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ANNONA MURICATA LEAF EXTRACT AMELIORATES HAEMATOLOGICAL AND BIOCHEMICAL ABNORMALITIES IN TESTOSTERONE-INDUCED MALE WISTAR RAT MODEL OF BENIGN PROSTATIC HYPERPLASIA

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The study aimed to explore the effects of A. muricata leaf ethanol extract on the haematological and biochemical indices in testosterone-induced male Wistar rat model of benign prostatic hyperplasia (BPH). Thirty male Wistar rats (av. weight 90g) were divided into six groups (n=5): normal control (NC), testosterone-induced negative control (TC), Finasteride treatment control (FT; 0.5mg/kg b.w), and the A. muricata treatment low (100 mg/kg), medium (200 mg/kg) and high (400 mg/kg) b.w doses denoted as AML, AMM and AMH respectively. BPH was induced by subcutaneous injection of testosterone propionate for 28 consecutive days. Oral treatments with finasteride and extract doses followed for 14 days. Results showed n-hexadecanoic with highest abundance (32.60%) using GC-MS. Leukocytosis, platelet aggregation, hyperlipidemia and hyperuricemia were significant observations in the TC. The extract doses significantly ($p<0.05$) modulated these states. This study suggests that A. muricata could improve haematological and biochemical alterations associated with benign prostatic hyperplasia

Keywords: *Benign prostatic hyperplasia, Annona muricata, liver, kidney, haematology*

INTRODUCTION

The stimulation of inflammation and cellular proliferation are believed to be key factors in the pathophysiology of BPH, according to compelling clinical and experimental evidence[1].Thus, oxidative stress, growth factor activity, hormonal system alterations, metabolic syndromes, and aging are all factors that contribute to the development of BPH[2]. Inflammation promotes prostate cells proliferation in benign situations, thus, combined with overall clinical development, increases the likelihood of urinary retention and the necessity for surgery[3,4].

As the major plasma androgen, testosterone plays a designated function as the prohormone of dihydrotestosterone (DHT) which is described as the key prostatic growth facilitator[5]. The conversion of circulating testosterone to the prostate-situated DHT is controlled by the enzyme 5-alpha reductase (5AR) and therefore, acts an important role in the pathogenesis of BPH[6]. Due to this mechanism, the basis for BPH therapy has focused on the targeted inhibition of 5AR activity[7]. Accordingly, 5-AR inhibitors (5ARIs) such as Finasteride are options mostly used by healthcare providers to ameliorate the impact of BPH and urinary retention in affected men[7].

However, the use of 5ARIs has not been without some overwhelming side effects including documented clinical trial reports of erectile dysfunction in about 38% of patients[8,9]. In addition, the inflammatory mechanisms linked with increased prostatic volume in BPH

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development have been associated with metabolic syndrome (MetS), which include low serum high density lipoprotein, high triacylglycerides and high body mass index[10]. Furthermore, elevated mean platelet volume may indicate the likelihood of inflammation caused by MetS in the course of BPH and related lower urinary tract symptoms [11]. Therefore, these associated health impacts of BPH raise the issue of alternative interventions.

Annona muricata has a wide range of ethnomedicinal uses, particularly exhibiting potent anticancer, anti-inflammatory, immunomodulating and anti-proliferative properties[12,13]. Its phytochemical profile including the acetogenins, alkaloids, flavonoids and phenolic compounds such as quercetin and gallic acid, are reported to be the compounds most responsible for the antioxidant and anti-inflammatory capacity of *A. muricata*[14,15]. Several studies suggest that the abundant presence of phytochemicals in whole foods contributes to several mechanisms that promote optimal health. Therefore, isolating a single or a group of beneficial compounds from whole foods may result in an ineffectual outcome, even when administered at high dosages [16,17].

Therefore, this study aims to investigate BPH treatment with *A. muricata* in a rat model of testosterone-induced BPH and evaluate the haematological and biochemical changes compared to a known 5-ARI, Finasteride.

MATERIALS AND METHODS

Plant materials and extraction

Healthy leaves of *A. muricata* were collected from farms at Eziobodo, Owerri West Local Government Area of Imo State, Nigeria. The plant was identified at the Department of Forestry and Wildlife Technology, Federal University of Technology, Owerri, Nigeria (voucher no. FUTO/FWT/ERB/2022/71). The plant samples were air-dried at room temperature until a constant weight was obtained. The dried leaves were weighed and pulverized to fine powder. About 1.3kg of the ground plant material of *A. muricata* was extracted in of 70% v/v ethanol for 48 hours by constant agitation. The extracting solvent extract was decanted, filtered and the filtrate evaporated in a water bath at 70C to obtain a molten extract of constant weight. The stock extract was kept at a cool temperature throughout the study.

GC-MS chemical characterization

The leaf ethanolic extract of *A. muricata* was analyzed quantitatively for phytochemical content via chemical characterization using Gas Chromatography Mass spectrometry (GC-MS). 2ul of the Sample Extract was injected into the GC column for analysis. The GC (Agilent 7890N) and MS (5975B MSD) is equipped with DB-5ms capillary column (30 m×0.25 mm; film thickness 0.25 µm). The initial temperature was set at 40°C which increased to 150°C at the rate of 10°C/min. The temperature again increased to 230°C at the rate of 5°C/min. The process continued till the temperature reached 280°C at the rate of 20°C/min which was held for 8 minutes. The injector port temperature remained constant at 280°C and detector temperature was 250°C then. Helium was used as the carrier gas with a flow rate of 1 mL/min. Split ratio and ionization voltage were 110:1 and 70 eV respectively. To identify the unknown components, present in the extract, their individual mass spectral peak value was compared with the database of National Institute of Science and Technology 2014.

Animals

Male Wistar rats (30; 6 weeks; avg. 90 g) were used for the study. The animals were kept in metal cages in a well–ventilated room of temperature 24 ± 2°C and relative humidity of 55–65% with a diurnal 12 h light cycle. The rats had access to water, pelletized standard mesh ad libitum and a two-week acclimatization period. All rats received humane care in accordance with animal handling guidelines. Ethical approval (NAU/AREC/2024/0020) was obtained from the Animal Research Ethics Committee, Nnamdi Azikiwe University Awka, Nigeria.

Experimental design

The animals were divided into six groups (n=5). Group 1 - normal control (NC), Group 2 – testosterone-induced negative control (TC), Group 3 - Finasteride standard drug treatment control (FT), Group 4 – *A. muricata* low dose AML (100 mg/kg *b.w*), Group 5 - *A. muricata* medium dose AMM (200 mg/kg *b.w*) and

Group 6 - *A. muricata* high dose AMH (400 mg/kg *b.w*).

BPH induction and treatments

Group 1 received sub-cutaneous 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 described with slight modification[18]. The animals were treated with Finasteride orally (0.5mg/kg b.w) (Group 3) for 14 days[19] as well as the *A. muricata* leaf ethanolic extract doses.

Blood Sample collection

At the end of each category experimental period, the rats were fasted for 12 hours and then sacrificed. Using the method of cardiac puncture, fresh unclotted blood samples were collected in both EDTA bottles for whole blood analysis and to plain bottles which were centrifuged at 4000 rpm for 15mins to obtain clear sera.

Determination of haematological parameters

Haematological analysis of the blood samples was performed in an automated haematology analyzer (BC-2300 model, Mindray Medical Co., China) with the procedure carried out as specified by the producer. The parameters which were evaluated included red blood cell count (RBC), total white blood cell count (TWBC), platelets (PLT), packed cell volume (PCV %) and haemoglobin (Hb) were obtained at once for each blood sample.

Determination of biochemical changes

Clear sera obtained following centrifugation were used for evaluating biochemical changes including liver function, lipid profile and renal function. Spectrophotometric methods using commercial test kits (Randox Laboratories Ltd, UK) were used as described[20]. Liver function parameters include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, total bilirubin, albumin, and globulin. Renal function parameters include serum creatinine, urea, electrolytes, bicarbonate, and uric acid. Electrolyte levels studied included serum sodium, potassium, and chloride. Total cholesterol, triacylglycerol (TAGs), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were assayed as lipid profile markers. (Catalogue numbers: Randox AL146, AS101, AP3803,TP245, BR8377, AB8000, CR2337, UR8070, NA 8327, PT3852, UR3824, CH3810, CH2655 and CH2656).

Statistical analysis

Data were expressed as Mean ± SD. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 19. One-way analysis of variance ANOVA were adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). Values at $p<0.05$ were considered significant for all the results obtained.

RESULTS AND DISCUSSION

Results

GC-MS evaluation of *A. muricata* leaf ethanol extract

At varied retention times (RT 4.128-29.767), the GC-MS result showed twenty-eight (28) peaks as shown in Fig.1, identifying a total of twenty-five (25) compounds. Predominantly revealed compounds with large peak areas include n-Hexadecanoic acid (32.60%), Oleic Acid (24.35%) and γ-Sitosterol (9.10%) at RT 17.48, 19.46 and 29.77 respectively **(Fig. 2)**.

Haematological results of testosterone-induced rats treated with A. muricata leaf ethanol extract

As shown in **Fig. 3**, significantly elevated ($p<0.05$) values of RBC, TWBC, platelet count, PCV and Hb were observed in the TC compared to the normal control. The *A. muricata* treatment groups showed no significant difference ($p>0.05$) from the TC in RBC levels. However, the FT and the *A. muricata* treatment groups showed significant reductions ($p<0.05$) in TWBC. The AMH exhibited the most significant reduction ($p<0.05$) in platelet count. The FT, AML and AMH showed no significant variation ($p>0.05$) with the normal control in PCV (%) levels.

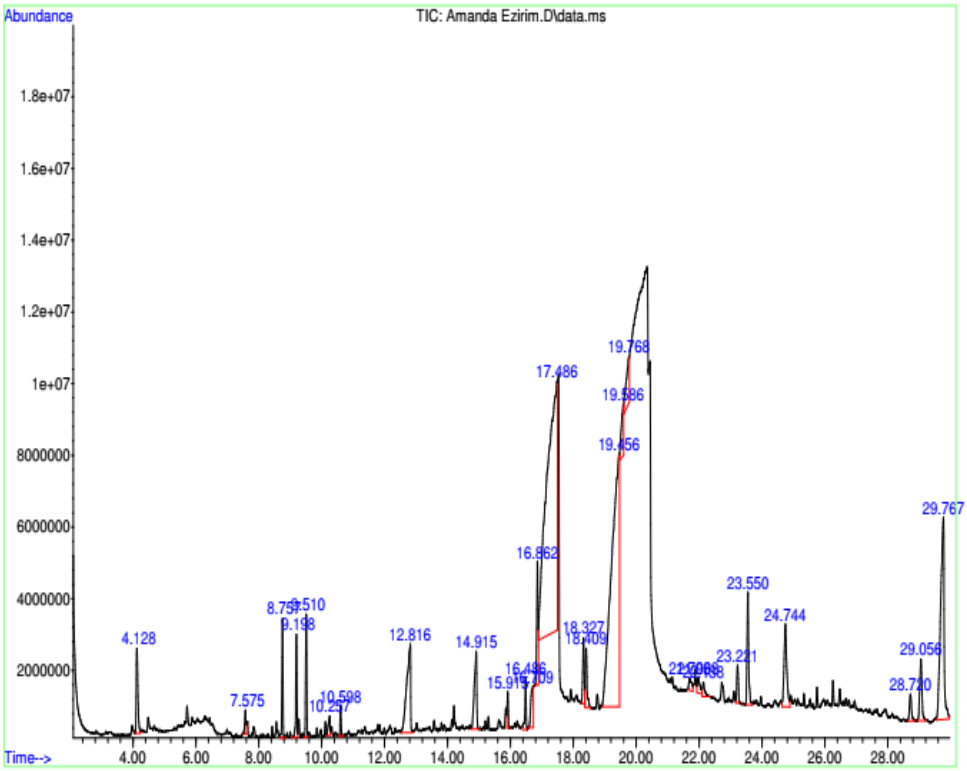

Fig. 1: Chemical characterization chromatogram of *A. muricata* via GC-MS**.**

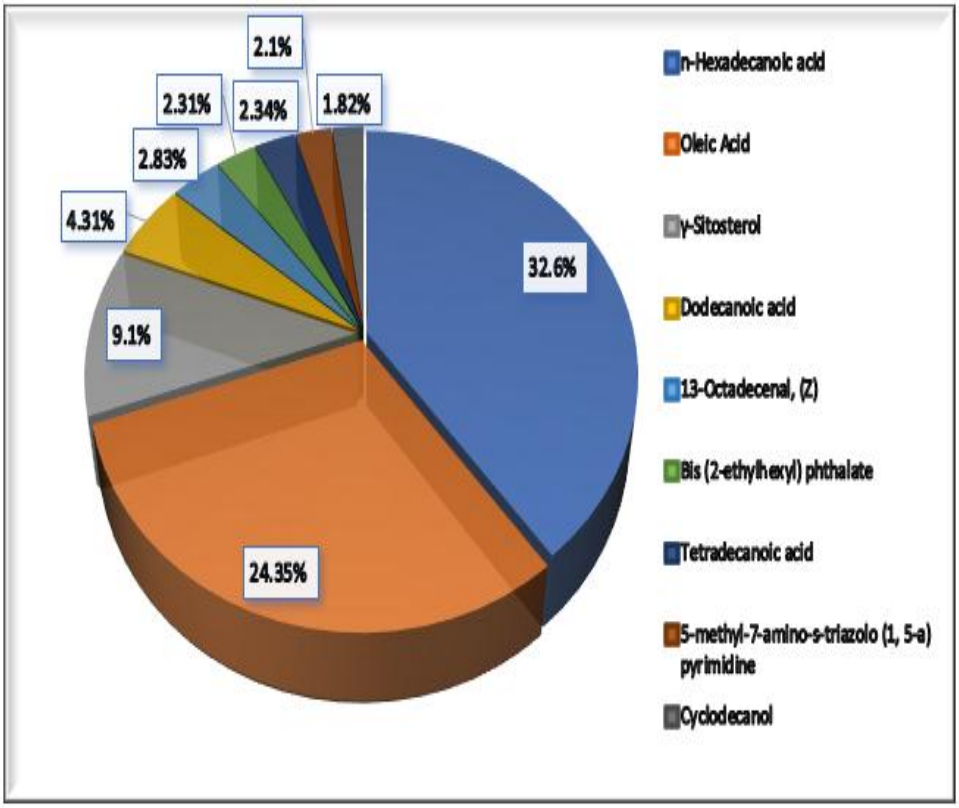

Fig. 2: Percentage chemical composition of major phytochemicals generated from *A. muricata* leaf ethanol extract by GC-MS.

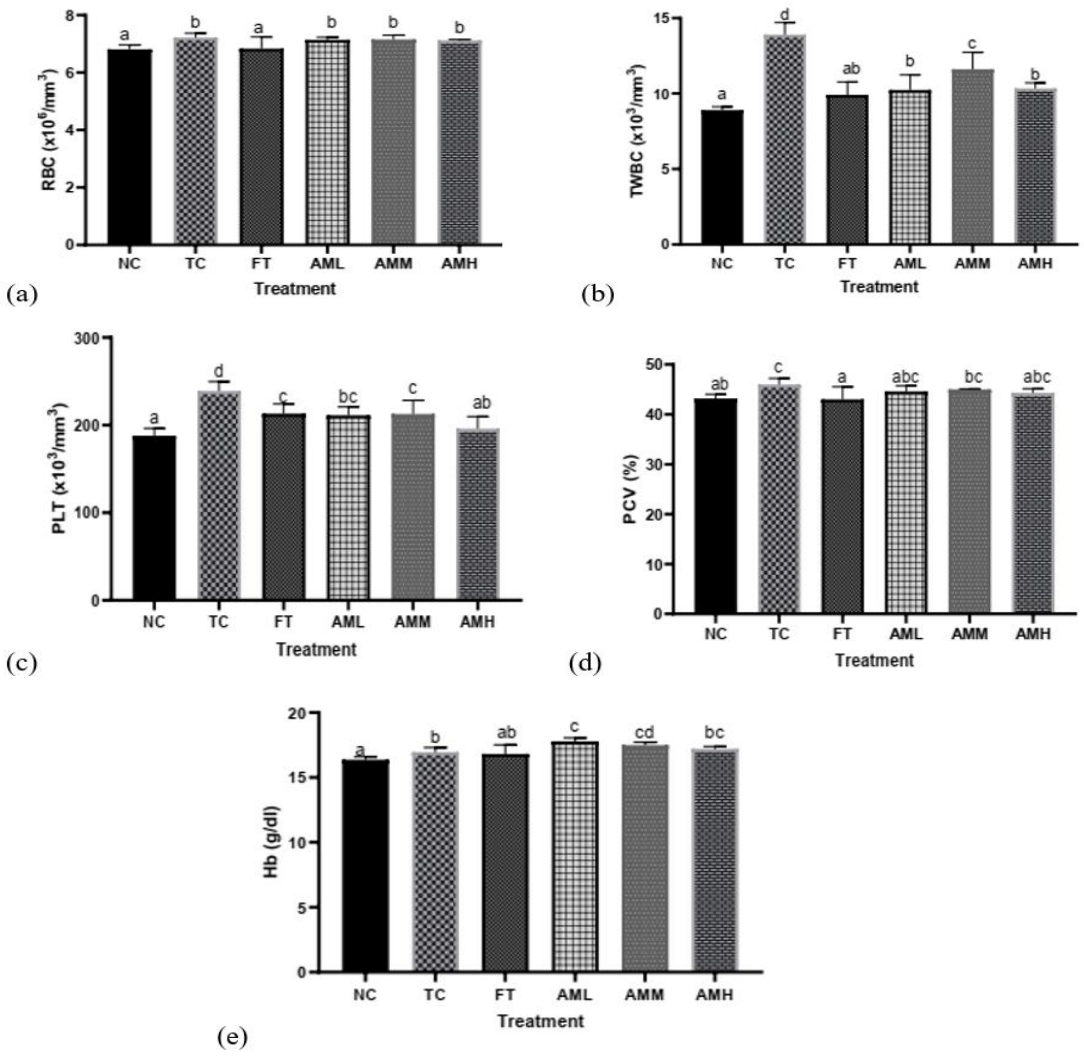

Fig. 3: Haematological analysis results of testosterone-induced rats treated with *A. muricata*. Haematological indices were investigated for (a) RBC (b) TWBC (c) PLT (d) PCV% and (e) Hb. Data are presented as the mean ± SD (n = 5). The Duncan one-way analysis of variance test was used to determine significant differences between the means of the individual groups. Values with same letters indicate no significant difference ($p>0.05$). Values with different letters indicate a significant difference ($p<0.05$). NC, Normal control; TC, testosterone-induced negative control; FT, Finasteride control; AML, *A. muricata* low dose (100 mg/kg b.w); AMM, *A. muricata* medium dose (200 mg/kg b.w); AMH *A. muricata* high dose (400 mg/kg b.w).

Liver function results of testosterone-induced rats treated with A. muricata leaf ethanol extract

As shown in **Fig. 4**, the AMM and AMH normalized values in the impaired serum AST levels seen in the TC. However, both groups showed significant increase ($p<0.05$) in serum ALT concentration and no significant variation ($p>0.05$) in serum ALP concentrations to the normal control. A decline in serum globulin levels and total protein levels observed in the treatment groups was significant as the TC showed no significant change ($p>0.05$) to the normal control. A markedly significant decrease ($p<0.05$) in serum albumin was observed in the FT group. However, the *A. muricata* treatment groups exhibited a significant elevation ($p<0.05$) in serum albumin levels compared to the FT yet were still significantly low ($p<0.05$) compared to the normal control. The AML normalized serum total bilirubin levels that were significantly decreased ($p<0.05$) in the TC group.

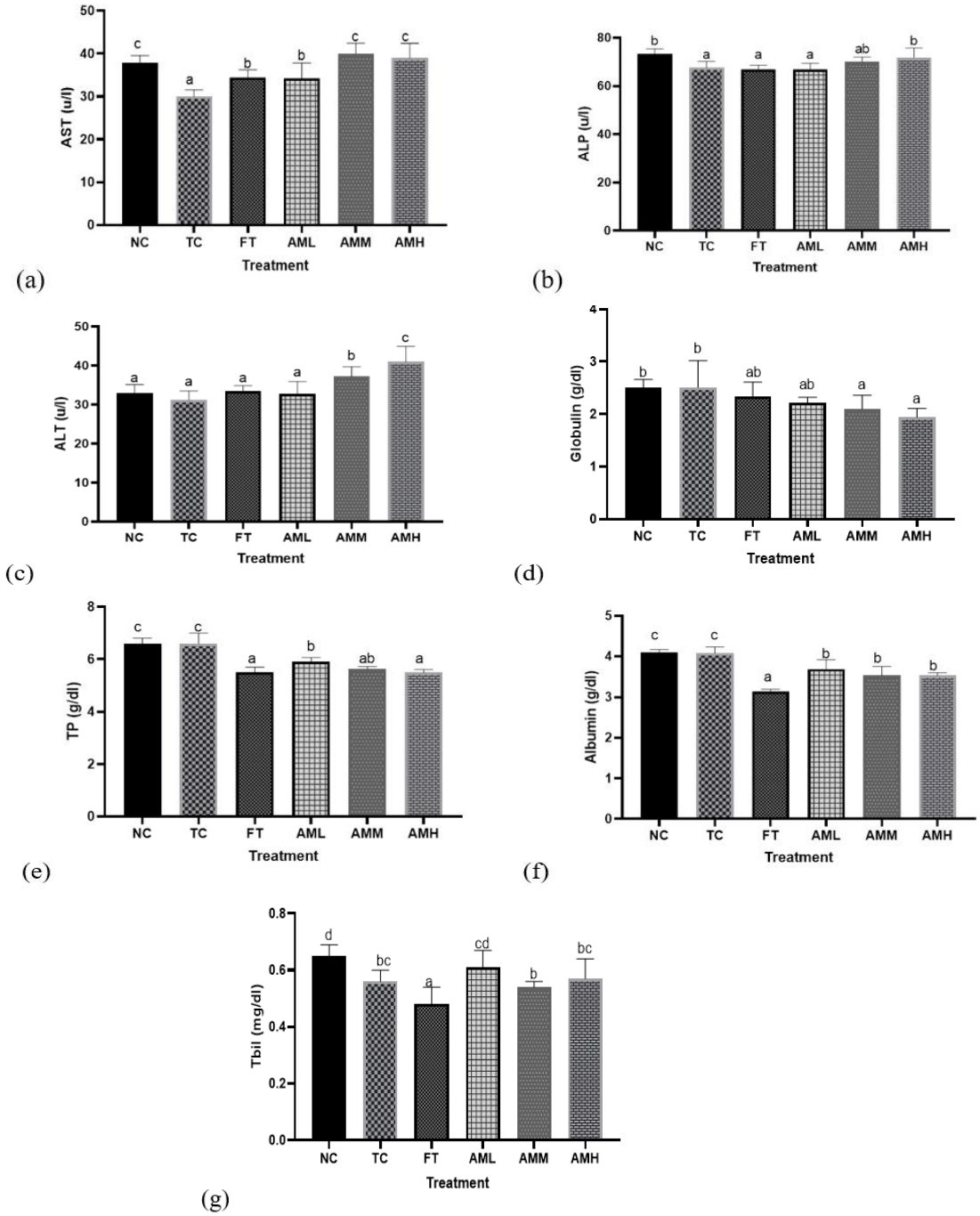

Fig. 4: Liver function results of testosterone-induced rats treated with *A. muricata* leaf ethanol extract. Liver function analysis was assessed for serum (a) AST (b) ALT (c) ALP (d) Globulin (e) Total protein (f) Albumin and (g) Total bilirubin. Data are presented as the mean ± SD (n = 5). The Duncan one-way analysis of variance test was used to determine significant differences between the means of the individual groups. Values with same letters indicate no significant difference ($p>0.05$). Values with different letters indicate a significant difference ($p<0.05$). NC, Normal control; TC, testosterone-induced negative control; FT, Finasteride control; AML, *A. muricata* low dose (100 mg/kg b.w); AMM, *A. muricata* medium dose (200 mg/kg b.w); AMH *A. muricata* high dose (400 mg/kg b.w).

Lipid profile results of testosterone-induced rats treated with *A. muricata* leaf ethanol extract

As shown in **Fig. 5**, serum total cholesterol, TAGs, and LDL-c were significantly elevated ($p<0.05$) in the TC. The Finasteride control, AMM and AMH exhibited significant decrease in serum total cholesterol. The increased values of HDL-c from the normal control in the *A. muricata* treatment groups and the dose-dependent reduction in LDL-c were significant ($p<0.05$). Serum VLDL-c levels were significantly elevated ($p<0.05$) in the experimental groups compared to the normal control, with the AML showing significantly higher ($p<0.05$) value.

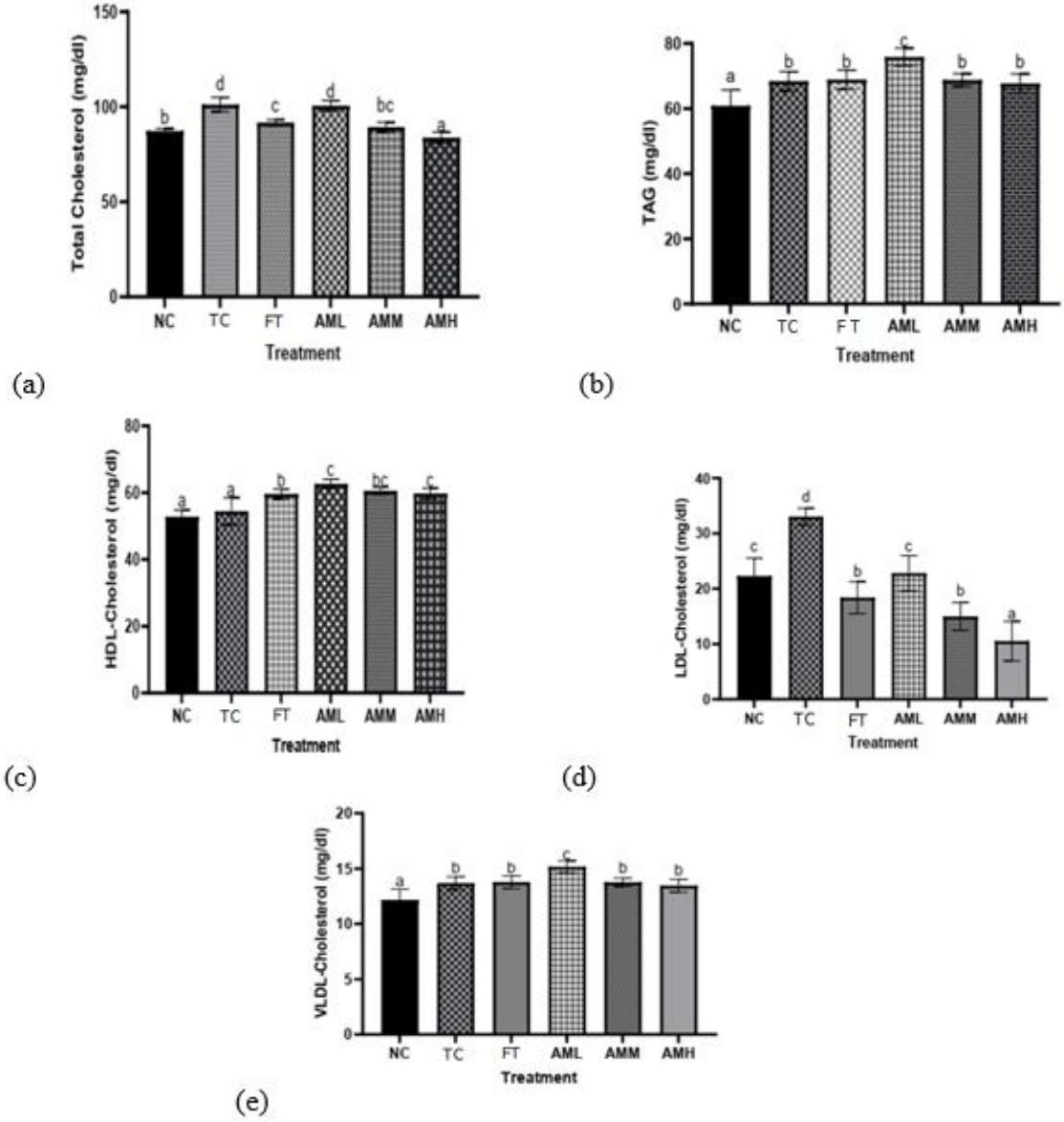

Fig. 5: Lipid profile results of testosterone-induced rats treated with *A. muricata* leaf ethanol extract. Lipid profile was evaluated for serum (a) Total cholesterol (b) TAG (c) HDL-c (d) LDL-c (e) VLDL-c. Data are presented as the mean ± SD (n = 5). The Duncan one-way analysis of variance test was used to determine significant differences between the means of the individual groups. Values with same letters indicate no significant difference ($p>0.05$). Values with different letters indicate a significant difference ($p<0.05$). NC, Normal control; TC, testosterone-induced negative control; FT, Finasteride control; AML, *A. muricata* low dose (100 mg/kg b.w); AMM, *A. muricata* medium dose (200 mg/kg b.w); AMH *A. muricata* high dose (400 mg/kg b.w).

Renal function results of testosterone-induced rats treated with *A. muricata* leaf ethanol extract

As shown in **Fig. 6,** serum creatinine, sodium and potassium were significantly elevated in the TC compared to the normal control. However, the treatment groups, including FT, AML, AMM and AMH significantly normalized ($p<0.05$) these values. A marked decrease ($p<0.05$) in serum urea was observed in the FT, compared to the TC and normal control. However, the AMM and AMH showed significant increase ($p<0.05$) compared to the FT. Bicarbonate levels were unchanged in all the experimental groups showing no significant difference ($p>0.05$) to the normal control. The AMH showed the most significant reduction in serum uric acid concentrations elevated in the TC.

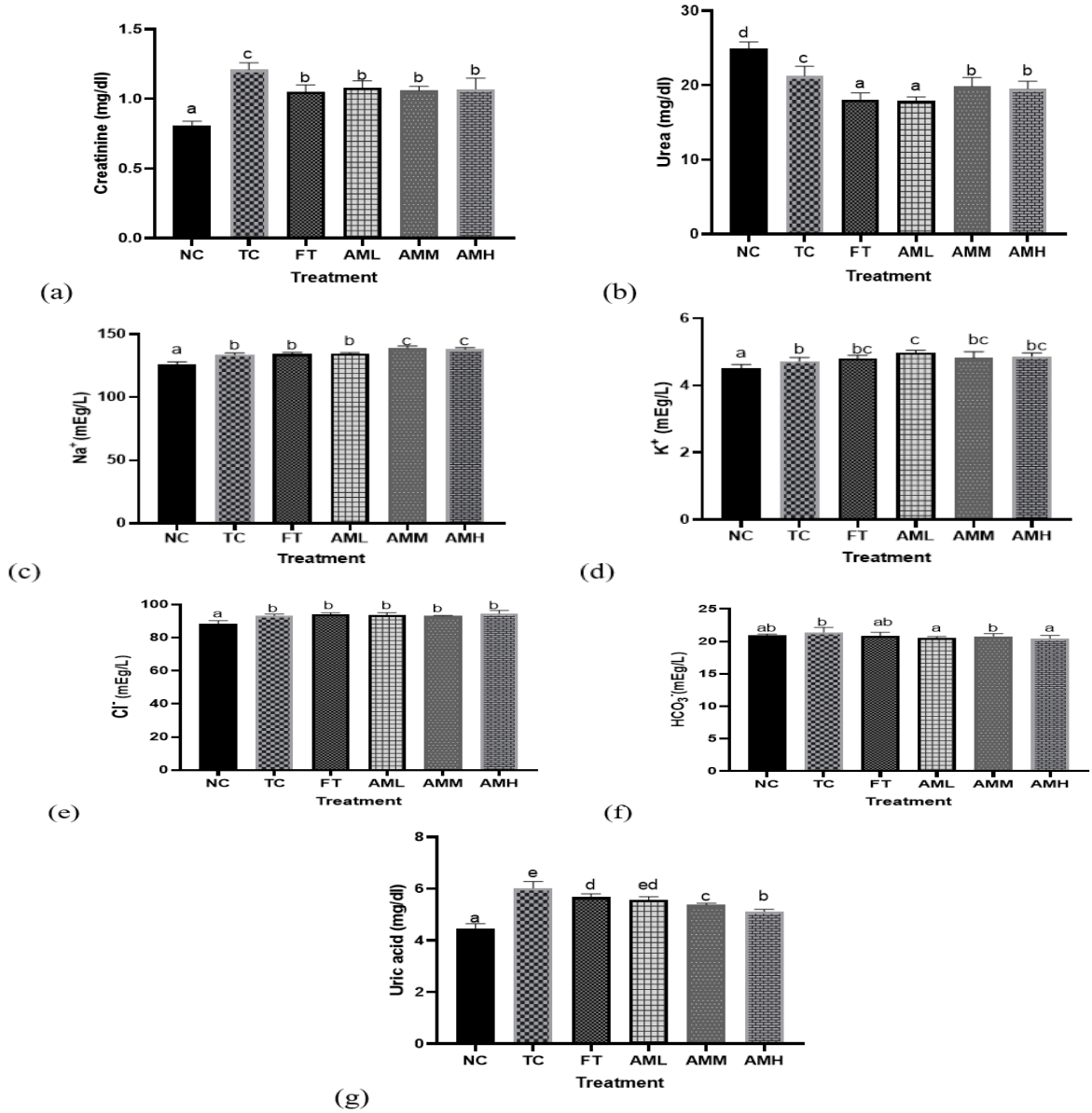

Fig. 6: Renal function results of testosterone-induced rats treated with *A. muricata* leaf ethanol extract. Renal function analysis was assessed for serum (a) Creatinine (b) Urea (c) Sodium (d) Potassium (e) Chloride (f) Bicarbonate and (g) Uric acid. Data are presented as the mean ± SD (n = 5). The Duncan one-way analysis of variance test was used to determine significant differences between the means of the individual groups. Values with same letters indicate no significant difference ($p>0.05$). Values with different letters indicate a significant difference ($p<0.05$). NC, Normal control; TC, testosterone-induced negative control; FT, Finasteride control; AML, *A. muricata* low dose (100 mg/kg b.w); AMM, *A. muricata* medium dose (200 mg/kg b.w); AMH *A. muricata* high dose (400 mg/kg b.w).

Discussion

The reliance on herbal alternatives has persistently attracted interests because they are accepted, relatively cheap, accessible, and with minimal side effects when compared to modern medicine[21,22]. Despite the demands for scientific proof and justification of the use of herbs for medicinal purposes, Annona muricata leaf extracts have demonstrated antioxidant, antibacterial, anti-protozoan, anti-cancer, anti-inflammatory and antitumor properties in different scientific interventions [23,24,25,26,27].

The GC-MS chemical characterization of the *A. muricata* leaf ethanol extract revealed the presence of different volatile compounds including fatty acids, heterocyclic compounds, and esters among others **(Fig. 1)**. n-Hexadecanoic acid, oleic Acid and γ-sitosterol were the major compounds observed. Others include dodecanoic acid, 13-octadecenal (Z), bis (2-ethylhexyl) phthalate, tetradecanoic acid, 5-methyl-7-amino-s-triazolo (1, 5-a) pyrimidine and cyclodecanol **(Fig. 2)**. n-Hexadecanoic acid commonly known as palmitic acid possess antioxidant, anti-inflammatory and hypocholesterolemic features[28]. According to some studies, oleic acid, a monounsaturated fatty acid induces a low incidence of inflammatory diseases via the modulatory mechanisms on the immune system[29]. γ – sitosterol, a phytosterol present in many plants, exhibits strong anti-diabetic, anticancer, anti-inflammatory, antifungal, antibacterial and anti-angiogenic activity[30].

Haematological parameters are important in the diagnosis and monitoring of diseases[31]. The RBC increase in the untreated control **(Fig. 3)** could be attributed to findings which stated that an expected but unreported side effect of exogenous testosterone is erythrocytosis[32]. It can be deduced that the given time frame of therapy possibly limited the amelioration of the erythrocytic condition by the *A. muricata* treatment groups unlike the finasteride control which showed no significant variation ($p>0.05$) with the normal control. However, the surge in total white blood cell (TWBC) counts in the untreated control was significantly attenuated by the *A. muricata* extract which corroborates with another study[33]. TWBC and the degree of systemic inflammation in the pathogenesis of BPH appear to be correlated[34].

Elevated platelet levels were observed in the untreated control. Previous studies have implicated exogenous testosterone as a regulator in the expression of human platelet TXA_2 receptors which are key factors in vasoconstriction and platelet aggregation that could influence cardiovascular disease emergence[35]. In the present study, the AMH exhibited the capacity to stabilize the impaired platelet counts to the normal control. Compared to the AMH, the platelet level of the finasteride control was still significantly higher ($p<0.05$). The increased packed cell volume (PCV%) levels exhibited in the untreated group also gives credence to the prior observation of the markedly increased platelet levels. A plausible reason is evident in reports that following vascular damage, an elevated PCV encourages platelet accumulation[36]. The AML and the AMH showed no significant variation with the finasteride control. These outcomes suggest that *A. muricata* exerts a modulatory impact on testosterone-induced inflammatory effects in the blood via its antioxidant capacity.

Hepatocyte enzymes released into the blood could accurately represent the existence of liver injury[37]. The AMM and AMH reversed the decreased concentration of serum AST levels in the untreated control while the finasteride control was observed to be significantly lower ($p<0.05$) than both AMM and AMH **(Fig. 4)**. As AST plays important roles in gluconeogenesis, amino acid metabolism, synthesis of purines/pyrimidines, its low levels may be negligible, but it reflects increased cardiovascular risk associated with advanced chronic renal or liver disorders, inflammation, and vitamin B6 deficiency[38]. Since ALT, an important enzyme in gluconeogenesis, is largely found in the liver cell cytosol, it is thought to be a more sensitive marker of liver inflammation or injury than AST and, within certain bounds, can offer a quantitative evaluation of the extent of liver damage[39]. The AMM and AMH showed slight, yet significantly increased ($p<0.05$) ALT levels to the normal control, whereas the finasteride control showed no significant deviation from normal control values. However, both AMM and AMH showed no variation to the normal control in serum ALP concentration indicating that no biliary tract lining injury had occurred.

This study reports a marked decline in serum albumin and total bilirubin in the finasteride control. Studies suggest that a low serum albumin level in the presence of normal liver function may indicate either inadequate protein intake (malnutrition) or protein loss (nephrotic syndrome, malabsorption, or protein-losing enteropathy)[40].

The *A. muricata* treatment groups showed marked significant increment ($p<0.05$) of the reduced serum albumin, yet they were significantly lower ($p<0.05$) than the normal control. Both outcomes on serum globulin and albumin were reflected in reduced total protein levels of the treatment groups. Current hepatological results in this study demonstrate that the leaf ethanol extract treatment with *A. muricata* exhibited no deleterious effect on the liver neither did it provide significant hepatoprotective evidence in testosterone induced BPH.

Pathologic studies have linked inflammation and the risk for cardiovascular disease as atherosclerotic lesions have infiltrates associated with inflammation[41]. The observed increase in serum total cholesterol, TAG, LDL-c and VLDL-c corroborate with reports of the hyperlipidemic effects of exogenous testosterone which aggravate the progression of BPH[42]. However, the *A. muricata* treatment groups displayed a significant reduction in serum total cholesterol and LDL concentrations in a dose-dependent manner, with the AMM showing no significant variation with the finasteride group **(Fig. 5)**. This could be owing to the the anti-hyperlipidemic capacity of *A. muricata* bioactive components by mechanism of plasma fatty acid synthase activity modulation.

As seen in this study, the increased levels of HDL-c substantiate findings which demonstrated the hypolipidemic and anti-inflammatory properties of *A. muricata*[43,44,45]. Though the finasteride group exhibited a significant reversal ($p<0.05$) of diminished HDL levels, it was the least observed compared to AML and AMH groups. The consistent imbalance of high plasma LDL to low HDL concentrations has been associated with metabolic syndrome, which increases chances of prostate disorders in men[46]. Furthermore, the preference of fatty acids to glucose uptake as an energy source is a predominant feature of prostate cells[47]. Therefore, this study suggests that *A. muricata* possessed anti-hyperlipidemic function which could curtail metabolic associated risks of BPH.

Studies have established the substantial association between BPH, presenting symptoms of urinary retention, and renal dysfunction[48]. In the present study, serum creatinine levels were significantly increased in the untreated control, but the *A. muricata* treatment groups reduced this effect without any variation to the finasteride group **(Fig.** 6). It is suggested that the antioxidant mechanisms of the bioactive components found in *A. muricata* leaf ethanol extract could be responsible. This study reports a markedly low level of serum urea in the testosterone-induced control. However, the AMM and AMH exhibited significant improvement ($p<0.05$), much more than the finasteride group. According to research, reduced serum urea concentrations are present in conditions of malnutrition, opioid use, starvation, surgery, severe liver disease and anabolic steroid utilization[49].

In addition, the accumulation of testosterone propionate presents a high risk of peripheral oedema which is associated with the retention of serum electrolytes, manifested by weight gain that is more prominent in earlier time of use[50]. This was reflected in the untreated group showing significantly elevated levels of sodium, potassium and chloride compared with the normal control. Findings from this study indicate that within the two-week treatment, all the treatment groups could not attenuate the impact of the BPH inducer on the serum electrolyte retention state. However, a non-alteration of the acid-base balance function of the kidneys was maintained as shown in the bicarbonate levels **(Fig. 6)**.

Recent reports suggest an associated link between increased uric acid levels and the occurrence of BPH[51,52,53]. The finasteride group and the AML showed the least effect in reducing the impact of the BPH inducer on serum uric levels. The AMH group most evidently reversed the significantly increased levels of uric acid observed in the untreated control which correlates with another finding[54]. Thus, it is touted that bioactive compounds with promising multiple therapeutic properties: anti-inflammatory, antioxidant and

antiproliferative, could reduce serum uric acid levels through the inhibition of xanthine oxidase activity and the control of the IL-6/JAK1/STAT3 inflammatory signalling cascade[55].

Conclusion

The *A. muricata* leaf ethanol extract sustained its ability to reverse leukocytosis, platelet aggregation, hyperlipidemia and hyperuricemia, which were the negative impacts of testosterone-induced BPH. The findings from this study further validates the potentials of *A. muricata* to avert associated cardiovascular diseases arising from inflammatory processes. This study therefore suggests that the multiple therapeutic properties of *A. muricata* - anti-inflammatory, antioxidant and antiproliferative - could serve as a multifactorial treatment approach against BPH.

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استخلاص أوراق أنونا موريكاتا يحسن الاضطرابات في الدم والكيمياء الحيوية في نموذج ذكور جرذان ويستار المصابين بتضخم البروستاتا الحميد الناتج عن التستوستيرون

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هدفت الدراسة إلى استكشاف تأثيرات مستخلص أوراق أنونا موريكاتا الإيثانولي على المؤشرات في الدم والكيمياء الحيوية في نموذج ذكور جرذان ويستار المصابين بتضخم البروستاتا الحميد (BPH) الناتج عن التستوستيرون. تم تقسيم ثلاثين من ذكور جرذان ويستار (بمتوسط وزن ٩٠ جرام) إلى ست مجموعات (ن=٥): مجموعة التحكم الطبيعية ((NC، مجموعة التحكم السلبية المحفزة بالتستوستيرون ((TC، مجموعة التحكم في العلاج بالفيناستراید (FT؛ ٠.٥ ملج/كج وزن جسم(، ومجموعات العلاج بمستخلص أنونا موريكاتا بجرعات منخفضة (١٠٠ ملج/كج)، متوسطة (٢٠٠ ملج/كج)، وعالية (٤٠٠ ملج/كج) وزن جسم، والتي تم التسمية بها كـ AML، AMM، وAMH على التوالي. تم تحفيز تضخم البروستاتا بحقن التستوستيرون بروبيونات تحت الجلد لمدة ٢٨ يوماً متتالياً. تلتها علاجات فموية بالفيناستراید وجرعات المستخلص لمدة ١٤ يوماً. أظهرت النتائج أن n-hexadecanoic كان الأكثر وفرة (٣٢.٦٠%) باستخدام جهاز التحليل الطيفي بالكروماتوغرافيا الغازية-الكتلة. لوحظت زيادة عدد الكرات البيضاء، تكتل الصفائح الدموية، ارتفاع نسبة الدهون في الدم، وارتفاع مستوى حمض اليوريك بشكل ملحوظ في مجموعة التحكم السلبية المحفزة بالتستوستيرون. وقد عملت جرعات المستخلص على تعديل هذه الحالات بشكل ملحوظ (.($p<0.05$تقترح هذه الدراسة أن أنونا موريكاتا يمكن أن تحسن التغيرات في الدم والكيمياء الحيوية المرتبطة بتضخم البروستاتا الحميد.