college of veterinary medicine, university of Al-Qadisiayh (Ref. No. 57/2020).

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CONFLICTS OF INTEREST STATEMENT

The authors announce that the publishing of this article is not a conflict of interest.

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