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Research Article

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Comparing Various Protocols for Experimental Induction of Benign Prostate Hyperplasia in Albino Wistar Rats

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a nonmalignant enlargement of the prostate has been associated with an increased prostate cancer risk. Spontaneous BPH rarely occurs outside humans, dogs, and chimpanzees, limiting suitable animal models. Researchers have developed induction protocols, but conflicting data on optimal duration affects consistency. This study compared protocols for inducing BPH in albino Wistar rats to determine the most effective method. Fifty-four mature male rats (11–12 weeks old) were randomly assigned to six groups (n=9). Group I was the control; groups IV and V underwent bilateral orchiectomy; and Group VI was the sham control. BPH induction used testosterone propionate (6 mg/kg, subcutaneously) in groups II and IV, or testosterone plus oestradiol valerate (0.6 mg/kg, subcutaneously) in groups III and V, every other day for 28 days. Sacrifices occurred on days 14, 21, and 28. Assessments included relative prostate weight, serum prostatic acid phosphatase (PAP), prostate-specific antigen (PSA), and histology. Non-castrated rats showed higher induction rates with both testosterone alone and hormone combination from day 14, with the non-castrated testosterone-oestradiol group (NCTE) producing the strongest response—relative prostate weight 0.26±0.01 vs. 0.16±0.02 in controls ($p<0.05$). PAP rose in all induction groups and peaked in non-castrated testosterone-treated animals. Histology revealed glandular hyperplasia, enlarged acini, and congestion in intact rats given hormones; castrated rats showed minimal changes. The testosterone-oestradiol protocol in intact rats was most effective, producing significant hyperplasia within 14 days, providing a reliable model for BPH research.

Keywords: Castration, Estradiol Valerate, Testosterone, Model Validation, Prostatic Acid Phosphatase, Prostate Index

List of Abbreviations

BPH	:	Benign Prostatic Hyperplasia
PAP	:	Prostatic Acid Phosphatase
PSA	:	Prostate-Specific Antigen
DHT	:	Dihydrotestosterone
ELISA	:	Enzyme-Linked Immunosorbent Assay
NC	:	Normal Control
NCT	:	Non-Castrated + Testosterone
NCTE	:	Non-Castrated + Testosterone + Estradiol
CT	:	Castrated + Testosterone
CTE	:	Castrated + Testosterone + Estradiol
SH	:	Sham Control
MCH	:	Mean Corpuscular Hemoglobin
MCV	:	Mean Corpuscular Volume

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MCHC	:	Mean Corpuscular Hemoglobin Concentration
Hb	:	Hemoglobin
PCV	:	Packed Cell Volume
TRBC	:	Total Red Blood Cell Count
TWBC	:	Total White Blood Cell Count
ANOVA	:	Analysis of Variance
SPSS	:	Statistical Package for Social Sciences
SEM	:	Standard Error of Mean
MOUAU	:	Michael Okpara University of Agriculture, Umudike
EEC	:	Experimental Ethics Committee
H&E	:	Hematoxylin and Eosin (staining)
KAU	:	King-Armstrong Units (for PAP measurement
IU/L	:	International Units per Liter
ng/mL	:	Nanograms per Milliliter
pg/mL	:	Picograms per Milliliter
mg/kg	:	Milligrams per Kilogram
s/c	:	Subcutaneous

Introduction

Benign prostatic hyperplasia (BPH) is a non-cancerous enlargement of the prostate gland that significantly affects a large proportion of the aging male population globally, often leading to lower urinary tract symptoms and negative impacts on quality of life [1,2]. Extensive research has sought to clarify the pathogenesis of this condition, yet the precise mechanisms underlying BPH remain incompletely understood. The disease has been closely linked to age-related hormonal changes, particularly involving androgens such as testosterone and its more potent form, dihydrotestosterone (DHT), as well as oestrogens, both of which play crucial roles in prostate growth through complex receptor-mediated pathways [2-4].

Due to ethical and practical limitations in conducting human studies, experimental animal models have become a cornerstone for understanding BPH biology and testing therapeutic interventions [3,5]. Rats, especially Wistar and Sprague-Dawley strains, are among the most commonly used species for experimentally induced BPH, as their prostate responses to hormonal manipulation, such as administration of testosterone and oestradiol, closely mirror those seen in humans [6]. Typically, experimental protocols involve either castrated or intact male rats subjected to subcutaneous injections of testosterone, with or without estradiol, over periods ranging from 14 to 28 days, leading to the development of prostatic enlargement and histological changes characteristic of BPH [6-8].

BPH is both a clinical and public health concern, with prevalence rates rising significantly with age; nearly half of men in their 50s and up to 90% of those over 80 years demonstrate histologic BPH [9-11]. The associated healthcare burden is substantial, with millions of diagnoses annually and high management costs. Experimental models are critical for improving the understanding and management of the disease; they enable investigation into mechanistic pathways, identification of biomarkers, and evaluation of new pharmacological treatments [12,13]. Key biomarkers measured during experimental BPH induction include prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) [14]. These molecules serve as indicators of prostatic activity and pathology, with PSA remaining the cornerstone for diagnosis and monitoring, albeit with acknowledged limitations in specificity [14-16]. Recent advances have also highlighted molecular and genetic tools, including transgenic and xenograft models, that further refine the study of BPH and its therapeutic modulation [3,5,17,18].

The present study was positioned within this research landscape, aiming to systematically compare and validate protocols for BPH induction in Wistar rats using various combinations of testosterone and oestradiol, both in castrated and intact animal models. By assessing relative prostate weights, serum biomarkers, and histological outcomes across different induction regimens and durations, this study sought to establish a robust, reproducible model of BPH that can facilitate future therapeutic and mechanistic investigations. This research not only addresses methodological gaps regarding duration, hormonal combinations, and animal status (castrated versus intact) in BPH modelling, but also contributes empirical data to guide experimental design and harmonise protocols in preclinical studies worldwide.

Materials and Methods

Study Location

The experimental procedures were conducted at the Department of Veterinary Theriogenology and Department of Veterinary Biochemistry and Animal Production, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. The study site is located in the South Eastern Zone of Nigeria at latitude 5°29'N and longitude 7°32'E, with an altitude of 123 meters above sea level. The region experiences an annual rainfall of 2177mm, average ambient temperature of 22.32°C, and relative humidity ranging from 50-90% [19].

Ethical Clearance

Ethical approval for this animal study (MOUAU/CVM/REC/202123) was obtained from the Research Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. All surgical procedures were conducted in strict compliance with the institutional ethical guidelines for laboratory animal care and use, following the Experimental Ethics Committee (EEC) guidelines.

Experimental Animals

Fifty-four (54) sexually mature male Wistar rats, aged 11-12 weeks and weighing–216-278 grams, were obtained from the Laboratory Animal House of the Department of Physiology and Pharmacology, College of Veterinary Medicine, MOUAU. The experimental rats were dewormed with albendazole (broad-spectrum anthelmintic) three weeks prior to study commencement. All animals underwent a one-week acclimatization period under standard laboratory conditions before experimental procedures began.

Experimental Design

This study employed a randomised controlled experimental design to compare six different protocols for BPH induction.

The 54 male Wistar rats were randomly divided into six groups (I-VI) with nine animals per group. The experimental design is illustrated in the flowchart below.

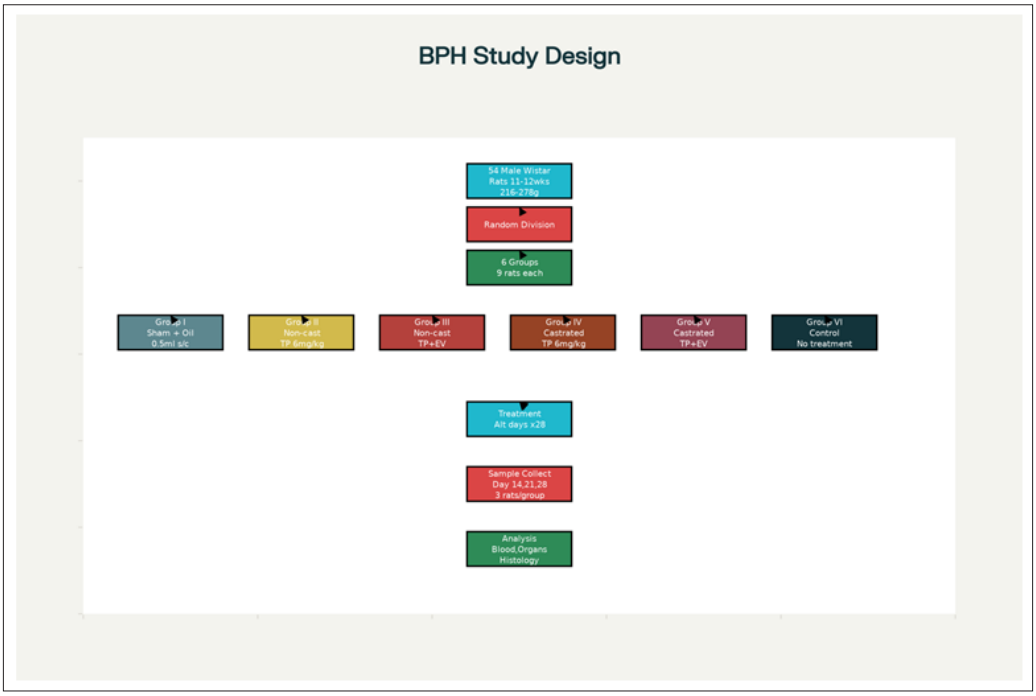

Plate 1: Experimental design flowchart for BPH induction study using different protocols in male Wistar rats

Group Assignments and Treatments

- **Group I (Sham Control, n=9):** Animals underwent anaesthesia and scrotal incision without castration, receiving corn oil (0.5 ml subcutaneously) as vehicle control.
- **Group II (Non-castrated + Testosterone, n=9):** Intact rats administered testosterone propionate (6 mg/kg subcutaneously) alone.
- **Group III (non-castrated + testosterone + oestradiol, n=9):** Intact rats receiving testosterone propionate (6 mg/kg s/c) combined with oestradiol valerate (0.6 mg/kg s/c).
- **Group IV (Castrated + Testosterone, n=9):** Bilaterally castrated rats administered testosterone propionate (6 mg/kg s/c).
- **Group V (Castrated + Testosterone + Oestradiol, n=9):** Bilaterally castrated rats receiving testosterone propionate (6 mg/kg s/c) plus oestradiol valerate (0.6 mg/kg s/c).
- **Group VI (Normal Control, n=9):** Intact rats receiving no treatment.

Surgical Procedures

Bilateral Orchiectomy

Castration was performed on Groups IV and V under general anaesthesia using xylazine (0.5 mg/kg intraperitoneally) and ketamine HCl (25 mg/kg intramuscularly). Following anaesthetic induction, both testes were excised via small scrotal incisions after ligation of the blood vessels and spermatic cords. The incision sites were closed with two suture stitches, and animals were housed individually in sterile metal cages for one week post-operatively to allow for complete recovery before BPH induction commenced [20,21].

Sham Surgery

Group I animals underwent identical anaesthetic protocols and scrotal incisions, but without testicular removal, serving as surgical controls [3].

BPH Induction Protocol

BPH induction was initiated using established hormonal protocols. The treatment regimen involved subcutaneous administration of induction agents every other day for 28 days [3,5,22]. The experimental timeline is as follows.

Pharmaceutical Agents

- **Testosterone Propionate:** 6mg/kg subcutaneously (Testost®, Laborate, India)
- **Estradiol Valerate:** 0.6mg/kg subcutaneously (Progymon Depot®, Kwality Pharmaceuticals Ltd, India)
- **Vehicle Control:** Corn oil 0.5ml subcutaneously (Cardinals Agrofoods Oils Ltd, Abuja, Nigeria)

All hormonal preparations were diluted every other day in corn oil and administered using tuberculin syringes with 25-gauge needles. The hormonal preparations were all administered in the morning hours (8 am – 10 am).

Sample Collection and Processing

Blood Sample Collection

Blood samples were collected from three randomly selected rats per group on days 14, 21, and 28 via retro-orbital plexus puncture under light anaesthesiausing using heparinised capillary tubes. [23].

- **EDTA tubes:** For hematological analysis
- **Plain tubes:** For serum biochemical and hormonal assays.

Serum Preparation

Non-heparinised samples (3 mL) were clotted at room temperature (3 h) before centrifugation. Serum was separated into clean labeled tubes and stored at -80°C until analysis [24].

Organ Harvesting

Following blood collection, the animals were humanely euthanized by cervical dislocation. The prostate gland and

seminal vesicles were carefully dissected, cleaned of connective tissue, weighed using an analytical balance (Mettler, Citizen ISO 900-1, China), fixed in 10% neutral buffered formalin for 24 h before it was processed for histological examination [25].

Laboratory Investigations

Prostatic Indices Calculation

Relative organ weights were calculated using the following formula:

Prostate Index (%) = (Prostate weight/Live body weight) × 100

Seminal Vesicle Index (%) = (Seminal vesicle weight/Live body weight) × 100 [26].

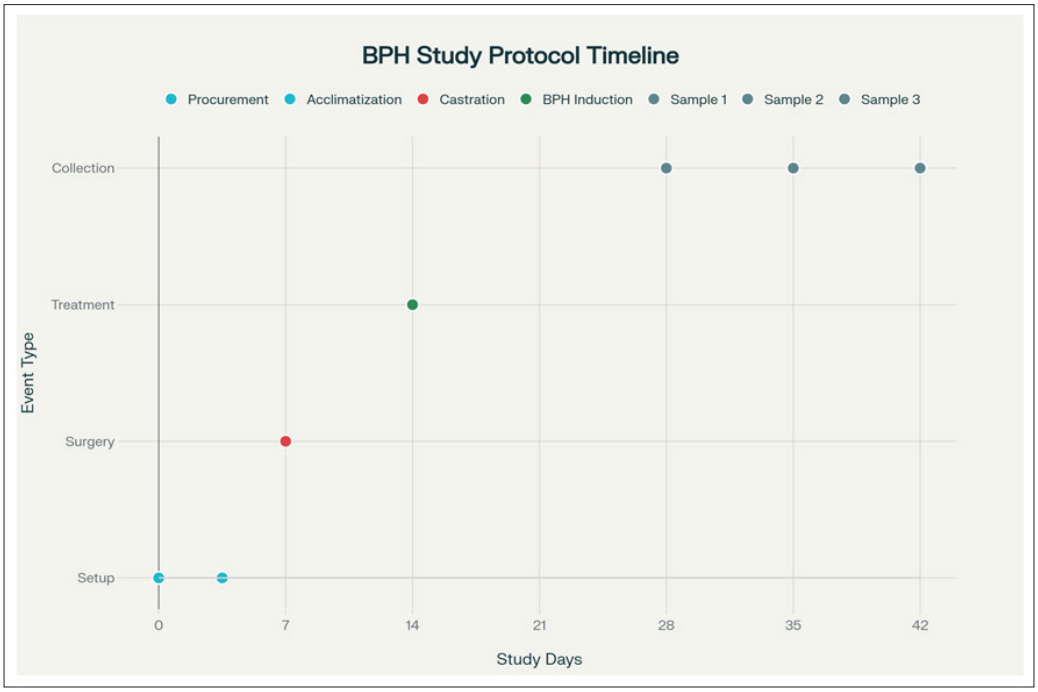

Plate 2: Timeline of experimental procedures for BPH induction study showing key milestones from animal procurement to final sampling

Hematological Analysis

Complete blood counts were performed using standard laboratory techniques, with red blood cell counts determined via dilution method using isotonic diluents and a Neubauer counting chamber, packed cell volume measured through the microhematocrit method employing non-graduated capillary tubes, and hemoglobin concentration quantified using the acid hematin method with N/10 hydrochloric acid. White blood cell counts were obtained through Turk reagent dilution with hemocytometer counting, and derived indices including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulas [27].

Biochemical Assays

Testosterone Assay

Serum testosterone levels were determined using quantitative Enzyme-Linked Immunosorbent Assay (ELISA) with microwell kits from Syntro Bioresearch Inc., California, USA. The competitive binding assay utilised a testosterone-conjugated reagent and anti-testosterone antibodies, with colour development measured at 450 nm using an automated microwell reader [28].

Prostatic Acid Phosphatase (PAP) Assay

PAP levels were measured using the modified Benotti method based on the Gutman and Gutman version of the King-Armstrong procedure. The assay principle involves the hydrolysis of phenyl phosphate in the presence and absence of L-tartrate, with the difference representing prostatic acid phosphatase activity [29,30].

Prostate Specific Antigen (PSA) and Estradiol

These parameters were analysed using commercially available ELISA kits following the manufacturer's protocols [16].

Histoarchitectural Studies

Prostate tissue samples were processed for histological examination using standard procedures:

1. **Fixation:** Tissues were fixed in 10% neutral buffered formalin
2. **Processing:** Routine paraffin embedding and sectioning at 5µm thickness
3. **Staining:** Hematoxylin and eosin (H&E) staining following established protocols
4. **Microscopic Examination:** Histological evaluation was performed using light microscopy to assess prostatic architecture, glandular hyperplasia, and cellular changes

The H&E staining protocol involved nuclear staining with hematoxylin (blue-purple coloration) and cytoplasmic/extracellular matrix staining with eosin (pink coloration) [31].

Statistical Analysis

Data obtained at each collection time point were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS, version 23). Duncan's multiple range test was employed for mean separation when significant differences were detected. Results are presented as mean ± standard error of mean (SEM), with statistical significance set at $p<0.05$ [32].

Ethical Clearance

Ethical approval for this animal study (MOUAU/CVM/REC/202123) was obtained from the Research Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

Results

Effect of Protocols on Prostate Index

Treatment Group	Day 14	Day 21	Day 28
NC	0.16 ± 0.02	0.15 ± 0.01	0.10 ± 0.01
NCT	0.25 ± 0.01*	0.22 ± 0.04*	0.23 ± 0.02*
NCTE	0.26 ± 0.01*	0.25 0.01*	0.27 ± 0.03*
CT	0.09 ± 0.02*	0.11 ± 0.01*	0.19 ± 0.01*
CTE	0.13 ± 0.02*	0.14 ± 0.01*	0.14 ± 0.01*
SH	0.15 ± 0.02*	0.15 0.01*	0.15 ± 0.01*

Significantly higher than control ($p<0.05$)

(NC: Non-castrated control; NCT: Non-castrated + testosterone propionate; NCTE: Non-castrated + testosterone propionate + oestradiol valerate; CT: Castrated + testosterone; CTE: Castrated + testosterone + oestradiol valerate; SH: Sham-operated rats)

Administration of testosterone propionate alone (NCT) or in combination with oestradiol valerate (NCTE) to non-castrated (intact) rats resulted in a significant increase in the prostate index as early as day 14 post-treatment compared to the control (NC) and castrated groups. On day 14, prostate indices were 0.25 ± 0.01 (NCT) and 0.26 ± 0.01 (NCTE) vs. 0.16 ± 0.02 (NC; $p<0.05$). Castrated rats with testosterone (CT) or combination therapy (CTE) showed indices of 0.09 ± 0.02 and 0.13 ± 0.02, respectively, which were lower ($p<0.05$) than controls. Sham-operated rats (SH) had indices similar to NC (0.15 ± 0.01). This pattern persisted with further administration; by day 21, NCTE (0.25 ± 0.01), NCT (0.22 ± 0.04), and CT (0.21 ± 0.02) remained significantly higher than NC/SH, with the highest in NCTE. At day 28, indices peaked in NCTE (0.27 ± 0.03) and NCT (0.23 ± 0.02), with CT (0.19 ± 0.01) lower and CTE (0.14 ± 0.01) comparable to the control (0.10 ± 0.01).

Seminal Vesicle Index

The relative weights of seminal vesicles were significantly higher in the induction groups (especially NCTE and CT) than in the control and sham groups at every time point. Notably, castrated rats (CT) and non-castrated rats receiving combination therapy (NCTE) displayed pronounced enlargement at day 14 and maintained this elevation through day 28, implicating an androgen-driven response amplified by co-administered oestradiol.

Serum Markers (PAP, PSA, Hormones)

(I will ask for the discretion of the editorial body to let me know where to place all the Bar charts that cover PAP, PSA and Hormones; kindly help me place them at positions you deem fit.)

Prostatic acid phosphatase (PAP) levels were significantly elevated in all induced groups at every time point compared with controls, with the highest PAP in NCT at days 21 and 28 (e.g. day 28: NCT 3.20 ± 0.02, CT 2.61 ± 0.02, NCTE 1.69 ± 0.01, CTE 1.30 ± 0.02, SH 0.90 ± 0.01, NC 0.70 ± 0.01; $p<0.05$). PSA levels and serum testosterone were markedly increased in induction groups, with NCTE rats recording the highest PSA (3.06 ± 0.12 IU/L) on day 28 followed by NCT (2.40 ± 0.01 IU/L), both above the normal value (1.60 ± 0.10 IU/L). Testosterone was highest in the NCT group (6.20 ± 0.78 ng/mL) and lowest in controls, aligned with the administered protocol. NCTE rats displayed dramatically elevated oestradiol (114.66 ± 12.11 pg/mL) compared to all other groups.

Histological Outcomes

Prostates from all rats treated with testosterone and/or oestradiol showed evidence of hyperplasia after 28 days. In NCTE, glandular hyperplasia is characterised by back-to-back acinar enlargement, exaggerated intra-acinar papillary convolutions, and increased secretory activity, corroborating the largest increases in relative prostate weight. NCT revealed mild acinar enlargement and stromal congestion. In contrast, the CT and CTE groups showed compressed or atrophied acini with architectural distortion and reduced secretory material; despite some qualitative changes, these did not result in statistically significant mass increases. Sham rats exhibited preserved histoarchitecture similar to that of NC rats.

Haematology

Red cell indices (Hb, PCV, TRBC, MCV, MCH, MCHC) remained largely within control ranges except for increases in MCH/MCHC in CTE on day 14 and significant reductions of Hb, PCV, and TRBC in NCTE, CTE, and SH on day 28, potentially reflecting cumulative systemic effects of the protocol but not confounding primary prostatic outcomes. The total white blood cell count (TWBC) was the lowest in the sham group across multiple points.

Discussion

The results of this study demonstrate that the efficacy of various protocols for the experimental induction of benign prostate hyperplasia (BPH) in Albino Wistar rats is highly dependent on both hormonal manipulation and the physiological integrity of the animals, with significant implications for preclinical modelling of BPH and future therapeutic screening.

Protocols utilising testosterone propionate alone or in combination with oestradiol valerate proved most efficient in inducing BPH in intact (non-castrated) male Wistar rats, as evidenced by significant increases in relative prostate gland weights, elevated serum levels of Prostatic Acid Phosphatase (PAP), and Prostate-Specific Antigen (PSA) across multiple evaluated timepoints. The onset of measurable hyperplasia was observed as early as 14 days post-induction, with progressive organ and biochemical marker changes throughout days 21 and 28, thus supporting a time-dependent induction effect [33,34].

In contrast, protocols incorporating bilateral orchiectomy (castration) prior to hormonal induction produced a markedly reduced hyperplastic response, as reflected by the absence of significant prostate enlargement and milder glandular changes on histological assessment. This highlights the crucial role of endogenous androgens and the complementary action of administered exogenous hormones in driving BPH pathogenesis, consistent with the established viewpoint that dihydrotestosterone (DHT) is a primary mediator of BPH via androgen receptor activation and gene expression [35,36].

Histopathological examination further confirmed that intact rats exposed to combined testosterone and oestradiol displayed pronounced glandular hyperplasia, architectural distortion, stromal infiltration, and congestion, findings representative of advanced BPH in humans [37-39]. Conversely, castrated rats, even after similar hormonal induction, exhibited atrophic changes with reduced secretory activity and acinar compression, but lacked the overt hyperplastic features seen in intact animals. These results provide empirical evidence supporting the superiority of intact hormonal status for establishing clinically relevant BPH models in rodents and suggest the necessity of direct monitoring of both androgen and oestrogen axes during induction [40-42].

Serum marker analyses showed parallel increases in PAP and PSA correlating with organ enlargement in the induction groups, further confirming the reliability of these markers for monitoring BPH development and experimental progression. The observed elevations in testosterone and oestradiol, particularly in the group receiving both hormones, underlines the synergistic hormonal influence in driving pathology and aligns with the theory that altered androgen-oestrogen ratios favour prostatic cellular proliferation [43,44].

A notable secondary finding was the conspicuous enlargement of seminal vesicles in all induction groups, which occasionally exceeded the degree of prostatic enlargement. This phenomenon, although less emphasized in prior literature, may have important implications for understanding lower urinary tract symptoms and bladder outlet obstruction in BPH [13,45,46].

Haematological indices generally remained within normal ranges across most groups and timepoints, except for slight deviations at later stages of induction, likely attributable to prolonged hormonal administration rather than direct procedure-related effects [47].

Conclusion and Recommendations

In summary, the most effective protocol for the experimental induction of BPH in Albino Wistar rats is the administration of testosterone, preferably in combination with oestradiol, to intact males. This induction method reliably produces morphological and serological hallmarks of BPH within 14 days, providing a robust and reproducible model for further pharmacological or mechanistic studies. Castration impaired the induction process, underscoring the need to preserve endogenous androgenic stimulation in the model.

Future experimental screening for putative BPH therapies should utilize protocols based on hormonal induction of intact rats, with careful attention to both prostate and seminal vesicular pathology.

These findings are consistent with previously published evidence and extend the current knowledge by establishing clear experimental guidelines for BPH induction and progression monitoring in preclinical settings. This model offers a practical foundation for evaluating novel pharmacological agents targeting androgen-oestrogen-driven prostatic growth.

Funding: NIL

Acknowledgement

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Histopathological Micrographs of the Epididymis

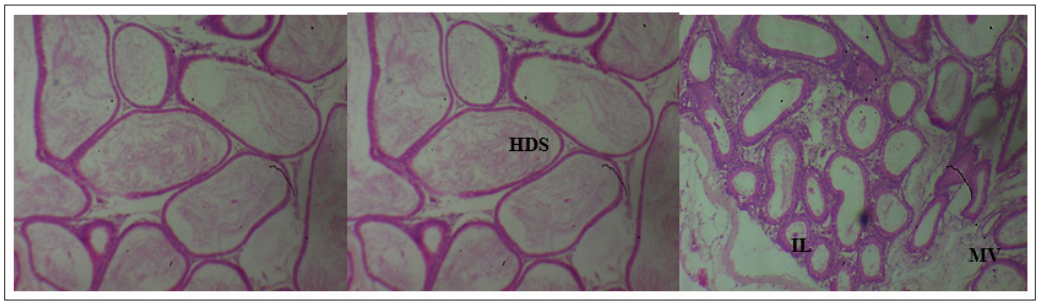

Figure 1a: Group 1 (Control) **Figure 1b:** Group 2 (T) **Figure 1c:** Group 3 (NCTE)

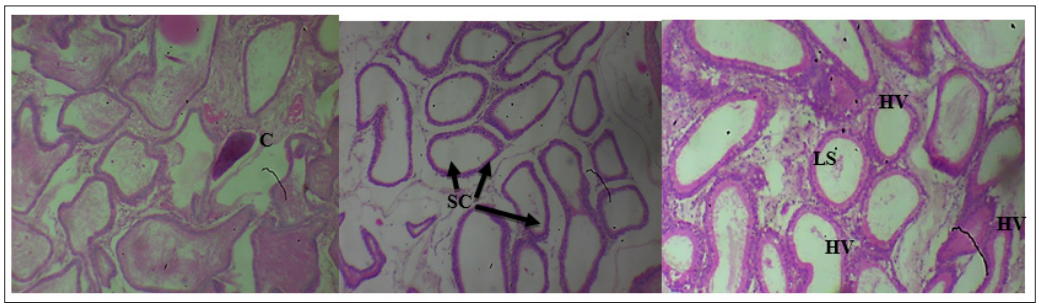

Figure 1d: Group 4 (CT) **Figure 1e:** Group 5 (CTE) **Figure 1f:** Group 6 (SH)

Note: T: non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham). **MV:** Mild Vacuolation; **IL:** Indented lumen; **SC:** Scanty sperm cells; **C:** Congestion; **LS:** Luminal secretion; **HV:** High vascularization; **HDS:** High dense spermatids

Histopathological Micrograph of the Testes

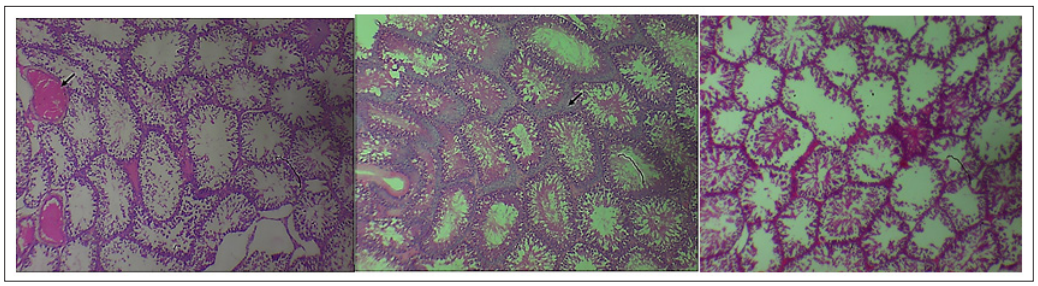

Figure 2a: Group 1 (Control) **Figure 2b:** Group 2 (T) **Figure 2c:** Group 3 (NCTE)

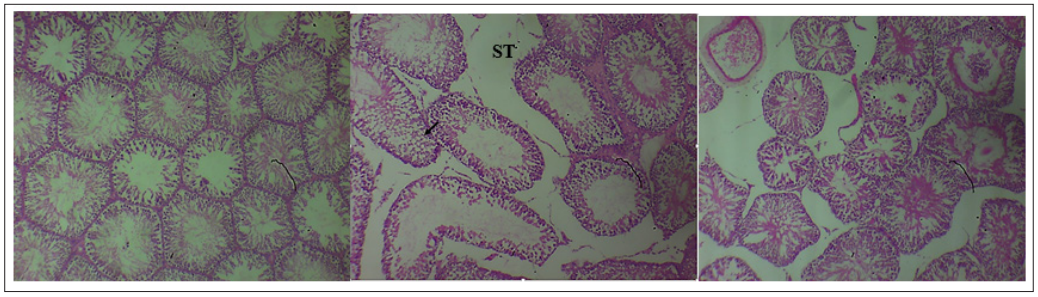

Figure 2d: Group 4 (CT) **Figure 2e:** Group 5 (CTE) **Figure 2f:** Group 6 (SH)

Note: T: non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** Castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham). **ST:** Scanty testicular tubules with extensive cell detachment and obliteration

Histopathological Micrograph of the Prostate Gland

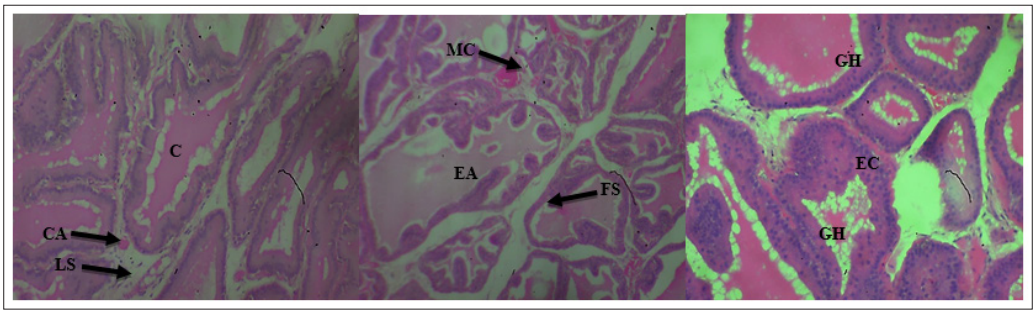

Figure 3a: Group 1 (Control) **Figure 3b:** Group 2 (T) **Figure 3c:** Group 3 (NCTE)

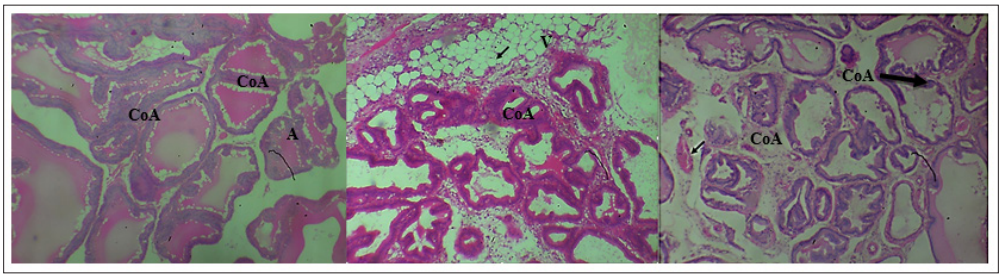

Figure 3d: Group 4 (CT) **Figure 3e:** Group 5 (CTE) **Figure 3f:** Group 6 (SH)

Note: T: non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham). **CA:** Corpora amylacea; **LS:** Luminal secretions; **C:** Convolution; **GH:** Glandular hyperplasia; **EC:** Extensive congestion; **CoA:** Compressed Acini (Atrophied)gland)

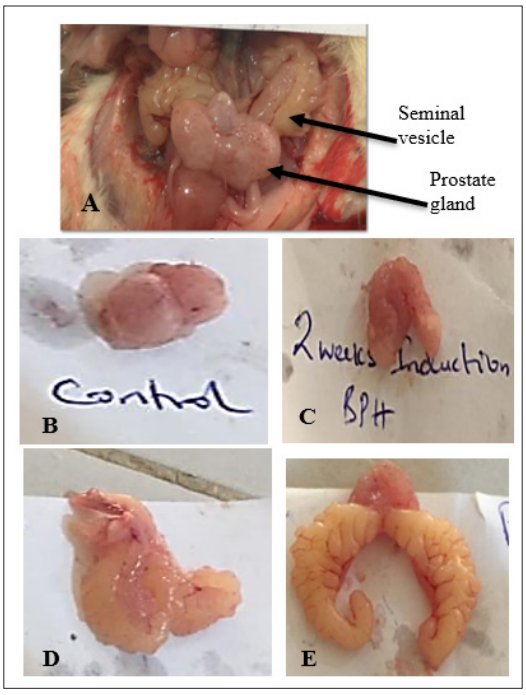

Plate 3: Images of normal and hyperplastic prostate gland and seminal vesicle after BPH induction.

A: The intact seminal vesicle and prostate gland in a Wistar rat (Arrows); **B:** Harvested prostate (Control); **C:** Harvested prostate (2 weeks induction BPH); **D:** Harvested seminal vesicle (Control); **E:** Harvested seminal vesicle (2 weeks induction BPH)

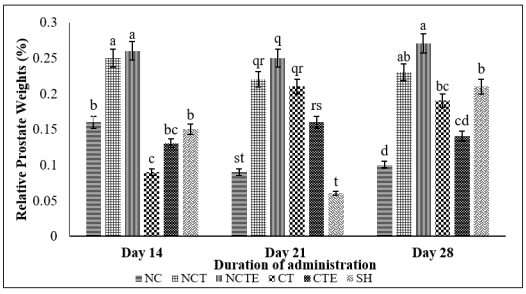

Figure 4: Relative prostate weights of BPH induced Wistar rats under different protocols. Different letters indicate significant differences ($p<0.05$) between groups, within each time point (n=3).

Legend: NC: Normal control; **NCT:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham).

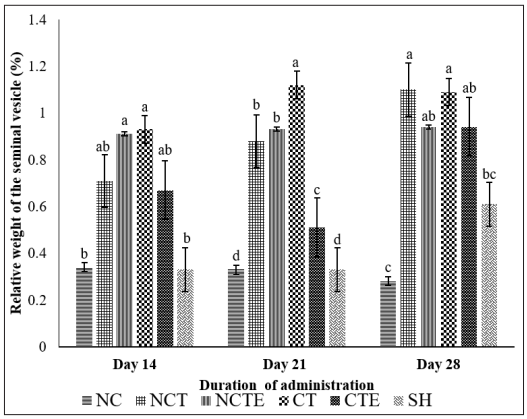

Figure 5: Relative weight of seminal vesicle of Wistar rats using different protocols of BPH induction. Different letters indicate significant differences ($p<0.05$) between groups, within each time point (n=3).

Legend: NC: Normal control; **NCT:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham).

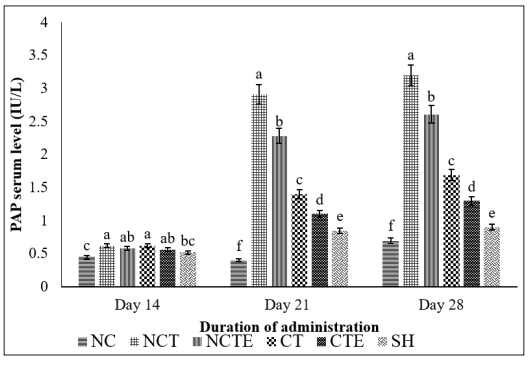

Figure 6: Serum PAP levels of BPH-induced Wistar rats using different protocols.
Different letters indicate significant differences ($p<0.05$) between groups, within each time point (n=3).

Legend: NC: Normal control; **NCT:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham).

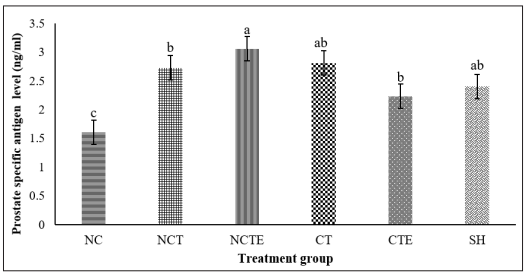

Figure 7: Serum PSA levels of BPH-induced Wistar rats using different protocol.

Note: Different letters indicate significant differences ($p<0.05$) between groups, (n=3).

Legend: NC: Normal control; **NCT:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham).

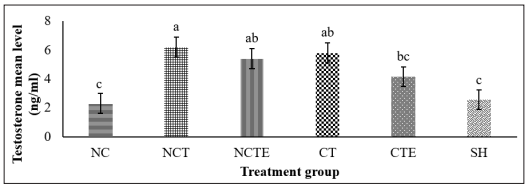

Figure 8: Testosterone level of BPH-induced Wistar rats using different protocol.

Note: Different letters indicate significant differences ($p<0.05$) between groups, (n=3).

Legend: NC: Normal control; **NCT:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham).

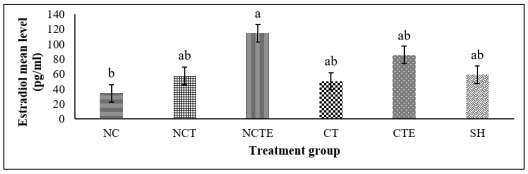

Figure 9: Estradiol level of BPH-induced Wistar rats using different protocol.

Note: Different letters indicate significant differences ($p<0.05$) between groups, (n=3).

Legend: NC: Normal control; **NCT:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham).

Table 1: Summary of Key Findings at Day 28

Group	Prostate Index	Seminal Vesicle Index	PAP (KAU/100ml)	PSA (IU/L)	Testosterone (ng/mL)	Estradiol (pg/mL)	Histology
NC	0.1 ± 0.01	0.28 ± 0.06	0.7 ± 0.01	1.6 ± 0.1	2.3 ± 1.05	34.53 ± 8.8	Normal
NCT	0.23 ± 0.02	1.1 ± 0.05	3.2 ± 0.02	2.4 ± 0.01	6.2 ± 0.78	57.63 ± 7.52	Mild hyperplasia
NCTE	0.27 ± 0.03	0.94 ± 0.09	1.69 ± 0.01	3.06 ± 0.12	5.4 ± 0.36	114.66 ± 12.11	Glandular hyperplasia
CT	0.19 ± 0.01	1.09 ± 0.11	2.61 ± 0.02	2.81 ± 0.19	5.8 ± 0.3	50.4 ± 23.08	Compressed/ atrophic
CTE	0.14 ± 0.01	0.94 ± 0.17	1.3 ± 0.02	2.23 ± 0.37	4.16 ± 0.32	85.66 ± 24.1	Distorted/ atrophic
SH	0.21 ±	0.61 ± 0.13	0.9 ± 0.01	2.73 ± 0.03	2.56 ± 0.14	59.4 ± 28.55	Preserved

Table 2: Effect of testosterone and or its combination with estradiol on the haematological parameters of induced wistar rats on day 14 of administration.

Parameters	Control	T	NCTE	CT	CTE	SH
Haemoglobin (g/dl)	17.53 ± 0.35^{ab}	18.33 ± 0.13^{a}	18.26 ± 0.17^{a}	18.06 ± 0.29^{a}	17.00 ± 0.52^{b}	18.06 ± 0.17^{a}
PCV (%)	44.00 ± 2.08^{ab}	46.66 ± 0.33^{a}	47.00 ± 0.57^{a}	45.67 ± 1.20^{a}	39.33 ± 3.17^{b}	46.00 ± 0.57^{a}
TRBC ($\times10^{6}mm^{3}$)	7.00 ± 0.30^{ab}	7.39 ± 0.06^{a}	7.49 ± 0.10^{a}	7.29 ± 0.19^{a}	6.30 ± 0.51^{b}	7.36 ± 0.09^{a}
TWBC ($\times10^{3}mm^{3}$)	12.90 ± 0.20^{a}	11.66 ± 0.40^{a}	13.56 ± 0.31^{a}	11.90 ± 0.25^{a}	12.20 ± 0.72^{a}	9.65 ± 1.23^{b}
MCV (fl)	63.74 ± 0.17^{ab}	64.01 ± 0.22^{a}	63.54 ± 0.27^{ab}	63.45 ± 0.09^{ab}	63.46 ± 0.13^{ab}	63.36 ± 0.01^{b}
MCH (Pg)	25.09 ± 0.57^{b}	24.81 ± 0.25^{b}	23.37 ± 0.17^{b}	24.77 ± 0.27^{b}	27.21 ± 1.40^{a}	24.55 ± 0.08^{b}
MCHC (g/dl)	39.95 ± 1.05^{b}	39.28 ± 0.28^{b}	38.86 ± 0.13^{b}	39.58 ± 0.41^{b}	43.57 ± 2.22^{a}	39.28 ± 0.14^{b}

Note: values are presented as Mean ± S.E (Standard Error of Mean). Different superscript letters across rows shows significant ($p<0.05$) differences. PCV: Packed Cell Volume; **TRBC:** Total Red Blood Cell; **TWBC:** Total White Blood Cell; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Haemoglobin; **MCHC:** Mean Corpuscular Haemoglobin Concentration. **T:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol combination; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol combination, **SH:** No induction, no castration incision only (Sham).

Table 3: Effect of testosterone and or its combination with estradiol on the PAP and relative organ weight of induced wistar rats on day 14 of administration.

Parameters	Control	T	NCTE	CT	CTE	SH
Live weight (g)	297.00 ± 9.29^{a}	257.00 ± 2.64^{bc}	306.00 ± 1.29^{a}	313.33 ± 11.72^{a}	237.33 ± 8.29^{c}	285.33 ± 14.76^{ab}
PAP (KAU/100ml)	0.45 ± 0.01^{c}	0.62 ± 0.05^{a}	0.58 ± 0.01^{ab}	0.62 ± 0.02^{a}	0.56 ± 0.01^{ab}	0.52 ± 0.01^{bc}
Prostate (%)	0.16 ± 0.02^{b}	0.25 ± 0.01^{a}	0.26 ± 0.01^{a}	0.09 ± 0.02^{c}	0.13 ± 0.02^{bc}	0.15 ± 0.01^{b}
SV (%)	0.39 ± 0.00^{b}	0.71 ± 0.01^{ab}	0.91 ± 0.15^{a}	0.93 ± 0.16^{a}	0.67 ± 0.18^{ab}	0.33 ± 0.03^{b}

Note: values are presented as Mean ± S.E (Standard Error of Mean). Different superscript letters across rows shows significant ($p<0.05$) differences **T:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol combination; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol combination, **SH:** No induction, no castration incision only (Sham). **PAP:** Prostate Acid Phosphatase; **SV:** Seminal vesicle

Table 4: Effect of testosterone and or its combination with estradiol on the haematological parameters of induced wistar rats on day 21 of administration.

Parameters	Control	T	NCTE	CT	CTE	SH
Haemoglobin (g/dl)	20.20 ± 0.20	18.40 ± 0.87	17.46 ± 1.27	19.00 ± 0.80	18.40 ± 0.92	18.00 ± 1.05
PCV (%)	52.66 ± 1.66	46.00 ± 3.21	41.00 ± 5.03	48.00 ± 3.00	46.00 ± 3.21	44.33 ± 3.75
TRBC ($\times10^{6}mm^{3}$)	8.18 ± 0.05	7.34 ± 0.50	6.55 ± 0.79	7.63 ± 0.47	7.33 ± 0.52	7.11 ± 0.58
TWBC ($\times10^{3}mm^{3}$)	13.71 ± 0.21^{a}	12.10 ± 0.73^{ab}	14.10 ± 0.32^{a}	13.03 ± 0.32^{ab}	11.96 ± 0.95^{ab}	10.35 ± 1.58^{b}
MCV (fl)	65.15 ± 1.64	63.53 ± 0.03	63.57 ± 0.04	63.69 ± 0.09	63.63 ± 0.30	63.16 ± 0.18
MCH (Pg)	24.69 ± 0.09	25.15 ± 0.70	26.96 ± 1.20	24.96 ± 0.55	25.18 ± 0.68	25.39 ± 0.70
MCHC (g/dl)	38.41 ± 0.81^{b}	40.14 ± 1.18^{ab}	43.08 ± 1.97^{a}	39.69 ± 0.89^{ab}	40.12 ± 0.93^{ab}	40.79 ± 1.20^{ab}

Note: values are presented as Mean ± S.E (Standard Error of Mean). Different superscript letters across rows shows significant ($p<0.05$) differences. **PCV:** Packed Cell Volume; **TRBC:** Total Red Blood Cell; **TWBC:** Total White Blood Cell; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Haemoglobin; **MCHC:** Mean Corpuscular Haemoglobin Concentration. **T:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol combination; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol combination, **SH:** No induction, no castration incision only (Sham).

Table 5: Effect of testosterone and or its combination with estradiol on the PAP and relative organ weight of induced wistar rats on day 21 of administration.

Parameters	Control	T	NCTE	CT	CTE	SH
Live weight	234.66 ± 8.29^{cd}	272.66 ± 17.33^{ab}	207.00 ± 0.00^{d}	288.33 ± 10.66^{a}	253.33 ± 13.66^{bc}	240.66 ± 0.33^{bcd}
PAP	0.64 ± 0.00^{f}	2.91 ± 0.01^{a}	1.40 ± 0.01^{c}	2.28 ± 0.01^{b}	1.10 ± 0.02^{d}	0.85 ± 0.01^{e}
Prostate	0.09 ± 0.02^{cd}	0.22 ± 0.04^{ab}	0.25 ± 0.01^{a}	0.21 ± 0.02^{ab}	0.16 ± 0.00^{bc}	0.06 ± 0.01^{d}
Seminal vesicle	0.33 ± 0.01^{d}	0.88 ± 0.07^{b}	0.93 ± 0.09^{b}	1.12 ± 0.01^{a}	0.51 ± 0.03^{c}	0.33 ± 0.04^{d}

Note: values are presented as Mean ± S.E (Standard Error of Mean). Different superscript letters across rows shows significant ($p<0.05$) differences. **T:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol combination; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol combination, **SH:** No induction, no castration incision only (Sham). **PAP:** Prostate Acid Phosphatase.

Table 6: Effect of testosterone and or its combination with estradiol on the haematological parameters of induced wistar rats on day 28 of administration.

Parameters	Control	T	NCTE	CT	CTE	SH
Haemoglobin (g/dl)	17.55 ± 0.24^{a}	17.46 ± 0.13^{a}	16.33 ± 0.29^{b}	17.46 ± 0.17^{a}	16.20 ± 0.34^{b}	16.60 ± 0.11^{b}
PCV (%)	43.33 ± 1.33^{a}	43.33 ± 0.33^{a}	38.33 ± 1.76^{b}	44.33 ± 0.33^{a}	37.00 ± 1.52^{b}	39.00 ± 0.57^{b}
TRBC ($\times10^{6}mm^{3}$)	6.89 ± 0.20^{a}	6.86 ± 0.08^{a}	6.07 ± 0.31^{b}	7.12 ± 0.01^{a}	5.86 ± 0.24^{b}	6.24 ± 0.06^{b}

TWBC ($\times 10^3 mm^3$)	13.93±0.74^{a}	14.10±0.30^{a}	12.45±0.27^{ab}	12.96±0.48^{ab}	11.93±0.93^{b}	8.88±0.56^{c}
MCV (fl)	63.78±0.22	64.07±0.33	64.26±0.51	63.09±0.37	64.16±0.05	63.44±0.43
MCH (Pg)	25.16±0.42^{cd}	25.45±0.31^{bcd}	27.01±0.98^{ab}	24.52±0.19^{d}	27.66±0.64^{a}	26.57±0.12^{abc}
MCHC (g/dl)	40.04±0.68^{c}	40.31±0.31^{bc}	42.72±1.25^{a}	39.40±0.15^{c}	43.86±1.01^{a}	42.57±0.33^{ab}

Note: values are presented as Mean ± S.E (Standard Error of Mean). Different superscript letters across rows shows significant ($p<0.05$) differences. **PCV:** Packed Cell Volume; **TRBC:** Total Red Blood Cell; **TWBC:** Total White Blood Cell; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Haemoglobin; **MCHC:** Mean Corpuscular Haemoglobin Concentration. **T:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol combination; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol combination, **SH:** No induction, no castration, incision only (Sham).

Table 7: Effect of testosterone and or its combination with estradiol on the hormonal profile and relative organ weight of induced wistar rats on day 28 of administration.

Parameters	Control	T	NCTE	CT	CTE	SH
Live weight	249.66±11.28^{b}	308.00±13.31^{a}	232.00±15.53^{b}	251.66±6.93^{b}	238.55±18.35^{b}	231.33±6.69^{b}
PAP	0.70±0.01^{f}	3.20±0.02^{a}	1.69±0.01^{c}	2.61±0.02^{b}	1.30±0.02^{d}	0.90±0.01^{e}
Prostate	0.10±0.01^{d}	0.23±0.02^{ab}	0.27±0.03^{a}	0.19±0.01^{bc}	0.14±0.01^{cd}	0.21±0.00^{b}
Seminal vesicle	0.28±0.06^{c}	1.10±0.05^{a}	0.94±0.09^{ab}	1.09±0.11^{a}	0.94±0.17^{ab}	0.61±0.13^{bc}
PSA	1.60±0.10^{c}	2.40±0.01^{b}	3.06±0.12^{a}	2.81±0.19^{ab}	2.23±0.37^{b}	2.73±0.03^{ab}
Testosterone	2.30±1.05^{c}	6.20±0.78^{a}	5.40±0.36^{ab}	5.80±0.30^{ab}	4.16±0.32^{bc}	2.56±0.14^{c}
Estradiol	34.53±8.80^{b}	57.63±7.52^{ab}	114.66±12.11^{a}	50.40±23.08^{ab}	85.66±24.10^{ab}	59.40±28.55^{ab}

Note: values are presented as Mean ± S.E (Standard Error of Mean). Different superscript letters across rows are significantly ($p<0.05$) different. **T:** Non-castrated, testosterone alone; **NCTE:** Non-castrated, testosterone and estradiol; **CT:** castrated, testosterone alone; **CTE:** Castrated, testosterone and estradiol, **SH:** Non-castrated incision only, no induction (Sham). **PAP:** Prostate Acid Phosphatase. **PSA:** Prostate Specific Acid.

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