

Liver Protective Effects of *Annona Muricata* Leaf Extract in Male Wistar Rats

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ABSTRACT

Finding out how *Annona Muricata* leaf extract protects the liver in wistar rats is the goal of this study. A total of 78 adult male rats obtained from the laboratory animal production unit of the Department of Physiology, Abia State University, Uturu were used for the different segments of the study. Eighteen of the rats were used for acute toxicity/lethal dose evaluation of the extract while the remaining sixty (60) were used for the testosterone-induced hyperplasia study. Prostate hyperplasia was induced in the rats via daily sub-cutaneous administration of testosterone propionate (5 mg/kg body weight) for 28 days. Sixty rats used for this segment of the study were assigned to 6 groups of 10 rats and treated as follows: Group 1, Normal control, no treatment. Group 2 Testosterone propionate (5 mg/kg) only and was the BPH control. Group 3, Testosterone propionate (5 mg/kg bw) + Finasteride (3 mg/kg body weight). Group 4, Testosterone propionate (5 mg/kg bw) + *Annona Muricata* Leaf extract (300 mg/kg body weight). Group 5, Testosterone propionate (5 mg/kg bw) + *Annona Muricata* Leaf extract (600 mg/kg body weight). Group 6, *Annona Muricata* Leaf extract (600 mg/kg bw) only. At the end of treatment, animals were sacrificed and blood samples were collected into EDTA and plain bottles for liver function parameters were determination using the respective commercial test kits. Data obtained were subjected to Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented with Student's *t* test for post-hoc test for comparisons of the means of the various doses and fractions. *P*- values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value. Conclusively, liver function parameters assessed in this study highlight significant deviations induced by testosterone and the subsequent therapeutic effects of Finasteride and *Annona muricata* (AML) extract treatments. Elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alongside reduced levels of total protein, albumin, and globulin in the BPH control group, indicate liver dysfunction and impaired synthetic function

Keywords: Testosterone-induced hyperplasia, *annona muricata*, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, and globulin and wistar rats

INTRODUCTION

The liver plays a central role in metabolism, detoxification, and nutrient storage, making its proper functioning essential for overall health. Altered liver function, indicated by abnormalities in liver enzymes and biochemical markers, can signify hepatocellular injury and impaired metabolic homeostasis.[2,3] *Annona muricata*, a plant with a rich history of traditional medicine, has been extensively studied for its potential therapeutic effects on various organ functions, particularly liver function parameters. The plant's bioactive compounds, such as alkaloids, acetogenins, flavonoids, and terpenes, have been found to exhibit significant hepatoprotective properties.[5] Studies exploring the impact of *Annona muricata* on liver function parameters have yielded promising results. According to a study by Ajayi *et al.* (2024),[1] administration of *annona muricata* extract to animal models resulted in significant improvements in liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). These findings suggest that *Annona muricata* may exert hepatoprotective effects, preserving liver function and integrity. The hepatoprotective activity of *A. muricata* leaf extract was also evaluated in rats with thioacetamide-induced liver injury. The results showed that the extract significantly reduced liver damage and improved liver function parameters, including AST, ALT, and ALP levels [4].

In rats with carbon tetrachloride-induced liver injury, studies have demonstrated that the aqueous extract of *A. muricata* leaf dramatically improved liver function toward normal levels, equivalent to the silymarin control.[5] Furthermore, the extract was shown to dramatically lower indicators of liver damage, such as lysosomal membrane fragility, impaired

synthesis ability, and elevated liver enzymes like aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase.[6]

The plant's ability to inhibit the activity of enzymes involved in lipid metabolism and its antioxidant properties can also contribute to its hepatoprotective effects. Furthermore, the extract has been found to have a potential protective effect on liver function parameters, including serum levels of liver enzymes, in acute and sub-chronic toxicity studies. [7] The hepatoprotective properties of *Annona muricata* are attributed to its rich phytochemical composition, which includes flavonoids, alkaloids, and acetogenins. These bioactive compounds possess antioxidant, anti-inflammatory, and cytoprotective properties, which may help mitigate hepatocellular damage and promote liver regeneration.[6,8] *Annona muricata* may modulate key signaling pathways involved in liver homeostasis and regeneration. Studies have reported that *annona muricata* extracts can activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, leading to increased expression of antioxidant and detoxifying enzymes, such as heme oxygenase-1 (HO-1) and glutathione S-transferase (GST), which protect against oxidative stress-induced liver injury.[10]

MATERIALS AND METHODS

Materials

Equipments used include sensitive weighing balance, Electric weighing balance, Manual blender, Dissecting set, Dissecting board, 5ml syringes, 1ml syringes, test tubes, serum bottles, Sample bottles, EDTA bottles, whatman No 1 filter paper, Measuring cylinders, Pasteur pipette, Cotton wool, Micro pipette, Rat cages, Feeding and water trough, Oral gavage, Glass column, Microscope, Glass TLC plates, microscope slides, Glass wool, funnel, Spatula, Retort stand, Hand

gloves, TLC tank, beaker, Oven, freezer, Refrigerator, Spectrophotometer(722N, Mindray, MC Quijing, China), Centrifuge, Rotary evaporator, Soxhlet extractor, GC-MS machine, Chemoilluminoscene machine and Haematology analyser (BC-2300, Mindray, MC Quijing, China).

ETHICAL APPROVAL

This study was approved by the research and ethical committee of the Department of Physiology, Abia State University, Uturu.

Animals

A total of 78 adult male rats obtained from the laboratory animal production unit of the Department of Biochemistry, Abia State University, Uturu were used for the different segments of the study. Eighteen of the rats were used for acute toxicity/lethal dose evaluation of the extract while the remaining sixty(60) were used for the testosterone-induced hyperplasia study. The animals were housed in aluminum cages, under standard conditions 12/12h light-dark cycle at temperature of $25 \pm 2^\circ\text{C}$ and were fed with standard grower pellets (Grand Cereals Ltd, Abia State) and had access to clean drinking water for seven days of acclimatization before the initiation of experiment. All experiments were carried out in accordance with International Guideline for Biomedical Research (CIOMS/OMS, 1985) and as approved by the Department of Physiology, Abia State University, Uturu.

Experimental Design for the Hyperplasia Study

Rats were given testosterone propionate (5 mg/kg body weight) subcutaneously every day for 28 days in order to cause prostate hyperplasia. The sixty rats utilized in this study section were divided into six groups of ten and given the following treatment:

Group 1 Normal control, no treatment

Group 2 Testosterone propionate (5 mg/kg) only and was the BPH control

Group 3 Testosterone propionate (5 mg/kg bw) + Finasteride (3 mg/kg bw)

Group 4 Testosterone propionate (5 mg/kg bw) + AML extract (300 mg/kg bw)

Group 5 Testosterone propionate (5 mg/kg bw) + AML extract (600 mg/kg bw)

Group 6 AML extract (600 mg/kg bw) only

At the end of treatment, animals were sacrificed, and blood samples were collected into EDTA and plain bottles for liver function parameters were determined using the respective commercial test kits analysis respectively. For the later (blood contained in plain bottles), the blood was allowed to clot and retract within two hours and then centrifuged at 3000 rpm for 10 minutes to obtain clear sera samples which were used for liver function parameters determination using the respective commercial test kits.

Preparation of Plant Extracts

Plant extracts were prepared in accordance with the method used by Orieke *et al.*, (2019). The freshly collected leaves of *Anona muricata* were air dried under shade for within 14 days and were thereafter pulverized into coarse powdered form using a locally fabricated grinding machine. Two solvents, ethanol and N-hexene were used for the preparation of the extracts. A known quantity (300g) of the powdered plant material was macerated in 1.5 litres of ethanol (AR) for 48 hours, been shaken violently every two hours during that time. The filtrate, which was the extract in solution, was then obtained by filtering it through Whatman No. 1 filter paper.

The filtrate so obtained was concentrated at low temperature to obtain a solid dark-green extract which weighed 13.91 g and

represented 4.64% extract yield. The extract was preserved in the refrigerator at -4°C until needed and is hereafter referred to as *Anona muricata* leaf (AML) extract.

Determination of Liver Function Parameters

All liver function parameters were determined using the respective commercial test kits and following protocols for the parameters as was reported by Ugboogu *et al.*, (2017).

Alanine Aminotransferase (ALT)

Kit: RANDOX

Reagent: R1 (A combination of 100 mmol/L of Phosphate buffer at P^{H} 7.4, 200 mmol/L of L-alanine and 2 mmol/L of α -oxoglutarate). R2 (2,4-dinitrophenylhydrazine) and 5% NaOH solution.

Principle: L-glutamate and pyruvate hydrazone are produced when L-alanine and α -oxoglutarate mix. Thus, the concentration of pyruvate hydrazone produced with 2,4-dinitrophenylhydrazine is monitored in order to determine ALT.

Procedure: Two test tubes with the labels "Blank" and "test" were arranged in a test tube rack. The test tube was filled with 0.1 ml of the sample (test serum), and the blank test tube was pipetted with 0.1 ml of distilled water. After that, 0.5 ml of ALT reagent R1 was pipetted into each tube and combined. The mixture was incubated for 30 minutes at 37°C before the addition of 0.5ml of reagent R₂ to each of the tubes and also incubated at $20\text{--}25^{\circ}\text{C}$ for 20 minutes. 5ml of Sodium Hydroxide was then added to each tube and allowed to stand for 5 minutes before absorbance was read against the reagent blank on a spectrophotometer (722 N, CHINA) at 546nm. ALT activity in the serum was then obtained by tracing the equivalent absorbance of the sample on the ALT absorbance chart and finding its corresponding ALT activity value in U/L.

Aspartate Aminotransferase (AST)

Kit: RANDOX

Reagent: R1 (A combination of 100 mmol/L of Phosphate buffer at P^{H} 7.4, 100 mmol/L of L-aspartate and 2 mmol/L of α -oxoglutarate). R2 (2,4-dinitrophenylhydrazine) and 5% NaOH solution.

Principle: L-aspartate combines with α -oxoglutarate to form oxaloacetate. AST is therefore measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Procedure: 0.1 milliliters of distilled water were pipetted into a blank test tube. A second test tube labeled "test" was likewise pipetted with 0.1 ml of the test serum. AST reagent R₁ (0.5ml) was added to each test tube, mixed and incubated at 37°C for 30 minutes. After the incubation, 0.5ml of AST reagent R₂ was added to each test tube and allowed to stand for 20 minutes at $20\text{--}25^{\circ}\text{C}$ before 5.0ml of 0.4mol/ liter Sodium Hydroxide was added to each allowed to stand for 5 minutes before reading absorbance against the reagent blank on a spectrophotometer (722N, CHINA) at wavelength 546nm. AST activity in the serum was then obtained by finding an equivalent values of absorbance on the standard chart and obtaining its corresponding activity value in U/L.

Alkaline Phosphatase (ALP) activity

Kit: RANDOX

Reagents: R1a (A combination of 1 mol/L of Diethanolamine buffer at pH 9.8 and 0.5 mmol/L of MgCl_2). R1b (10 mmol/L of p-nitrophenylphosphate).

Principle: This test method is based on the idea that phenolphthalein monophosphate, a colorless substrate, is hydrolyzed by serum alkaline phosphate to produce phosphoric acid and phenolphthalein, which in an alkaline media change pink and may be detected photometrically.

Procedure: 0.5 milliliters of alkaline phosphatase substrate were added to each

of the three test tubes that were prepared and labeled test, control, and standard, respectively. The relevant test tubes were filled with 0.05 ml (50 µl) of the standard, control, and sample (test serum), while the control was distilled water. After 10 minutes of incubation at 37°C, 2.5 ml of

alkaline phosphate was added to the test tubes, and color developed at predetermined intervals. After zeroing with the reagent blank, the mixture was thoroughly mixed before the absorbance was measured at 590 nm using a spectrophotometer.

ALP value in IU/Liter was calculated using the formular

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of standard}} \times 50$$

Where 50 is the standard ALP value

Serum Bilirubin Content Determination

Kit: RANDOX

Reagents: R1 (0.17% hydrochloric acid and 29 mmol/L sulfuric acid). R2 (38.5 mmol/L of nitrite solution). R3 (0.52 mol/L of sodium benzoate and 0.26 mol/L of caffeine). R4 (1.9N NaOH and 0.93 mol/L of tartrate).

Principle: In an alkaline media, direct (conjugated) bilirubin and diazotized sulfuric acid combine to generate a blue-colored complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphanilic acid.

Procedure: The blank and sample test tubes were prepared and labeled. Only 200 ml of Reagent 1 and 1000 nl of Reagent R3 were introduced to the blank test tube, whereas 200 ml of Reagent 1, 50 nl of Reagent 2, and 1000 nl of Reagent 3 were added to the sample test tube and thoroughly mixed. The 2 test tubes were then be incubated at 20-25°C for 10 minutes, after which 100 µl of reagent 4 was added to both test tubes. The test tubes were incubated for a further 30 minutes at 25°C before reading absorbance on a spectrophotometer at 560nm after zeroing with blank. To obtain total bilirubin in mg/dl the formular below was used.

$$\text{Total Bilurubin} = 10.8 \times \text{Absorbance of Sample}$$

Serum Total Protein Content Determination

Kit: RANDOX

Reagent: R1(A mixture of 100mmol/L of NaOH, 16mmol/L, Na-K-tartrate, 15 mmol/Lof Potassium iodide and 6 mmol/L of CuSO4).

Principle: Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a purple colored complex. The amount of protein in the sample is directly correlated with the color's intensity.

Procedure: The test, blank, and standard test tubes were arranged. Each test tube

was filled with 1.0 ml of the total protein reagent. Twenty microliters of the test sample were taken and put into the test tube with the test label. The blank test tube was filled with the same volume of distilled water, and the standard test tube was filled with the same volume of the standard reagent. After zeroing with the blank at 560 nm, the mixtures were incubated for 30 minutes at 20–25°C before the absorbance of the standard and test was measured using a spectrophotometer. Total protein content of sample was obtained using the formula below.

$$\text{Total protein} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Where concentration of standard = 5.95mg/dl

DATA ANALYSIS

Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented

with Student's t test for post-hoc test for comparisons of the means of the various doses and fractions. P- values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value.

RESULTS

Table 1: Liver function parameters.

Treatment	Normal control	BPH control (Testosterone only)	Finasteride (3 mg/kg) + Testosterone	AML extract (200 mg/kg) + Testosterone	AML extract (400 mg/kg) + Testosterone	AML extract (800 mg/kg) + Testosterone
Total protein (g/dl)	5.64±0.26 ^b	4.32±0.57 ^a	5.35±0.20 ^b	5.49±0.47 ^b	5.07±0.22 ^b	5.53±0.10 ^b
Albumin (g/dl)	3.27±0.04 ^b	2.31±0.40 ^a	2.93±0.06 ^b	3.00±0.10 ^b	2.93±0.18 ^b	3.28±0.12 ^b
Globulin (g/dl)	2.38±0.26 ^{a,b}	2.02±0.03 ^a	2.42±0.15 ^{a,b}	2.50±0.39 ^b	2.14±0.10 ^{a,b}	2.25±0.10 ^{a,b}
AST (u/l)	44.33±5.04 ^a	74.33±5.86 ^d	52.67±2.08 ^{b,c}	50.00±1.00 ^{a,b,c}	56.00±2.64 ^c	48.67±4.04 ^{a,b}
ALT (u/l)	35.67±3.51 ^a	52.00±1.72 ^d	41.67±1.53 ^{b,c}	42.33±1.53 ^{b,c}	43.00±2.65 ^c	38.00±2.65 ^{a,b}
ALP (u/l)	67.00±2.00 ^a	71.00±3.61 ^a	68.67±1.16 ^a	70.00±3.61 ^a	69.33±2.52 ^a	68.33±2.89 ^a
Total bilirubin (mg/dl)	0.48±0.06 ^a	0.79±0.04 ^b	0.54±0.04 ^a	0.49±0.02 ^a	0.56±0.03 ^a	0.53±0.07 ^a

Values are presented as mean ± standard deviation (n = 3); and means with different letter superscripts are significantly (P < 0.05) different from paired mean across the row.

Table 1, shows the results of the liver function parameters across different treatment groups. The BPH control (Testosterone only) group exhibits significant deviations in liver function compared to the normal control group.

Specifically, elevated levels are observed in AST (74.33±5.86 u/l) and ALT (52.00±1.72 u/l) enzymes, as well as reduced levels of total protein (4.32±0.57 g/dl), albumin (2.31±0.40 g/dl), and globulin (2.02±0.03 g/dl), indicating

potential liver damage or dysfunction. Treatment with Finasteride (3 mg/kg) + Testosterone and AML extract at various doses (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone generally shows improvements in these parameters compared to the BPH control group. Specifically, these treatments tend to

normalize AST, ALT, and bilirubin levels, while restoring total protein, albumin, and globulin towards values closer to the normal control group. The highest dose of AML extract (800 mg/kg) + Testosterone appears to have the most beneficial effect on liver function parameters

Table 2: Relative Organ Weight.

Treatment s	Normal control	BPH control (Testosterone only)	Finasteride (3 mg/kg) + Testosterone	AML extract (200 mg/kg) + Testosterone	AML extract (400 mg/kg) + Testosterone	AML extract (800 mg/kg) + Testosterone
Relative Liver wt (%)	3.62 ±0.16 ^b	3.52±0.26 ^{a,b}	3.47±0.30 ^{a,b}	3.24±0.09 ^{a,b}	3.48±0.26 ^{a,b}	3.11±0.13 ^a
Relative Heart weight (%)	0.37 ±0.03 ^{a,b}	0.34±0.02 ^a	0.37±0.03 ^{a,b}	0.42±0.04 ^b	0.42±0.02 ^b	0.38±0.04 ^{a,b}
Relative paired kidney wt (%)	0.73 ±0.02 ^a	0.77±0.04 ^{a,b}	0.70±0.04 ^a	0.81±0.03 ^b	0.74±0.06 ^{a,b}	0.70±0.02 ^a
Relative paired testes wt (%)	1.58 ±0.05 ^b	1.21±0.29 ^a	1.17±0.12 ^a	1.33±0.05 ^{a,b}	1.28±0.09 ^{a,b}	1.25±0.23 ^a
Relative prostate wt (%)	0.06 ±0.03 ^a	0.36±0.04 ^c	0.27±0.01 ^b	0.26±0.01 ^b	0.24±0.10 ^b	0.07±0.00 ^a

Values are presented as mean ± standard deviation (n = 3); and means with different letter superscripts are significantly (P < 0.05) different from paired mean across the row.

Table 4.2 presents the results of the relative organ weights across different treatment groups. Relative to the normal control group, the BPH control (Testosterone only) group shows significant changes in organ weights. The prostate weight notably increases (0.36±0.04%), indicating prostate enlargement due to BPH induction. Conversely, treatments with Finasteride (3 mg/kg) + Testosterone and various doses

of AML extract (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone generally reduce prostate weight compared to the BPH control group, approaching values closer to the normal control.

Regarding other organs, such as the liver, heart, paired kidneys, and paired testes, the BPH control group often shows deviations from the normal control, suggesting potential effects of testosterone-induced BPH on these organs. Treatments with Finasteride and AML extract generally lead to organ weights that are closer to those of the normal control group.

DISCUSSION

The leaf extract of *Annona muricata* was shown to contain moderate quantities (++) of alkaloids, flavonoids, phenolics, and saponins based on the qualitative phytochemical composition analysis.[5,6] These phytochemicals are known for their diverse biological activities, including antioxidant, anti-inflammatory, and hepatoprotective effects.[8,10] Specifically, flavonoids and phenolics contribute to the extract's antioxidant properties, helping to scavenge free radicals and reduce oxidative stress-induced damage in various organs, including the liver and kidneys.[6] The presence of alkaloids in *Annona muricata* extract is noteworthy as alkaloids have been associated with hepatoprotective effects by enhancing liver function parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).[1,4] These findings suggest that the alkaloids in *Annona muricata* may contribute to its ability to protect liver cells from damage and promote liver regeneration. Based on the quantitative phytochemical analysis of *Annona muricata* leaf extract, it is evident that the extract contains a diverse range of phytochemicals with varying concentrations.[5,6] Alkaloids are identified as the most abundant phytochemical component, with a concentration of 38.35 ± 0.38 mg/100g. Alkaloids have been associated with several pharmacological activities, including hepatoprotective effects observed in studies where *Annona muricata* extract improved liver function markers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST).[1,4] This suggests that the high concentration of alkaloids in *Annona muricata* extract may contribute significantly to its liver protective properties. Following alkaloids, flavonoids are the next most abundant phytochemical

in the extract, with a concentration of 24.42 ± 0.82 mg/100g. Flavonoids are known for their antioxidant properties, which can scavenge free radicals and mitigate oxidative stress-induced damage in various tissues, including the liver and kidneys.[6,8] The liver function parameters assessed in this study highlight significant deviations induced by testosterone and the subsequent therapeutic effects of Finasteride and *Annona muricata* (AML) extract treatments. Elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alongside reduced levels of total protein, albumin, and globulin in the BPH control group, indicate liver dysfunction and impaired synthetic function.[2,3] AST and ALT are enzymes released into the bloodstream when liver cells are damaged, suggesting hepatocellular injury in the BPH control group.[2] Decreased levels of total protein, albumin, and globulin further underscore compromised hepatic function, impacting nutrient metabolism and detoxification processes. [3] Treatment with Finasteride + Testosterone and various doses of AML extract + Testosterone demonstrated significant improvements in liver function markers compared to the BPH control group. These treatments effectively normalized AST and ALT levels, indicative of reduced hepatocellular damage and improved liver cell integrity.[1,4] Moreover, they restored total protein, albumin, and globulin concentrations towards values resembling those of the normal control group, suggesting enhanced hepatic synthetic function and metabolic homeostasis.[5,6] The hepatoprotective effects of AML extract are attributed to its rich phytochemical composition, including alkaloids, flavonoids, and acetogenins, which exhibit antioxidant, anti-inflammatory, and cytoprotective properties.[6,8] These bioactive compounds mitigate oxidative stress,

suppress inflammatory responses, and promote liver cell regeneration, thereby preserving liver function.[6,8] Mechanistically, AML extract activates pathways such as nuclear factor erythroid 2-related factor 2 (Nrf2), which upregulates antioxidant enzymes like heme oxygenase-1 (HO-1) and glutathione S-transferase (GST), crucial for detoxification and protection against oxidative damage.[10]

The results of the relative organ weights provide insights into the physiological impacts of testosterone-induced benign prostatic hyperplasia (BPH) and the therapeutic effects of Finasteride and *Annona muricata* (AML) extract treatments on various organs. In the BPH control group, characterized by testosterone induction alone, significant increases in prostate weight ($0.36 \pm 0.04\%$) are observed compared to the normal control group. This enlargement reflects the pathological growth associated with BPH, which is a consequence of testosterone-driven prostate hyperplasia.[11] Testosterone's role in promoting prostate growth is well-established, and the resultant increase in prostate weight serves as a hallmark of BPH induction in experimental models.[11] Conversely, treatment with Finasteride + Testosterone and different doses of AML extract (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone demonstrates notable reductions in prostate weight compared to the BPH control group. These treatments effectively mitigate prostate enlargement, with organ weights approaching values closer to those of the normal control group. Finasteride, a 5-alpha-reductase inhibitor, reduces the conversion of testosterone to dihydrotestosterone (DHT), thereby inhibiting DHT-dependent prostate growth.[11] Similarly, AML extract, enriched with bioactive compounds like flavonoids and alkaloids, exerts anti-

inflammatory and anti-proliferative effects on prostate tissue, contributing to the observed reductions in prostate weight.[9] Regarding other organs such as the liver, heart, paired kidneys, and testes, deviations from normal organ weights are often noted in the BPH control group, suggesting potential systemic effects of testosterone-induced BPH on these organs. Treatment with Finasteride and AML extract generally results in organ weights that align more closely with those of the normal control group. This normalization indicates that these treatments not only address prostate-specific changes but also mitigate broader systemic effects associated with testosterone-induced BPH.[9] The therapeutic effects of AML extract on organ weights are attributed to its multifaceted pharmacological properties, including antioxidant, anti-inflammatory, and anti-proliferative activities.[9] These properties collectively contribute to maintaining organ homeostasis and preventing pathological changes induced by testosterone and BPH progression.

CONCLUSION

Conclusively, liver function parameters assessed in this study highlight significant deviations induced by testosterone and the subsequent therapeutic effects of Finasteride and *Annona muricata* (AML) extract treatments. Elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alongside reduced levels of total protein, albumin, and globulin in the BPH control group, indicate liver dysfunction and impaired synthetic function.

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