Stem bark extract of *Mangifera indica* prevents testosterone-induced benign prostate hyperplasia in rats

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ABSTRACT

Background: Benign prostate hyperplasia (BPH) is one of the most common conditions affecting up to 80% of men in their 8th decade of life. *Mangifera indica* Linn stem bark is used in traditional African medicine for the treatment of cancer, prostate hyperplasia, prostatitis and diabetes.

Objective: This study sought to investigate the effect of hydroethanolic stem bark extract of *Mangifera indica* (MI) on testosterone-induced BPH in rats.

Methods: BPH was induced in male albino rats (200-250g; n=8) through subcutaneous injection of testosterone propionate (3 mg/kg, in 10% olive oil) for 4 weeks. MI (100, 200 or 400 mg/kg, p.o.) or vehicle (10 ml/kg, p.o.) was administered 15 min before testosterone injection. On day 29, the animals were anaesthetized and blood collected for estimation of serum testosterone and prostate specific antigen (PSA). The prostates were excised, weighed and subjected to biochemical and histological studies.

Results: subcutaneous injection of testosterone caused significant increase in prostate weight (2.83 folds), prostatic index (3.24 folds), serum testosterone (2.29 folds) and PSA (1.83 fold) levels indicative of BPH which was ameliorated by pretreatment of rats with MI. Testosterone also increased malondialdehyde (MDA) level but decreased glutathione, superoxide dismutase and catalase activities indicating oxidative stress which was attenuated by MI treatment. The alteration in the morphology of the prostate induced by testosterone was prevented by MI administration.

Conclusion: The results obtained from this study showed that *Mangifera indica* stem bark extract prevented testosterone-induced BPH through enhancement of antioxidant defense mechanisms. Thus, could be a potential phytotherapeutic agent in the management of BPH.

Keywords: antioxidant; prostatic index; glutathione; malondialdehyde; testosterone

L'extrait d'écorce de tige de *Mangifera indica* prévient l'hyperplasie bénigne de la prostate induite par la testostérone chez le rat

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RESUME

Contexte : L'hyperplasie bénigne de la prostate (HBP) est l'une des affections les plus courantes affectant jusqu'à 80% des hommes au cours de leur huitième décennie de vie. L'écorce de tige de Mangifera indica Linn est utilisée en médecine traditionnelle africaine pour le traitement du cancer, de l'hyperplasie de la prostate, de la prostatite et du diabète.

Objectif : Cette étude visait à étudier l'effet de l'extrait hydroéthanolique d'écorce de tige de Mangifera indica (MI) sur l'HBP induite par la testostérone chez le rat.

Méthodes : Une HBP a été induite chez des rats albinos mâles (200-250g; n=8) par injection sous-cutanée de propionate de testostérone (3 mg/kg, dans 10% d'huile d'olive) pendant 4 semaines. Un MI (100, 200 or 400 mg/kg, p.o.) ou un véhicule (10 ml/kg, p.o.) a été administré 15 minutes avant l'injection de testostérone. Au jour 29, les animaux ont été anesthésiés et du sang a été prélevé pour estimer la testostérone sérique et l'antigène spécifique de la prostate (PSA). Les prostates ont été excisées, pesées et soumises à des études biochimiques et histologiques.

Résultats : une injection sous-cutanée de testostérone a entraîné une augmentation significative du poids de la prostate (2,83 fois), de l'indice prostatique (3,24 fois), de la testostérone sérique (2,29 fois) et du PSA (1,83 fois) indiquant une HBP améliorée par le prétraitement des rats avec MI. La testostérone a également augmenté le taux de malondialdéhyde (MDA), mais a diminué les activités du glutathion, de la super-oxyde dismutase et de la catalase, indiquant un stress oxydatif atténué par le traitement au MI. L'administration de MI a permis d'éviter l'altération de la morphologie de la prostate induite par la testostérone.

Conclusion : Les résultats de cette étude ont montré que l'extrait d'écorce de tige de Mangifera indica empêchait l'HBP induite par la testostérone en renforçant les mécanismes de défense antioxydants. Ainsi, pourrait être un agent phyto-thérapeutique potentiel dans la gestion de l'HBP.

Mots-clés : antioxydant ; index prostatique ; le glutathion ; malondialdéhyde; testostérone

INTRODUCTION

Benign prostate hyperplasia (BPH) is characterized by non-malignant enlargement of the prostate and is one of the most common urological diseases in elderly men, with an incidence of approximately 50% in those aged 60 or older, and more than 90% in men older than 85.[1] BPH is characterized by increased number of both stromal and epithelial cells in the transition zone of the prostate, which leads to obstruction of urine flow and significant morbidity in the majority of older men.[2,3] Complications of BPH such as acute urinary retention, urinary tract infection and renal failure can lead to death in some cases. Development and maintenance of the normal prostate, as well as development of BPH, depend on a functional androgen-signaling axis, components of which include: (1) testosterone synthesis in the testes and adrenal glands; (2) conversion of testosterone to DHT; (3) transport of DHT to target tissues; and (4) binding of DHT to the androgen receptor with consequent modulation of genes.[4] DHT plays a beneficial role in the developing prostate but it can be detrimental in the adult prostate in that it causes pathologic prostate growth[4]. Moreover, Pejčić et al.[5] showed strong correlation between amount of testosterone and DHT accumulated in the stroma of enlarged prostates and prostate volume. In addition, several studies have shown increased levels of oxidative stress in plasma and urine of patients with BPH.[3,6,7]

Conventional steroid 5-alpha reductase inhibitors, such as finasteride and dutasteride, were successful in the treatment of hyperplastic growth of prostate, however, these drugs were responsible for adverse effects, such as gynecomastia, dizziness, upper respiratory infections, headache, and chest pain.[8,9] Such effect may limit the use of conventional drugs for BPH. Hence, medicinal plants are potential alternative of safer drugs for the treatment of BPH.

Mangifera indica Linn (Anarcardiaceae) widely known as Mango is used in traditional African medicine in the treatment of cancer, prostate hyperplasia, prostatitis and diabetes,[10, 11] It possesses anti-inflammatory, [12] nephroprotective, [13] hepatoprotective, [14] anxiolytic/antidepressant, [15] anticancer[16] and antimicrobial. [17] The main polyphenolic compound present in this extract is mangiferin with C-glucosyl linkage and polyhydroxy component, which have been reported to be a potent antioxidant and polypharmacologic properties. [18] This study sought to evaluate the protective effect of hydroethanolic stem bark extract of *M. indica* in benign prostate hyperplasia in rats as well as the involvement of antioxidant defense system in its possible ameliorative effect.

METHODS

Laboratory animals

Male Sprague-Dawley (SD) rats (200-250 g) were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos and were randomly divided into 5 groups (n= 8). The animals were allowed to acclimatize for a week prior to experiments. All animals were housed in a well-ventilated room under a 12 h light/dark cycle and had access to feed and water *ad libitum*. The experimental procedures adopted in this study were in accordance with the United States National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (2011).

Drugs and chemicals

Testosterone propionate (TESTOST™, Laborate Pharmaceuticals Ltd, India), celecoxib (Celebrex ™, Pfizer Pharmaceuticals Ltd, lllertissen, Germany), Olive oil (Goya En Spana S.A.U, Spain), testosterone ELISA kit (Cayman, Ann Harbor, USA), prostate specific antigen (PSA) (Enzo Life Sciences, Farmingdale, NY, USA), ethanol, thiobarbituric acid, bovine serum albumin, 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), trichloroacetic acid, glacial acetic acid, sodium chloride, and sodium hydroxide were purchased from Sigma Aldrich (St, Loius MO, USA)

Plant materials

The stem bark of *Mangifera indica* was collected in Abatadu Village, Osun State, Nigeria by Mr. T.K Odewo (a Forestry expert) formerly with Forestry Research Institute of Nigeria (FRIN) now with the Department of Botany, Faculty of Science, University of Lagos, Lagos, Nigeria. A voucher specimen with reference number LUH 6061 was deposited in the Herbarium of the Department of Botany, University of Lagos, Nigeria.

Preparation of plant extract

The stem bark of *Mangifera indica* was cut into small pieces and air-dried at room temperature for 8 days. The dried stem bark (1.89 kg) was pulverised into powder by FT2 Laboratory Hammer mill (Armfiled Limited, Ringwood, England). The powdered plant material was soaked in 2.5 L of 70%v/v ethanol at 4°C for 72 h with intermittent shaking and the procedure was repeated twice. The filtrate obtained were pooled together and concentrated with Heidolph® Rotavapor (Switzerland) at 40°C, the concentrate obtained was oven dried at 40°C giving a dark-brown powder with a yield of 6.91%.

Quantitative phytochemical analysis

The preliminary quantitative analysis of phytochemical constituents of MI were assayed using earlier reported protocols; total phenolic content (TPC),[19] total flavonoid content (TFC)[20] and total antioxidant capacity (TAC).[21]

***In vitro* antioxidant assay**

1,1 Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of stem bark extract of *Mangifera indica* (MI) was assayed against DPPH radicals. Five hundred microlitre of DPPH was mixed with aliquots of gallic acid or MI (0-100 mg/ml) with dimethylsulfoxide (DMSO) used as control. The mixture was incubated for 30 min at 37°C in the dark. The absorbance was read at 517 nm.[21]

Nitric oxide scavenging assay

The ability of MI to inhibit nitric oxide radicals was estimated using the Griess reaction method[22]. Griess reagent comprises: 1% sulphanilamide in 5%□*v/v* phosphoric acid and 0.01% naphthylethylenediamine in distilled water in equal volumes. Sodium nitroprusside (5µM) in phosphate buffer (0.025M, pH 7.4) was added to different concentrations of MI or gallic acid (0-100 mg/ml) and incubated for 5h at 37°C. Equal amount of methanol was taken as control. Five hours later, 500µL of Griess reagent was added. The absorbance of chromophore formed during the digitization of nitrite with sulphanilamide and its subsequent coupling with napthylethylenediamine was read at 546 nm.

Reducing power assay

The ferric ion reducing power of MI was determined according to the method Oyaizu[23]. Briefly, 1 ml of MO (0-100 mg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 %). The reaction mixture was incubated for 20 min at 50°C. Then, 2.5 ml of trichloroacetic acid (10 %) was added and centrifuged for 10 min. An aliquot 2.5 ml was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1 %). The absorbance of all solutions was measured at 700 nm and expressed as mg of gallic acid equivalent per g of powder (mg GAE/g powder).

Acute toxicity test

Acute oral toxicity test was carried out in 10 female albino mice (18-25 g) using the limit dose test of up and down procedure according to the OECD (TG_{425})[24] procedure for acute toxicity, three doses (maximum tolerated (5000 mg/kg, p.o.; n=5), medium (500 mg/kg., p.o.; n=2), and low dose (50 mg/kg., p.o.; n=2) levels) of MI or vehicle control (10 ml/kg, p.o.; n=1) were assay (which did not cause any unacceptable sign of toxicity or death). Animals were closely observed for signs of toxicity during the first 30 min, hourly for 3h. Then during the first 24 h, and then daily for 14 days for delayed toxicity or mortality.

Experimental design

After an acclimatization period of 7 days, 40 male albino rats were randomly divided into 5 groups (n=8) and treated for 28 consecutive days as follows: Group 1- olive oil (vehicle control) (10 ml/kg, p.o., normal), group 2- olive oil (test group) (10 ml/kg, p.o.) + testosterone, groups 3-5- MI (100, 200 or 400 mg/kg, p.o.). Fifteen minutes post-treatment, animals in groups 2-5 were given testosterone propionate in olive oil (3 mg/kg, s.c., 10%v/v). Weights of animals in different treatment groups were recorded weekly. Twenty four hours after last administration (day 29), animals were anaesthetized with chloral hydrate (300 mg/kg, i.p.) and blood collected through ocular puncture into plain bottle. The serum was separated by centrifuge at 3000 ×*g* for 20 min at 4°C. The serum was stored at -80°C until further analysis. The animals were sacrificed, prostate tissue carefully removed, weighed and washed with PBS (pH 7.4). The prostate index was calculated as the ratio of prostate weight (PW) (mg) to body weight (BW) (100 g). The prostate tissue was divided into two halves; one half was fixed in 10% formalin and embedded in paraffin for histological analysis, the other half was stored at -80°C for oxidative stress parameters.

Measurement of serum testosterone and prostate specific antigen (PSA)

The level of PSA in the serum was assayed using sandwich enzyme-linked immunosorbent assay (ELISA) system (Elabscience, Bethesda, MD, USA) while serum testosterone was assayed with testosterone ELISA kit (Cayman, Ann Arbor, MI, USA). Each assay was performed in triplicate using individual serum samples of six selected rats per group according to the manufacturer's protocol.

Histological examination

The dorso-lateral lobes of the prostates were fixed in 10% formo-saline for 24 h, then dehydrated in ascending grades of ethanol, cleared in xylene, then infiltrated with, and embedded in paraffin. Each was sectioned at 5µm and stained with hematoxylin and eosin. The sections were subsequently viewed and photomicrographs taken (Omax digital microscope).

Assessment of Oxidative Stress Markers

A 10% (w/v) homogenate of prostate samples (0.03M

sodium phosphate buffer, pH 7.4) was prepared using an Ultra-Turrax T25 homogenizer (USA) at a speed of 9500 rpm and stored at -20°C.

Estimation of MDA level

MDA, which is an indicator of lipid peroxidation, was spectrophotometrically measured using the thiobarbituric acid assay procedure as previously described by Ohkawa *et al.*[25] To 500µl of prostate homogenate in phosphate buffer (pH 7.4), 300µl of 30% trichloroacetic acid (TCA), 150µl of 5N HCl and 300µl of 2% (w/v) 2-thiobarbituric acid (TBA) were added and then the mixture was heated for 15 min at 90◦ C. The mixture was centrifuged at 12,000×*g* for 10 min. Pink colour supernatant was obtained, which was measured spectrophotometrically at 532 nm. A calibration curve was constructed using MDA as standard and the results were expressed as U/mg protein.

Estimation of glutathione (GSH) level

GSH, the endogenous antioxidant, was determined by its reaction with 5, 5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically.[26] The prostate homogenate was mixed with an equal amount of 10% trichloroacetic acid (TCA) and centrifuged (Remi cold centrifuge) at 2000× *g* for 10 min at 4°C. To 100 µl of processed tissue sample, 200 µl of phosphate buffer (pH 8.4), 500 µl of 5, 5′-dithiobis (2-Nitrobenzoic acid) (DTNB) and 400 µl of double-distilled water were added and the mixture was shaken vigorously on a vortex mixer. The absorbance was read at 412 nm within 15 min.

Estimation of superoxide dismutase (SOD) activity

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was assayed according to the method described by Winterbourn et al[27]. Each 1.5 mL reaction mixture contained 100 mM TRIS/HCl (pH 7.8), 75 mM nitro-blue-tetrazolium (NBT), 2 µM riboflavin, 6 mM EDTA, and 200 µL of supernatant. The absorbance was read at 560 nm. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50%. The enzyme activity is expressed as units/mg protein.

Protein estimation

Protein was measured in all prostate samples using the method of Lowry *et al*.[28] and bovine serum albumin (BSA) (1 mg/ml) used as standard.

Statistical analysis

Data are expressed as the mean±SEM. Statistical level of significance analysis was done using one way ANOVA followed by Tukey's *post hoc* multiple comparison tests.

RESULTS

Preliminary quantitative phytochemical analysis of *M. indica*

The results of total phenolic contents (TPC) assay showed that MI exhibited 16.20±0.72mg/100g gallic acid (GAE). The total antioxidant capacity of MI revealed 46.89±0.23 mg/100g GAE, total flavonoid contents assay showed that MI exhibited 34.15 ± 0.19 mg/100g GAE. (GAE- gallic acid equivalent)

In vitro antioxidant assay

Free radical scavenging capacity of *M. indica*

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical was used to determine hydrogen donating ability of MI. DPPH reacts with hydrogen donors (free radical scavengers) to yield a stable product 1, 1-diphenyl-2-picrylhydrazine resulting in a color change from purple to yellow. The median inhibitory concentration (IC_{50}) obtained for DPPH assay are 52.02 µg/ml [Y=0.4284*X+27.71] and 41.42µg/ml [0.5778*X+26.07] for MI and ascorbic acid, respectively. The results showed that the hydrogen donating ability of MI was lower than that of ascorbic acid. The results of the nitric oxide scavenging activity showed a median inhibitory concentration of 53.51 [Y=0.5891*X+18.48] and 41.77 [Y=0.5243*X+28.10] µg/ml for MI and ascorbic acid, respectively. Similarly, the median effective concentration (EC_{50}) 91.02 [Y=0.0039*X+0.04525] and 65.73 [Y=0.0060*X+0.0035] µg/ml, respectively of MI and ascorbic acid required to reduce Fe^{3+}/ferricyanide complex to the ferrous form.

Effect of *M. indica* on prostate weight and prostatic index

As shown in Fig. 1A-B, subcutaneous injection of testosterone significantly increased prostate weight by 2.83 folds [F(4, 25)=32.11,p<0.001] (Fig. 1A) and prostatic index by 3.24 folds [F(4, 25)=73.25,p<0.001] (Fig.1B) when compared with vehicle-treated control. Interestingly, the pretreatment of rats with *MI* (200 and 400 mg/kg) significantly (p<0.05, p<0.001) reduced the increase in prostate weight and prostatic index induced by testosterone by (1.29 and 1.73 folds), (1.14 and 1.38 folds), respectively.

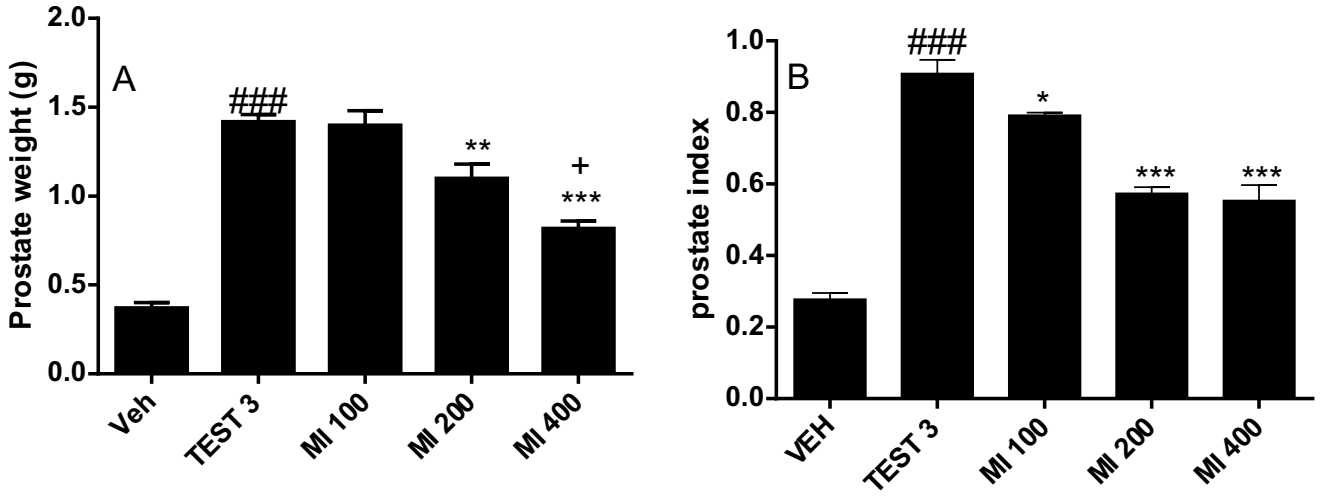

Fig. 1A-B: effects of MI on (A) prostate weight and (B) prostate index in testosterone-induced BPH rats. Values are expressed as mean±SEM (n=6), level of significance; ###p<0.001 versus vehicle-treated control; *p<0.05; ***p<0.001 versus testosterone treated; +p<0.05 versus MI 100 mg/kg treated.

Effect of *M. indica* on serum testosterone and PSA levels

Subcutaneous injection of testosterone significantly (p<0.001) increased the serum testosterone by 2.29 folds compared with the vehicle-control treated. However, the pre-treatment of rats with MI (100, 200 or 400 mg/kg) significantly [F(4, 25)=16.31,p<0.001] attenuated testosterone levels by (1.94, 1.56, 1.79 folds), respectively, compared with the vehicle-testosterone treated (Fig. 2A). Similarly, testosterone injection significantly (p<0.001) increased the serum prostate specific antigen was by 1.83 folds when compared with vehicle-control (Fig. 2B). In contrast, the pretreatment of rats with MI (100, 200 or 400mg/kg) significantly [F(4, 25)=27.28;p<0.001] attenuated the increase in PSA levels by (2.37, 5.48, 2.19 folds), respectively, compared with the vehicle-testosterone treated.

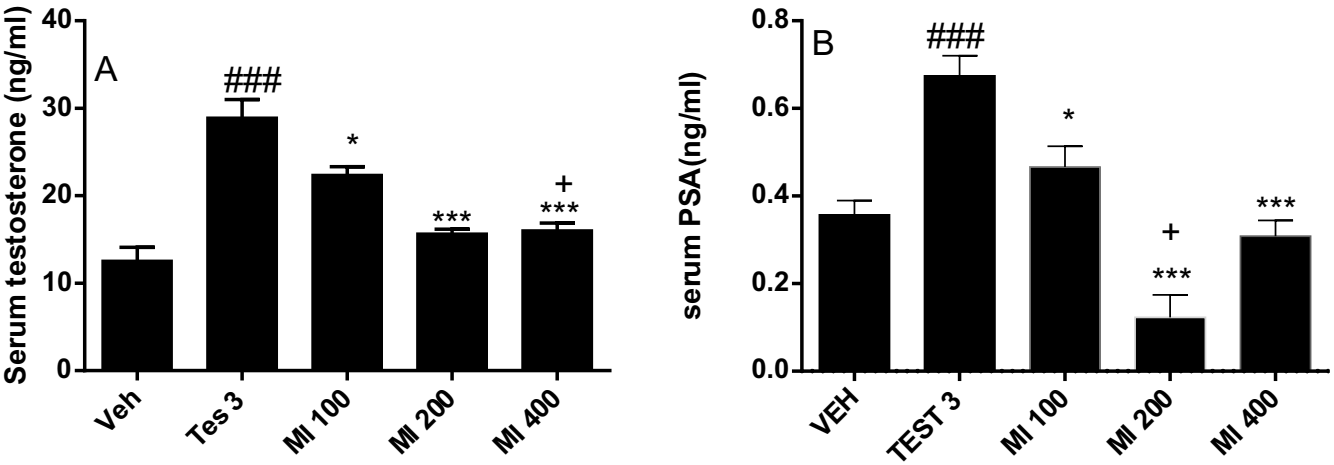

Fig. 2A-B: effects of MI on (A) serum testosterone and (B) serum prostate specific antigen in testosterone-induced BPH rats. Values are expressed as mean±SEM (n=6), level of significance; ###p<0.001 versus vehicle-control treated; *p<0.05; ***p<0.001 versus vehicle-testosterone treated; +p<0.05 versus MI 100 mg/kg treated. One-way ANOVA followed by Tukey *post hoc* multiple comparison tests.

Effect of M. indica on prostate oxidative stress markers

Subcutaneous injection of testosterone significantly ($p<0.05$) increased prostate MDA generation by 1.83 folds. The increase in MDA level was attenuated by pretreatment of rats with *MI* [$F(4, 25)=9.98$, $p<0.001$)] with peak effect observed at 400 mg/kg treated group (2.76 folds) (Fig. 3A). Testosterone caused marked deficit in prostate GSH level (2.62 folds) compared with vehicle-control. The pretreatment of rats with MI (200 and 400 mg/kg) significantly [$F(4,25)=19.02$, $p<0.001$], enhanced GSH level by 2.34 and 2.65 folds, respectively (Fig. 3B). Testosterone also reduced the activity of SOD in the prostate by 1.37 folds. However, the pretreatment of rats with *MI* (200 and 400 mg/kg) [$F(4,25)=22.07$, $p<0.001$] enhanced SOD activity in the prostate by 1.96 and 1.71 folds, respectively, compared with vehicle-testosterone treated (Fig. 3C).

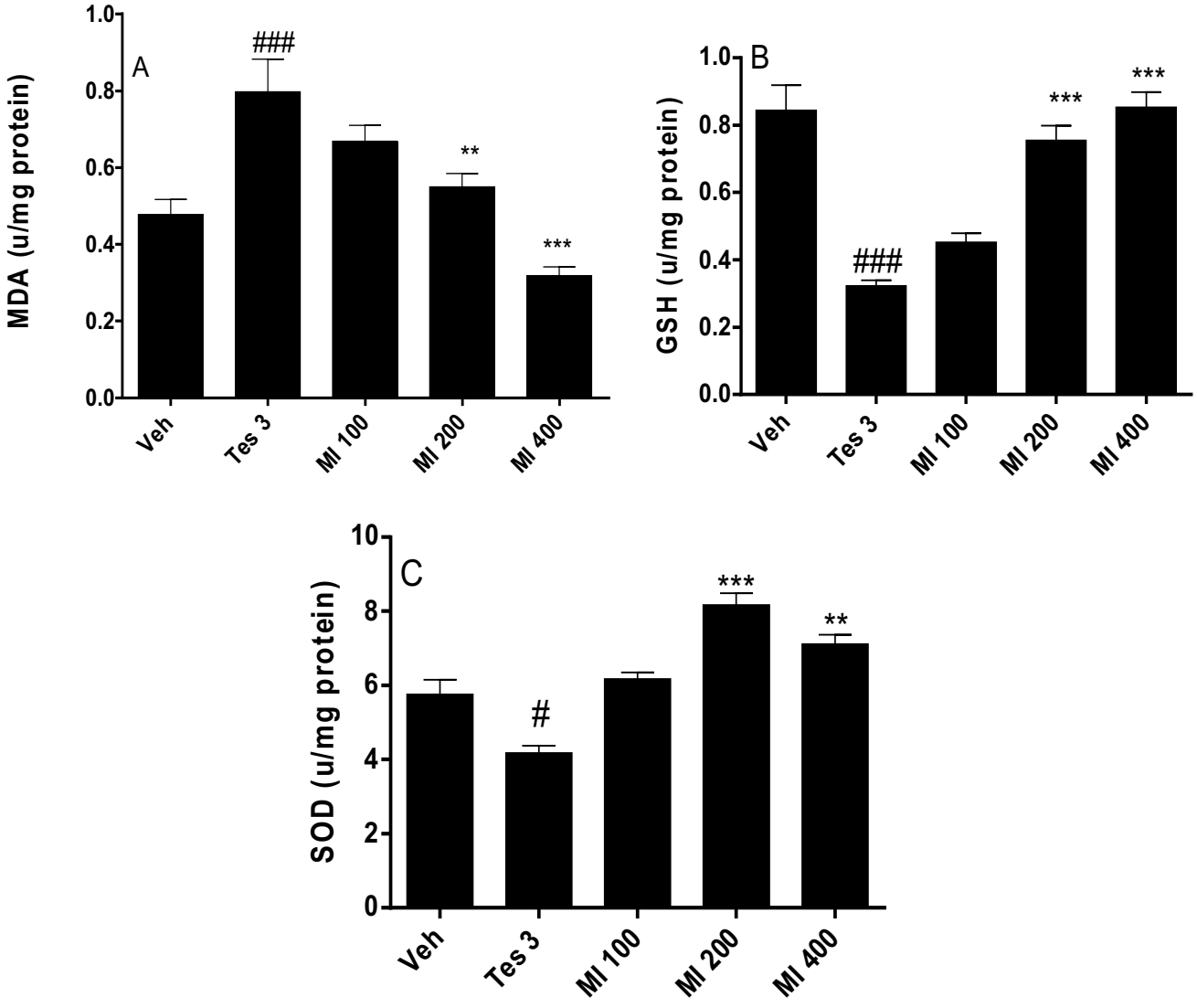

Fig. 3A-C: effects of MI on prostate levels of (A) malondialdehyde; (B) GSH and (C) SOD activity in testosterone-induced BPH rats. Values are expressed as mean±SEM (n=6), level of significance; $^{\#}p<0.05$; $^{\#\#\#}p<0.001$ versus vehicle-control treated; $^{**}p<0.01$; $^{***}p<0.001$ versus vehicle-testosterone treated. One-way ANOVA followed by Tukey *post hoc* multiple comparison tests.

Effect of *M. indica* on histological examination

In vehicle control treated, normal histological features of prostate were visible showing tubules of variable diameter and irregular lumen. The acini were filled with intraluminal secretions. The connective tissue, blood vessels, and the matrix were normal. The prostate gland is surrounded by capsule; a thick layer of involuntary muscle (Fig. 4A). Figure 4B shows the histology of vehicle-testosterone treated group, the epithelial cells lining crowded with tall columnar cells, the walls of the tubules thickened and enlarged with very large involutions observed reducing the volume of the lumen compared with the control. Thin connective tissue (fibrous stroma) was observed due to compression by enlarged tubules, cells were basophilic and signs of nodular hyperplasia. In MI (100 mg/kg) treated, normal recovery in the texture of the tubules is seen but less than the control group. Tubules showed morphological improvement and the lumen contained secretions with few debris. Connective tissue, although thicker than the testosterone group is distributed between the tubules. Epithelial cells present with fewer involutions compared to the testosterone group (Fig. 4C). MI (200 mg/kg) group showed normal recovery in the texture of the tubules but less than the control group. Ductular spaces appear more visible with intra-luminar secretions seen in acini. Involutions were thicker but fewer when compared with the testosterone group (Fig. 4D). In MI (400mg/kg), the shape and size of the tubules had recovered with the ducts appearing visible, coagulating glands appear hyper-basophilic with concretions present. Simple layered epithelium was observed with very few involutions compared to the testosterone group (Fig. 4E).

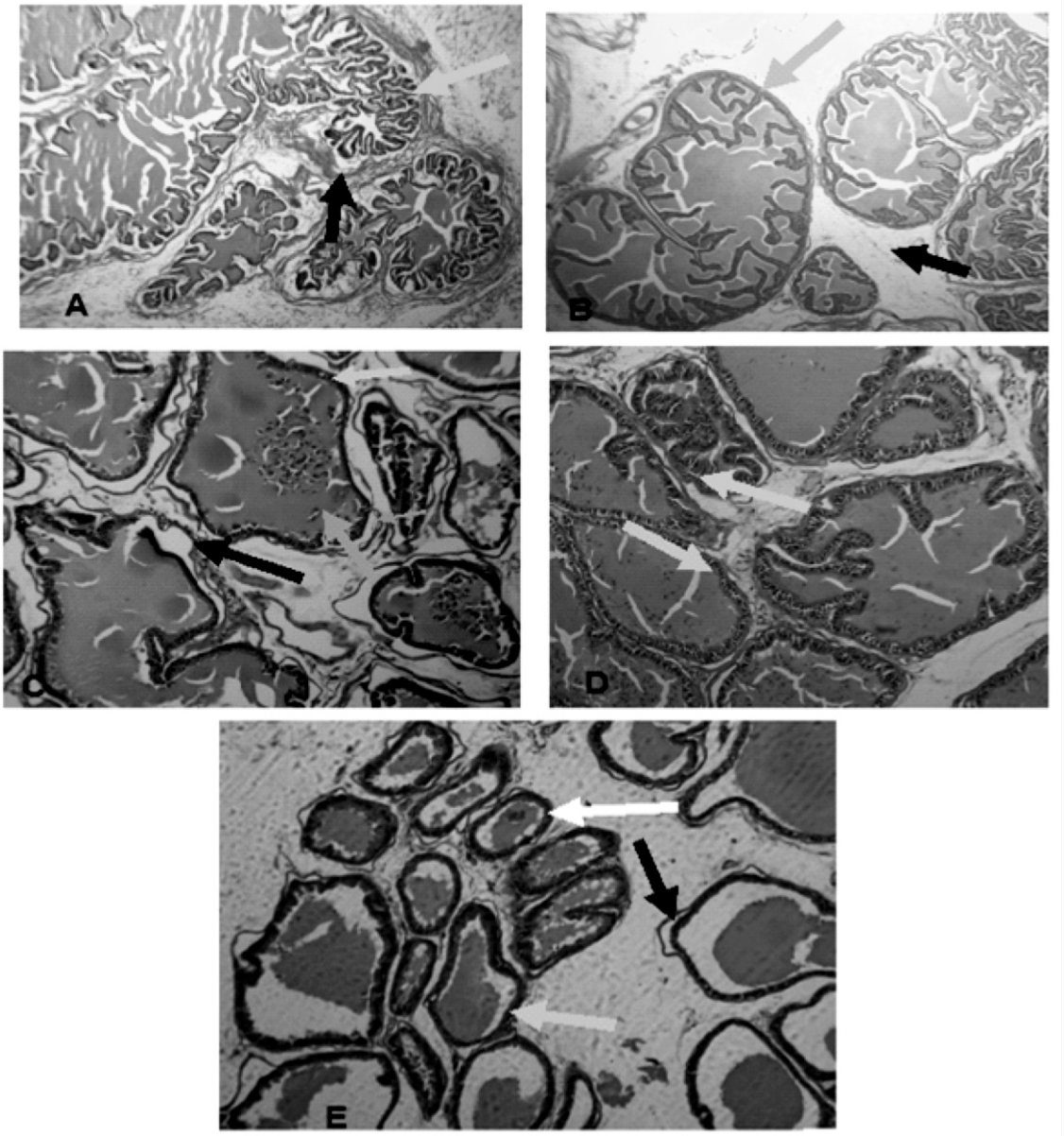

Fig. 4A-E: representative photomicrographs of prostate samples of (A) Vehicle control; (B) Vehicle testosterone treated, (C) MI (100 mg/kg) treated + testosterone, (D) MI (200 mg/kg) treated + testosterone and (E) MI (400 mg/kg) treated + testosterone. [A]- a normal histological appearance of the prostate; the stroma and acini are indicated by black and orange arrow, respectively; [B]- increased epithelial cell involutions with increased secretion (green arrow) and thin fibrous stroma (black arrow); [C] - thick fibro muscular tissue (black arrow) and attenuated basal layer (green arrow). [D]- ductular spaces visible with increased secretion (orange arrow), simple layered epithelial cells with involutions (green arrow). [E]- single layered epithelium (green arrow) with distinct acini, concretions present (white arrow) some are dilated which compresses gland, black arrow indicates thin fibrous muscle.

DISCUSSION

BPH is the most common proliferative disorders affecting older men and androgen plays a significant role its development. Hence, the mainstay in the management of BPH is inhibition of androgens. In the present study, BPH was induced through subcutaneous injection of testosterone, a widely used model to induce BPH in animals.[5,29,30] In agreement with previous studies, subcutaneous administration of testosterone for 4 weeks increased the prostate weight, prostatic index (prostate weight/body weight ratio), serum testosterone and prostate specific antigen levels.[30,31] The relative prostate weight is an important marker of BPH development, interestingly, the pretreatment of rats with *M. indica* ameliorated testosterone-induced BPH markers. Moreover, *M. indica* attenuated the increase in peroxidation of prostate lipid membrane and deficit in glutathione level and superoxide dismutase activity induced by subchronic injection of testosterone. These enhancement of antioxidant defenses markers in the prostate was corroborated with our findings from the in vitro assay, evidenced in the ability of *M. indica* to scavenged DPPH and nitric oxide radicals as well as effective reductive ability on ferrous ion possibly due to its richness in flavonoids and phenolic compounds

BPH involves epithelial and stromal hyperplasia of the prostate resulting in an increase in prostate weight. The prostate index is used as an important biomarker of BPH development.[29] In the present study, animals with BPH showed an increased prostate weight and prostate index compared to the control group. In contrast, pretreatment of rats with *M. indica* after 4 weeks showed a significant reduction in prostate weight and prostate index compared to the BPH group.[30] These results indicate that *M. indica* attenuated the prostatic enlargement induced by testosterone. Additionally, these findings were supported by histological examination of the prostate tissue. The animals with BPH experienced stromal proliferation and glandular hyperplasia in the prostate, whereas the animals with BPH that had been treated with *M. indica* showed mild glandular hyperplasia. These results suggested that *M. indica* inhibits the progression of BPH induced by testosterone.

The two main classes of drugs used in BPH treatments are α_1- adrenoceptor blockers which inhibit smooth muscle contraction[31], and inhibitors of type II 5α-reductase, an enzyme responsible for the conversion of testosterone to the more potent dihydrotestosterone (DHT).[32] The conversion of testosterone to the more potent dihydrotestosterone by the enzyme 5α- reductase is implicated as a causative factor in BPH. *M. indica* in a dose dependent manner significantly suppressed the serum levels of testosterone compared to the BPH groups with 400 mg/kg being the most effective. The results indicate that *M. indica* has important anti-proliferative effects and could probably suppress the DHT levels.

Several studies have demonstrated a clinically useful relationship between serum prostate specific antigen (PSA) and prostate volume in men with benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS).[33,34] Both prostate volume and serum PSA predict certain aspects of the natural history of LUTS and BPH, and men with higher PSA and larger glands in general have a higher rate of progression measured by various parameters.[34] Serum PSA also predicts the response to certain types of medical therapy in men with LUTS and BPH and is thus useful in the evaluation and management of these patients[35]. In this study, subcutaneous injection of testosterone for 28 consecutive days increased serum PSA level which was significantly attenuated by *M. indica* indicating possible efficacy in the treatment of BPH.

The reactive oxygen species (ROS) have been implicated in the etiology of BPH.[6,36] Oxidative stress is defined as an abnormal ratio of free radicals and reactive metabolites known as ROS due to their overproduction, to the ratio of cellular antioxidants responsible for their destruction. This imbalance results in injury to the cells, tissues, and possibly to all

body organs through damaging critical cellular macromolecules including DNA, resulting in altered cellular function.[37] In this study, testosterone induced an increased malondialdehyde in the prostate indicating lipid peroxidation, decreased GSH and superoxide dismutase activities in the prostate. However, the pretreatment of rats with *M. indica* attenuated the increase in malondialdehyde level and enhanced the activities of GSH and superoxide dismutase. These findings were corroborated by the results of in vitro antioxidant reductive capacity of *M. indica*. DPPH assay is classified as single electron transfer reaction used to assess the antioxidant reductive capacity of the test extract to neutralize either by direct reduction through electron transfer or by radical quenching by hydrogen transfer.[38] *M. indica* exhibited significant DPPH scavenging activity. Similarly, the stem bark extract of *M. indica* prevented nitrite formation by directly competing with oxygen in the reaction with nitric oxide. Nitric oxide is well implicated in inflammatory processes. Moreover, its toxicity increases when it reacts with superoxide radicals forming peroxynitrite anion ($ONOO^-$).[39] In addition, the reducing capacity of *M. indica* confirms its potential antioxidant activity. Metal chelating capacity of *M. indica* corroborates its attenuation of lipid peroxidation through reduction of transition metals that catalyzes lipid peroxidation.[38] Moreso, the results showed that *M. indica* contains significant amounts of flavonoids and phenolic compounds well reputed for their antioxidant properties[40]. More importantly, *M. indica* as a wide margin of safety, the extract up to 5000 mg/kg did not induce mortality nor behavioral toxic effect when administered orally. However, further studies are required to isolate the active constituents responsible for the observed effect as well as cellular mechanistic studies.

CONCLUSION

Findings from this study showed that the hydroethanolic stem bark extract of *Mangifera indica* prevented testosterone induced BPH possibly through enhancement of antioxidant defense mechanisms. Thus, confirms its folkloric uses and could be a potential phytotherapeutic agent in the treatment of BPH.

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