The Bioscientist Journal Vol. 12 (1): 12 -23, March 2024
Journal of the faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria
Available online at *http://www.bioscientistjournal.com*

DOI 10.54117/the_bioscientist.v12i1.162



OPEN ACCESS

Full Length Research Article

Effect of *Annona muricata* leaf ethanol extract on testosterone-induced benign prostatic hyperplasia in Wistar rats

Ezirim Amanda U. [*1], Ezekwesili-Ofili Josephine. O.[2] and Igwilo Innocent. O.[2]

[1] Department of Biochemistry, Federal University of Technology Owerri, Nigeria.

[2] Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University Awka, Nigeria.

*Corresponding author email: amanda.ezirim@futo.edu.ng +2348131533419

Article History: Submitted, 03/01/2024, Accepted, 31/03/2024.

Abstract

Benign prostatic hyperplasia (BPH) has become the most common urological disease among aging men. This study investigated the inhibitory effect of *Annona muricata* leaf ethanol extract on a BPH animal model. Thirty male Wistar rats with weight range from 80-100g were divided into six groups: normal control (NC), testosterone-induced negative control (TC), Finasteride treatment control (FT), and the *A. muricata* low, medium, and high dose treatment groups: AML (100 mg/kg), AMM (200 mg/kg) and AMH (400 mg/kg) *b.w* respectively. BPH was induced by subcutaneous injection of testosterone propionate (5.0 mg/kg/*b.w*) for 28 consecutive days. Plant extract *in vitro* antioxidant activities were determined. After 14 days post-treatment, a 40% increase in prostate weight in the TC reflected a significant increase ($p<0.05$) in prostate index. The AMM and AMH groups showed significant reductions ($p<0.05$) in the prostate index, at approximately 50%. Prostate inhibition percentage of the FT at 45.26% and the AML, AMM and AMH at 43.4%, 42.1% and 44.4% were non-significant ($p>0.05$). *In vitro* antioxidant capacities were exhibited at 94.4% DPPH and 60% FRAP inhibition. This study therefore suggests that *A. muricata* is a potential candidate for the treatment of BPH.

Keywords: Benign prostate hyperplasia, prostate index, antioxidant, *Annona muricata*

Introduction

Benign prostatic hyperplasia (BPH) is a prevalent urological condition that affects older men and has become a global public health issue. This complication occurs in approximately 50% of 50–60-year-old men, 55% of men aged 60 to 70 years and 90% of the male population aged >80 years, following anatomic evidence at autopsy (Mahapokai *et al.*, 2000; Fibbi *et al.,* 2010). According to Madersbacher *et al.* (2019), the mechanism underlying the hyperproliferation of affected cells that results in the progression of BPH is still uncertain. However, strong clinical and experimental evidence suggests that the pathophysiological mechanisms of BPH involve the activation of inflammation and cellular proliferation (Devlin *et al.,* 2021).

Oxidative stress, hormonal pathway alteration, aging, metabolic syndrome, inflammation, and growth factor activity are known causative factors of BPH (Wang *et al.,* 2018; Raafat *et al.,* 2022). Taken together, these findings result in enhanced cellular events involving normal epithelial cell apoptosis inhibition, and general apoptotic proliferation disorders, which further cause increased proliferation of cells, hyperplasia and subsequent prostate cancer (Cai *et al.*, 2019). Inflammation has been proven to be a recognized risk factor for BPH development and progression revealing what is regarded as prostate tissue remodelling (Untergasser *et al.,* 2005). Additionally, inflammation promotes prostate cell proliferation in benign circumstances, which, together with the overall clinical development, increases the risk of urinary retention and the need for surgical intervention (Cai *et al.,* 2019; Madersbacher *et al.,* 2019). Hence, the high prevalence of BPH and its consequential health implications necessitate its prevention and the development of safe pharmaceutical therapeutics.

Currently, botanical products with proven therapeutic and medicinal capacity are being investigated as alternative sources of BPH intervention (Ejike and Ezeanyika, 2011; Ekeyi *et al.*, 2021). Studies show that 30% of BPH patients rely on local medicines to treat this condition (Singh, 2015). Among the many plant-based therapeutics that have attracted the interest of researchers in providing alternative strategies is *Annona muricata* L, widely known as Sour sop and found in tropical regions of Central and South America, West Africa, and Southeast Asia (da Rocha *et al.*, 2001; Wahab *et al*., 2018). Several investigations suggest that the leaves of *A. muricata* are the most utilized parts for a wide array of ethnomedicinal uses. In particular, *A. muricata* leaves exhibit potent anticancer, anti-inflammatory, immunomodulatory and antiproliferative properties (Sun *et al.,* 2016; Afzaal *et al*., 2022). In addition, numerous lines of evidence suggest that the leaf extract of *A. muricata* represses tumour growth *in vivo* in animal models as well as induces apoptosis in various cancer cells *in vitro* (Adewole and Ojewole, 2009; Ezirim *et al.,* 2013; Liu *et al*., 2016). Given the increased reliance on various ethnobotanical preparations to ameliorate disease conditions, this study aimed to investigate the potential of *A. muricata* leaf ethanol extracts for the treatment of BPH in an established disease model.

Materials And Methods

Plant Material and Extraction

Apparently healthy leaves of Sour sop were obtained from farming areas at Eziobodo, Owerri West Local Government Area of Imo State, Nigeria. Identification of the leaves as that of *Annona muricata* was carried out at the Department of Forestry and Wildlife Technology, Federal University of Technology, Owerri, (FUTO) Imo State with voucher number FUTO/FWT/ERB/2022/71. The collected leaves were air-dried at room temperature until a constant weight was obtained indicating that the leaves were completely dehydrated. Dried *A. muricata* leaves were weighed, ground to a fine powder and subsequently stored in airtight containers for further use. The maceration extraction method which involves simple soaking, filtering and evaporation was applied. About 1.3kg of ground plant material from *A. muricata* was weighed into conical flasks and extracted in 6.2L of 70% v/v ethanol for 48 hours with constant stirring. The extracting solvent was subsequently decanted and filtered using a Whatman No. 1 filter paper. The filtrate was evaporated in a water bath at 70°C to obtain a molten extract of constant weight. The stock extract was kept at a cool temperature throughout the study.

Quantification of total phenol content

The amount of total phenols in the sample was estimated by the Folin-Ciocalteu method proposed by Mallick and Singh (1980). The concentration of phenols is expressed as mg/100g GAE (gallic acid equivalent).

***In vitro* antioxidant assays**

In vitro antioxidant assays that were conducted on the leaf ethanol extract of *A. muricata* included:

i. **Nitric oxide scavenging activity assay:** The extent of nitric oxide radical generation inhibition *in vitro* was determined according to methods of Green *et al.* (1982). The concentrations were obtained with gallic acid as standard.

ii. **DPPH (2,2-diphenyl-2-picryl hydrazyl hydrate) spectrophotometric assay:** The ability of the natural antioxidants from the leaf ethanol extract to scavenge the stable free radical DPPH was measured by the method of Mensor *et al*. (2001). The radical scavenging activity was calculated as a percentage while butylated hydroxytoluene served as a reference.

iii. **Ferric reducing antioxidant power (FRAP) assay:** The ferric reducing antioxidant power of the leaf ethanol extract was determined as described by Pulido *et al*. (2000). The reducing ability was expressed as a percentage compared to the gallic acid standard.

iv. **Acute toxicity (LD_{50} determination):** The index of acute toxicity is the LD_{50} which is the dose of a substance capable of causing death in 50% of the population of animals exposed to the substance. Lorke's (1983) method was used to determine the extract LD_{50}.

Animal model

A total of thirty (30) apparently healthy, male 6-week-old albino Wistar rats of body weights between 80 and 100 g were procured for the study. The animals were kept in metal cages in a well-ventilated room with a temperature of 24 ± 2°C and a relative humidity of 55–65% with a diurnal 12 h light cycle. The rats had access to water

and were fed *ad libitum* with pelleted standard mash. Acclimatization to their new environment was allowed for two weeks before the experiments commenced. All rats received humane care in accordance with animal handling guidelines and approval (NAU/AREC/2024/0020) from the animal ethics committee of Nnamdi Azikiwe University, Nigeria.

Study design

Thirty male Wistar rats with a mean weight 90 g were divided into six groups (n=5). Group 1 was the normal control (NC) which was not induced nor treated. Group 2 was the testosterone-induced negative control (TC) and Group 3 was the induced Finasteride standard drug control (FT). Groups 4, 5 and 6 were induced and served as *A. muricata* low, medium and high dose: AML (100mg/kg), AMM (200mg/kg) and AMH (400 mg/kg) *b.w* respectively.

BPH induction and treatments

Group 1 was subjected to subcutaneous olive oil (vehicle) throughout the study. BPH was induced by subcutaneous injection of testosterone propionate (5.0 mg/kg per *b.w*) for 28 consecutive days as reported by Ekeyi *et al*. (2021). The animals were then treated orally with 0.5mg/kg *b.w* of Finasteride (group 3) and the *A. muricata* leaf ethanolic extract doses for 14 days. The finasteride treatment was adapted from Alamoudi *et al*. (2022).

Body and prostate weights

At the end of each experimental period, the rats were fasted for 12 hours, weighed and euthenized following anaesthesia by trichloromethane vapour exposure. The prostrates from each dissected rat were carefully excised, blotted with filter paper and weighed on a sensitive balance.

Determination of the Prostate Index (PI) Percentage

The mean body weight and prostate index (PI) were calculated for each group. Following the immediate excision and weighing of the rat prostates, the prostate index percentage was calculated as: Prostate index percentage = (prostate wet weight/body weight) × 100%.

Determination of the Prostate Inhibition Percentage

The prostate inhibition percentage was calculated for each group. Following the immediate excision and weighing of the rat prostate, the inhibition percentage was calculated as:

Prostate inhibition percentage = 100 - [(prostate wet weight of BPH rats treated with *Annona muricata* / prostate wet weight of BPH rats) × 100]

Prostate histopathology

Histological examination was carried out on prostate samples harvested from the controls and treatment groups. The prostate tissues were rapidly dissected, blotted, and weighed. Sections of the ventral lobes were fixed in 10% formal saline for 48 hours for histological examination which was performed according to the methods of Clayden 1967); John and Alan (1977), and Ajonuma *et al*. (2005).

Data analysis

The data obtained were expressed as the mean of three replicates ± SD. Data analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 19. Two-way and one-way analyses of variance ANOVA were used for comparison, and the results were subjected to post hoc tests using least square deviation (LSD) tests. Values at $p<0.05$ were considered significant for all the results obtained.

RESULTS

Total phenol content of *A. muricata* leaf ethanol extract

When *A. muricata* leaf ethanol extract was subjected to quantitative analysis of total phenol content, the total phenol content was observed to be 272.02 mg/100g GAE.

Nitric oxide scavenging activity of *A. muricata* leaf ethanol extract.

The nitric oxide scavenging activity was determined in relation to that of gallic acid from the standard curve with the linear equation y = 0.0496x + 0.02. At 20 mg/ml concentration, the nitric oxide concentration in the leaf ethanolic extract of *A. muricata* was determined at 2.18 ± 1.7ug/ml against 3.47±0.2ug/ml of the gallic acid standard.

Ferric reducing antioxidant power (FRAP) of *A. muricata* leaf ethanol extract.

The ferric reducing antioxidant power was expressed as FRAP % inhibition (Figure 1). The leaf ethanol extract of *A. muricata* exhibited 60% FRAP inhibition compared to the gallic acid standard at a concentration of 20 mg/ml.

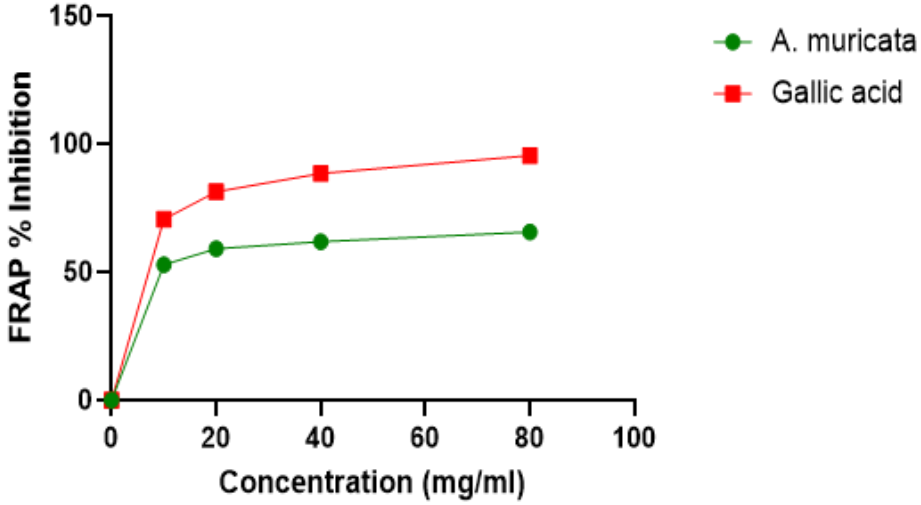


Figure 1. Ferric reducing antioxidant power (FRAP) of *A. muricata* leaf ethanol extract.

DPPH assay results of *A. muricata* leaf ethanol extract

The quantification of antioxidant activity using the DPPH method was expressed as a percentage inhibition (Figure 2) At a concentration of 40 mg/ml, the leaf ethanol extract of *A. muricata* exhibited strong free radical scavenging activity as indicated by a peak percentage inhibition of 94.4% which was comparable with that of the BHT standard at 98.50%.

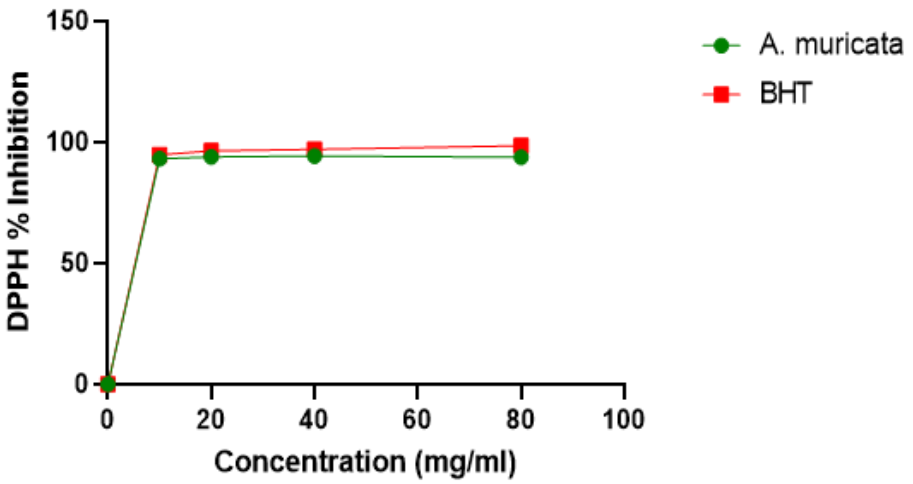


Figure 2. DPPH spectrophotometric assay results of *A. muricata* leaf ethanol extract

Acute toxicity assessment of *A. muricata* leaf ethanol extract

The acute toxicity study of the leaf ethanolic extract of *A. muricata* showed that the plant leaf is toxic at doses above 2000 mg/kg, as the acute toxicity index obtained in this study was 2236.06 mg/kg.

Effect of *A. muricata* leaf ethanol extract on body weight and prostate indices

The animals in the TC exhibited a significant reduction ($p<0.05$) in their mean final body weight (188.95 g) compared to that of the normal control (224.61g) as shown in Table 1. Among the treatment groups, only AMH significantly reversed ($p<0.05$) the recovery of body weight (221.54 g). Compared with the normal control, the TC exhibited a significant increase ($p<0.05$) in prostate weight which led to an increase in the prostate index. However, the AMM, AML and AMH significantly lowered ($p<0.05$) the elevated prostate indices, showing no significant difference from those of the normal control. The prostate inhibition percentage of the *A. muricata* groups, showed no significant variation ($p>0.05$) from that of the FT.

Table 1. Effect of *A. muricata* leaf ethanol extract on the final body weight and prostate parameters.

Group	Final body weight (g)	Prostate weight (mg)	Prostate index (%)	Prostate inhibition (%)
NC	224.61 ± 5.05^{a}	0.29 ± 0.03^{a}	0.13 ± 0.00^{a}	38.66 ± 3.24^{a}
TC (T; 5.0 mg/kg *b.w*)	188.95 ± 14.47^{b}	0.48 ± 0.09^{b}	0.24 ± 0.04^{b}	0 ± 0.00^{c}
FT (F; 0.5mg/kg *b.w*)	192.78 ± 16.61^{b}	0.26 ± 0.05^{a}	0.15 ± 0.01^{a}	45.26 ± 2.63^{b}
AML (100mg/kg *b.w*)	180.85 ± 25.46^{b}	0.27 ± 0.05^{a}	0.14 ± 0.02^{a}	43.41 ± 2.41^{b}
AMM (200mg/kg *b.w*)	181.51 ± 20.04^{b}	0.28 ± 0.11^{a}	0.12 ± 0.02^{a}	42.15 ± 2.55^{b}
AMH (400mg/kg *b.w*)	221.54 ± 16.62^{a}	0.27 ± 0.03^{a}	0.12 ± 0.02^{a}	44.44 ± 2.54^{b}

The data (n=5) are presented as the mean ± SD. Values with different letters are significantly different at $p<0.05$. NC (normal control), TC (testosterone negative control), FT (Finasteride standard drug control), AML (*A. muricata* low dose), AMM (*A. muricata* medium dose), AMH (*A. muricata* high dose).

Histological evaluation

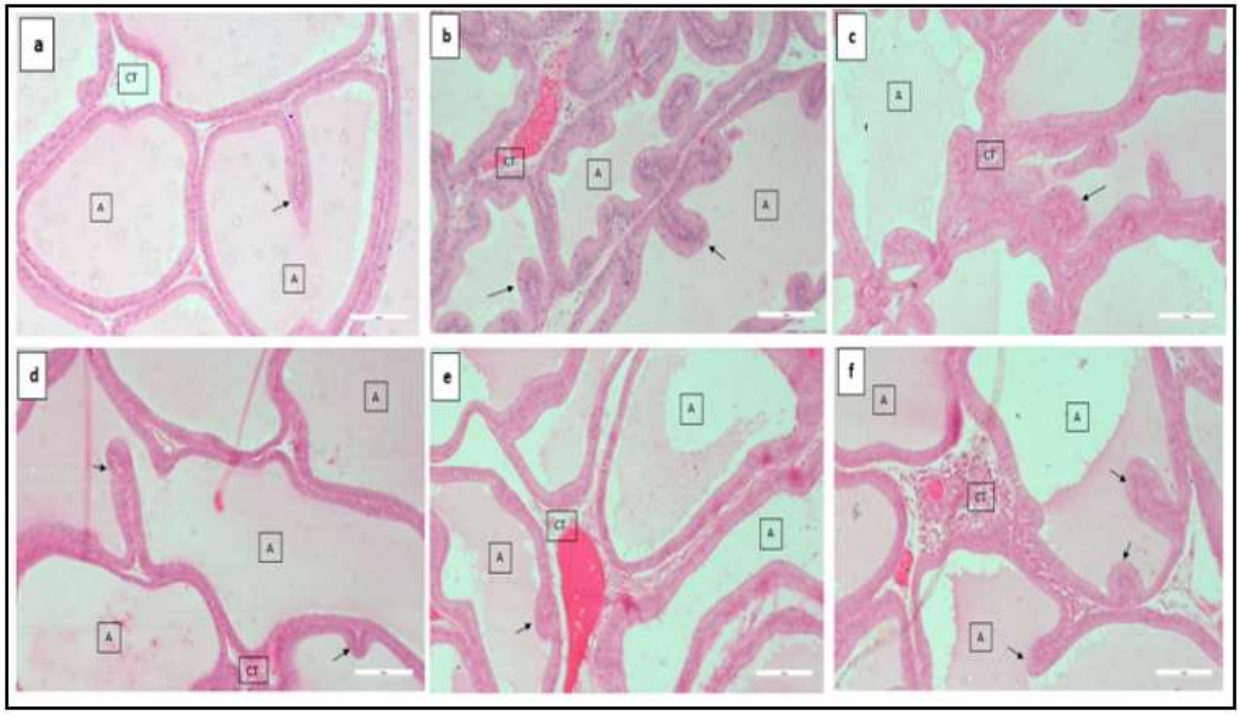


Figure 3. Photomicrographs of prostate tissue samples from the normal control (**a**), testosterone negative control (TC) (**b**), Finasteride standard drug control (FT) (**c**), *A. muricata* low dose (AML) (**d**), *A. muricata* medium dose (AMM) (**e**) and *A. muricata* high dose (AMH) (**f**). A, acinar; CT, inter-acinar connective tissue. Arrows (→) indicate epithelial projections into the lumen. Eosine stain. Magnification 400x.

As shown in Fig. 3.a, the normal histomorphology of the prostate lobe in the normal control showed normal histoarchitecture and regular acini. However, the TC exhibited architectural disruption of the prostate gland as evidenced by the irregular acinar shape lined with cuboidal to low columnar epithelium with occasional papillary infolding, papillary projections into the lumen and multifocal areas with hyperplastic nodules (Fig. 3b). After the treatment duration, the FT (Fig. 3c.) showed sections with fewer multifocal areas of epithelial hyperplasia. However, Fig. 3d-f shows the effects of *A. muricata* (100, 200 and 400 mg/kg *b.w* respectively) including widespread acinar dilation, occasional epithelial infoldings, eosinophilic secretions and mild infiltration of inflammatory leukocytes in the inter-acinar connective tissue (Fig. 3f) compared to those in the untreated TC (Fig 1c), indicating marked reductions in hyperplasia and hypertrophy.

Discussion

Phenolic compounds and flavonoids are responsible for the antioxidant capacity of medicinal plant extracts (Orak *et al.*, 2019). In a previous report (Ezirim *et al.*, 2013), the leaf ethanolic extract of *A. muricata* was shown to exhibit potent antioxidant properties and the capacity to induce apoptosis in a leukaemia cell line. In the

present study, the total phenolic content of *A. muricata* leaf ethanol extract was analyzed at 272.02 mg/100g GAE. This finding provides insight into the potential ethnobotanical roles played by phenolic compounds in the activity of this plant. Gavamukulya *et al*. (2015) observed a slightly greater total phenolic content in ethanolic extracts but reported a greater level of phenolics in aqueous extracts of *A. muricata*.

The ability of a molecule to undergo reduction reactions can be a valuable predictor of its potential as an antioxidant of which mechanisms could involve scavenging of active oxygen species and hydroxyl radicals, inhibition of lipid peroxidation and chelation of metal ions (Dorman *et al.*, 2003). The *in vitro* quantification of antioxidant activity using three methods was carried out in this study. The degree of nitric oxide radical generation inhibition by the leaf ethanol extract of *A. muricata* was 2.18 ± 1.7 ug/ml against 3.47±0.2 ug/ml gallic acid standard at a concentration of 20 mg/ml. The ferric reducing antioxidant power (FRAP) was quantified by calculating the percentage inhibition of FRAP (Fig. 1). The ethanol extract of *A. muricata* leaves demonstrated a 60% suppression of FRAP compared to the gallic acid standard at a dosage of 20 mg/ml.

The DPPH method was used to determine the percentage inhibition and the results showed that the *A. muricata* leaf ethanol extract exhibited strong free radical scavenging activity depicted by a peak percentage inhibition of 94.4% which competed favourably with the BHT standard at 98.50% at a concentration of 40mg/ml (Fig. 2). According to Leon-Fernandez *et al.* (2017), *A. muricata* ethanol and chloroform extracts, including acetogenin fractions, had strongly prominent DPPH activity owing to the high phenolic content and acetogenin concentration found in the extract. Further studies suggest that the synergistic effects of the metabolites found in *A. muricata* could be attributed to its antioxidant and anti-inflammatory potential (Baskar *et al.*, 2007; Nwaehujor *et al.*, 2020).

In this study, the acute toxicity study result of ethanol leaf extract of *A. muricata* showed that the plant leaf is toxic in doses above 2000mg/kg as the index of acute toxicity obtained in this study is 2236.06mg/kg. The outcome of the LD50 determination resonates with de Sousa *et al*. (2010) which reported that the leaf ethanolic extract of *A. muricata* had displayed a LD50 of >2g/kg and which, according to OECD guidelines, is considered nontoxic. In addition, further studies have suggested the possible synergy of the components of *A. muricata* crude leaf extracts in exhibiting less toxicity than the more toxic acetogenin enriched extracts (Yang *et al.*, 2015).

The enlargement of prostate nodules is a pathological manifestation of BPH and is a result of stromal and epithelial cell hyperplasia in addition to subsequent urethral narrowing, thus triggering approximately 80% of lower urinary tract symptoms (LUTS) (Kapoor, 2012; Maghsoudi *et al.*, 2018). As shown in Table 2, after 28 days of induction, the TC showed a significant increase ($p<0.05$) in the mean prostate weight at 0.48 ± 0.09g, which was significantly greater than the mean prostate weight of normal control rats (0.29±0.03g) which was a prostate weight increase by about 40%. Compared with those in the normal controls, the weights of the prostate in the FT AML, AMM and AMH were significantly lower ($p<0.05$).

Furthermore, the reported increase of prostate weight in the TC consequently reflected the significant increase ($p<0.05$) in the prostate index (~1.84-fold) compared to the normal control. However, compared to the TC, the treatments in the FT and AML significantly lowered ($p<0.05$) the prostate index by 37% and 41.6% respectively, whereas both AMM and AMH peaked at 50% (Table 2). There were significant increases ($p<0.05$) in prostate inhibition (43.4%, 42.1% and 44.4%) in the AML, AMM and AMH compared to the normal control and notably, not dose dependent. These values were not significantly different ($p>0.05$) from the FT at 45.26%.

It is of interest to note that these results were consistent with histological examination of the prostate tissues as indicators of hyperplasia and hypertrophy were evident in the untreated TC group, which were observed to be markedly reduced in the experimental groups treated with *A. muricata* leaf ethanol extract (Fig. 3). These findings are consistent with similar studies by Mbaka *et al*. (2017), which confirmed that medicinal plant extracts with proven efficacy can repair prostate structural changes caused by aging and in addition, decrease prostate weight.

Several *in vivo* models have been established to investigate BPH growth behaviour and various treatment agents have been tested. These include xenograft models, the use of transgenic mice and BPH induction (Mahapokai *et al.*, 2000). Studies have shown that the prostates of rats and mice respond to experimental carcinogens and hormonal induction. According to Campolina-Silva *et al.* (2020), the rat strain plays an important role in the development of BPH following treatment with testosterone from an exogenous source, in both castrated and non-castrated conditions, as the Wistar strain was found to be most responsive in this regard. In addition, several studies have suggested that an increase in the prostate index is an established indicator of the development of the BPH model of induction in non-castrated rats through an increase in the prostate index (Wang *et al.*, 2018; Choi *et al.*, 2020; Shabani *et al.*, 2021; Ekeyi *et al.*, 2021). Therefore, these reported corroborative data suggest that the BPH model was successfully established in this study.

Conclusion

Prostatic indicators of the BPH animal model using exogenous testosterone were established in this study and the effects of the ethanol extract were explored. The prostate index and prostate inhibition values of the extract-treated groups demonstrated the potential to mitigate BPH development compared with the standard drug Finasteride. It is therefore plausible that *A. muricata* could be an inexpensive and safe alternative for the treatment of prostate diseases in developing countries.

References

Adewole, S. and Ojewole, J. (2009). Protective effects of Annona muricata Linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. African. Journal of Traditional Complementary and Alternative Medicine. 6, 30–41.

Afzaal, M., Saeed, F., Asghar, A., Shah, Y. A., Ikram, A., Ateeq, H., Hussain, M., Ofoedu, C. E. and Chacha, J. S. (2022). Nutritional and therapeutic potential of soursop. Journal of Food Quality, Article ID 8828358.

Ajonuma, L. C., Ng, E. N. Y., Chang, L. N., Chow, P. H., Kung, L. S., Chang, A. N. Y.,

Ho, L. S., Briton-Jones, C., Lok, I. H., Haines, C.J, and Chan, H. C. (2005). Ultrastructural characterization of whole hydrosalpinx from infertile Chinese women. Cell Biology International, 29: 849–856.

Alamoudi, A. J., Alessi, S. A., Rizg, W. Y., Jali, A. M., Safhi, A. Y.; Sabei, F.Y., Alshehri, S., Hosny, K. M. and Abdel-Naim, A. B. (2022). Cordycepin attenuates testosterone-induced benign prostatic hyperplasia in rats via modulation of AMPK and AKT Activation. Pharmaceutics, 14 (8):1652.

Baskar, R., Rajeswari, V. and Kumar, T. S. (2007). In-vitro antioxidant studies in leaves of Annona species. Indian Journal of Experimental Biology, 45 (5): 480 – 485.

Cai, T., Santi, R., Tamanini, I., Galli, I. C., Perletti, G., Bjerklund-Johansen, T. E. and Nesi G. (2019). Current knowledge of the potential links between inflammation and prostate cancer. International Journal of Molecular Sciences, 20 (15): 3833.

Campolina-Silva, G. H., Werneck-Gomes, H., Maria, B. T., Barata, M. C., Torres, M. J., Contreras, H. R., Mahecha, G. A. B., Oliveira, C. A. (2020). Targeting Wistar rat as a model for studying benign, premalignant and malignant lesions of the prostate. Life Sciences, 242: 117149.

Choi, Y. J., Lee, J. I., Fan, M., Tang, Y., Yoon, E. J., Ryu, Y. B. and Kim, E. K. (2020). Metabolomic analysis of Morus cultivar root extracts and their ameliorative effect on testosterone-induced prostate enlargement in Sprague Dawley rats. International Journal of Molecular Sciences, 21 (4): 1435.

Clayden, E. C. (1967). Practical section cutting and staining, 4th edn. J and E ChurchHill Ltd, UK.

da Rocha, A. B., Lopes, R. M. and Schwartsmann, G. (2001). Natural products in anticancer therapy. Current Opinion in Pharmacology, 4 (1): 364-369.

de Sousa O.V., Vieira G. D., Pinho J. J. R., Yamamoto, C.H., and Alves, M.S. (2010). Antinociceptive and anti-inflammatory activities of the ethanol extract of Annona muricata L. leaves in animal models. International Journal of Molecular Sciences, 11 (5): 2067-2078.

Devlin, C. M., Simms, M. S. and Maitland, N. J. (2021). Benign prostatic hyperplasia–what do we know? British Journal of Urology International, 127 (4): 389-399.

Dorman, H. J., Kosar, M., Kahlos, K., Holm, Y., and Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. Journal of Agricultural and Food Chemistry, 51: 4563-4569.

Ejike, C.E.C. and Ezeanyika, L.U.S. (2011). Management of experimental benign prostatic hyperplasia in rats using a feedbased therapy containing Telfairia occidentalis seeds. African Journal of Traditional, Complementary, and Alternative Medicine. 8 (4): 398–404.

Ekeyi, Y., Uchendu, N. O., Anaduaka, E. G. and Ezeanyika, L.U.S (2021). Ethanol extract of Cassia sieberiana leaves ameliorates deviances associated with benign prostatic hyperplasia in rats. All Life, 14 (1): 473-483.

Ezirim, A. U., Okochi, V. I., James, A. B., Adebeshi, O. A., Ogunnowo S. and Odeghe, O. B. (2013). Induction of apoptosis in myelogenous leukemic K562 cells by ethanolic leaf extract of Annona muricata. Global Journal of Research on Medicinal

Plants & Indigenous Medicine, 2 (3): 142-151.

Fibbi, B., Penna, G., Morelli, A., Adorini, L., and Maggi, M. (2010). Chronic inflammation in the pathogenesis of benign prostatic hyperplasia. International Journal of Andrology, 33 (3): 475-488.

Gavamukulya, Y., Abou-Elella, F., Wamunyokoli, F., and El-Shemy, H. A. (2015). GC-MS analysis of bioactive phytochemicals present in ethanolic extracts of leaves of Annona muricata: A further evidence for its medicinal diversity. Pharmacognosy Journal, 7 (5): 300-304.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. and Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite and 15N in biological fluids. Analytical Biochemistry, 126: 131–136.

John, D. B. and Alan, S. (1977) Theory and practice of histological techniques. Churchill Livingstone, Edinburgh.

Kapoor, A. (2012). Benign prostatic hyperplasia (BPH) management in the primary care setting. Canadian Journal of Urology, 19 (1): 10-17.

Leon-Fernandez, A. E., Sayago-Ayerdi, S. G., Velazquez-Estrada, R. M., Zepeda-Vallejo, L. G., Yahia, E. and Montalvo-Gonzalez E. (2017). In-vitro antioxidant capacity of crude extracts and acetogenin fraction of soursop fruit pulp. Pharmaceutica Analytica Acta, 8 (6): 550 – 556.

Liu, N., Yang, H. L., Wang, P., Lu, Y. C., Yang, Y. J. and Wang, L. (2016). Functional proteomic analysis reveals that the ethanol extract of Annona muricata L. induces liver cancer cell apoptosis through endoplasmic reticulum stress pathway. Journal of Ethnopharmacology, 189: 210–217.

Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of Toxicology, 54: 275–87.

Madersbacher, S., Sampson, N. and Culig, Z. (2019). Pathophysiology of benign prostatic hyperplasia and benign prostatic enlargement: A Mini-Review. Gerontology, 65:458-464.

Maghsoudi, H., Hallajzadeh, J., and Rezaeipour, M. (2018). Evaluation of the effect of polyphenol of escin compared with ibuprofen and dexamethasone in synoviocyte model for osteoarthritis: an in vitro study. Clinical Rheumatology, 37 (9): 2471-2478.

Mahapokai, W., Van Sluijs, F. J., and Schalken, J. A. (2000). Models for studying benign prostatic hyperplasia. Prostate Cancer and Prostatic Diseases, 3 (1): 28-33.

Mallick, C. P. and Singh, M. B. (1980). Plant enzymology and plant histoenzymology. Kalaya Publishing, New Delhi.

Mbaka, G., Anunobi, C., Ogunsina, S. and Osiagwu, D. (2017). Histomorphological changes in induced benign prostatic hyperplasia with exogenous testosterone and estradiol in adult male rats treated with aqueous ethanol extract of Secamone afzelii. Egyptian Journal of Basic and Applied Sciences, 4: 15–21.

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. D., Coube, C. S., and Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research, 15 (2): 127-130.

Nwaehujor, I., Olatunji, G., Oluwatoyin, F. and Samuel, A. (2020). Antioxidant and anti-inflammatory potential, and chemical

composition of fractions of ethanol extract of Annona muricata leaf. Ruhuna Journal of Science. 11, 131.

Orak, H. H., Bahrisefit, I. S. and Sabudak, T. (2019). Antioxidant activity of the extracts of soursop (Annona muricata L.) leaves, fruits pulps, peels and seeds. Polish Journal of Food and Nutrition Sciences, 69 (4): 359 – 366.

Phua, T. J. (2021). The etiology and pathophysiology genesis of benign prostatic hyperplasia and prostate cancer: A New Perspective. Medicines, 8 (6):30.

Pulido, R., Bravo, L., and Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry, 48: 3396–3402.

Raafat, M., Kamel, A. A., Shehata, A.H., Ahmed, A. S. F., Bayoumi, A. M. A., Moussa, R. A., Abourehab, M.A.S. and El-Daly, M. (2022). Aescin protects against experimental benign prostatic hyperplasia and preserves prostate histomorphology in rats via suppression of inflammatory cytokines and COX-2. Pharmaceuticals, 15, 130.

Shabani, E., Kalantari, H., Kalantar, M., Goudarzi, M., Mansouri, E. and Kalantar, H. (2021). Berberine ameliorates testosterone-induced benign prostate hyperplasia in rats. BMC Complementary Medicine and Therapies, 21(1): 1-10.

Singh, R. (2015). Medicinal plants: A review. Journal of Plant Sciences, 3 (1):50–55.

Sun, S., Liu, J., Zhou, N., Zhu, W., Dou, Q. P., and Zhou, K. (2016). Isolation of three new annonaceous acetogenins from graviola fruit (Annona muricata) and their anti-proliferation on human prostate cancer cell PC-3. Bioorganic & Medicinal Chemistry Letters, 26: 4382–4385.

Untergasser G., Madersbacher S., and Berger P. (2005). Benign prostatic hyperplasia: age-related tissue-remodeling, Experimental Gerontology, 40 (3): 121-128.

Wahab, S. M., Jantan, I., Haque, M. A. and Arshad, L. (2018). Exploring the Leaves of Annona muricata L. as a source of potential anti-inflammatory and anticancer agents. Frontiers in Pharmacology, 9: 661.

Wang, C. T., Wang, Y. Y., Liu, W. S., Cheng, C. M., Chiu, K. H., Liu, L. L., Liu, X. Z., Wen, Z. H., Chen, Y. H., and Chen, T. M. (2018). Rhodobacter sphaeroides extract Lycogen™ attenuates testosterone-induced benign prostate hyperplasia in rats. International Journal of Molecular Sciences, 19 (4): 1137.

Yang, C., Gundala, S. R., Mukkavilli, R., Vangala, S., Reid, M. D., and Aneja, R. (2015). Synergistic interactions among flavonoids and acetogenins in Graviola (Annona muricata) leaves confer protection against prostate cancer. Carcinogenesis, 36: 656–665.