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***Research Article***

# Spermatogenic and Androgenic Potential of Nephrolepis biserrata on Testosterone- Propionate Induced Benign Prostatic Hyperplasia In Male Wistar Rats

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## Article Info

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## Abstract

Infertility is one of the most serious and growing problems in the world. Numerous external and internal factors can lead to male infertility. Although a basal level of free radicals produced is essential for sperm capacitation, hyperactivation and sperm-oocyte fusion, their overproduction can cause sperm damage by overcoming the antioxidant defense system. This condition is a common place in testosterone propionate induced benign prostatic hyperplasia. *Nephrolepis biserrata* possesses pro-infertility properties that can be exploited in management and promotion of fertility health of BPH patients. This study aims to study the effect of *Nephrolepis biserrata* on the fertility indices of testosterone-propionate induced BPH in male Wistar rats. Male Wistar rats were randomly placed in sixteen groups normal control, three groups treated with TP for 11, 22 and 33 days. Another 3 groups treated with TP for 11, 22 and 33 days and thereafter administered with 5mg/kg b.wt of Finasteride and 1mg/kg b.wt of Doxazosin. 9 groups were administered with 100mg/kg b.wt, 200mg/kg b.wt and 300mg/kg b.wt of the plant extract for 11, 22 and 33 days after TP induction. The hormonal assay was determined by colorimetric method using ELISA kit microplate and semen analysis was done by microscopic assessment. *N.biserrata.* extract significantly decreased the prostate weight and prostatic index in rats with TP-induced BPH. There was also a significant ($p < 0.05$) decrease in the level of testosterone for the extract treated groups showed a marked increase at $p < 0.05$ in comparison to the BPH group. The extract treated groups showed a significant ($p < 0.05$) increase in the total sperm count when compared to the BPH groups. From the results of the current study, it could be said that *N.biserrata.* enhanced the spermatogenic and androgenic functions in testosterone propionate induced benign prostatic hyperplasia. Hence it could be advanced as a pro-fertility agent for men with infertility condition resulting from BPH.

## Abbreviation

TP-Testosterone propionate; TEST-Testosterone; FSH-Follicle Stimulating Hormone; LH-Luteinizing Hormone; TSC-Total Sperm Cells

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# 1. Introduction

Infertility is one of the most serious and growing problem in the world today [1]. Numerous external and internal factors can lead to male infertility cases. [2]. While free radicals are produced at basal level and are essential for sperm capacitation, hyperactivation, and sperm-oocyte fusion, overproduction can cause sperm damage by overcoming the antioxidant defense system [3, 4]. The prostate is a small, muscular gland about the size of a walnut. It is an essential male reproductive organ, so when problems with the prostate arise, they can affect a man's infertility. A larger sized prostate not only causes problems with urination, but it is also known to obstruct the flow of semen or alter the direction of semen-backwards into the bladder. Men with enlarged prostate may therefore become candidates for infertility problems. [5] asserted given the functional role of the prostate in male reproduction, unhealthy prostate as in the case of BPH can affect spermatozoa functioning and hence male fertility.

Benign prostatic hyperplasia (BPH) is the topmost prominent disease affecting the prostate gland in older men and approximately one half of all men aged above 50 years will have this disease [6]. The BPH condition is characterized by an abnormally enlarged prostate gland, which induces urethral constriction, resulting in increased frequency, urgency and hesitancy of urination as well as disrupted urine flow, which eventually affects the quality of life of the patients. Even though the pathway associated with prostatic enlargement is not fully understood, androgens are regarded as one of the most significant factors. Although androgens do not directly cause BPH, testicular androgens play an important role in the development of prostatic growth in men [7]. Dihydrotestosterone (DHT) is likely to be the most vital factor associated with the enlargement of prostate. It has been reported that the DHT concentrations in the serum are higher in BPH patients than in unaffected men of similar age [8]. Experiments have also revealed that estrogen levels rise with age, potentially increasing the expression of DHT, the progenitor of BPH [9].

The affordability of herbs over expensive pharmaceutical drugs to treat diseases among non-industrialized societies is fast becoming revolutionized. In some countries, it has been integrated into the health scheme despite advances in orthodox medicine [10]. Nephrolepis biserrata (giant sword fern) is a large, erect spreading, evergreen fern forming beautiful mounds of long, narrow, arching fronds that can reach 8ft (240cm) [11]. It creates a hush, tropical effect; its tall fronds quickly spreading from horizontal stolons. The medicinal properties of the plant have been widely reported. Studies have shown its antimicrobial, hepatoprotective, antioxidant, chemopreventive, cytotoxic and phytoremediative properties [12–14]. Other medicinal values have been reported of its effect on hematological indices of Wistar albino rats, lipid peroxidation amelioration and cardiovascular disease reduction [15]. Against this backdrop, the current study was carried out to study the fertility indices by investigating the effect of Nephrolepis biserrata aqueous leaf extract on the spermatogenic and androgenic function of adult male Wistar rats induced with testosterone propionate.

# 2. Materials and Methods

## Collection and Identification of Study Plant

The fresh leaves of Nephrolepis biserrata (NB) were collected from Choba river bank, in Choba community, Obio/ Akpor Local Government Area of Rivers State, Nigeria. The plant sample was identified and authenticated by Dr Ekeke Chimezie at the Herbarium Unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt. The voucher specimen was registered with the number UPH/P/393 and deposited in the herbarium.

## Extraction Process

The aqueous extract was prepared according to the method described by Sofowara (1993). The fresh leaves of Nephrolepis biserrata was washed over running water and was allowed to air dry. It was blended into powder. 25g of the powder was macerated in 100ml of deionized water for 24 hours using mechanical agitation at room temperature. The suspension was filtered using Whatman filter paper and concentrated in a water bath at approximately 55°C. The crude extract was obtained and stored in the refrigerator at 4°C.

## Phytochemical Screening of the Plant

The fresh leaves of the plant were subjected to phytochemical analysis according to the method outlined by [16, 17]. The phytochemical analysis was done to detect the presence of secondary metabolites, such as alkaloids, glycoside, saponins, tannins, flavonoids, phenolic compounds, steroids, polyphenols and cardiac glycosides.

## Study Animals

The animal model employed for this study were male Wistar albino rats which were eight weeks old (weighing 180-250g). They were housed in clean plastic cages with temperature between 25°C-28°C. The rats were allowed access to water and normal rat chow freely throughout the duration of the experiment. The rats were also acclimatized for 2 weeks before the commencement of the study. The procedure for animal handling and the study methodology conformed to the ARRIVE Guideline as stipulated, guideline for Laboratory Animal Care (NIH Publication 1996, No 85-23).

## Ethical Approval

The experimental protocol of the present study was thoroughly reviewed and approved with reference no UPH/CEREMAD/REC/MM98/048 by the University of Port Harcourt ethical Committee, University of Port Harcourt, Choba, Rivers State, Nigeria.

### Experimental Design

BPH was induced in rats through daily subcutaneous injection (S.C) of testosterone-propionate (8mg/kg b.wt), adopting the method of [18, 19] with modification.

Rats were randomized into six groups; Group 1 received feed and water only (normal control), Group 2 received TP injection only and were divided into 3 subgroups (for durations of 11days, 22days and 33days). Group 3 (Positive control) were divided into 3 subgroups and each subgroup received 5mg/kg b.wt Finasteride + 1mg/kg b.wt Doxazosin for 11days, 22days and 33days. Group 4 (Low dose extract) was divided into three subgroups and each subgroup separately received 100mg/kg b.wt of the plant extract for 11days, 22days and 33days. Group 5 (medium dose extract) was divided into three subgroups and each subgroup separately received 200mg/kg b.wt of the plant extract for 11days, 22days and 33days. Finally, Group 6 (high dose extract) was also divided into three subgroups and each subgroup separately received 300mg/kg b.wt of the plant extract for 11days, 22days and 33days. The animal grouping and treatment protocol is summarized in Table 1.

**Table 1:** Grouping of animals and treatment protocol.

| | Groups | Treatment |
|---|---|---|
| Group 1 | | Normal rats received feed and water only |
| Group 2 | Subgroup 1 | TP induced only for 11 days |
| | Subgroup 2 | TP induced only for 22 days |
| | Subgroup 3 | TP induced only for 33 days |
| Group 3 | Subgroup 1 | TP induced + 5mg/kg b.wt Finasteride + 1mg/kg b.wt Doxazosin for 11 days |
| | Subgroup 2 | TP induced + 5mg/kg b.wt Finasteride + 1mg/kg b.wt Doxazosin for 22 days |
| | Subgroup 3 | TP induced + 5mg/kg b.wt Finasteride + 1mg/kg b.wt Doxazosin for 33 days |
| Group 4 | Subgroup 1 | TP induced + 100mg/kg b.wt of extract for 11 days |
| | Subgroup 2 | TP induced + 100mg/kg b.wt of extract for 22 days |
| | Subgroup 3 | TP induced + 100mg/kg b.wt of extract for 33 days |
| Group 5 | Subgroup 1 | TP induced + 200mg/kg b.wt of extract for 11 days |
| | Subgroup 2 | TP induced + 200mg/kg b.wt of extract for 22 days |
| | Subgroup 3 | TP induced + 200mg/kg b.wt of extract for 22 days |
| Group 6 | Subgroup 1 | TP induced + 300mg/kg b.wt of extract for 11 days |
| | Subgroup 2 | TP induced + 300mg/kg b.wt of extract for 22 days |
| | Subgroup 3 | TP induced + 300mg/kg b.wt of extract for 33 days |

## 3. Determination of Prostatic Index

At the terminal sacrifice of the study, the body weight (BW) and prostate weight (PW) of each animal were recorded. Following this, group mean body weights were calculated. For each individual study group animal, the prostatic index (PI) was calculated by dividing the prostate weight by the animal's body weight i.e.,

$$\text{Prostatic Index (PI)} = \frac{\text{Weight of prostate (in grams)}}{\text{Body Weight of Rat (in grams)}}$$

### Determination of Hormonal Parameters

The rats underwent fasting 24 hours prior to sacrifice. Blood samples were carefully collected in plain bottles, spinned for 15mins at 3000 rpm to obtain serum. The serum TEST, FSH and LH levels were estimated using Elabscience kit (Elabscience Biotechnology Co., Ltd., China) following manufacturer's instructions.

### Semen Analysis

The rat seminal fliud was extracted from the epididymis into a clean microscope slide, a few drops of normal saline was added and this was used for evaluation of sperm quality (ie sperm count, sperm motility and sperm morphology). The concentration of spermatozoa was determined using the improved Neubauer Improved Counting Chamber (Deep 1/10 mm, LABART, Germany).

### Statistical Analysis

Data collected were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's (HSD) multiple comparison test in GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). The significance of the data was assessed using a p-value of less than 0.05 and reported as Mean $\pm$ Standard Error of Mean (SEM).

## 4. Results

### Qualitative Phytochemical Analysis of the Leaves of Nephrolepis biserrata

Qualitative Phytochemical Screening of the leaves is shown in Table 2. The phytochemicals present include alkaloids, flavonoids, saponins, phenols, steroids, tannins and terpenoids.

**Table 2:** Qualitative Phytochemical Composition of the Leaves of *N.biserrata*.

| Phytochemical | Status |
|---|---|
| **Alkaloids** | + |
| **Flavonoids** | + |
| **Saponins** | + |
| **Phenols** | + |
| **Steroids** | + |
| **Tannins** | + |
| **Terpenoids** | + |

### Therapeutic Effect of Nephrolepis biserrata Aqueous Extract on Hormonal Profile

In Figures 1,2,4 the effect of treatment with various doses of the aqueous extract of *Nephrolepis biserrata* on testosterone-propionate treated rats are presented. There was an increase in TEST level from 2.27 ± 0.48ng/ml in the normal control (group 1) to 7.27 ± 0.66ng/ml, 6.67 ± 1.16ng/ml and 13.0 ± 1.73ng/ml for group 2 (negative control, which received TP only). At low, medium and high doses, the levels of TEST was observed to be significantly ($p < 0.05$) lowered when compared to group 2. The levels of LH and FSH for group 2 were significantly ($p < 0.05$) reduced when compared to the normal control (group 1). From 2.73 ± 0.88mμ/ml to 0.97 ± 0.12mμ/ml, 0.77 ± 0.09mμ/ml and 0.90 ± 0.21mμ/ml for LH. And for FSH, the values decreased from 9.00 ± 2.08mμ/ml (for group 1) to 2.67 ± 0.24mμ/ml, 1.90 ± 0.06mμ/ml and 2.30 ± 0.44mμ/ml (for group 2). Group 4, 5 and 6 which received low, medium and high doses of the plant extract had values which compared favorably with the normal control (group 1).

### Effect of Aqueous Extract of *N.biserrata* on Semen Parameters

Following exposure to testosterone propionate, there was a significant ($p < 0.05$) decrease in the total sperm count, sperm motility, activeness and viability of the negative control (group 2) when compared to the normal control (group 1). However, treatment with 100mg/kg b.wt, 200mg/kg b.wt and 300mg/kg b.wt (group 4, 5 and 6 respectively) significantly abated the pathological conditions by restoring the sertoli and leydig cells of the testes and the stroma cells of the prostate. This is demonstrated by the levels of LH and FSH for groups 4, 5 and 6 which compared favorably to the normal control, group 1.

### Effect of Aqueous Extract of *N.biserrata* on Prostate Index

Relative prostate weight is normally used to evaluate the growth of BPH. The rats in the TP-induced BPH group recorded a significantly ($p < 0.05$) increased weight of prostate (1.07 ± 0.01g, 1.25 ± 0.20g and 2.10 ± 0.29g) when compared to the normal control (0.70 ± 0.06g). Similarly the prostate index for the negative control, group 2 ($5.17 \pm 0.18x10^{-3}, 5.07 \pm 0.54x10^{-3} and 9.55 \pm 1.31x10^{-3}$) was significantly ($p < 0.05$) elevated when compared to the normal control ($3.27 \pm 0.50x10^{-3}$). The standard drugs treated groups and the extract treated groups had values which compared favorably to the normal control (group 1).

## 5. Discussion

Male fertility requires the cooperation of the different organs of the male urogenital system each carrying out its assigned function. Through their interactions, the testes (which contain germ cells, Sertoli cells and Leydig cells), the epididymis and the male accessory glands (prostate, seminal vesicles and bulbourethral glands) simultaneously contribute to the production of the human seminal fluid [5, 20] in their review, the role of the prostate in male fertility, health and disease reported that male fertility is controlled by a $Zn^{2+}$ dependent short circuit of the Krebs cycle within the prostate epithelial cells. Homeostasis and pathophysiological status of the prostate epithelium depends on the ultra-cellular androgen-dependant accumulation of $Zn^{2+}$, citrate and Kallikreins, and the semenogelin-enriched seminal vesicle secretion. The proliferative disorders of the prostate gland will negatively impart male fertility function.

The burden of clinical BPH still remains a significant health concern especially among elderly men worldwide [21]. The present study sought to evaluate the anti-proliferative effects of *N.biserrata* aqueous extracts on the development of BPH using a TP-induced model in rats. The rats with TP-induced BPH displayed hormonal dysregulation and elevated prostate weights. The semen parameters also were negatively imparted with TP-treatment. The total sperm count (TSC), viable sperm cells, and motility of the sperm cells were also witnessed low in the TP-only treated groups. This study however reveals that *N.biserrata* aqueous extract ameliorated the hormonal dysregulation, reduced the induced increment in prostate weight and improved the sperm quality parameters. Testosterone is involved in the pathogenesis of BPH and plays an important role in the development of male reproductive organs [6]. The serum concentration of testosterone may vary with age [22]. Testosterone is converted to dihydrotestosterone (DHT) in the prostate, hair follicles and testes via the enzymatic action of 5 $\alpha$-reductase. DHT has a greater affinity towards androgen receptors when compared to that of testosterone and other adrenal androgens [23]. Elevated levels of testosterone in the induced groups have direct correlation with increased level of DHT and hence the prostatic enlargement noticed in the current study. Hence the reduction in the testosterone concentration for groups treated with the plant extract suggests the plant extract had the potential to ameliorate prostatic proliferation. This can be surmised to have followed the same mechanism as the $\alpha$ -blocker agents such as finasteride. Increased levels of LH and FSH is an indication of the plant extract potential to restore and improve sexual function and hence fertility [24]. Increased luteinizing hormone will contribute to the increased production of testosterone by the interstitial cells besides the other mechanism for its production via increased cholesterol synthesis [25]. With increased FSH, spermatogenesis will be enhanced by the sertoli cells leading to the increased production of viable and motile spermatozoa.

Several research reports have proven that an increase in the prostate is a vital indicator of the development of BPH [19, 26]. The findings of our study agrees with these reports. Significant enlargement in the size of the prostate gland was observed in testosterone propionate only

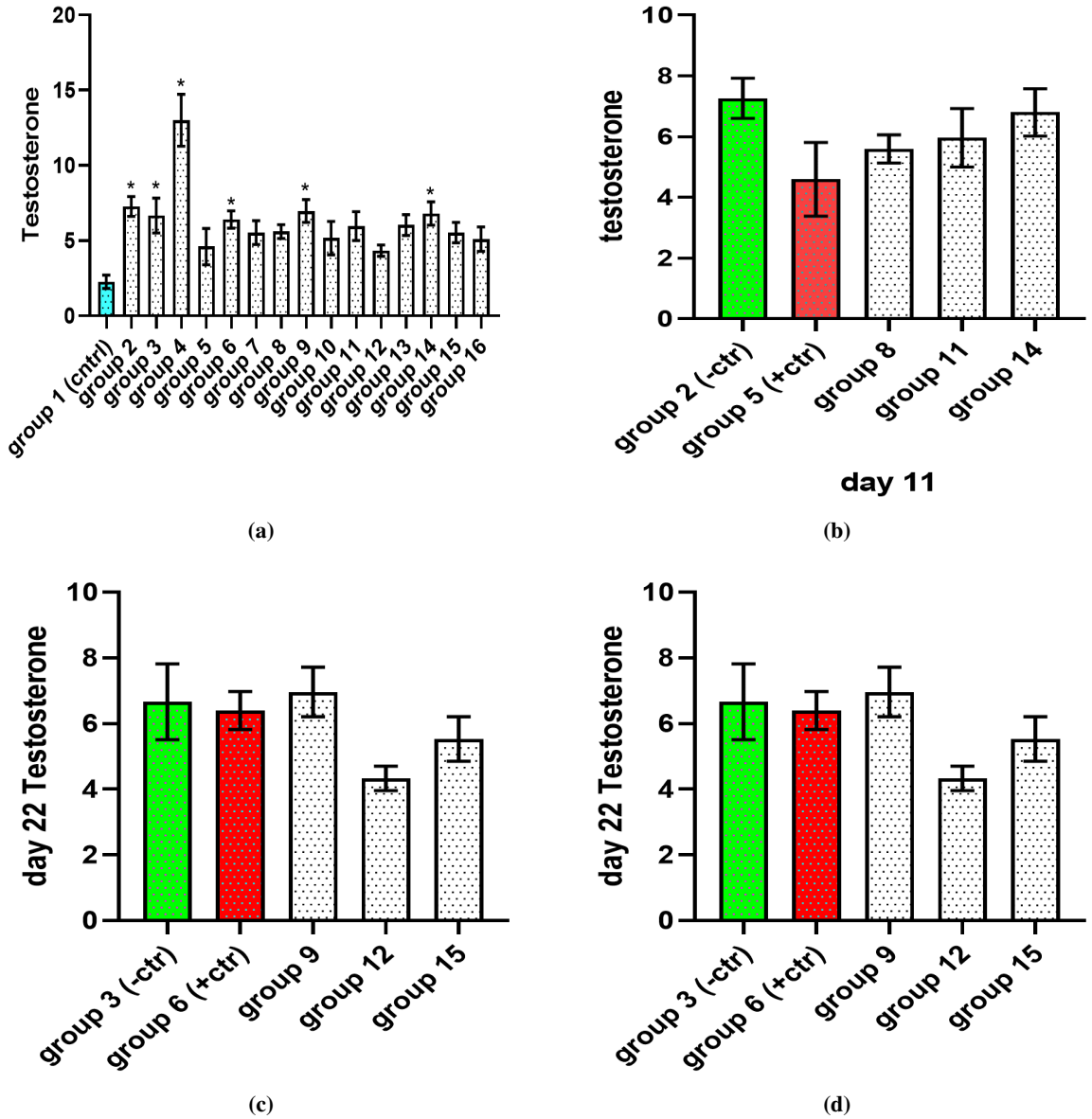


**Figure 1:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on Testosterone Concentration of TP-induced BPH in male Wistar rats. Values are mean ± SEM. Values in the bar are significantly different at (*$p<0.05$ with negative, $p<0.05$ with positive control)

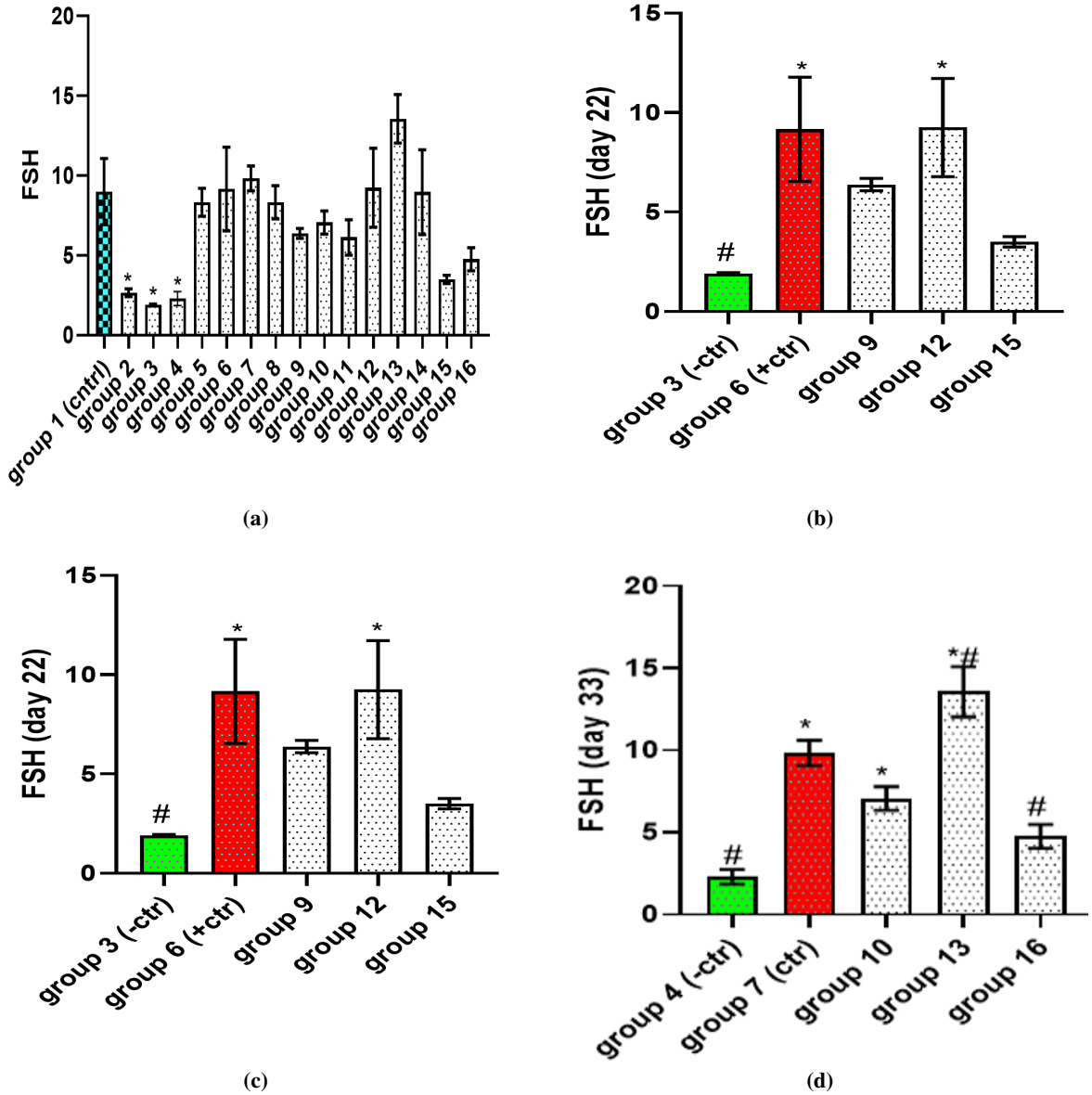


**Figure 2:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on Follicle Stimulating Hormone Concentration of TP-induced BPH in male Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*$p<0.05$ with negative, $p<0.05$ with positive control)

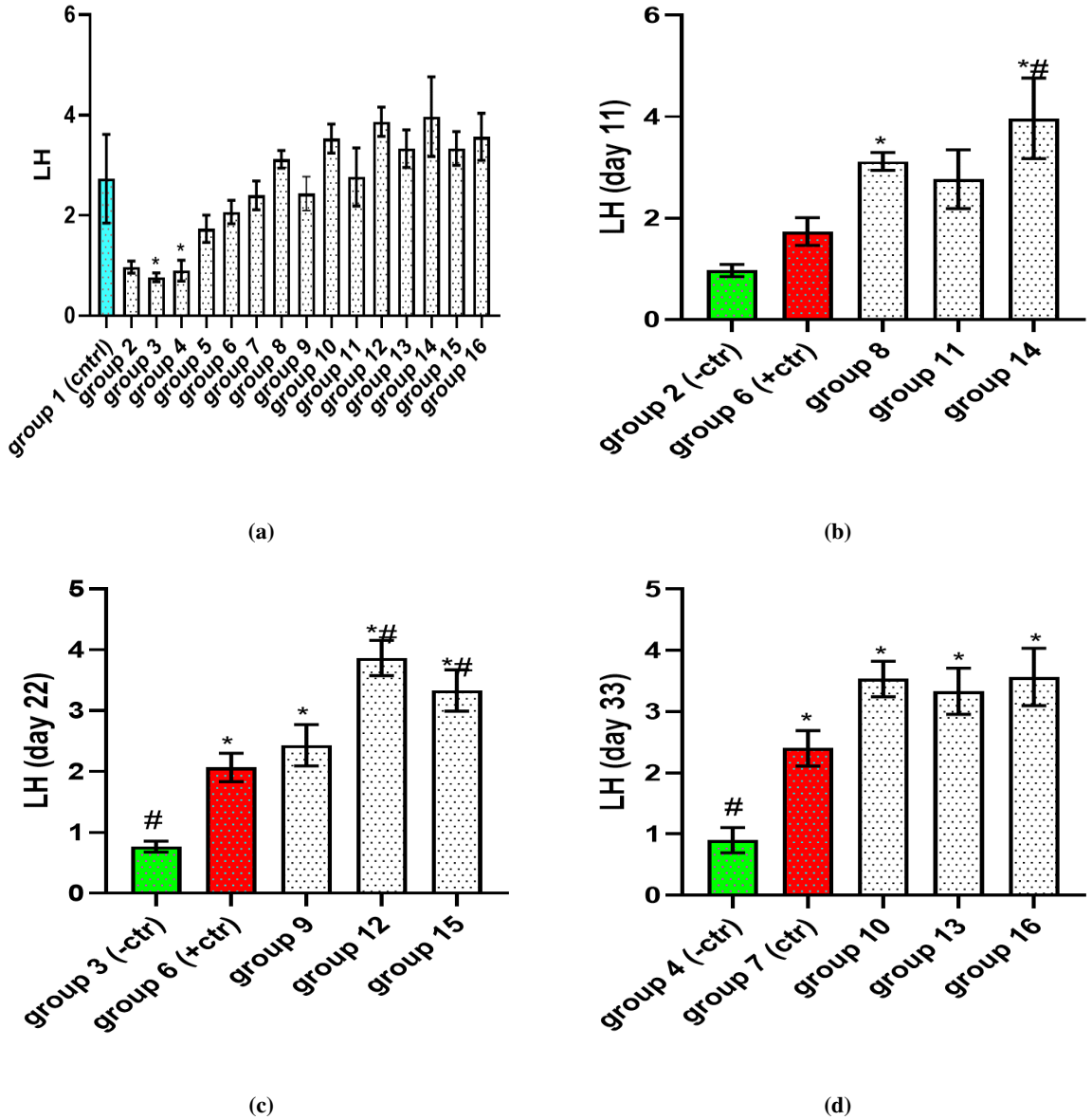


**Figure 3:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on Luteinizing Hormone Concentration of TP-induced BPH in male Wistar Rats. Values are mean ± SEM.Values in the bar are significantly different at (*p<0.05 with negative, p<0.05 with positive control)

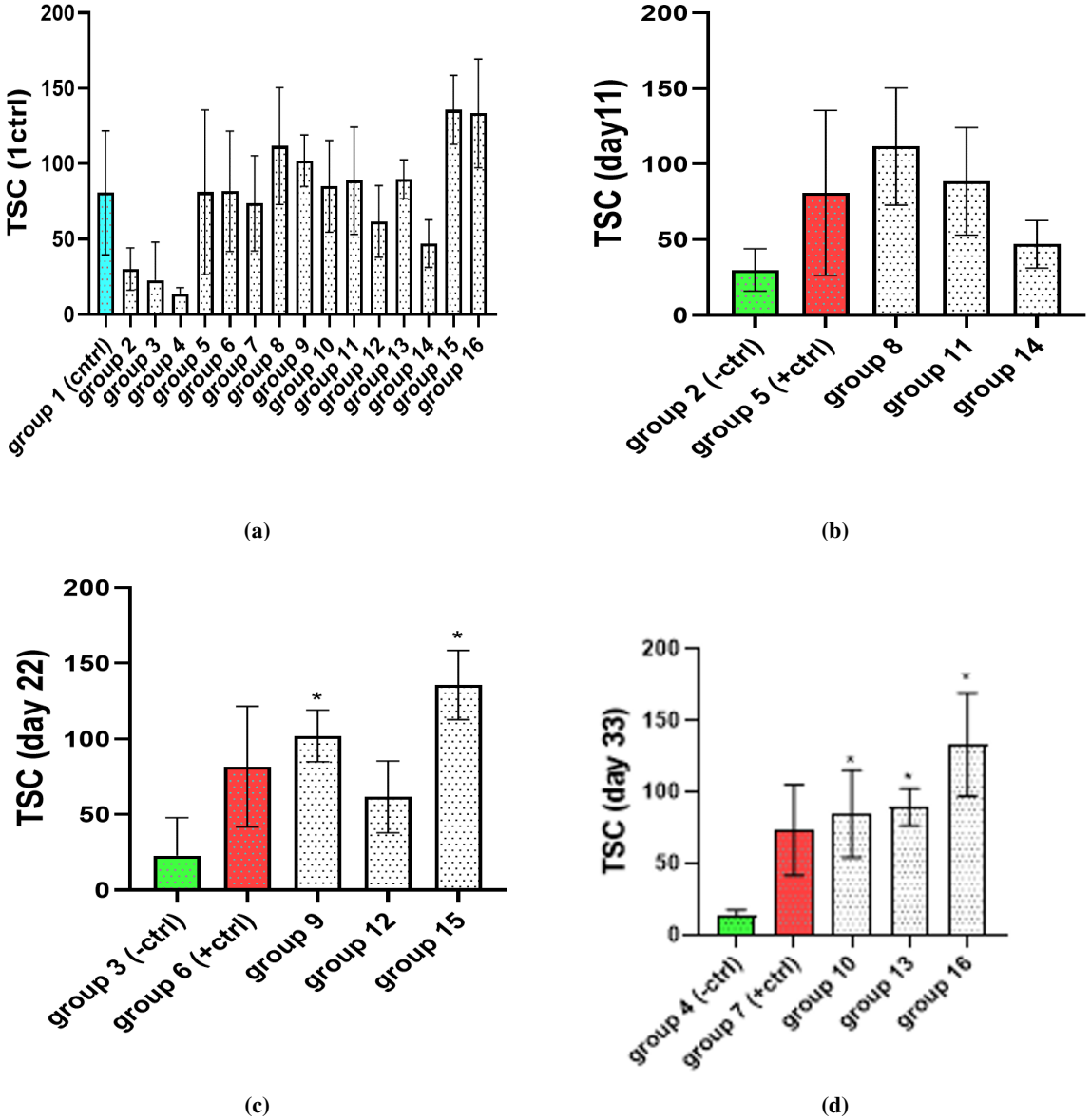


**Figure 4:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on the Total Sperm Count of TP-induced BPH in male Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*p<0.05 with negative, p<0.05 with positive control)

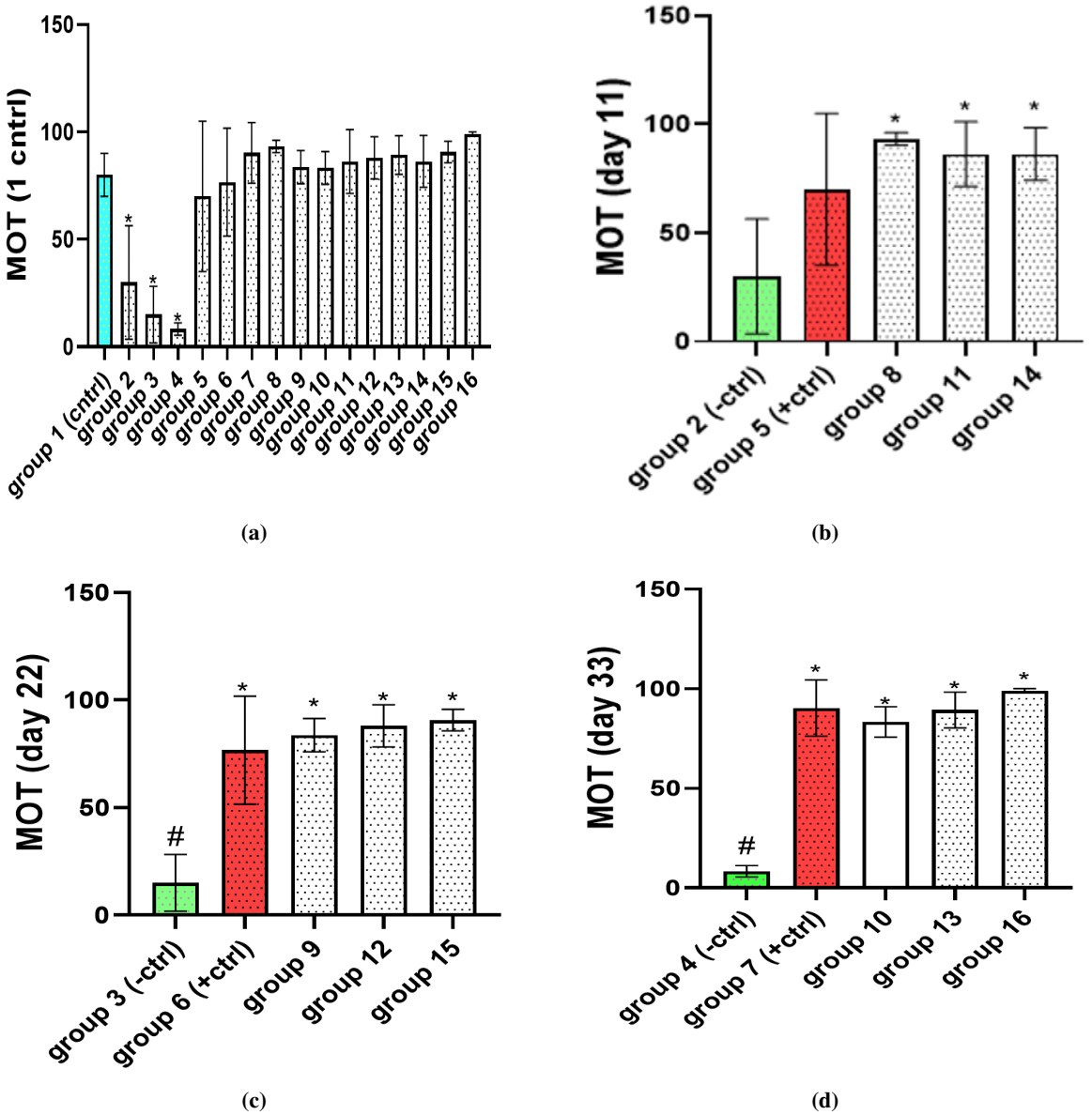


**Figure 5:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on Sperm motility of TP-induced BPH in male Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*p<0.05 with negative, p<0.05 with positive control)

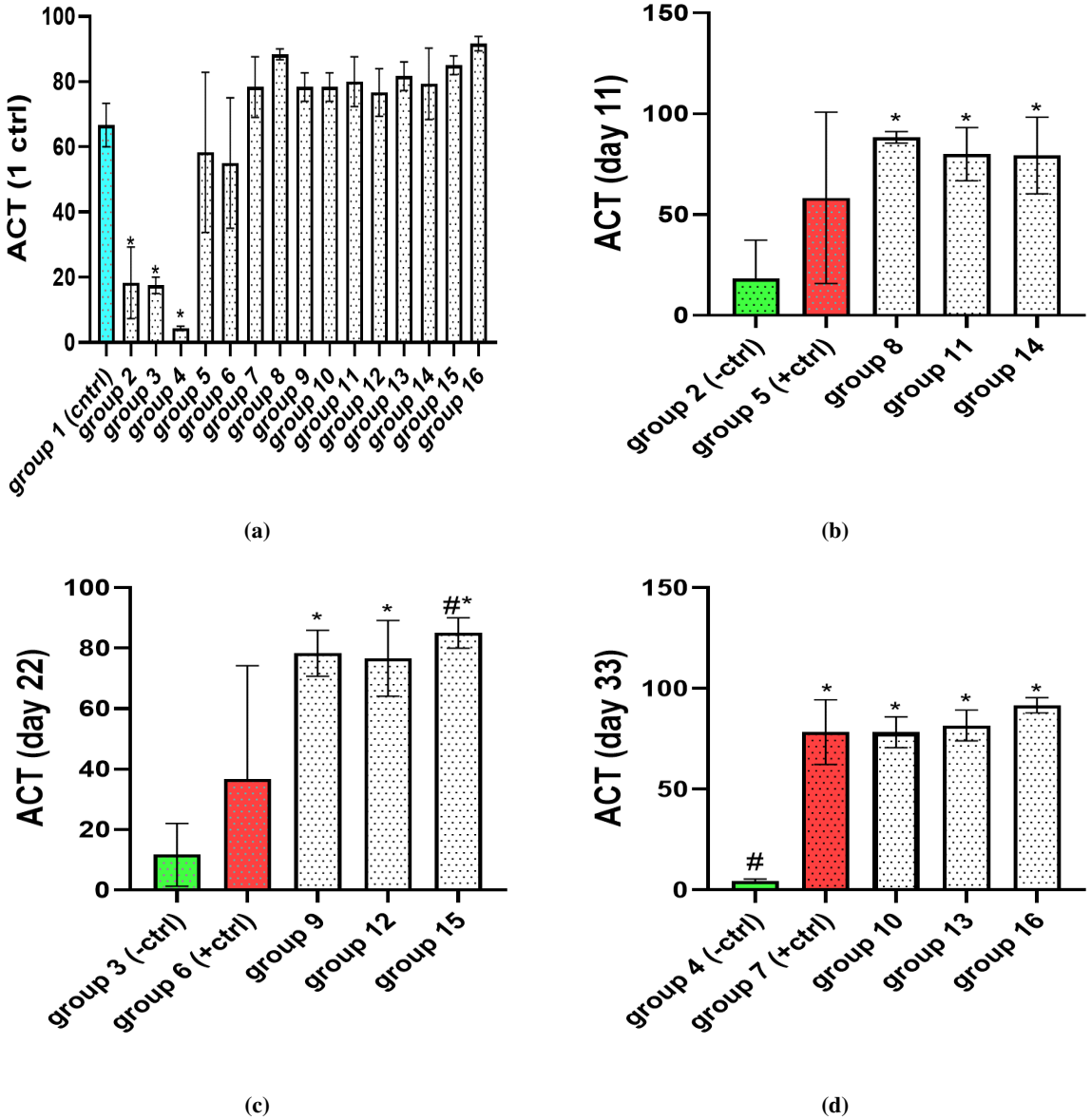


**Figure 6:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on Activeness of Sperm cells of TP-induced BPH in male Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*p<0.05 with negative, p<0.05 with positive control)

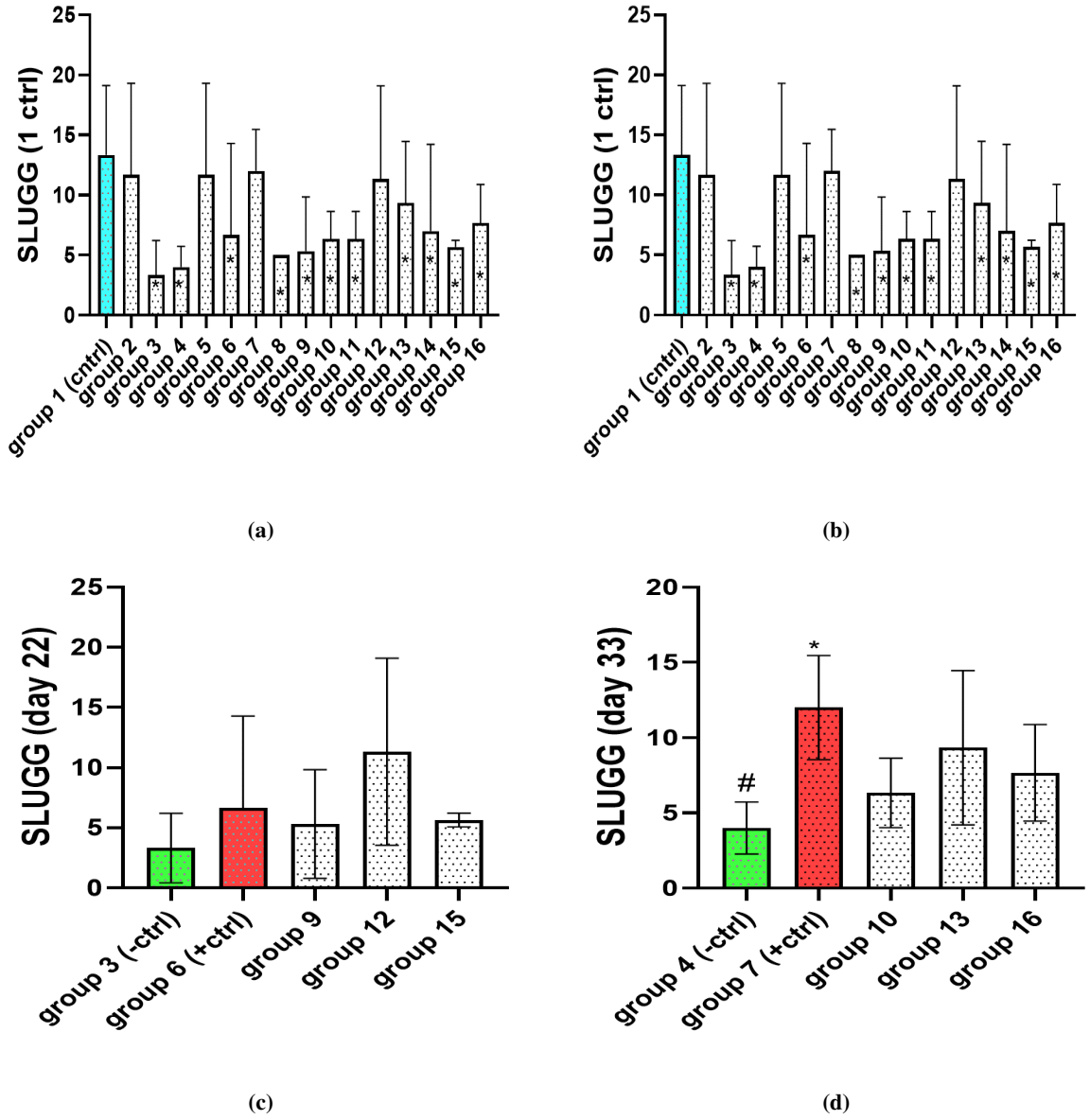


**Figure 7:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on Sluggishness of Sperm cells of TP-induced BPH in male Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*$p<0.05$ with negative, $p<0.05$ with positive control)

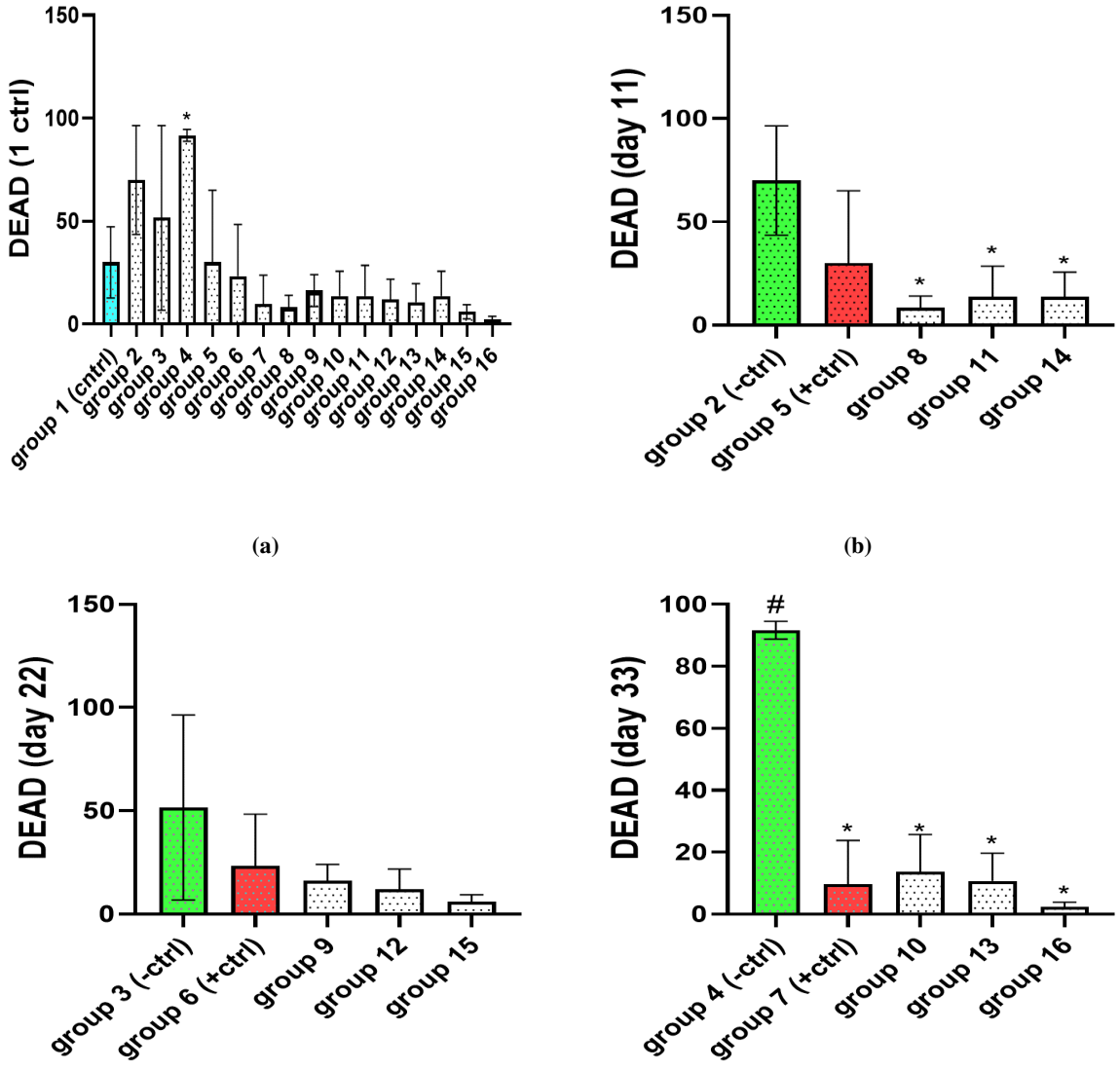


**Figure 8:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on the number of dead sperm cells of TP-induced BPH in male Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*$p<0.05$ with negative, $p<0.05$ with positive control)

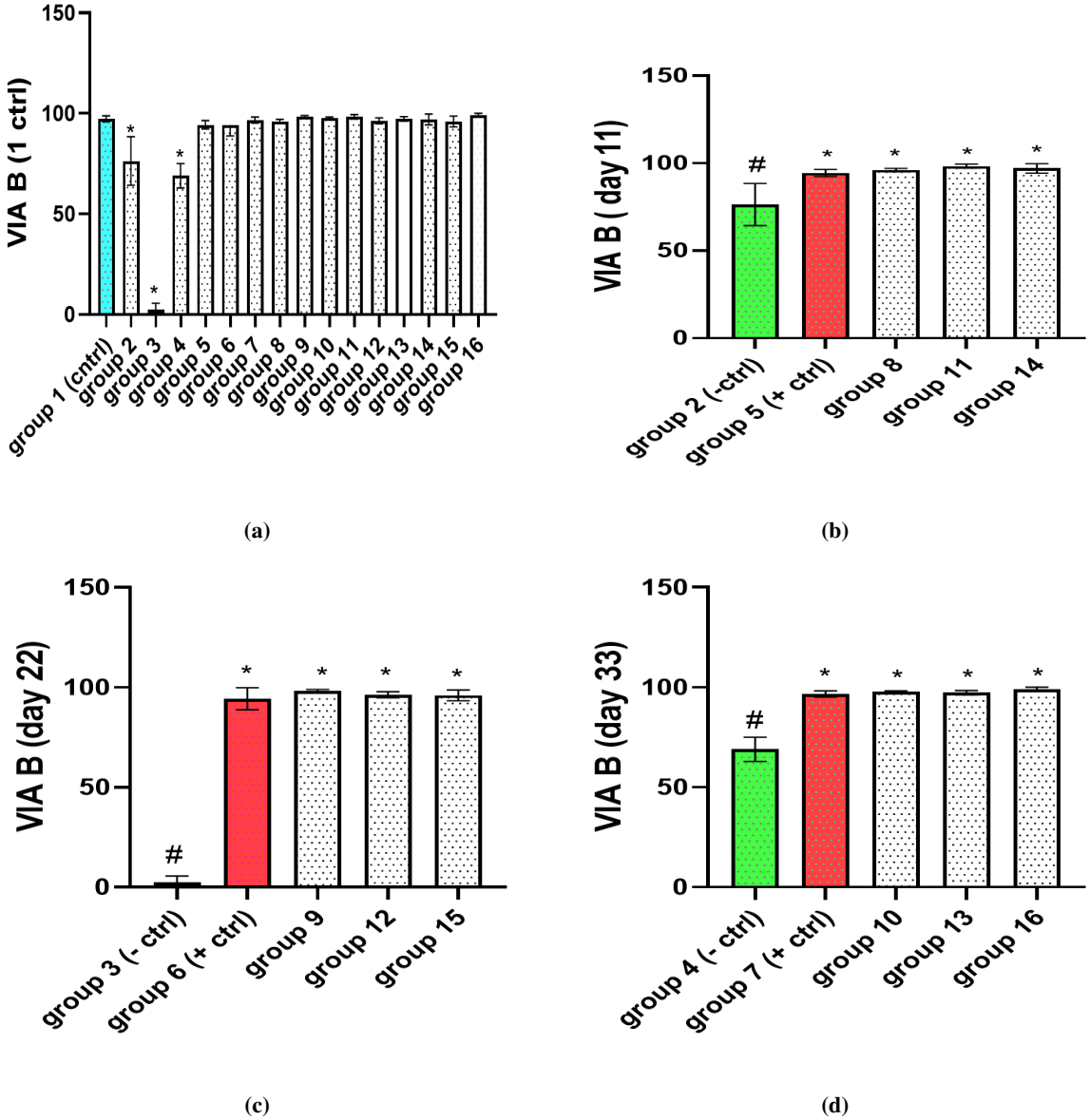


**Figure 9:** The Effect of Aqueous Extract of *Nephrolepis biserrata* on Sperm Motility of TP-induced BPH in male Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*$p<0.05$ with negative, $p<0.05$ with positive control)

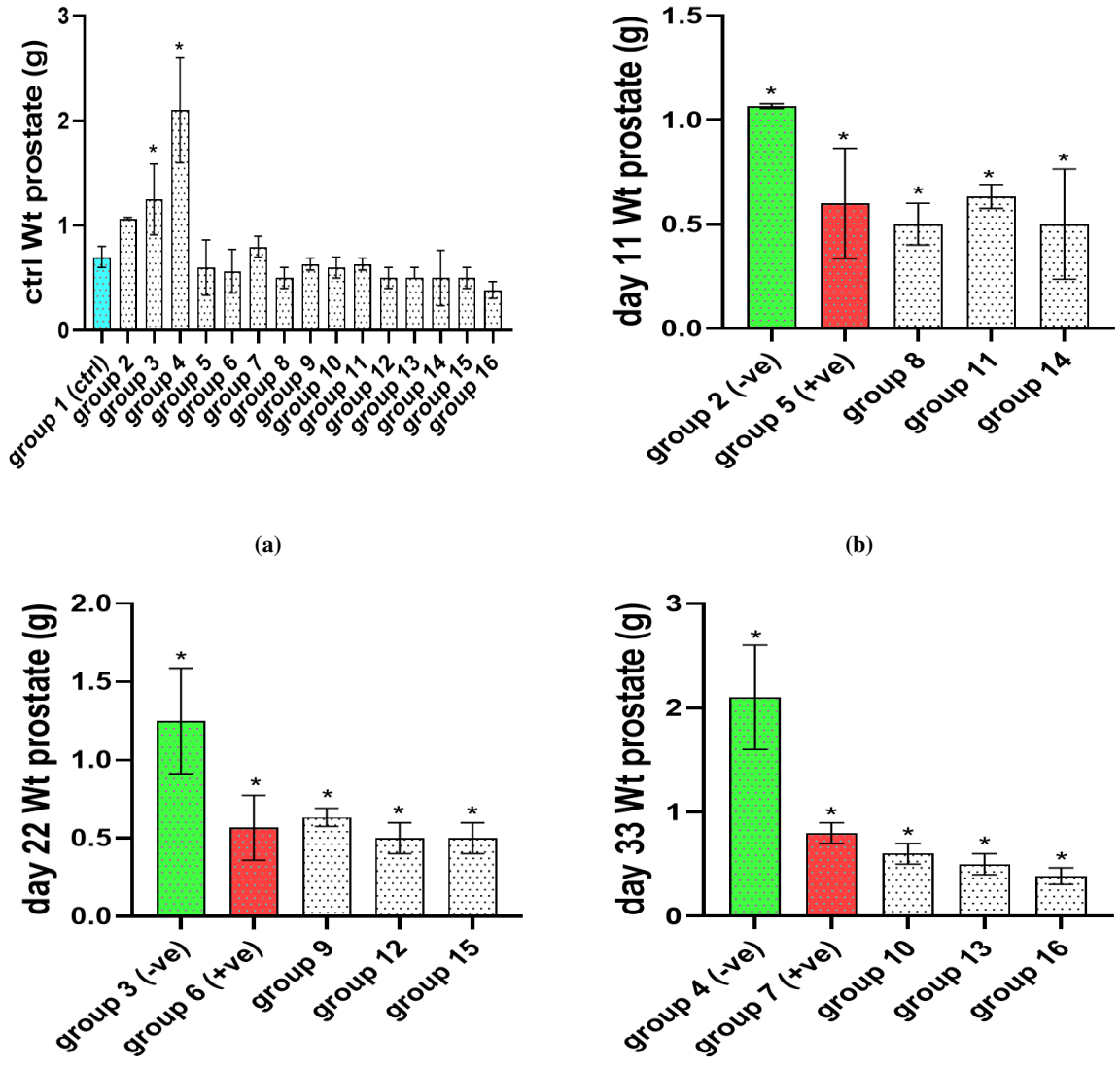


**Figure 10:** The effect of aqueous extract of *Nephrolepis biserrata* on the weight of prostate of TP-induced benign prostatic hyperplasia in Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*p<0.05 with negative, p<0.05 with positive control)

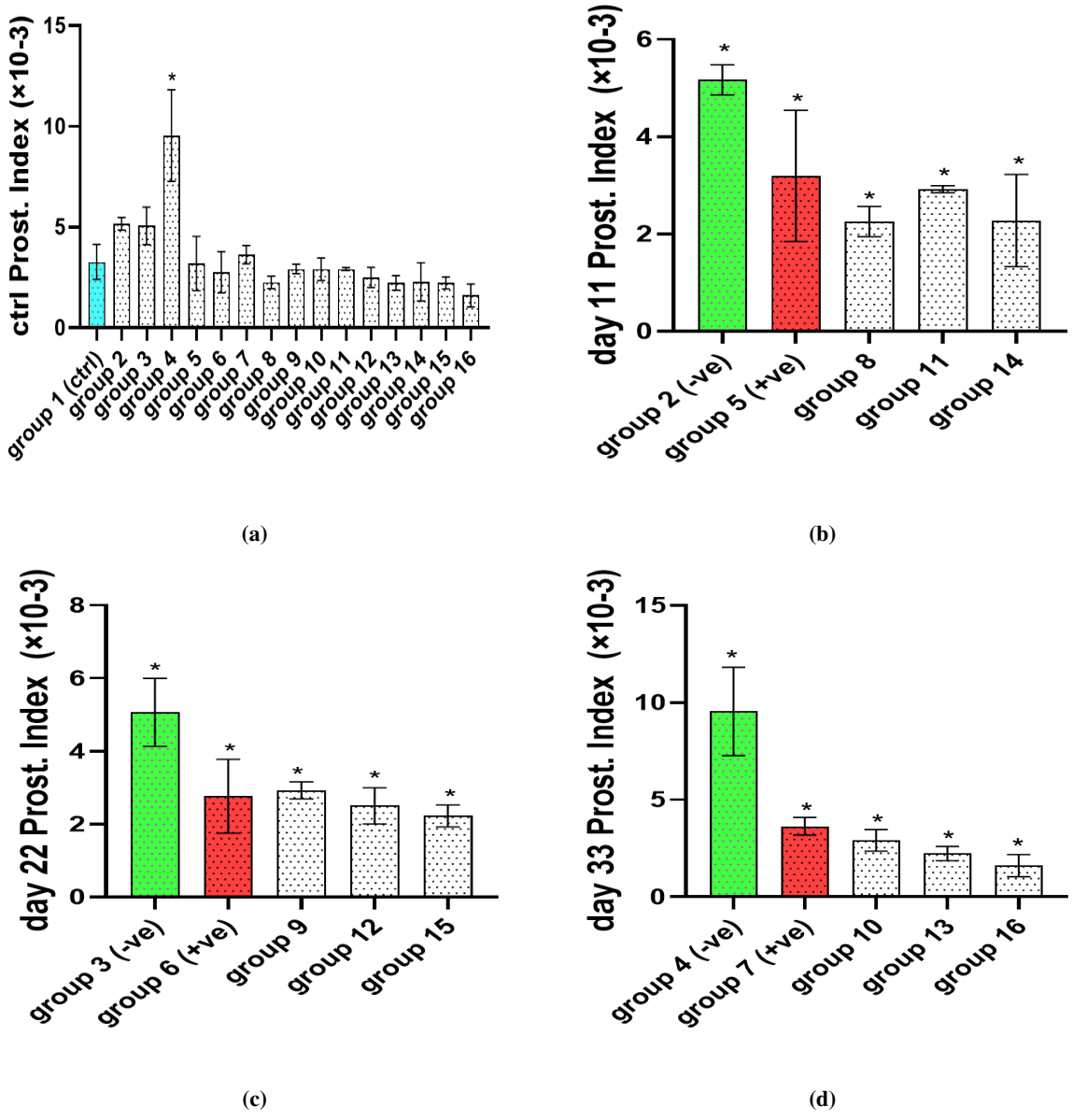


**Figure 11:** The effect of aqueous extract of *Nephrolepis biserrata* on prostatic index of TP-induced benign prostatic hyperplasia in Wistar Rats. Values are mean ± SEM. Values in the bar are significantly different at (*p<0.05 with negative, p<0.05 with positive control)

treated groups. An increase in the size of the prostate results in urethral canal constriction, resulting in partial or complete urinary canal obstruction. According to [27], increased relative prostate weight is used as one of the significant marker indicating the development of BPH. BPH is characterized by epithelial and stromal hyperplasia of the prostate, which results in an increase in prostate weight. When the prostate dilates, it results in the constriction of urethral canal, causing partial or complete obstruction. Treatment of BPH will therefore correspond with the reduction in prostate weight. Many studies have undertaken to study the effect of various natural products in the treatment of BPH by investigating the volume/size of the prostate. Treatment with the aqueous extract of Nephrolepis biserrata in the current study at the both the low, medium and high doses showed a significant reduction in the prostate weight of BPH-induced rats. The deduction is that positively imparted the BPH condition and brought about a restoration of normal physiological function.

The high death rate of the sperms witnessed in the groups administered with testosterone propionate only could be attributed to the inflammatory response elicited by administration with TP. This also corresponded with the observed reduction in total sperm death recorded. Abnormal head defects, midpiece defects and tail defects in the cells must have significantly effected the decline in spermatogenesis and fecundity. Other works have stated that factors which damage the testes, and imparts semen parameters could hamper fertility [28]. In addition, the previous report and several others [29] have associated sperm count, sperm motility and morphology with spermatogenic and androgenic properties of plant extracts. In our study, compared to the negative control, the extract treatment groups showed an improved sperm count, sperm motility and morphology. The ameliorative properties attested to by the observations of the current study may be attributed to the phytochemical constitution present in the plant which could have significantly improved the spermatogenic and androgenic activities.

## 6. Conclusion

The results of this investigative study revealed that Nephrolepis biserrata aqueous leaf extract had a curative effect against the prostate and testis alteration caused by testosterone propionate induced benign prostatic hyperplasia by restoring and bringing a balance to hormonal control. A restoration of normal spermatogenic parameters also gives credence to the possible androgenic and spermatogenic potential of the plant extract.

## Article Information

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**Conflict of Interest Disclosure:** The authors affirm that they have no conflict of interest.

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