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Rice bran oil ameliorates the symptoms of benign prostatic hyperplasia in male Wistar rats

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Abstract

Objective: Benign prostatic hyperplasia (BPH) is the proliferation of prostatic cells and the growth of the prostate gland in elderly men. Oils that are rich in free fatty acids have been reported to be potentially effective in treating BPH. In the present study, we investigated the effect of rice bran oil (RO) on the treatment of testosterone enanthate (TE)-induced experimental BPH in male Wistar rats.

Materials and Methods: An animal model of BPH was established by subcutaneous administration of TE to Wistar rats for 28 days. RO was administered by oral gavage daily before TE/corn oil injection at a dose of 400 and 800 mg/kg body weight. All the rats were sacrificed at the end of the experiment and we measured prostate index (PI), histological changes, and activities of antioxidant enzymes. Moreover, we assessed the level of prostate-specific antigen (PSA) in the serum.

Results: Our findings indicate that RO significantly inhibited the development of BPH in experimental rats, reduced PI ($p<0.0001$), decreased PSA in serum ($p<0.01$), increased antioxidant enzyme activities (Glutatione (GSH), super oxide dismutase (SOD), and catalase (CAT); $p<0.0001$), and decreased concentration of oxidative stress biomarkers advanced oxidation protein products (AOPP), and nitric oxide (NO) ($p<0.0001$) compared to positive controls. Hematoxylin & Eosin staining demonstrated that RO decreased pathological changes in the prostate of experimental animals. The treatment effect of RO was better exhibited in rats receiving RO at high dose.

Conclusion: These results suggest that RO may be used as a therapeutic agent for BPH as oral administration of RO significantly halted the disease progression via anti-proliferative, antioxidant, and anti-inflammatory activity.

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Introduction

Benign prostatic hyperplasia (BPH) is the benign and uncontrolled growth of the prostate gland that leads to bladder outflow obstruction and lower urinary tract symptoms (Yoo and Cho 2012). The prevalence of BPH is approximately 2500 per 100,000 people which is prominent in men over 50 years of age, and the frequency increases with age (Awedew et al. 2022; Barry and Roehrborn 2001; Bhargava et al. 2004). Hormonal changes in the aging man are hypothesized to be the prime factor of this disease although the etiology is not yet fully understood. Hyperplasia of stromal and glandular epithelium is a common feature of BPH and it occurs in the periurethral transition zone of the prostate that surrounds the urethra. This pathological change leads to an uncomfortable lower urinary tract symptom (Alonso-Magdalena et al. 2009).

Testosterone, a male sex hormone, is highly involved in developing BPH (Altwein and Baur 1992). This hormone is transformed into dihydrotestosterone (DHT) via the enzymatic action of 5α-reductase in the prostate and DHT is associated with the enlargement of the prostate gland's size (Sciacqua et al. 2023). Androgen hormones, mainly DHT control the growth and development of the prostate gland. This hormone binds to androgenic receptors and initiates protein synthesis and cellular growth (Silver et al. 1994). Elevated prostatic DHT level promotes excessive prostatic epithelial and stromal cell growth, resulting in prostate hyperplasia (Bartsch et al. 2002; Carson and Rittmaster 2003). Moreover, BPH causes augmented adrenergic tone in prostate smooth muscle by α_1 adrenoreceptors (Michel 2002). Finasteride, a 5α-reductase inhibitor, blocks the conversion of testosterone to DHT by deactivating the activity of 5α-reductase (Ishola et al. 2017; Pais 2010). and thus, reduces the enlarged prostate and associated complication (Lam et al. 2003; Sandhu and Te 2004). Apart from finasteride, other treatment options are surgical methods, α_1- receptor antagonists, and other 5α-reductase inhibitors like dutasteride, and they are the currently used medications to treat BPH (Al-Trad et al. 2019). However, these drugs have severe side effects including headache, loss of libido, gynecomastia, and erectile dysfunction (Mostafa et al. 2019). Therefore, there is an unmet need for developing medications that could prevent and manage BPH with a better treatment profile i.e. having less harmful side effects (Al-Trad et al. 2019). Numerous studies proved that natural substances can be used as therapeutic agents with minimal adverse outcomes that could help treat various diseases (Lin et al. 2013).

The fatty acids found in natural edible oils play a vital role in the treatment and management of BPH and this could be due to their inhibitory capacity on the activity of the enzyme 5α-reductase. Therefore, phytotherapeutic agents such as Saw palmetto (*Serenoa repens*) lipid extracts, olive oil, and coconut oil have been shown to be effective in the treatment of BPH (de Lourdes Arruzazabala et al. 2010; Oyelowo et al. 2019). Among the fatty acids, lauric acid is the most effective (Liang and Liao 1992; Niederprüm et al. 1994) whereas similar properties were exhibited by myristic acid, and oleic acid. Lauric and oleic acid inhibited both isoforms of 5α-reductase (de Lourdes Arruzazabala et al. 2010) whereas myristic acid has been reported to be effective in the inhibition of the type 2 isoform of the enzyme (Raynaud et al. 2002). Therefore, oils that contain high amounts of these free fatty acids have the potential to be effective in the treatment of BPH.

In recent times, rice bran oil (RO) has emerged as a popular alternative to traditionally used oils such as soybean oil and mustard oil in Bangladesh because of its balanced fatty acid profile and high antioxidants (Ali et al. 2019). It contains a high amount of oleic, linoleic acid, and myristic acid (Dunford 2019). Moreover, it

contains a relatively higher proportion of monounsaturated and polyunsaturated fatty acids compared to saturated fatty acids (Abbas Ali et al. 2019). Therefore, we can hypothesize that RO consumption may reduce the risk of BPH or be effective in the management of BPH in elderly men.

To the best of our knowledge, no study has been conducted so far on the treatment effect of RO on BPH. We aim to investigate the effect of rice bran oil on testosterone enanthate-induced experimental BPH in male Wistar rats.

Materials and Methods

Chemicals and drugs

Testosterone enanthate (TE; Panpharma, Germany) was purchased from Evercare Hospital, Dhaka, Bangladesh. Each ampoule contained 250 mg/ml of testosterone which was diluted to 25 mg/ml using corn oil to induce BPH in rats. Raw finasteride powder was generously donated by Square Pharmaceuticals PLC, Dhaka, Bangladesh. Tween 20 was used as a vehicle in this study, which was prepared by suspending it in water (4%). Rice bran oil (ACI Edible Oils Ltd.) was purchased from the local market.

Gas chromatographic (GC) analysis of rice bran oil

The fatty acid composition of RO was assessed by gas chromatography (GCMS-QP2050, Shimadzu, Kyoto, Japan). Briefly, the methyl-esters of fatty acids were prepared by methylation, using acetyl chloride: methanol (10:90 v/v). Gas chromatography was conducted, with a flame ionization detector and a Solgelwax column (30 m × 0.25 mm film thickness, 0.25 m internal diameter). The oven was heated from 80°C to 180°C at the rate of increasing 20°C per min, and from 180°C to 280°C at the rate of increasing 4°C per min. The injector and detector were set at 270°C and 275°C, respectively. Hydrogen at 1.3 ml/min was used as the carrier gas. Standards of each fatty acid were used to identify each peak observed. Concentrations were calculated from peak areas, using tridecanoic acid as the internal standard. The GC analysis was carried out at the laboratories of Bangladesh Council of Scientific and Industrial Research, Dhaka. The composition of the RO determined by this process is mentioned in Table 1.

Table 1. Compositions of fatty acids in rice bran oil used in this research study determined by gas chromatography

Serial No.	Fatty Acid	Lipid Numbers	Results (% of total fatty acid)
1	Myristic acid	(C14:0)	0.2503
2	Palmitic acid	(C16:0)	20.1022
3	Stearic acid	(C18:0)	3.2122
4	Oleic acid	(C18:1)	42.3756
5	Linoleic acid	(C18:2)	30.6658
6	Linolenic acid	(C18:3)	1.2448
7	Arachidic acid	(C20:0)	0.9373
8	Eicosenoic acid	(C20:1)	0.4911
9	Behenic acid	(C22:0)	0.3082
10	Erucic acid	(C22:1)	Not detected
11	Lignoceric acid	(C24:0)	0.4125

Animals

Male Wistar rats, weighing 250–300 g, were collected from the animal resource center of the North South University. They were kept in the animal house for proper adaptation to laboratory conditions (temperature 25±3°C, relative humidity 60±5%, light/dark cycles of 12 hr) for 7 days. Free access to food and water was provided. Animal handling was conducted in accordance with the regulations for the use of laboratory animals and ethical principles of animal management. All experiments were approved by the Institutional Animal Care and Use Committee of the North South University (approval number: 2021/OR-NSU/IACUC/1105).

Experimental BPH induction and euthanization

A total of thirty (30) male Wistar rats were used in this study and they were randomly assigned to five groups of six animals each, as follows:

Group 1 (Control): Received subcutaneous injection (sc) of corn oil and oral administration (p.o.) of phosphate-buffered saline (PBS);

Group 2 (BPH model): Received sc injection of testosterone enanthate, TE (5 mg/kg bw to induce BPH and oral administration of PBS;

Group 3 (BPH+Finasteride): Received TE (5 mg/kg) subcutaneously and finasteride (10 mg/kg bw, p.o.);

Group 4 (BPH+RO 400): Received TE (5 mg/kg) subcutaneously and rice bran oil (400 mg/kg bw, p.o.) and

Group 5 (BPH+RO 800): Received TE (5 mg/kg) subcutaneously and rice bran oil (800 mg/kg bw, p.o.).

The animals were subcutaneously injected with TE (5 mg/kg bw) daily for 28 days for the induction of BPH and all other materials were administered by oral gavage to the rats once a day. The lower dose of RO was selected on the basis that Saw palmetto lipid extracts (400 mg/kg) administered orally reduced BPH symptoms in rats (Carbajal et al. 2004). Following a 4-week course of treatment with rice bran oil, the rats fasted for 24 hr and were anesthetized and euthanized using ketamine (300mg/kg) and xylazine (30mg/kg). Next, histopathological and biochemical studies were performed to study the treatment effects of rice bran oil testosterone-induced BPH in male Wistar rats.

Tissue collection

Blood was collected from the caudal vena cava. Serum was separated using centrifugation and stored at -80°C until further analysis. Next, the ventral lobe of the prostate was split into two halves. One half was collected in formalin solution for histological analysis and the other half was stored at -80°C for biochemical analysis.

Measurement of prostate index (PI)

Whole prostate gland was removed and weighed. Before euthanizing, the animal's whole-body weight was measured and prostate index (PI) (Atawia et al. 2014) was determined by dividing prostate weight/ total body weight.

Measurement of prostate-specific antigen (PSA)

The prostate-specific antigen level in serum was measured by a commercially available enzyme linked immunosorbent assay kit (Rat PSA ELISA kit; Elabscience, TX, USA) following the manufacturer's protocol.

Preparation of prostate tissue homogenates

Prostate tissue was homogenized in tissue lysis reagent using a homogenizer (Homogenizer analog, SH-HZA, SH Scientific, Korea) at 16,000 g for 20 mins at 4°C. Total protein concentration was measured by Lowry's method (Lowry et al. 1951).

Biochemical assay

Measurement of glutathione (GSH) level

The level of GSH in prostatic tissue was measured using a previously described protocol (Al-Amin et al. 2015; Ellman 1959). In brief, 2.7 ml of phosphate buffer and 0.2 ml of Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) was mixed with 1 ml of prostate homogenate. The progression of color was determined promptly at 412 nm. Results are expressed in µmol/mg of protein.

Measurement of superoxide dismutase (SOD) and catalase (CAT) activity

The level of SOD and CAT activity was quantified using commercially available kits following the manufacturer's protocol (Jeon et al. 2014). Results are expressed in U/mg of protein.

Measurement of advanced oxidation protein products (AOPP) and nitric oxide (NO)

The level of AOPP and NO in prostate homogenate was quantified using previously described protocols (Jeon et al. 2014). Results are expressed in mmol/mg and µmol/ml for NO and APOP, respectively.

Tissue histology

The prostate tissue was fixed in 10% neutral buffered formalin and then embedded in paraffin wax. Next, the embedded tissue was sectioned at 5 µm and fixed in a slide. The slides are then stained with Hematoxylin and Eosin (H&E) stains for further observations. At 200x magnification, 3 sections per tissue sample were examined. Additionally, five fields per section were examined. Photographs of the prostate tissues were captured using light microscopy (Zeiss Axio Scope A.1).

Statistical analysis

Comparison between different groups was performed using one-way ANOVA followed by Dunnett's test for multiple comparisons. The data are presented as mean± standard deviation. $p<0.05$ was considered statistically significant. Statistical analysis was performed and graphs were prepared using GraphPad Prism 9.

Results

The effect of RO on prostate index (PI)

Firstly, we measured the effect of RO in PI of diseased rats as this parameter is considered to be an important indicator to assess the degree of BPH development (David and Leslie 2024). The rats of BPH group displayed a notably elevated PI ($p < 0.0001$) compared to the rats of the control group whereas the treatment with a standard drug (finasteride) reduced elevated PI (Figure 1). Similar to the standard treatment, the rats receiving rice bran oil demonstrated a notably lower PI compared to the diseased rats in both doses. Interestingly, the higher dose of RO exhibited a significant reduction compared to the standard treatment of finasteride.

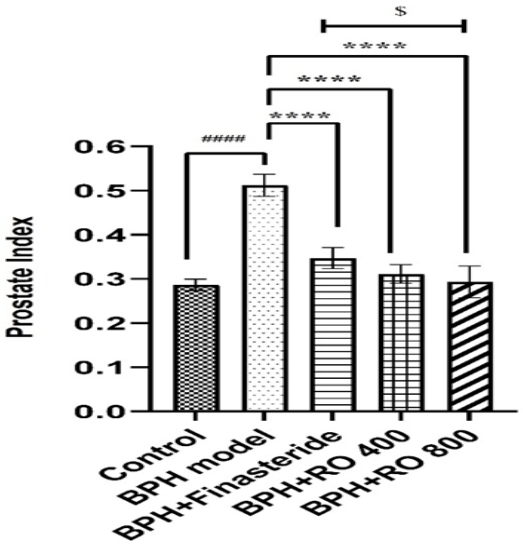

Figure 1. Prostate Index. Effect of Rice Bran Oil on prostrate to body weight ratio (prostate index). Values are mean±SD (n=6). Control: Healthy rats, BPH model: Rats with BPH, BPH+Finasteride: Rats with BPH treated with 10 mg/kg finasteride, BPH+RO (400): Rats with BPH treated with 400mg/kg rice bran oil, BPH+RO (800): Rats with BPH treated with 800mg/kg rice bran oil. #### significant difference with the control group ($p< 0.0001$), and **** significant difference with the BPH group ($p<0.0001$), $= $p<0.05$. By following Dunnett's test, One-way ANOVA was conducted to identify variance between the means of different groups with the BPH model.

The effect of RO on PSA concentration

Next, we assessed the level of PSA in serum of rats with experimental BPH. Elevated PSA in serum is an indicator of developing BPH (David and Leslie 2024) and we exhibited a similar increase in the serum of rats receiving TE compared to control rats (Figure 2). This elevated level of PSA in serum due to TE was reduced by the standard treatment, and RO at the dose of 800 mg/kg bw exhibited similar effects. Interestingly, RO at the lower dose did not affect the rise of PSA in serum of TE-exposed rats.

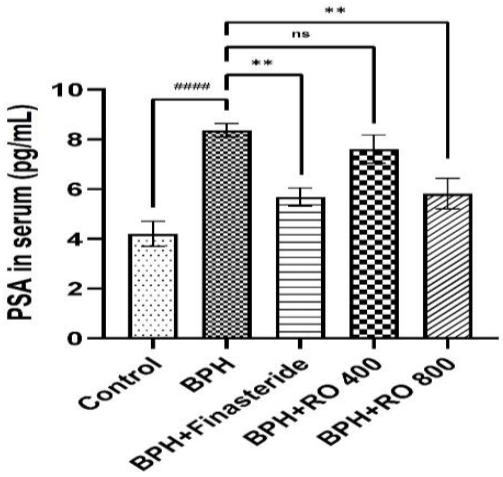

Figure 2. Prostate-Specific Antigen. Effect of rice bran oil on PSA in serum. Values are mean ± SD (n=6). Control: Healthy rats, BPH model: Rats with BPH, BPH + Finasteride: Rats with BPH treated with 10mg/kg finasteride, BPH+RO (400): Rats with BPH treated with 400 mg/kg rice bran oil, BPH+RO (800): Rats with BPH treated with 800mg/kg rice bran oil. #### significant difference with the control group ($p< 0.001$) and ** and ** significant modification compared with the BPH group ($p<0.002$ and $p<0.003$, respectively), ns= not significant variance compared with the BPH group. One-way ANOVA, was conducted to identify variance between the means of different groups with the BPH model.

Antioxidant property of RO benefits in BPH

Several studies have suggested a positive correlation between oxidative stress and BPH (Jena et al. 2016), therefore, we measured the concentration of GSH, and SOD, and CAT activity in the prostate tissue of experimental animals of BPH. Firstly, we exhibited lower levels of GSH in the prostate tissues of BPH rats compared to control rats indicating an imbalance in the redox homeostasis in these mice due to subcutaneous administration of TE (Figure 3A). The standard treatment finasteride restored the concentration of GSH significantly and RO exhibited a similar effect at both doses, although high dose (800mg/kg) showed significantly better response compared to finasteride (Figure 3A).

Next, we assessed the activity of SOD and CAT in prostate tissues of experimental BPH animals that are speculative of neutralization of oxidative stress (Nandi et al. 2019). Subcutaneous TE administration reduced the activity of these enzymes in the enzymes compared to control rats and RO elevated the activity of these enzymes significantly (Figure 3B and 3C), a phenomenon similar to the standard treatment. Although these beneficial activities were significantly demonstrated by the higher dose of RO (800 mg/kg bw), the lower dose of RO failed to restore the enzymatic activity of CAT (Figure 3C).

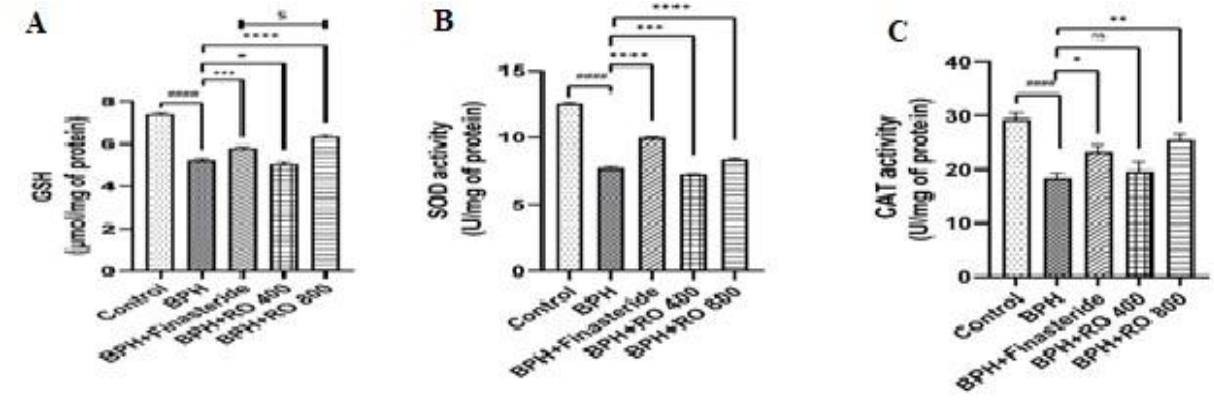

Figure 3. Effect of Rice Bran Oil on GSH(A), SOD(B) and CAT(C) concentrations. Control: Healthy rats, BPH model: Rats with BPH, BPH + Finasteride: Rats with BPH treated with 10 mg/kg finasteride, BPH+RO (400): Rats with BPH treated with 400 mg/kg rice bran oil, BPH+RO (800): Rats with BPH treated with 800 mg/kg rice bran oil. GSH level, SOD, and CAT activity were expressed in µmol/mg, U/mg, and U/mg of protein respectively. Values are mean ± SD (n=6). #### significant difference with the control group ($p<0.0001$), and ****, ***, ***, **, and * Significant difference with BPH group ($p<0.0001$, $p<0.0002$, $p< 0.0007$, $p<0.002$, and $p<0.03$, respectively), $= $p<0.05$, ns = not significant. One-way ANOVA followed by Dunnett's test, was conducted to identify variance between the means of different groups with the BPH model.

Anti-inflammatory potential of RO benefits in BPH

AOPP is mainly the oxidative marker of inflammation and stress and NO plays an essential role in correlating BPH and inflammation (Roehrborn 2004). Therefore, we determined the concentration of NO and AOPP in the prostate tissues of rats of experimental BPH. Elevated levels of NO and AOPP concentration indicated increased inflammation in the prostate of TE-exposed rats compared to control rats (Figure 4A, 4B). These escalated levels of TE-induced inflammation were reduced by RO (at both doses) and finasteride significantly suggesting beneficial anti-inflammatory activity of these compounds.

RO reduces TE-induced prostatic hyperplasia

The effect of RO on the development of TE-induced epithelial hyperplasia in rat prostate was evaluated by H & E staining of prostate tissue (Figure 5). The epithelial cell layer got thickened, and an increase in prostatic secretion and intraluminal projection was observed in prostates of experimental animals of the BPH model compared to control ones (Figure 5A and 5B). Rats from the finasteride-treated group demonstrated mild epithelial hyperplasia, prostatic secretion, and intraluminal projection compared to the BPH group (Figure 5C). Additionally, rats from RO groups exhibited reduced epithelial hyperplasia, prostatic secretion, and intraluminal projection compared to the BPH group at both doses (Figure 5D and 5E). Interestingly, prostate tissue of rats treated with RO showed some traits of prostate glandular hyperplasia, but there was a significant decrease in the prostate's stromal spaces, formation of papillary projections, and epithelial proliferation.

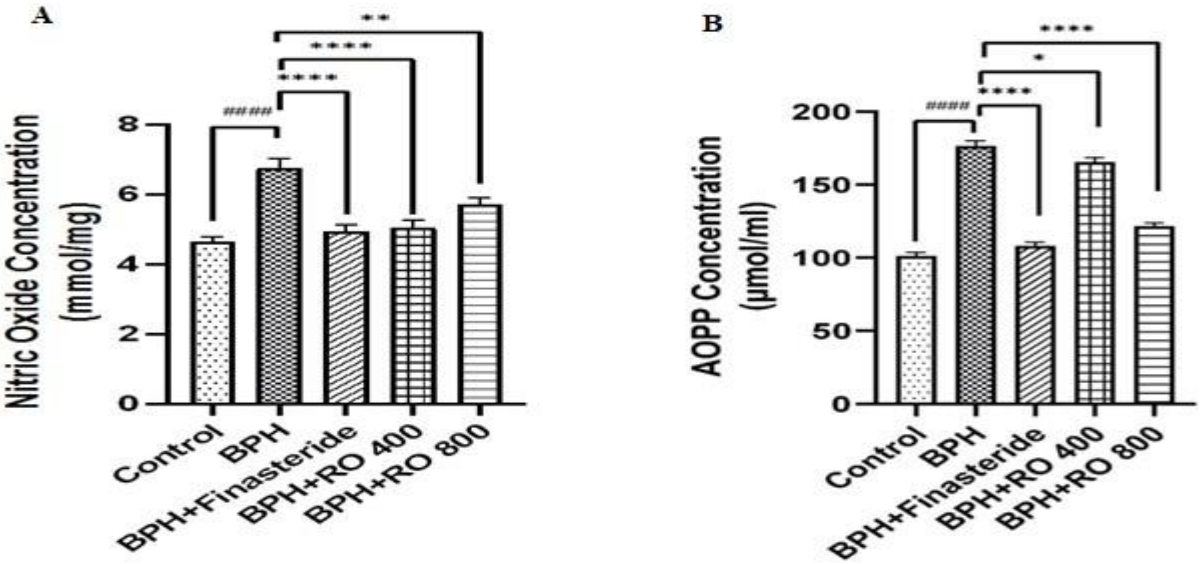

Figure 4. Effects of Rice Bran Oil on concentrations of NO (A) and AOPP (B). Control: Healthy rats, BPH model: Rats with BPH, BPH + Finasteride: Rats with BPH treated with 10mg/kg finasteride, BPH+RO (400): Rats with BPH treated with 400 mg/kg rice bran oil, BPH+RO (800): Rats with BPH treated with 800 mg/kg rice bran oil. NO and AOPP concentrations were expressed in µmol/ml and mmol/mg respectively. Values are mean ± SD (n=6). #### significant difference with the control group, ($p<0.0001$), and ****, **, and * ($p<0.0001$, $p<0.009$, and $p<0.03$, respectively). One-way ANOVA followed by Dunnett's test, was conducted to identify variance between the means of different groups with the BPH model.

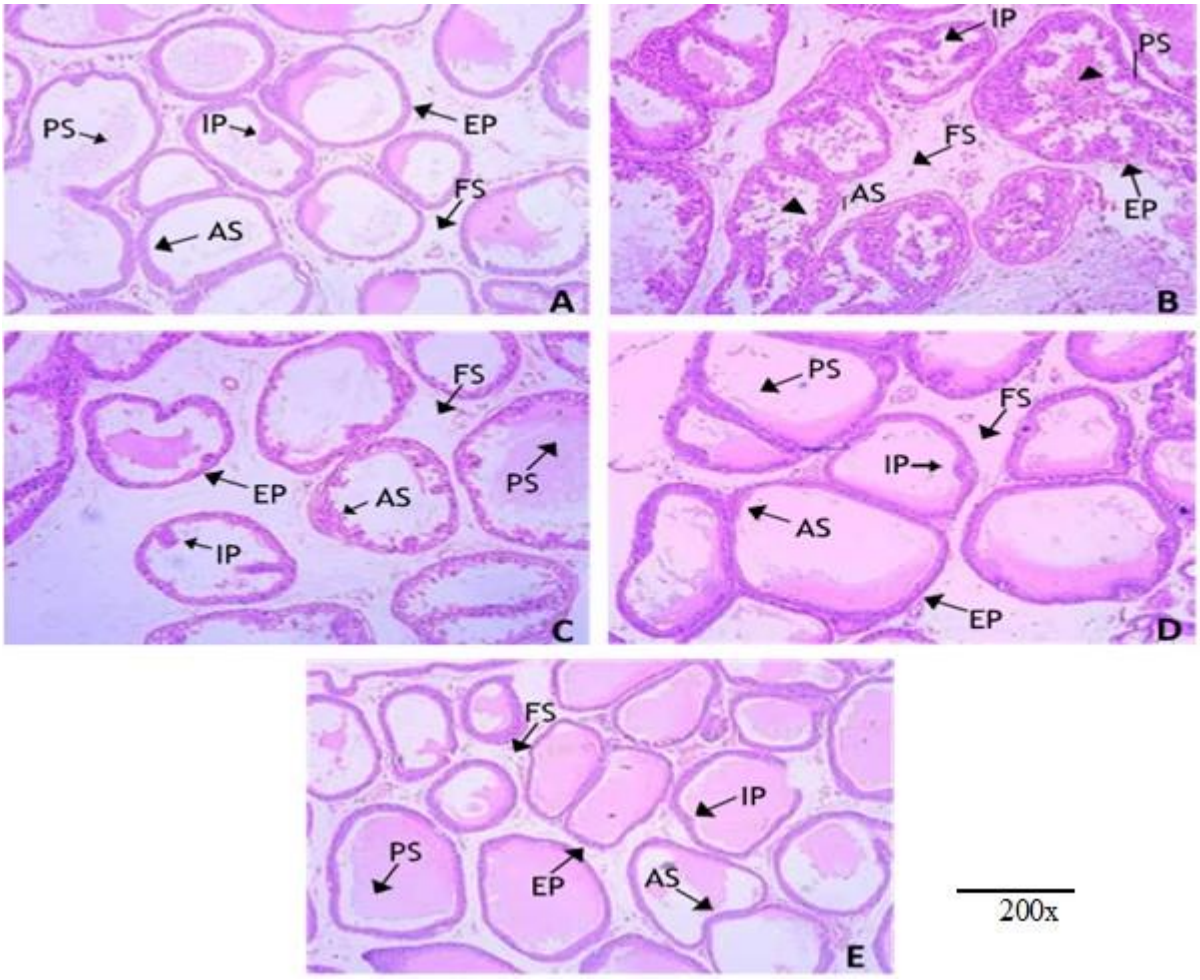

Figure 5. Histopathological observations (prostate tissue was sectioned at 4µm thickness and stained with hematoxylin and eosin, magnification, X10) showing effects of Rice Bran Oil and Finasteride on BPH. (A) Control (B) BPH (C) BPH+ Finasteride (D) BPH+ Rice Bran Oil 400 mg/kg (E) BPH+ Rice Bran Oil 800 mg/kg. EP (Epithelial Lining), FS (Fibromuscular Stroma), IP (Intraluminal Projection), PS (Prostatic Secretion), and AS (Acinar Shape).

Discussion

This research was conducted to evaluate the treatment effect of rice bran oil (RO) on TE-induced development of experimental BPH in male Wistar rats. In this study, RO considerably exhibited beneficial effects on the antioxidant biomarkers (GSH, CAT, and SOD), reduced histological changes, and decreased inflammatory markers in the prostate tissue of BPH rats. Moreover, administration of RO resulted in reduced prostate index and prostate-specific antigen (PSA) levels in serum.

Prostate enlargement is generally caused by benign proliferation of the gland's cellular components (Bostwick 2002). Previous research has confirmed that androgen hormones stimulate the enlargement of the prostate gland (Mirone et al. 2006), especially those hormones associated with testosterone are involved in the underlying process. These hormones stimulate growth factors to become active, which in turn, affect prostate cell division and proliferation, leading to hyperplasia (Acheampong et al. 2019). Thus, prolonged testosterone therapy is frequently used to induce BPH in rats experimentally. This results in a significant increase in prostate weight and, in turn, PI. We have observed elevated TE-induced PI in experimental animals of our BPH model (Figure 1). Similar results have been reported previously by various research groups

(Akbari et al. 2022; Gossell-Williams et al. 2006). Histological analysis of prostate tissue found increased stromal proliferation, glandular hyperplasia, and thickened epithelial layers among the TE-induced BPH rats (Figure 5). Finasteride and both doses of RO demonstrated a reduction in the TE-induced PI in rats of our experimental model. Histologically, the prostates of rats that received finasteride and RO exhibited mild epithelial hyperplasia, prostatic secretion, and intraluminal projection. As finasteride reduces the activity of testosterone by inhibiting conversion to DHT, thus, resulting in lowering hyperplasia as well as PI, RO could exhibit similar properties via a similar mechanism. Further analysis on determining DHT levels in the prostate tissue of RO-treated rats can help in elucidating the mechanism.

In a normal physiological state, a serine protease glycoprotein named PSA is produced in minute amounts by the cells of the epithelium of prostates. In pathological conditions like BPH or prostate cancer, the level of PSA in serum is increased (Lee et al. 2017) and therefore, elevated PSA is designated as an important biomarker for BPH. Additionally, reducing the serum level of PSA is indicative of the effectiveness of a trial drug in managing BPH (Lee et al. 2017). We demonstrated an increased level of PSA in the serum of rats due to the administration of TE. Interestingly, the elevation was reduced by finasteride and RO (at both doses). The mean serum PSA level of the finasteride-treating group was not substantially different than the group receiving the higher dose (800 mg/kg) of RO, attesting to the effectiveness of the RO. Testosterone and DHT can influence the expression of PSA via binding with androgen receptors (Bennett et al. 2010; Kim et al. 2018) . Thus, RO can suppress the level of TE-induced serum PSA following the same mechanism of finasteride i.e. inhibiting the conversion of DHT from testosterone. In addition, polyphenols can reduce the level of expression of PSA genes (Stevanin et al. 2000) and RO contains various polyphenolic compounds (Kusumawardani and Luangsakul 2024). Therefore, RO can exert the PSA lowering activity via various phenolic compounds it contains.

RO is comprised of various compounds with potent antioxidant, antiproliferative, and anti-inflammatory properties like tocotrienols, tocopherols, and ferulic acid (Ali and Devarajan 2017; Roehrborn 2004). These compounds have been reported to provide anti-cancer effects regulating cell proliferation and controlling benign BPH. In addition, the combination of γ-tocotrienol and ferulic acid has been reported to reciprocally prevent the propagation of a prostate cancer cell line (Gupta et al. 2009). In normal physiological conditions, GSH, SOD, and CAT prevent oxidative damage in the tissues by various mechanisms, such as balancing redox homeostasis, neutralizing reactive oxygen species, and removing H_2O_2 (Adaramoye et al. 2019; Forman et al. 2009; Nandi et al. 2019). These antioxidant enzymes usually protect prostatic cells and these cells activate the body's defense mechanism against oxidative stress (Ammar et al. 2015). The study demonstrated a decreased level of these enzymes in the prostates of rats receiving TE. This phenomenon implied the overproduction of reactive oxygen species, and elevated peroxidative activity i.e. oxidative stress in the prostate (Aryal et al. 2007; Aydin et al. 2006). The experimental BPH rats treated with RO/ finasteride recorded a significant increase in the level of these enzymes in the prostate. Interestingly, the higher dose (800 mg/kg bw) exhibited better results than the standard drug finasteride in reestablishing the level of these enzymes in the prostate tissue. Phytochemical screening of the RO resulted in the presence of tocotrienols, tocopherols, and ferulic acid-like compounds with established antioxidant properties. Therefore, preventing the decreasing trend of GSH, SOD, and CAT is suggestive of the antioxidant property of

RO and this property could be due to the presence of tocotrienols, tocopherols, and ferulic acid-like compounds.

According to our study, some inflammatory factors contribute to the pathophysiology of BPH. That causes the levels of beta (IL-8RB), interleukin 8 receptor, and interleukin 10 receptor subunit alpha (IL-10RA) to rise in the BPH. However, Rice bran oil contains anti-inflammatory property that contributes to decreasing the expression of inflammatory factors. Rice bran oil contains tocotrienols which are regarded as antioxidants and have offered anti-cancer effects as well as helping to regulate cell proliferation and control benign prostatic hyperplasia (BPH).

Apart from these antioxidant enzymes, several biomarkers are influenced due to BPH. For example, elevated levels of AOPP in the prostate tissue have been recorded in TE-treated rats of our experimental model. AOPPs are mainly produced by the reaction of plasma proteins which raises the level of oxidative stress in BPH. The measurement of the level of AOPPs indicates the oxidative damage of proteins (Uygur et al. 2009) as well as the level of inflammation as AOPP is a marker of inflammation and stress (Roehrborn 2004). Treating the TE-exposed rats with RO significantly reduced the level of AOPP at both of the doses in our experimental model. Both the antioxidant and potential anti-inflammatory effects of RO may have played crucial roles in managing and reducing oxidative stress-related markers like AOPPs in BPH (Roehrborn 2004). AOPPs are mainly produced by the reaction of plasma proteins which raises the level of Oxidative stress in BPH. The measurement of the level of AOPPs indicates the oxidative damage of proteins (Uygur et al. 2009). During BPH treatment, the antioxidants reduce the level of AOPPs and thus prevent oxidative damage of proteins to reduce oxidative stress and inflammation. So, AOPP's impacts have been significantly recovered by the treatment of RO. In the case of NO, both doses of rice bran oil (RBO) did not show remarkable change compared to the standard drug but showed significant change compared to the positive control group. However, for AOPP, rice bran oil at a dose of 800 mg/kg showed a significant decrease compared to the positive control group. However, both doses did not show remarkable change compared to the standard drug (finasteride) group. We suggest that RBO at even higher doses might be able to decrease the levels of AOPP and NO even further which needs to be investigated in future studies.

Levels of various pro-inflammatory cytokines and chemokines are found to be elevated in the pathogenesis of BPH. Reports of increased levels of tumor necrosis factor-α, Interleukin (IL)-6, IL-8, IL-10, transforming growth factor-β and cyclooxygenase-2 like pro-inflammatory cytokine and chemokines in prostate tissue of BPH patients have been published previously (Abdel-Aziz et al. 2020). NO is responsible for playing an important role in establishing the association between inflammation and prostate hyperplasia (Chughtai et al. 2011). Elevated levels of NO in the prostate have been correlated with abnormal growths of the organ in a clinical setting (İŞMan et al. 2003). In addition, levels of pro-inflammatory cytokines like IL-16 and IL-8 were reported to be elevated on prostate cell lines by exposure to NO (Burke et al. 2022). Therefore, escalated concentrations of NO in the prostate tissue of TE-induced rats were suggestive of elevated oxidative stress and inflammatory response in our experimental model. Treating the BPH rats with RO reduced the levels of NO in prostatic tissue resulting in an indication of the anti-inflammatory potential of the oil in inhibiting the progression of BPH. Various researchers have reported regarding attenuating inflammatory responses by RO in various settings (Lee et al. 2019; Park et al. 2021; Shin et al. 2017). Therefore, RO can ameliorate the TE-induced inflammations by following these

mechanisms. Measuring the cytokines and chemokines in the prostate tissue that promote inflammation in the pathogenesis of BPH might become helpful in exploring the anti-inflammatory action of RO.

Researchers have found that altered degenerations in the prostate of BPH patients can be reversed by preventing the activity of 5α-reductase (Tostes et al. 2016). Moreover, this inhibition can be attained by the presence of both unsaturated and short-chain free fatty acids (Tostes et al. 2016). The RO has been reported to be enriched in antioxidant, unsaturated, and saturated fatty acids (Shoieb et al. 2018). In addition, we have demonstrated the composition of fatty acids of RO used in our study (Table 1). The results showed the presence of unsaturated fatty acids like oleic acid and linoleic acid as well as saturated fatty acids like palmitic acid and stearic acid in significant amounts. Therefore, we can speculate that these fatty acids can inhibit the activity of 5α-reductase, in turn, exerting the beneficial activity of declining the development of BPH. Future experiments designed to elucidate the mechanism of these fatty acids in inhibiting the activity of the enzymes can shed light on understanding the favorable properties of RO in managing BPH.

Current medications for BPH include α-blockers and α-reductase inhibitors (Gossell-Williams et al. 2006). Among them, finasteride is the most common one as it can reduce prostate size by 20-30%, though this drug has some severe side effects (Lowe et al. 2003). The other available options can improve the complications but do not affect the prostate size. Therefore, natural products have risen as an alternative option having less severe side effects with similar efficacy. For example, Saw palmetto is indicated in treating BPH in the Republic of Korea due to having a beneficial effect against the disease along with no side effects like sexual dysfunction (Fagelman and Lowe 2001). RO is gaining popularity as a cooking oil among others because of its quality, shelf-life, and health benefits (Ali and Devarajan 2017). Moreover, RO exhibited anti-proliferative, antioxidant, and anti-inflammatory properties, resulting in inhibiting the development of TE-induced BPH in Wistar rats in our experimental model. Therefore, RO can be rendered as a functional health food that benefits as a therapeutic target for managing BPH. Further researches on animals as well as humans are required to establish this argument.

We investigated the protective effect of RO in the development of experimental BPH on Wistar rats. RO demonstrated antiproliferative properties by decreasing PI as well as an antioxidant effect by modulating the levels of GSH, SOD and CAT activity, and NO, and AOPP levels. In addition, RO exhibited anti-inflammatory potential regarding BPH. These findings support the therapeutic prospects of RO for managing BPH.

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Conflicts of interest

The authors declare no conflicts of interest.

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Ethical Considerations

All experiments were approved by the Institutional Animal Care and Use Committee of the North South University.

Code of Ethics

(Ethical approval number: 2021/OR-NSU/IACUC/1105).

Authors' Contributions

Supervision and Fund acquisition: M.S.M, Conceptualization, and methodology: M.S.M, M.H, M.M.R, M.A.M, M.A.S; Data collection: M.A.M,

M,A.S, M.G.H, M.R, N.M; Data analysis: M.A.M, M,A.S, M.G.H, M.R, N.M; M.H; Resources and software: M.C.S, H.M.R, Writing-original draft: M.A.M, M,A.S; Review and editing: M.C.S, H.M.R, M.H, M.M.R, M.S.M.

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