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Annona muricata (Soursop) Mitigated Testicular Toxicity and Prostatic Impairment in Testosterone-Propionate-Induced BPH in Male Rats

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Abstract

Benign prostate hyperplasia (BPH) is an age-associated prostate gland enlargement in men that can lead to urination difficulty. The exact etiology of BPH is unknown. Several unscientific reports with few documentations, supporting *Annona muricata* (soursop) fruit against benign prostatic hyperplasia (BPH). This study aims to evaluate the mitigating mechanisms of *A. muricata* fruit phytochemicals on prostatic indices in BPH-induced male Wistar rats. The fruits of soursop were processed to obtain juice extract and lyophilized. A preliminary trial was conducted with six doses of the soursop fruit juice (10, 100, 1000, 1500, 2900 and 5000 mg/kg) to determine the LD_{50}. The experiment involved forty male rats divided into five groups of eight animals each; groups 3 – 5 had 3mg/kg body weight of testosterone propionate (TP) intravenous for two days. Groups 3 and 4 had 1600 and 2900 mg/kg body weight of soursop fruit juice, while the effects finasteride was tested against BPH in the 5th group. The effects of the treatments were evaluated on body weight, prostatic weight, testicular weight, testosterone, DHT, prostate-specific antigen (PSA), testicular glycogen, testicular cholesterol, testicular zinc, 3β-hydroxysteroid dehydrogenase concentration and 17β-hydroxysteroid dehydrogenase levels. The soursop fruit juice significantly ($p \leq 0.05$) decreased prostate weight and modulated indices of testicular functions positively. This study suggests that soursop fruit ameliorated prostatic hyperplasia in rats and may be considered as an affordable and non-invasive management option for benign prostatic hyperplasia in men.

Keywords: *Annona muricata*, Testosterone Propionate, Benign prostatic hyperplasia, 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase.

1.0 Introduction

Benign prostatic hyperplasia (BPH) is an age-related prostate gland enlargement and uncontrolled growth of prostate cells (Kappor, 2012). The abnormal rapid increase of prostatic stromal cells caused the formation of discrete nodules in the peri-urethral region that developed into acute and chronic urinary retention, bladder outlet obstruction, urinary tract infection, urosepsis, bladder stones and hematuria (Jadallah *et al*., 2012).

Many studies have strongly linked the causes of BPH to an imbalance in steroid hormone metabolism, remodelling in the ageing prostate, systemic inflammation and oxidative stress associated with metabolic syndrome, among other factors (Ejike and Eze, 2015 and Briganti *et al*., 2009) The most common treatment options for BPH include medical treatment with α-blockers or 5α-reductase inhibitors, surgery and the use of plants for treatment (Phytotherapy) (Kim *et al*., 2019). 5α-reductase inhibitors are type of medication that inhibit the conversion of

testosterone to dihydrotestosterone (DHT), thereby leading to the contract of the prostate tissue, while α-blocker loosen up smooth muscles of the prostate and the bladder neck to relieve urinary obstruction caused by an enlarged prostate and allow free flow of urine.

DHT (Dihydrotestosterone) is an active metabolic product from the decrease of testosterone by 5α-reductase. It plays an important role in the growth of the prostate by binding to the nuclear androgen receptor, thereby actuating the synthesis of growth factors that act on prostatic epithelia and stroma, ensuing in prostate enlargement (Nahata and Dixit, 2011). Hence, inhibitors of 5α-reductase that block the production of DHT ultimately delay the development of BPH. Common inhibitors of 5α-reductase are pharmacological agents like dutasteride and finasteride. Side effects, such as reduced libido, erectile defective, dizziness, retrograde ejaculation and postural hypotension are always associated with the existing BPH drugs (Heidenreich *et al.,* 2011; Kalu *et al.,* 2016) which have increased the interest and research activities on the use of alternative treatment options.

The use of plants for treatment (phytotherapy) for the avoidance and treatment of BPH is drawing attention (Sharma *et al*., 2017) due to its feasible efficacy, more placid side effects and affordability compared to most other treatment options. Some plants possess anti-BPH properties which include *Saw palmetto*, *Pygeum africanum*, *Secale cereale* and *Phellodendron amurense,* which have been confirmed by several scientific explorations (Xu and Ventura, 2010) and are widely used for the avoidance and treatment of the disease.

Annona muricata, commonly called soursop, belongs to the Annonaceae family. The plant is well known for its anticancer properties (Coria–Tellez *et al*., 2018). A large range of ethnic medicinal activities have been assigned to different parts of the *Annona muricata* plant due to some of its attributes such as anti-inflammatory, anti-proliferative, hypoglycemic, sedative, smooth muscle relaxant and antispasmodic effects (Adewole and Caxton-Martins, 2006; Moghadamtousi *et al.,* 2015). Some indigenous communities in Africa including Nigeria use *A. muricata* in their tribal medicine. The leaf extract of the *Annona muricata* plant is used to mitigate difficulty associated with urination in certain communities in the Eastern part of Nigeria. Although Asare *et al*. (2015) recorded that the aqueous extract of the plant leaf exhibited anti-proliferative activity against BPH-1 cells, there is still incomplete information on the possible use of this plant in the treatment of BPH. It has been shown that plant-derived medications expose their anti-BPH effect through different processes including anti-androgenic, anti-proliferative, anti-inflammatory and antioxidant activities (Dreikorn, 2000). Antioxidants are known to alleviate the detrimental effect of oxidative stress - a factor implicated in the development of age-related diseases such as BPH (MInciullo *et al.,* 2015). In addition to anti-proliferative effect, *in vitro* studies have indicated that *A. muricata* leaf extract also reveals a remarkable antioxidant activity (Roduan *et al.*, 2019). Thus, exploration of the effect of this plant on BPH alongside its antioxidant activity could give more useful information on its probable anti-BPH properties. Despite its pharmacological effectiveness, little is known about the role *A. muricata* in the management of BPH. Therefore, the effects of *A. muricata* fruit juice on testosterone propionate–induced benign prostatic hyperplasia and reproductive toxicity in male albino rats were investigated.

2.0 Materials and Methods

2.1 Chemicals

Trichloroacetic acid, ferric chloride, Conc. H_2SO_4, ferrous sulphate, ethanol, anthrone, thiourea, glucose, nitric acid, bovine serum albumin, sodium pyrophosphate, dihydroxy epiandrosterone was obtained from Sigma Chemical Company, St Louis, USA, and ELISA use was obtained from Thermo Fisher Scientific, Waltham, MA USA. Other reagents were of analytical grade.

2.2 Sample Collection and Extraction

The ripe fruits of *A. muricata* were purchased from Molege market, Owo, Ondo State, Southwestern Nigeria. The fruits were authenticated at the Centre for Research and Development, The Federal University of Technology, Akure, Nigeria. The fruits of the soursop were washed and peeled, and the seeds were separated from the pulp and the pulp was blended without adding water to it. The extract was dried under vacuum by a rotary evaporator followed by a freeze drying (McCartan and Gosling, 2013). The dried extract was poured into a special container and stored in a refrigerator.

2.3 Experimental Design

Forty (40) male adult Wistar rats weighing 130 ± 20g were obtained from McTemmy Animal Farm, Ogbomoso, Nigeria and were kept under adequate conditions (12-h light-dark cycle and room temperature) in clean cages for 14 days and the animals had free access to standard food and water. After two weeks of acclimatization, the rats were divided into five groups (1, 2, 3, 4 and 5 respectively) using a completely arbitrary design with eight rats in each group.

Group 1: Normal (Negative control)

Group 2: Received 3 mg/kg body weight (BW) of TP intravenous.

Group 3: Received 3 mg/kg of TP and treated with 1600 mg/kg BW of *Annona muricata* juice, Group 4: Received 3 mg/kg of TP and treated with 2400 mg/kg BW *Annona muricata* juice

Group 5: Received 3 mg/kg of TP and treated with 10 mg/kg BW of Finasteride.

A preliminary trial was conducted with six doses of the mixture (10, 100, 1000, 1500, 2900 and 5000 mg/kg) to identify the optimum safe dose for treating BPH. Based on this result, a dose was utilized to reduce BPH related parameters, and the treatment was conducted for 21 days after induction. Rats were given an oral injection of phosphate buffer saline (PBS) and were considered the control group (CONT).

The BPH group was given an oral injection of PBS and testosterone propionate at 3mg/kg Body weight (BW) intravenous. The finasteride group received finasteride (10 mg/kg BW) (FINA) orally and intravenous injection of testosterone propionate. Rats were sacrificed and blood was collected. Blood was centrifuged, and serum was stored at 4°C until analysis. Prostates were carefully dissected by removing surrounding connective tissues and weighed.

2.4 Body Weight and Prostate Weight

Body weights were measured at the beginning and the end of the experiment. Also, prostate weights were measured after having been sacrificed and removing prostate glands. The prostate index is a formula that can be used to obtain the prostate weight to body weight ratio. When differentiating each group from normal and disease groups, any changes in this index indicate the percentage of inhibition of the progression of the disease. Using the two measured weights and these formulas, prostate index, inhibition prostate weight and inhibition prostate index are calculated.

$$\text{Prostate index} = \frac{\text{Prostate weight}}{\text{Body weights}}$$

2.5 PSA Content

After anesthetizing the rats, blood samples were collected from their hearts and sent to an associate laboratory to evaluate free prostate specific antigen (PSA) in the blood using the PSA ELISA kit.

2.6 Estimation of Testicular Cholesterol

Testicular total cholesterol was estimated according to the method of Zlatkis, Zak, and Boyle (1953) with slight modification. Briefly, testicular tissue (50 mg/mL) was homogenised in the ether: alcohol mixture (1:3), centrifuged at 10,000 × g for 10 min, and 0.1 mL of the supernatant was mixed with 4.9 mL of ferric chloride solution (50 mg $FeCl_3 \cdot 6H_2O$, dissolved in 100 mL Acetic acid) in a screw-capped centrifuge tube and allowed to stand for 15 min. The mixer was centrifuged at 10,000 × g for 10 min, and 1.5 mL of conc. H_2SO_4 was added to 2.5 mL of the clear supernatant and

incubated for about 30 min at room temperature for colour development. A stock concentration of 0.25 µg/µl (250mg/ml) of standard cholesterol was prepared. The colour intensity of the unknown and standard was read against blank at 560 nm in a spectrophotometer. The result was expressed in mg/g tissue.

2.7 Estimation of Testicular Glycogen

Testicular glycogen was determined using the method of Nicholas, Robert, and Joseph (1956) with a little modification. Briefly, 1 ml of 5% Trichloroacetic acid (TCA) was added to 50 mg of testicular tissue, homogenised and centrifuged at 3,000 rpm for 15 min. In a centrifuge tube, 1 ml of the supernatant and 5 ml 95% ethanol were mixed and allowed to stand in the water bath at 37–40°C for 3 hr. After completion of precipitation, tubes were centrifuged at 3,000 rpm for 15 min. The supernatant was gently decanted from packed glycogen, and the tubes were allowed to drain in an inverted position for 10 min. The packed glycogen was dissolved by adding 2 ml of distilled water, this was considered as a sample, and the blank was prepared with 2 ml of water. The standard was prepared with 2 ml of a solution containing 0.1 mg of glucose. Ten millilitres of anthrone reagent (0.05% anthrone, 1% thiourea and 66% H_2SO_4 v/v) was added into each tube with vigorous but consistent blowing and good mixing. All the tubes were kept in cold water and then immersed in a boiling water bath for 15 min. The tubes were removed again to a cold water bath and cooled to room temperature. The reading of the unknown (sample) and the standard was taken against blank at 620 nm in a spectrophotometer. The value of glycogen content was expressed in mg/g tissue.

2.8 Estimation of Testicular and Epididymal Zinc Content

Testicular and epididymal zinc was estimated according to the method of Mas, Romeu, Alemany, and Arola (1985). Briefly, testicular (0.5 g/5 ml) tissue was digested with concentrated nitric acid at 100°C for 2 hr in a boiling water bath and after 24hr, the clear digest was diluted to 10 ml with deionised water. The zinc level was estimated by atomic absorption (Model no. A Analyst- 200, PerkinElmer Make) and was expressed in mg/g of wet tissue.

2.9 Serum testosterone assay

Serum-free testosterone was determined using an enzyme immunoassay (EIA) kit (Immunometrics, UK Ltd). This method is based on the competition of serum testosterone and phosphatase-labelled testosterone in binding to a limited amount of fluorescein-labelled polyclonal anti-testosterone antibody (Biswas *et al*., 2001). The reaction was terminated by the addition of EIA stop buffer (glycine buffer pH 10.4 containing sodium hydroxide and a chelating agent), and the optical density was measured at 550 nm. The testosterone concentration of the test sample is interpolated from a calibration curve using testosterone EIA standards.

2.10 Determination of 3β-hydroxysteroid dehydrogenase (3β -HSD) Activity

3β - Hydroxysteroid dehydrogenase was measured by mixing 250µl of the supernatant with 250µl of 100 lM sodium pyrophosphate buffer, pH 8.9, 10µl ethanol containing 30µl of dihydroxyl epiandrosterone (DHEA) (Sigma) and 240µl of 25% BSA (Bangalore Genei, India). Enzymatic activity was measured after the addition of 50µl of 0.5µm NAD to the mixture in a spectrophotometer at 340 nm against a blank (without NAD). One unit of enzyme activity was the amount causing a change in absorbance of 0.001/min at 340 nm.

2.11 Determination of 17β - hydroxysteroid dehydrogenase (17β - HSD) Activity

17β - HSD was measured by mixing 250µl of the supernatant with 250µl of 440 lM sodium pyrophosphate buffer (pH 10.2), 10µl ethanol containing 0.3µm testosterone (Sigma) and 240µl of 25% BSA. Enzyme activity was measured after the addition of 50µl of 0.5µm NAD to the mixture in a spectrophotometer at 340 nm against a blank (without NAD). One unit of enzyme activity was

the amount causing a change in absorbance of 0.001/min at 340 nm.

2.12 Statistical Analysis

All values are expressed as mean standard deviation. Statistical evaluation was done using One-Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The significance level was set at $p<0.05$.

3.0 Results

The testicular weight of the BPH-induced group reduced significantly compared to the group without BPH induction (Figure 1a). The dose-dependent treatment with *Annona muricata* juice increased the weight of the testis in a dose-dependent manner compared to the untreated BPH-induced rat group.

Figure 1b showed the change in body weight of the Wistar rats. The rat group without BPH had a significant increase in weight while the rat group with induced-BPH groups had a significant body weight increase compared to the group without BPH induction. The rat group treated with 2900 mg/kg body weight had no significant increase in body weight, while 1600 mg/kg body weight treated rats had an average weight change which is significant but reduced compared to the BPH-induced and untreated group.

Prostatic weight was shown in Figure 1c. The result showed that experimental BPH-induction using testosterone propionate increased the average prostatic weight of rats in group 2. The treatment with 1600 and 2900 mg/kg body weight of the rats caused a dose-dependent reduction in the prostatic weight.

Treatment with *Annona muricata* fruit juice significantly ($p < .05$) reduced the testosterone levels when compared with the untreated BPH-induced group (Figure 1d). The untreated BPH group had an increased level of testosterone compared to the rat's group without BPH.

The effect of *Annona muricata* fruit juice on testicular glycogen in BPH-induce rats was shown in Figure 2a. The induction increased the level of testicular glycogen compared to the negative control (group 1). The treatment with doses of *Annona muricata* fruit juice reduced the level of testicular glycogen in a dose-dependent manner. A similar outcome was obtained for testicular cholesterol (Figure 2b), testicular zinc (Figure 2c) and the steroidogenic enzymes-17β- and 3β-hydroxysteroid dehydrogenase (Figures 2d and 2e respectively)

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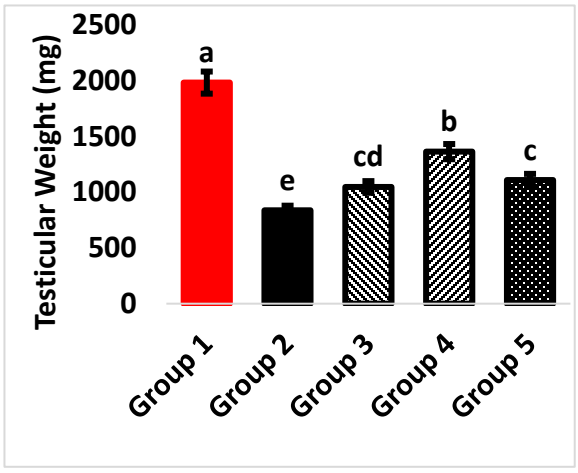

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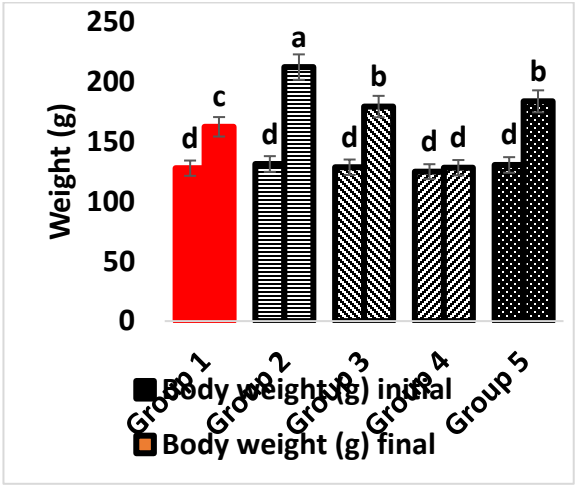

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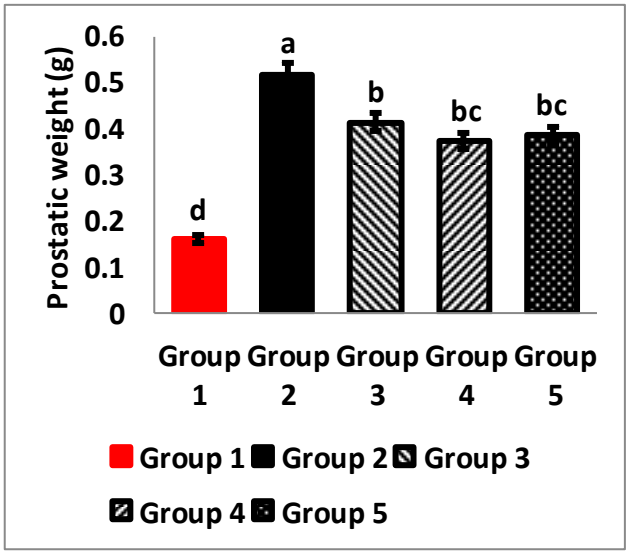

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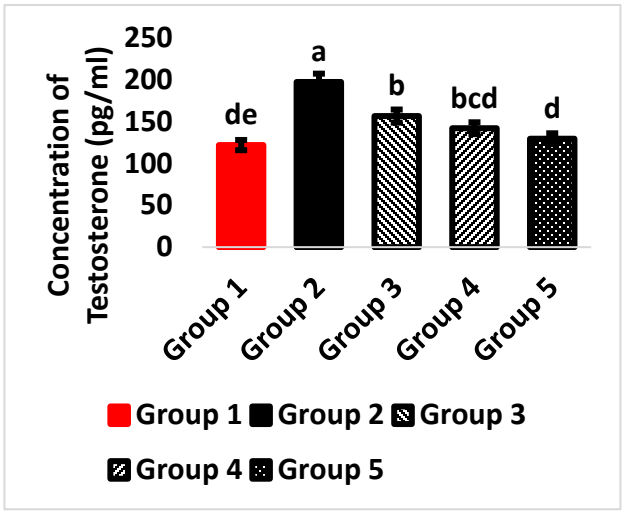
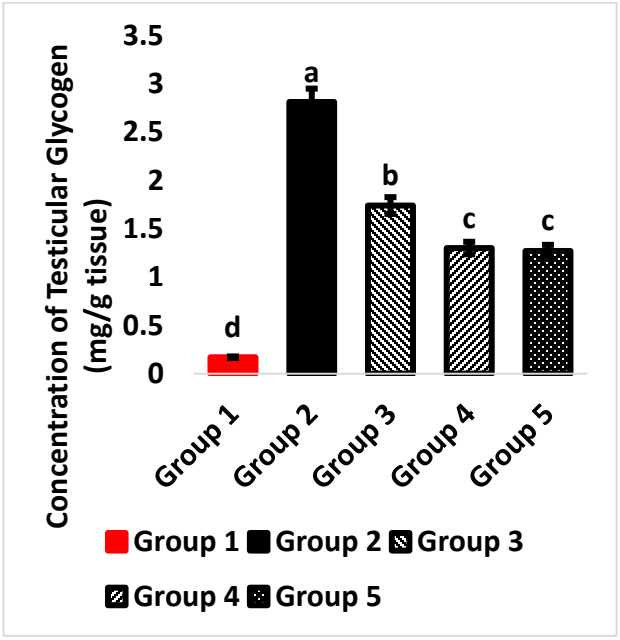


(d)

Figure 1: Effect of *Annona muricata* juice on the testicular weight (a); initial and final body weight (b); prostatic weight (c) and the testosterone concentration of male rats induced with BPH. Each value is represented as the mean of 8 replicates. Bars with the same alphabetic superscript are not significantly different ($p < 0.05$).



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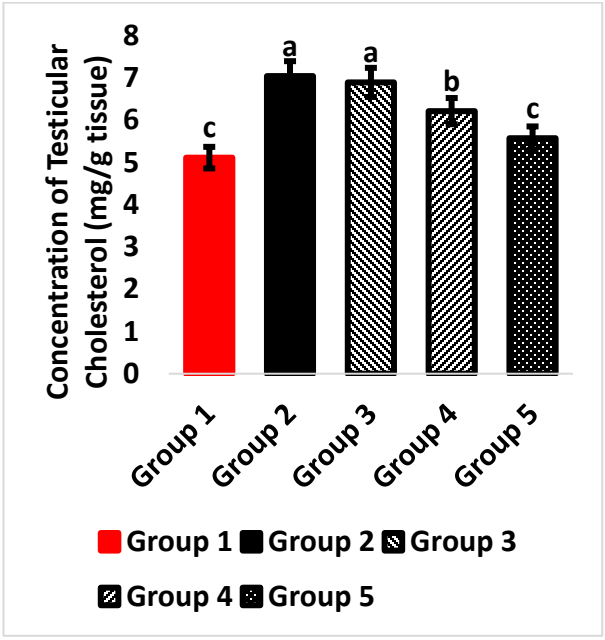

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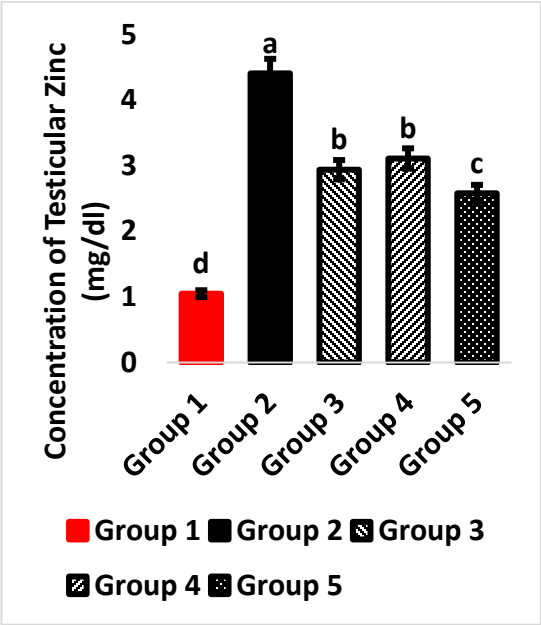

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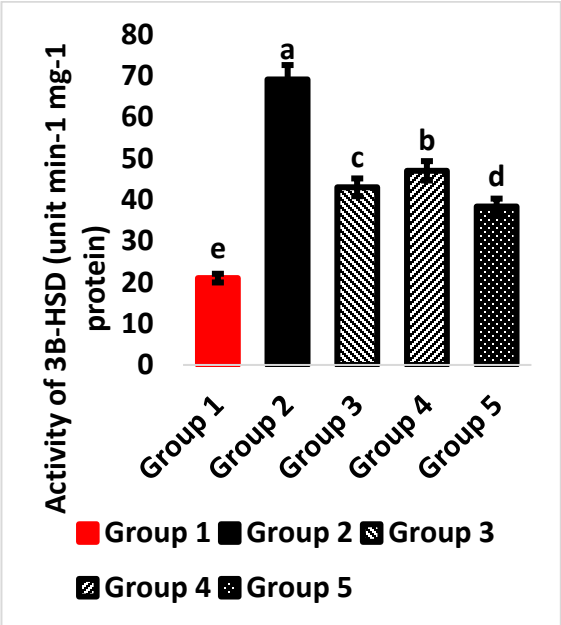

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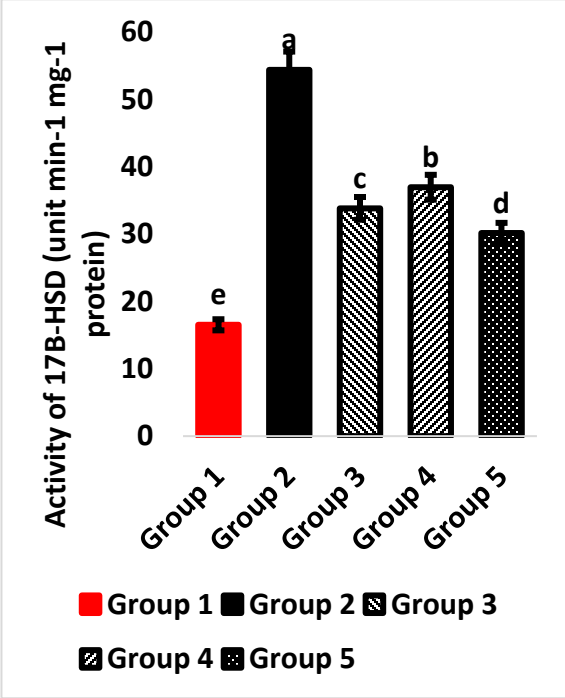

(e)

Figure 2: Effect of *Annona muricata* juice on the concentrations of testicular glycogen (a), on the concentrations of testicular cholesterol (b), on the concentrations of testicular zinc (c), on the activities of 3β- hydroxysteroid dehydrogenase (d) and on the activities of 17β- HSD activity in male rats induced with BPH. Each value is represented as the mean of 8 replicates. Bars with the same alphabetic superscript are not significantly different ($p < 0.05$).

4.0 Discussion

Irrespective of the progression in the diagnosis and management of BPH, it remains the most dominant urologic health problem troubling elderly men worldwide (Yu *et al*., 2020). The present study investigates the potential efficacy of *Annona muricata* fruit juice against experimental benign prostatic hyperplasia. The study reveals that BPH cause an increase in relative prostate weights increased DHT and PSA levels and prostatic epithelial hyperplasia compared to normal rats. It was discovered that *Annona muricata* fruit juice reduced the testosterone propionate-induced increase in prostate weight with evident improvement in the histology of prostate tissue of the *Annona* muricata treated groups. Furthermore, the fruit juice decreased the expression of DHT and PSA. Previous research studies have established that an increase in the prostate is a vital indicator of the development of BPH (Morcos and Afifi, 2011), and this study confirmed the findings since BPH rats' recorded significant enlargement in the size of the prostate gland. It is well understood that when the prostate enlarges, the urethral canal constricts, resulting in partial or complete urinary canal obstruction.

Testosterone and DHT are involved in the pathogenesis of BPH and play an important role in the development of male reproductive organs in the body (Hwangbo *et al.,* 2018). The serum concentrations of testosterone and DHT may vary with age (Izumi *et al.*, 2013). The levels of DHT in the serum of BPH patients are significantly higher than those of unaffected men of comparable age (Cannarella *et al*., 2021). DHT is primarily synthesized from circulating testosterone in the prostate, hair follicles, and testes via the enzymatic action of 5α-reductase. Interestingly, DHT binds to androgen receptors more strongly than testosterone and adrenal androgens. This is due to the greater affinity of DHT towards androgen receptors when compared to that of testosterone and adrenal androgens (Hwangbo *et al*., 2018). Because of this, a number of studies have been conducted to investigate how 5α-reductase regulates DHT levels. Finasteride is a known 5α-reductase inhibitor commonly used to manage BPH conditions. It reduces testosterone and DHT levels in serum and the prostate gland, contracting prostate size and BPH-related symptoms like LUTS (Arena *et al*., 2019). Scientists are seriously investigating finding an alternative medicine for finasteride to treat and manage BPH because long-term use of this drug has serious adverse effects (Cauci *et al*., 2017). In our studies, the testosterone-induced increase in the production of serum DHT was significantly inhibited by oral administration of *Annona muricata* extracts in a dose-dependent manner. This suggests that the inhibitory potential of *Annona muricata* extracts in our TP-induced rat was due to the down regulation of DHT production in serum and prostate. These results suggest that *Annona muricata* extracts can be a viable alternative to finasteride to manage BPH conditions in the future.

An increased level of testicular glycogen shows the availability of nutrients in the development of spermatozoa. Indrani *et al* 2014 reported that a decrease in glycogen and fructose contents in testis and seminal vesicles can decrease the number of mature and motile spermatozoa. In support of that, glycogen can also serve as a nutrient for the development of spermatozoa, which can now increase the number of mature and motile spermatozoa (Niknam *et al.,* 2014).

Increased testicular zinc level in response to the treatment has positive effects on serum testosterone concentration, as Zn controls the testosterone metabolism, semen volume (Altaher and Abdrabo, 2015), sperm maturation and maintenance of germinal epithelium (Alsalman *et al.,* 2013). Chia *et al*., 2000 reported that low sperm count, atrophy of the seminiferous tubules, failure of spermatogenesis and abnormal sperm production are associated with decreased zinc content and from this study increased testicular zinc levels in the treated rat show a beneficial effect on the *Annona muricata* fruit juice on the BPH rats following administration of *Annona muricata* fruit juice (both doses) shows that the potential of this fruit in the management of BPH.

It is well known that cholesterol is an essential precursor to produce testosterone (Traish and Kypreos, 2011) which is actually responsible for

maintaining the normal activities of the male reproductive system (Ruige *et al.*, 2013). The increased testicular cholesterol indicates that cholesterol was used to produce testosterone (Biswas and Deb, 1965) which can be supported by the increased activities of two testicular steroidogenic key enzymes 3β -HSD and 17β-HSD in all the treated groups.

Annona muricata extract increased the activities of 3β-HSD and 17β-HSD in rats' testicular tissues. The observed increase in activity of these steroidogenic enzymes may be due to the synthesis of gonadotrophin production in the pituitary gland. Furthermore, testosterone levels in the blood of *Annona muricata* fruit juice-treated rats were high, this suggests inhibition or suppression of spermatogenesis and reproductive disorder. From previous reports, it has been established that a decrease in 3β-HSD and 17β-HSD activities may be associated with low levels of serum testosterone. This finding implies *Annona muricata* fruit juice may increase sexual hormone (testosterone) levels which may increase the fertility rate in the male reproductive system. Testosterone is an important hormone involved in spermatogenesis. The observed increase in serum testosterone levels in rats treated with *Annona muricata* fruit juice prevents hypogonadism and improves sexual function.

5.0 Conclusion

The administration of AM inhibited the regulatory enzymes (3β- and 17β- hydroxysteroid dehydrogenase) in the biosynthesis of testosterone. Although the effects of *Annona muricata* on 5α-reductase was not determined, it is evident that *Annona muricata* fruit inhibited the enzyme by the reduction in the concentration of DHT. *Annona muricata* fruit juice reduced the level of PSA to near normal, PSA is a major indicator used in monitoring the prognosis of BPH in clinical practices.

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