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Modulatory effects of *Saccharomyces cerevisiae* var. *boulardii* on experimentally induced benign prostatic hyperplasia in rats

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Benign prostatic hyperplasia (BPH) is an age-related non-neoplastic disease of the prostate gland in men that has become a global health issue in recent years. Due to the side effects of conventional treatment options, attention is now focused on phytotherapeutics for its management. We investigated the possible protective effect of *Saccharomyces cerevisiae* var. *boulardii* in a rat model of testosterone propionate (TP) induced BPH. Rats were divided into five groups: Gr. I, untreated control group; Gr. II, TP group; Gr. III, TP + finasteride; Gr. IV, TP + *S. cerevisiae* var. *boulardii*; and Gr. V, *S. cerevisiae* var. *boulardii* group. Treatments were given daily for 28 days. At the end of the experiment, all rats were weighed and the prostatic indices, prostate specific antigen, serum testosterone concentration as well as the histological and histomorphometric changes were evaluated. *Saccharomyces cerevisiae* var. *boulardii* significantly (P <0.05) reduced prostate weight, prostatic index, serum prostate specific antigen, prostatic epithelial thickness and increased luminal diameter. Thus, the results of this study suggest that *S. cerevisiae* var. *boulardii* is a potential pharmacological candidate for management of benign prostatic hyperplasia.

Keywords: Dihydrotestosterone, Probiotic, Prostate gland, Rodents, Testosterone propionate, Yeast

Benign prostatic hyperplasia (BPH) is an age-related disease in men that lead to prostate enlargement which constrict the urethra to cause urinary outflow obstruction. It is a common disease in elderly men that affects over 42% of men in their 50s and more than 80% of octogenarians[1]. The etiology of BPH is still largely unresolved though the patho-etiologic mechanism is known to be endocrine controlled and involves alterations in the delicate balance between androgens and estrogens[2]. Typical symptoms include increased frequency of urination, nocturia, urgency, hesitancy, and weak urine stream[3-6].

Treatment options for BPH include watchful waiting, medical therapy with alpha blockers or 5 alpha reductase inhibitors, hormone therapy and surgery[5-8]. The problem with these conventional treatment methods is that they are associated with severe side effects like decreased libido and ejaculation, or erectile dysfunction[9-11]. Thus, alternative therapy such as plant extracts is now frequently used as a complementary treatment of BPH[12,13]. However, the effects of probiotics such as *Saccharomyces cerevisiae* var. *boulardii* (SC_b) on benign prostatic hyperplasia (BPH) have not been investigated.

Probiotics are live microorganisms that confer health benefits on the host when administered in adequate amounts[14-17]. It is reported that *Saccharomyces cerevisiae* var. *boulardii* is a type of yeast that can synthesize β-glucan in their cell wall and thus possess immunomodulatory, antioxidant and anti-inflammatory effects[18]. Thus, in this study, we investigated the possible inhibitory effects of *Saccharomyces cerevisiae* var. *boulardii* in a rat model of testosterone propionate (TP) induced Benign prostatic hyperplasia (BPH).

Materials and Methods

Chemicals

Finasteride, the standard drug used in the treatment of BPH and testosterone propionate used to induce BPH were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA).

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Source of *Saccharomyces cerevisiae* var. *boulardii* (SC_b)

Commercial probiotic (FloraNorm®) containing 250 mg of 5 billion live yeast cells of the fungus *Saccharomyces cerevisiae* var. *boulardii* manufactured by Biotech International Limited, Hyderabad, India was obtained from Prisma Pharmaceutical Limited and used for this study.

Animals

A total of 35 male 10-12 weeks old healthy Sprague-Dawley outbred albino rats (*Rattus norvegicus*) weighing between 150-200 g were purchased from a commercial breeder. The rats were housed 7 per cage in standard polypropylene cages of size 60×45×45 cm with wood shavings as bedding in the Experimental Animal Unit and acclimatized at a temperature of 25±4°C and relative humidity of 65±5% with an alternating 12 h light and dark cycle for two weeks. They were fed pelleted diet and given water *ad libitum.* The experiment was performed in compliance with the NIH Guidelines on the Care and Use of Laboratory Animals[19], with institutional approval number-FVM/UNN2020-0246.

Experimental design

After an acclimatization period of two weeks, the rats were randomly divided into five groups of seven rats each based on body weight in a completely randomized design as follows: Gr. I, control group given PBS orally and corn oil S.C. once daily; Gr. II, TP group (3 mg/kg S.C. once daily); Gr. III, TP @3 mg/kg S.C. + finasteride 10 mg/kg orally once daily; Gr. IV, TP @3 mg/kg S.C. + 500 mg/kg SC_b orally once daily; and Gr. V, 500 mg/kg SC_b group given orally once daily for 28 days. The technologist and histopathologist who analyzed the samples were blinded to the group allocation. The effective dose of finasteride for the treatment of BPH and testosterone used to induce BPH was determined from a previous study[20]. *Saccharomyces cerevisiae var boulardii* was administered to rats at the dosage of 500 mg/kg[18]. The volume for oral administration of PBS, finasteride, and SC_b orally once daily was 5 and 2 mL/kg for S.C. injection of corn oil and TP, respectively.

Body and prostatic weights

Baseline body weights (BW) were taken on the first day of the treatment and on the final day of the study while the prostates were dissected out and weighed after sacrificing the animals under anesthesia on the day of sample collection. The prostatic index was then calculated for each group using the formula:

$$\text{Prostatic index} = \frac{\text{Prostate weight (PW)} \times 100}{\text{Body weight (BW)}}$$

Sample collection

At the end of the experiment, the rats were fasted overnight, weighed and 2 mL of blood was collected in the morning via the retro-orbital plexus and into tubes without EDTA in order to obtain serum, after anaesthesia by intraperitoneal injection of 100 mg/kg body wt. ketamine hydrochloride (Laborate Pharmaceutical, India) and 5 mg/kg body wt. xylazine (Kepro Holland)[21-22]. The samples were centrifuged at 1000 *g* for 10 min for serum collection which was used for assay of serum testosterone and prostate specific antigen (PSA). Thereafter, the animals were dissected and the prostates were removed, weighed and fixed in 10% buffered formalin.

Assay for serum testosterone

ELISA kits (Shanghai Yi Li Biological Technology, Shanghai, China) were used for the quantitative determination of testosterone concentration according to the manufacturer's instructions. In this technique, the samples were added to micro-plate wells and absorbance was read in duplicates at 405 nm. The results were expressed in ng/mL.

Assay for prostate-specific antigen (PSA)

The PSA ELISA kit (Cusabio Biotech Co. Ltd) was used for the quantitative determination of total PSA based on the direct sandwich technique[23]. The samples were incubated with anti-PSA monoclonal antibody and horseradish peroxidase labelled anti-PSA monoclonal antibody in streptavidin-coated microtitre stripes. Buffered substrate (TMB-HRP substrate) that contains hydrogen peroxide and chromogen reagent (3, 3¢, 5, 5¢ tetra methyl benzidine) was added to each well after washing, and the enzyme reaction was allowed to proceed. The microtitre plate spectrophotometer was used to determine the color intensity at 620 nm. Thereafter, calibration curves were constructed for each assay from which the concentration of PSA was read. The values were expressed in ng/mL

Histopathology

The prostate glands were prepared for histopathological studies after fixing in 10% buffered formalin. The samples were then dehydrated in ascending grades of ethanol gradient (70, 80 and 90%) for 1 h 30 min each, 100% absolute ethanol for 1 h 30 min (two changes) before they were cleared in chloroform overnight, followed by infiltration with paraffin in a bath at 60°C for 1 h 30 min (two changes), after which they were embedded in paraffin. Five-micrometer thick sections of the samples were cut using a rotary microtome (Shandon, Finesse 325,

ThermoFisher Scientific, Luton, England). The sections were then floated in a water bath at a 40°C to spread before they were mounted on glass slides coated with egg albumin. Thereafter, sections were deparaffinized in xylene (two changes) for 15 min each and rehydrated through descending grades of ethanol (100, 90, 80 and 70%) for10 min each and then changed to distilled water for 15 min. Sections were then stained with haematoxylin and eosin, and Periodic Acid Schiff (PAS), respectively, for light microscopy[24]. Photomicrographs of the sections were captured using a Motic Images plus 2.0 cameras (Motic China Group Ltd. 1999-2004).

Histomorphometry

A random selection of 10 acini from the haematoxylin and eosin stained sections were used to measure the luminal diameter (LD) and epithelial thickness (ET) with the aid of Motic Images software in micrometers.

Statistical analysis

Statistical analysis of the data was done using SPSS statistical software. Multiple comparisons were performed using one-way ANOVA followed by *Duncan's* multiple range post-hoc tests (P value <0.05). Values were expressed as means ± standard error of the mean (S.E.M).

Results

Body and prostatic weights

The mean body weight, prostate weight and prostatic index of untreated TP-induced BPH group was significantly (P <0.05) higher compared to the normal control. Also, the mean body weight, prostate weight and prostatic index of TP + finasteride (Gr. III), TP + SC_b (Gr. IV) and SC_b group (Gr. V) was significantly (P <0.05) lower compared to the untreated TP-induced BPH group (Gr. II), but were not significantly (P >0.05) lower than that of the normal control (gr. I) as shown in Table 1.

Testosterone

Serum testosterone concentrations are presented in Fig. 1A. The mean serum testosterone concentration of untreated TP-induced BPH group, TP + finasteride and TP + SC_b groups were significantly (P <0.05) higher compared to the control group while the mean

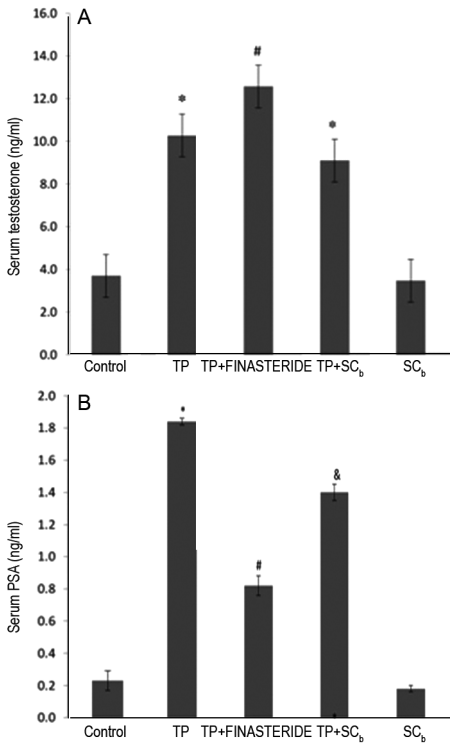

Fig. 1 — Mean serum (A) testosterone; and (B) prostate-specific antigen (PSA) concentration of the experimental groups: Gr. I, control; Gr. II, 3 mg/kg testosterone propionate (TP) only; Gr. III, 3 mg/kg TP+10 mg/kg. finasteride; Gr. IV, 3 mg/kg TP+500 mg/kg SC_b; and Gr. V, 500 mg/ kg SC_b only). [Values are expressed as mean ± SEM (n=7). A value of P <0.05 was considered to be statistically significant. Significance was determined by one-way ANOVA followed by Post-hoc test. *P <0.05, significantly higher than the normal control, TP + finasteride, TP + SC_b and SC_b treated group. $^{\#}P$ <0.05, significantly lower than TP and TP + SC_b group but higher than the control and SC_b treated group. $^{\&}P$ <0.05, significantly lower than the TP group but higher than the control, TP + finasteride and SC_b group]

Table 1 — Effect of *Saccharomyces cerevisiae var boulardii* on body weight, prostate weight and prostatic index of rats with experimentally induced BPH

Group	Initial body weight (g)	Final body weight (g)	Weight change (g)	Prostate weight (g)	Prostatic index (%)
Control	151.36±3.09	224.70±8.0[a]	73.34±1.02[a]	0.40±0.04[a]	0.18±0.02[a]
TP	153.84±0.36	250.50±11.80[b]	96.66±0.34[b]	1.10±0.10[b]	0.44±0.03[b]
TP+ finasteride	153.84±0.36	221.10±11.15[a]	67.26±5.21[a]	0.50±0.10[a]	0.22±0.04[a]
TP + SC_b	153.92±0.57	216.70±12.09[a]	62.78±9.45[a]	0.65±0.05[a]	0.30±0.03[a]
SC_b	153.68±0.39	221.70±7.53[a]	68.02±4.42[a]	0.64±0.04[a]	0.29±0.02[a]

[Values are expressed as mean ± SEM (n = 7). Values bearing different superscripts in the same column differ significantly at P <0.05. Significance was determined by one-way ANOVA followed by Post-hoc Test. SC_b, *Saccharomyces cerevisiae var boulardii*; TP, Testosterone propionate. [a] Significantly lower than the untreated TP group. [b] Significantly higher than the control, TP + finasteride, TP + SC_b and SC_b group]

serum testosterone concentration of the SC_b group was not significantly (P >0.05) different from the control group.

Prostate-specific antigen

The mean serum PSA value of TP-induced BPH group was significantly (P <0.05) higher compared to the normal control. However, TP+ finasteride treated group had a significantly (P <0.05) lower level of PSA in serum compared to the TP-induced BPH group. Also, TP + SC_b group had a significantly (P <0.05) lower PSA levels compared to the TP-induced BPH group while mean serum PSA value of the SC_b group was not significantly (P >0.05) different from the control (Fig. 1B).

Histopathology

The normal control group showed normal histological features of variable sized acinar diameter and lumen filled with eosinophilic prostatic secretions. These were separated by a thin connective tissue stroma with blood and lymph vessels as well as nerves (Fig. 2Ai). The prostates of TP-induced BPH group rats showed severe epithelial hyperplasia and irregular acinar shapes with papillary projections towards the lumen which was filled with increased eosinophilic secretion. There was compression of the connective tissue with dilation of the blood vessels and inflammatory cells infiltration compared to the control. Some of the tubules were also obliterated (Fig. 2Aii). The TP + finasteride group showed a decreased glandular lesion as normal stroma was seen with reduced papillary projections compared to TP-induced BPH group in Fig. 2AiiiC while the TP + SC_b group showed moderate restoration of glandular structures compared to the TP-induced BPH group. There were few thick tubular epitheliums with mild to moderate involutions. Restoration of acinar diameter and reduction in fibrous tissue proliferation and cell infiltration were seen (Fig. 2Aiv). However, SC_b group showed no lesion (Fig. 2Av).

Histochemistry

Histochemical staining with PAS showed a strong positivity for neutral mucin in the intra-luminal secretions of the prostate in the TP-induced BPH group (Fig. 2Bii) compared to the normal control (Fig. 2Bi). However, the positivity for neutral mucin in the secretions was reduced in the TP + finasteride group (Fig. 2Biii), while the TP + SC_b group had moderate positivity to PAS compared to TP-induced BPH group (Fig. 2Biv). The PAS positivity of the SC_b group was comparable to that of the control (Fig. 2Bv).

Histomorphometry

Histomorphometric evaluation showed that the epithelial thickness of the prostates was significantly (P <0.05) higher in the TP-induced BPH group compared to the control. The TP + finasteride group had a significantly (P <0.05) lower epithelial thickness compared to the TP-induced BPH group. The epithelial thickness of the prostates of the TP + SC_b group was lower than the TP-induced BPH group but not significantly (P >0.05) different. However, the epithelial thickness of the SC_b group was not significantly (P >0.05) different from the control (Fig. 3A). Consequently, the luminal diameter of the prostate in the TP-induced BPH group was significantly (P <0.05) lower compared to the control while the luminal diameter of the TP + finasteride

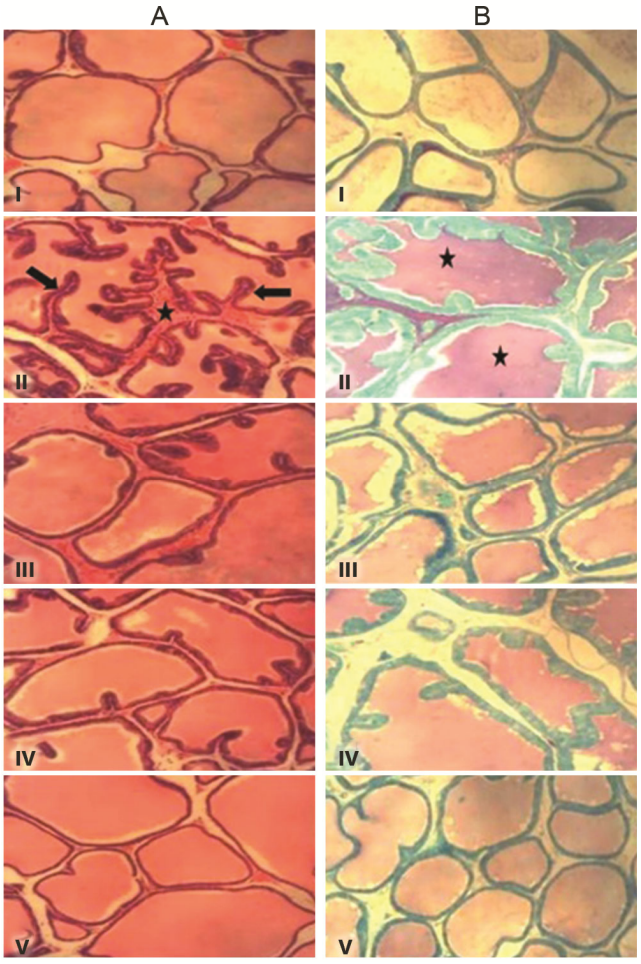

Fig. 2 — Photomicrographs of the cross-section of (A) prostate; and (B) PAS stained prostate of rats (n= 7) from: the experimental groups: Gr. I, (control); Gr. II, (3 mg/kg TP only); Gr. III, (3 mg/kg TP + 10 mg/kg finasteride); Gr. IV, (3 mg/kg TP + 500 mg/kg SC_b); and Gr. V, (500 mg/ kg SC_b only). [Note the strong PAS positivity in B (asterisks), PAS stain.100X]

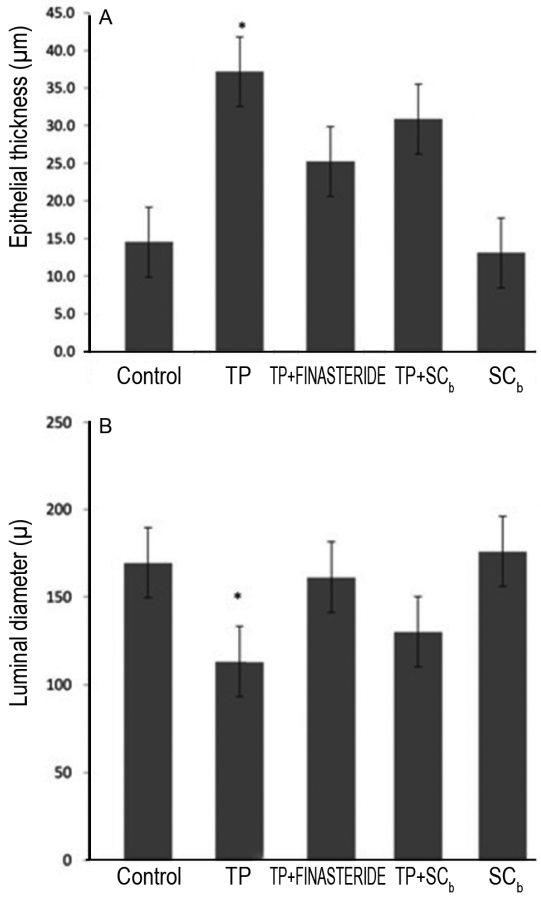

Fig. 3 — Mean (A) epithelial thickness; and (B) luminal diameter of prostatic acini of the experimental groups: Gr. I, control; Gr. II, 3 mg/kg testosterone propionate (TP) only; Gr. III, 3 mg/kg TP + 10 mg/kg finasteride; Gr. IV, 3 mg/kg TP + 500 mg/ kg SC_b; and Gr. V, 500 mg/ kg SC_b only). [Values are expressed as mean ± SEM (n=7). A value of P <0.05 was considered to be statistically significant. Significance was determined by one-way ANOVA followed by Post-hoc test. *P <0.05, significantly different from normal control and SC_b treated group]

group was significantly (P <0.05) higher compared to the TP-induced BPH group. Also, the luminal diameter of the TP + SC_b group was significantly (P <0.05) higher compared to the TP-induced BPH group. The luminal diameter of the SC_b group was not significantly (P >0.05) different from the control (Fig. 3B).

Discussion

At birth, the prostate is small (1.5 g) until puberty when its size increases through an androgen dependent activation to an average weight of 20±6 g in young adults. This first phase of growth and remodelling of the entire prostate gland (peripheral, central and transitional zones) is followed by a second growth phase which specifically involves the transitional zone in more than 50% of men by age 50 or 90% of men above 80 years of age[25]. This form of the second growth phase is known in pathology as BPH and medically recognized as benign prostatic obstruction or benign prostatic enlargement (BPE), which is associated with increased prostate weight and lower urinary tract symptoms (LUTS) as earlier stated. In addition to aging, a number of modifiable factors, such as obesity, diet, dyslipidemia, hypertension, smoking, metabolic syndrome, alcohol, and hormonal imbalance also play a role in the development of benign prostatic hyperplasia[26]. Although, spontaneous BPH is common in man than other species, it has been described in the chimpanzee and dog[27]. Due to the resemblance of many features of BPH in dogs to that in man, old dogs with spontaneous benign prostatic hyperplasia have been used for the study and evaluation of anti-BPH drugs. However, due to the low availability and high cost of old dogs several *in vivo* experimental BPH models have been developed in other species by hormonal induction for the comprehension and development of new preventive and therapeutic measures to combat this disease. Thus, animal models such as rats are commonly regarded as the best model for the study of BPH as they are relatively cheap with well-known physiology and genetics.

We evaluated the inhibitory effects of *Saccharomyces cerevisiae* var. *boulardii* on experimentally-induced prostatic hyperplasia in rats. The study showed that the induction of BPH significantly increased the body weight as well as the prostate weight and prostatic index compared to the normal control. It is well known that increased prostate weight and prostatic indices are reliable and vital indicators of BPH[28-31]. For these reasons, many studies have evaluated the inhibitory effects of various materials on the development of BPH by measuring prostate weight[32]. The significant increase in prostate weight and prostatic index observed in the TP- induced model group compared to the control confirmed our successful induction of BPH in this study. Although, the influence of testosterone on BPH is not fully elucidated , it is reported that it activates an androgen receptor (AR) on prostatic cells to induce growth when it is converted to its more active metabolite dihydrotestosterone (DHT), by the prostate isoenzymes 5α-reductase type 1 (5AR1) and type 2 (5AR2), which is responsible for activating the AR[25]. In this study, treatment with finasteride significantly

reduced the body weight as well as the prostate weight and prostatic index compared to the control. This is similar to previous reports on the effect of finasteride on BPH[33].

Finasteride is a 5α-reductase inhibitor which blocks the conversion of testosterone to dihydrotestosterone. This reduces the prostatic and serum dihydrotestosterone concentration as well as prostate size and weight[34]. The oral administration of *Saccharomyces cerevisiae* var. *boulardii* to TP-induced BPH moderately reduced the prostate weight and prostatic index in this study. The significant increase in expression of serum testosterone in the untreated TP-induced BPH group Gr. III) as observed in our experiment could be attributed to the exogenous testosterone administration. The finasteride treated BPH group had increased serum testosterone concentration compared to the control. This high level of testosterone in finasteride treated groups compared to the control is due to its inhibition of 5α-reductase, the enzyme responsible for the conversion of testosterone to dihydrotestosterone, which promotes excessive prostatic stromal and epithelial cell growth[34]. Although, the serum testosterone concentration in *Saccharomyces cerevisiae* var. *boulardii* treated BPH group was lower than the untreated TP-induced BPH group, it was still higher than that of the control which points to its 5a-reductase inhibitory activity leaving free testosterone in serum.

The prostate specific antigen (PSA) is a protein produced by the normal prostate cells. However, its increase in blood or serum beyond normal is indicative of prostatic disease[35]. Thus, the level of PSA in serum is abnormally elevated in patients with prostate inflammatory conditions, prostate cancer and BPH. In this study, exogenous testosterone administration increased the PSA levels in the TP-induced BPH group which is an indication of prostatic hyperplasia[36]. However, treatment with finasteride and *Saccharomyces cerevisiae* var. *boulardii* significantly reduced the serum PSA levels suggesting their ameliorative effects.

Histological examination of the prostate of TP-induced BPH group in this study showed severe epithelial hyperplasia and irregular alveolar shapes with papillary projections towards the lumen which was filled with increased eosinophilic secretion. There was compression of the connective tissue with dilation of the blood vessels and inflammatory cells infiltration. This is in agreement with previous reports on the lesions of experimental BPH in rats[37]. The *Saccharomyces cerevisiae* var. *boulardii* treated BPH group showed moderate recovery, decreased epithelial thickness and increased luminal diameter with absence of inflammation compared to the untreated TP-induced BPH group, suggesting that *S. cerevisiae* var. *boulardii* has the potential to ameliorate these lesions of BPH. The mechanism of action of *S. cerevisiae* var. *boulardii* in ameliorating these lesions of BPH could be attributed to its ability to produce vanillin which is oxidized to vanillic acid, a dihydroxybenzoic derivative reported to have an anti-inflammatory and antioxidant effect[38]. This acid is reported to have inhibitory effect on BPH through the downregulation of 5α-reductase (5AR), androgen receptors (AR) and estrogen receptors[39].

Also, yeasts are macrofungi, which are known to possess β-glucans that are reported to have ameliorative effects on BPH[40]. Probiotic yeasts and their metabolites have also been reported to regulate cellular proliferation, differentiation, apoptosis and inflammation[41-44]. The finding of this study confirms the hypothesis that *S. cerevisiae* var. *boulardii* has the potential of ameliorating the negative effects of benign prostatic hyperplasia.

Conclusion

In this study, the therapeutic potential of *Saccharomyces cerevisiae* var. *boulardii* was carried out on experimental benign prostatic hyperplasia in rats through biochemical, histopathological and histochemical studies. Treatment with *S. cerevisiae* var. *boulardii* ameliorated increased serum PSA as well as histopathological alterations in the prostate. *Saccharomyces cerevisiae* var. *boulardii* is a rich source of proteins, antioxidants, vitamins and contain a large number of bioactive compounds such as polysaccharides (beta-glucans) and polyphenolic metabolites (vanillic acid, cinnamic acid) which might be responsible for the observed bioactivity of the yeast. Results obtained have shown protective potential of *S. cerevisiae* var. *boulardii* in the management of prostatic hyperplasia in rats. However, there is need to isolate and identify the specific active principle responsible for the ameliorative effect and to establish its mechanism of action. Further research on its synergistic effects on BPH when used simultaneously with other medications is recommended.

Conflict of interest

Authors declare no competing interest

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