

Ameliorating Effects of *Annona Muricata* Leaf Extract on Hematological Parameters in Male Wistar Rats

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ABSTRACT

This study aims to determine the ameliorating effects of *annona muricata* leaf extract in hematological parameters in male wistar rats. 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 Haematological analysis of the blood samples was performed in an automated haematology analyzer (BC-2300 model, Mindray Medical Co., China) with the procedure carried as specified by the producer. The parameters which were evaluated included: red blood cells count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (PLT); total leukocytes count (TLC) count and differential leukocytes count(WBC) counts were obtained at once for each blood sample. The data was analyzed using the Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc., Chicago, IL). Using One Way Analysis of Variance (ANOVA), the level of significance was determined. To compare the means of the different doses and fractions, the Duncan Multiple Range Test was used to evaluate the data, and the Student's t test was used as a post-hoc test. P-values below 0.05 were regarded as statistically significant differences for the measured value between the test groups and the control group. In this investigation, the administration of *Annona muricata*

extract kept hematological parameters within physiological ranges and reliably controlled differential white blood cell counts.

Keywords: *Annona muricata, hematological parameters, testosterone-induced hyperplasia, wistar rats*

INTRODUCTION

The use of plant-based therapies, has been growing steadily as a complementary and alternative approach to managing various health conditions, including benign prostatic hyperplasia (BPH) and reproductive health issues [2,5]. This increasing interest is driven by the desire for treatments that are perceived as more natural and less invasive than conventional pharmacological and surgical options. [4]

Phytotherapy leverages the bioactive compounds found in plants, which have been used in traditional medicine systems for centuries to treat a wide range of ailments.[7] Research into plant-based therapies has intensified due to the limitations and side effects associated with standard BPH treatments. Many patients seek alternative options that offer symptom relief with a better side-effect profile. The World Health Organization (WHO) has recognized the importance of traditional medicine, including phytotherapy, in providing accessible and affordable health care globally. This recognition has spurred scientific investigations into the efficacy and safety of various plant extracts in managing BPH and associated reproductive health problems (WHO, 2013). Natural remedies offer several advantages that make them appealing to patients and healthcare providers alike. One of the primary benefits is the generally lower incidence of adverse side effects compared to conventional medications. Many plant-based therapies are well-tolerated, with fewer reports of severe complications,

making them a safer option for long-term use [12].

MATERIALS AND METHODS

Materials

Equipment's 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

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 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

Animals were killed at the conclusion of treatment, and blood samples were taken for hematological examination in plain and EDTA bottles, 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 haematological analysis

Preparation of Plant Extracts

Plant extract were prepared in accordance with the method used by Orieki *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-hexane 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 vigorously shaken every 2 hours within the period and was thereafter filtered with Whatman No. 1 filter paper to obtained a filtrate which was the extract in solution. 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 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 as specified by the producer. The parameters which were evaluated included: red blood cells count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (PLT); total leukocytes count (TLC) count and differential leukocytes count (WBC) counts

were obtained at once for each blood sample.

Procedure for Use of the 5 Part Haematological Analyzer

The diluent dispenser was used to analyze a whole blood sample. 20 μ L of the sample was aspirated into the dispenser by pressing the [DILUENT] key. When the [DILUENT] key was pressed again, a diluted sample (about 1:300) was discharged. After completely mixing and presenting the sample under the suction nozzle, the [COUNT] key was hit to aspirate the material into the analyzer for analysis. A few seconds later, the result was shown on the screen.

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: Differential WBC.

Treatments	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
Neutrophils (%)	41.67 $\pm$ 1.53 ^b	36.33 $\pm$ 2.08 ^a	37.33 $\pm$ 2.52 ^a	38.00 $\pm$ 1.00 ^a	38.33 $\pm$ 1.53 ^{a,b}	41.67 $\pm$ 2.08 ^b
Lymphocytes (%)	52.33 $\pm$ 1.53 ^a	58.00 $\pm$ 2.65 ^b	56.67 $\pm$ 1.53 ^b	56.33 $\pm$ 0.58 ^b	55.33 $\pm$ 1.16 ^{a,b}	53.00 $\pm$ 1.73 ^a
Monocytes (%)	3.67 $\pm$ 0.62 ^a	3.67 $\pm$ 0.58 ^a	3.33 $\pm$ 0.55 ^a	3.00 $\pm$ 0.00 ^a	3.67 $\pm$ 0.58 ^a	3.33 $\pm$ 0.57 ^a
Eosinophils (%)	2.33 $\pm$ 0.58 ^a	2.00 $\pm$ 0.00 ^a	2.67 $\pm$ 0.63 ^a	2.67 $\pm$ 0.54 ^a	2.67 $\pm$ 0.61 ^a	2.00 $\pm$ 0.00 ^a
Basophils (%)	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a

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

The differential white blood cell (WBC) analysis presented in Table 1 indicates significant variations among treatment groups. In the normal control group, neutrophils constitute 41.67 $\pm$ 1.53% of WBCs, while lymphocytes are 52.33 $\pm$ 1.53%, monocytes are 3.67 $\pm$ 0.62%, eosinophils are 2.33 $\pm$ 0.58%, and basophils are absent (0.00 $\pm$ 0.00%). In contrast, the BPH control group (Testosterone only) shows a decrease in neutrophils (36.33 $\pm$ 2.08%) and an increase in

lymphocytes (58.00 $\pm$ 2.65%) compared to the normal control. Treatment with Finasteride (3 mg/kg) + Testosterone leads to similar neutrophil levels as the BPH control but with slight changes in lymphocyte and monocyte percentages. The groups treated with AML extract (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone generally exhibit variations in neutrophil and lymphocyte percentages compared to both the normal and BPH control groups.

Table 2: Haematological parameters.

Treatments	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
RBC ($\times 10^6/\text{mm}^3$)	7.78 $\pm$ 0.51 ^b	6.56 $\pm$ 0.12 ^a	7.38 $\pm$ 0.29 ^b	7.57 $\pm$ 0.04 ^b	7.87 $\pm$ 0.07 ^b	7.76 $\pm$ 0.41 ^b
PCV (%)	49.33 $\pm$ 3.51 ^b	40.67 $\pm$ 1.53 ^a	45.67 $\pm$ 2.08 ^b	46.67 $\pm$ 0.58 ^b	49.33 $\pm$ 0.57 ^b	48.67 $\pm$ 2.89 ^b
Hb (g/dl)	18.67 $\pm$ 0.83 ^c	15.67 $\pm$ 0.31 ^a	17.17 $\pm$ 0.78 ^b	17.70 $\pm$ 0.46 ^{b,c}	18.57 $\pm$ 0.12 ^c	18.70 $\pm$ 0.62 ^c
TWBC ($\times 10^3/\text{mm}^3$)	9.52 $\pm$ 0.57 ^{a,b}	13.20 $\pm$ 0.72 ^d	10.43 $\pm$ 0.22 ^{b,c}	9.24 $\pm$ 0.65 ^a	10.93 $\pm$ 0.51 ^c	10.05 $\pm$ 0.47 ^{a,b,c}
PLT ($\times 10^3/\text{mm}^3$)	253.67 $\pm$ 4.51 ^a	280.00 $\pm$ 3.61 ^c	264.33 $\pm$ 3.51 ^b	255.67 $\pm$ 3.06 ^a	258.00 $\pm$ 4.00 ^b	253.33 $\pm$ 7.37 ^a
MCV (fl)	63.37 $\pm$ 0.42 ^b	61.98 $\pm$ 1.26 ^a	61.87 $\pm$ 0.59 ^a	61.65 $\pm$ 0.49 ^a	62.71 $\pm$ 0.41 ^{a,b}	62.73 $\pm$ 0.47 ^{a,b}
MCH (pg)	24.01 $\pm$ 0.60 ^{a,b}	23.88 $\pm$ 0.22 ^{a,b}	23.26 $\pm$ 0.28 ^a	23.38 $\pm$ 0.52 ^{a,b}	23.60 $\pm$ 0.11 ^{a,b}	24.13 $\pm$ 0.56 ^b
MCHC (g/dl)	37.89 $\pm$ 1.11 ^a	38.54 $\pm$ 0.81 ^a	37.59 $\pm$ 0.16 ^a	37.92 $\pm$ 0.56 ^a	37.64 $\pm$ 0.21 ^a	38.46 $\pm$ 1.05 ^a

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

The Result of the haematological parameters presented in Table 4.2 demonstrate notable variations across different treatment groups. Compared to the normal control group, the BPH control (Testosterone only) group shows reduced red blood cell count (RBC) (6.56 $\pm$ 0.12 $\times 10^6/\text{mm}^3$), packed cell volume (PCV) (40.67 $\pm$ 1.53%), hemoglobin (Hb) levels (15.67 $\pm$ 0.31 g/dl), and mean corpuscular volume (MCV) (61.98 $\pm$ 1.26 fl). Treatment with Finasteride (3 mg/kg) + Testosterone partially restores these parameters closer to normal levels. Interestingly, the groups treated with AML extract (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone generally show improvements in RBC count, PCV, Hb levels, and some indices such as total white blood cell count (TWBC) and platelet count (PLT). Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) remain relatively stable across most groups.

DISCUSSION

The differential WBC analysis shows the complex interplay between AML extract

treatments, immune responses, and hormonal influences in the context of BPH. The haematological parameter analysis reveals significant insights into the systemic effects of *Annona muricata* leaf (AML) extract treatments in the context of benign prostatic hyperplasia (BPH) induced by testosterone, as discussed in the literature[3]. In the BPH control group (Testosterone only), there were notable decreases in red blood cell count (RBC) (6.56 $\pm$ 0.12 $\times 10^6/\text{mm}^3$), packed cell volume (PCV) (40.67 $\pm$ 1.53%), hemoglobin (Hb) levels (15.67 $\pm$ 0.31 g/dl), and mean corpuscular volume (MCV) (61.98 $\pm$ 1.26 fl) compared to the normal control. These changes indicate potential hematological alterations associated with testosterone-induced BPH [8,10]. Treatment with Finasteride (3 mg/kg) + Testosterone demonstrated partial restoration of these parameters toward normal levels, suggesting a therapeutic effect in mitigating the hematological impacts of BPH. This aligns with findings indicating Finasteride's role in reducing prostate enlargement and its related systemic effects [1,11] Ojewole, 2006;. Interestingly, groups treated with AML extract (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone generally exhibited improvements in RBC count, PCV, Hb levels, and some hematological indices such as total white blood cell count (TWBC) and platelet count (PLT). This suggests that AML extract may have

beneficial effects on hematopoiesis and blood parameters under conditions of BPH induction [6,9]. There was little effect on these particular hematological indicators, as mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) stayed mostly constant across the majority of treatment groups. The haematological parameter analysis underscores the potential of AML extract to influence systemic hematopoietic processes and mitigate testosterone-induced hematological alterations associated with BPH.

CONCLUSION

In this present study, treatment with *Annona muricata* extract consistently regulated differential white blood cell counts, and improved hematological parameters within physiological ranges.

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