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Effects of Ethanol Extract of Unripe *Annona muricata* (l.) Fruits on the Haematological and Histopathological Parameters in Swiss Albino Rats Infected with *Salmonella typhi*

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Authors' contributions

This work was carried out in collaboration between both authors. Authors OSF and EOD designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author OSF managed the literature searches and various analyses performed during the study. Author OSF managed the experimental process and author EOD identified the species of plant. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: In addition to the problems of resistance to commonly used antibiotics, toxicity depicts the state of adverse effects caused by the interaction of toxicants with cells. Similarly, blood components are exposed to significant concentrations of toxic compounds as they form the medium for their transport.
Aims: To study the effects of ethanol extract of unripe *Annona muricata* fruits on the haematological and histopathological parameters in Swiss albino rats infected with *Salmonella typhi.*
Place and Duration of Study: Department of Microbiology Laboratory, Federal University of Technology, Akure, Ondo State, Nigeria, between June 2014 and December 2014.
Methodology: Matured unripe fruits of *A. muricata* were collected, dried, powdered and extracted using 70% ethanol. Eighteen rats of same age between 170-220 g in weight were selected and divided into six groups containing three each. The infectivity dose (ID) was determined using the

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clinical *Salmonella typhi* obtained from Don Bosco Catholic Hospital, Akure. The dose of the *A. muricata* fruit extract (400 mg/kg) used in this study was administered orally for 7 days. At the end of the treatment period, the rats were fasted overnight. Then blood samples were collected by cardiac puncture for haematological studies and there after sacrificed. Organs (liver, heart, kidney and spleen) were excised for relative organ weight analysis and histopathological studies.

Results: The infectious dose (ID) of *S. typhi* on experimental rats in this study was $6.8x10^6$ cfu/ml while the weights of liver, heart and spleen in all groups were not significantly different from the control. However, a significant increase in the heart was observed in group given the extract and antibiotics (ciprofloxacin). The Packed Cell Volume (PCV) and Haemoglobin (Hb) of rats treated with extract and ciprofloxacin increased significantly ($p<0.05$) compared to the control while a non-significant decrease was observed in the Red Blood Cell (RBC) of rats administered only extract and ciprofloxacin after treatment. White Blood Cells (WBC) of rats given the extract significantly reduced while there was no significant difference in the lymphocyte count of rat administered the extract of *A. muricata* and ciprofloxacin. Neutrophils of rats in all groups significantly increased ($p<0.05$). However, the monocyte and eosinophil level were not significantly different from the control. Minimal disruption and reduction in nuclear clumping were observed in the liver of rats infected/treated with the extract. Similarly, there were prominent improvements in the glomerula architecture as well as the white and red pulp of the spleen after treatment with the extract of *A. muricata*.

Conclusion: The extract stimulated blood production and possessed restorative effect on various organs examined. However, excessive use might be toxic to the heart.

Keywords: Anonna muricata; Salmonella typhi; haematological; histopathological; rats.

1. INTRODUCTION

Different plants and their parts are used all over the world for various purposes. They have been reported as an efficient constituents of mostly used antioxidant, antibacterial, antifungal, antiulcer, anti-inflammatory, antiviral and anticancer agents. They have also been used for the prevention and treatment of different type of disease conditions [1]. The employment of alternative traditional medicine in the treatment of different ailments has enormously expanded in both developed and developing countries of the world due to their cheapness, efficacy and easy to come-by advantage [2]. Some plants of medicinal value are believed to also promote positive health and maintain resistance against infection by re-establishing body equilibrium and conditioning the body tissues [3].

Annona muricata (L.) also referred to as graviola, soursop or guanabana belongs to a family called *Annonaceae.* It is popularly grown across the tropical regions of the world [4]. The plant is known to produce an edible fruit that is green in colour, large, heart-shaped and 15–20 cm in diameter with a white fleshy mesocarp. The fruit is sometimes irregular, lopsided or curved due to abnormal carper formation or injury caused by insects. Soursop has a long, rich historical use in herbal medicine as well as a lengthy recorded indigenous use. Different medicinal properties and uses have been attributed to various parts of the tree. Generally, the fruit and its juice are used to combat worms and parasitic organisms, to cool fevers, increase breast milk production after birth, and as an astringent for diarrhea and dysentery. In addition, internal and external parasites, head lice, and worms are treated with crushed seeds of the plant [5].

Salmonella typhi is a non-spore forming Gram-negative bacterium. It is the causative agent of a systemic infection called typhoid fever. The clinical manifestations of the disease vary from a mild illness with low-grade fever, malaise, and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications. The infection is usually contracted by consumption of contaminated food and water [6]. Every year the disease affects at least 17million persons world-wide most of whom resides in the developing countries of South East Asia and Africa [7]. The antibacterial resistance problems associated with commonly used antibiotics as well as the re-emergence of multi-antibiotic resistant strains of this pathogen has become a possible threat to public health. Therefore, the search for effective antimicrobials is necessary [8]. Besides, toxicity is a measure of how poisonous a substance is, it shows the degree of adverse effects caused by the interaction of the toxicants with body cells. The possible effects produced depend on the chemical properties of the toxic substance and the cell membrane [9]. Similarly, blood components are exposed to

significant concentrations of these toxic compounds as they form the medium for their transport. Hence, the evaluation of the toxic effects of unripe *Annona muricata* fruit extract on haematological and histopathological parameters in Swiss albino rats infected with *Salmonella typhi*.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Extraction of Bioactive Materials

Fresh unripe fruits of *Annona muricata* (Linn) were collected from a garden in Arimoro area of Ilesha, Osun State. The fruits were identified and authenticated by using the herbarium specimens of the Department of Crop, Soil and Pest management, Federal University of Technology, Akure (FUTA). Then the unripe *A. muricata* fruits were washed with sterile water. The peels and seeds were separated from the pulp and then cut into smaller pieces. The fruits were oven dried at 50℃ for 4 days. The dried fruits were then pulverized into fine powder by blending in a high-speed Philips Model blender. They were separately kept in an airtight container to avoid the absorption of moisture. Three hundred grams (300 g) of the powdered sample was soaked in two thousand, five hundred millilitres (2500 mL) of 70% ethanol as solvents to extract the bioactive compounds. The container was labeled appropriately and left for 72 hours (3 days). After which it was sieved using muslin cloth and then filtered using 0.45 µm micropore filter. The filtrates were vaporized to dryness using rotary evaporator and subsequently lyophilized to remove the extracting solvent. The ethanol extract was preserved in a sterile bottle at 4℃ until use [9,10].

2.2 Test Bacteria

Pure clinical isolate of *Salmonella typhi* was obtained from the stock culture of Don Bosco Catholic Hospital, Akure, Ondo State. The clinical *S. typhi* was isolated from a typhoid patient. The bacterium was further characterized in the laboratory using the methods of [11].

2.3 Experimental Animals

Forty-two Swiss albino rats (170-220 g) were obtained from Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria. The rats were fed with standard rat pellets (Livestock Feeds, Ikeja, Lagos State) and water *ad libitum*. The animals were housed under standard laboratory conditions and were acclimatized for 7days before the treatment started. The experimental procedures involving animals were conducted in conformity with international, national and institutional guidelines as reported by [12].

2.4 Inclusion and Exclusion Criteria

Both sexes of the albino rats between 5-7 weeks old, healthy and with no previous exposure to antibiotics were used while rats that are more 7 weeks old, infected and exposed to antibiotics were excluded from this study.

2.5 Preparation of Standard Inoculum of *S. typhi* for *In vivo* Assay

The method described by [11] was employed. A 0.238 g of sodium hydrogen phosphate was dissolved in 0.019 g of potassium dihydrogen phosphate and sodium chloride respectively. The mixture was made up to 100 mL with distilled water and pH was adjusted to neutral (7). Then standard inoculum of *S. typhi* was inoculated into 1000 mL of nutrient broth and incubated for 24 hrs. After incubation, the cells were centrifuged at 2000 rpm for 10 minutes and the supernatant was discarded. Pellets were re-suspended in PBS and centrifuged again for four times. The final cell button was resuspended in PBS and serially diluted 10^1 to 10^6.

2.6 Determination of Infectious Dose (ID)

The method described by [13] was adopted. Twenty four Swiss albino rats were used to determine the ID of *S. typhi*. The rats were divided into six groups of four rats. Each group was infected with different concentrations of *S. typhi* suspension. The groups were closely monitored for seven days. The concentration of *S. typhi* suspension that produces the signs like unformed stool, weak, scattered fur, falling of hairs, stool with mucous and weight loss in animals given is taken as the infectivity dose (ID_{50}) of *S. typhi*. Also, corresponding colony-forming units per millilitres (cfu/mL) of the bacterial dilutions was determined using plate count method on *Salmonella-Shigella* agar (SSA).

2.7 *In-vivo* Bioassay

The *in-vivo* bioassay was carried out using the methods reported by [13] and [14]. A total

number of eighteen rats were used and divided into six groups; Group 1 was infected with infective dose of *S. typhi* (calculated to be 6.8 X 10^6 cfu/mL) but was not treated with the extract while Group II was infected with the infective dose and then treated with the ethanolic extract of unripe *A. muricata* fruit after the infection had set in. Group III was given prophylactic dose 400 mg/mL extract of *A. muricata* for three days before being infected with *S. typhi.* Then Group IV was administered only extract of *A. muricata*; Group V was infected and treated with ciprofloxacin; while Group VI was fed with basal diet and water only. All the experimental rats in groups I, II, IV and V were observed for signs of infection before being treated.

2.7.1 Relative organ weight

After the experiment, all the animals were euthanized under chloroform anaesthesia. Organs such as heart, liver, kidney and spleen of the sacrificed animals were excised, washed with normal saline, examined for any lesions and weighed in grams to obtain the absolute organ weight [2]. The relative organ weights were calculated for each rat using;

$$Relative\ Organ\ Weight = \frac{Absolute\ organ\ weight\ (g)}{Body\ weight\ at\ Sacrifice\ (g)} \times 100$$

2.7.2 Haematological assay

Blood samples were collected before treatment through cardiac puncture while after the experiment, animals were sacrificed and incisions were quickly made in the sacrificed animal's cervical region and blood samples collected from the heart were dispensed into EDTA bottles for haematological analyses [2] and various analyses as described by [11] were carried out on the blood samples.

2.7.3 Histopathological assay

The histology of various organs was carried out using the method of [9]. The required organs were excised, weight and fixed in aqueous Bouin's solution for 48 hrs and were sequentially embedded in paraffin wax blocks according to the standard procedure, sectioned at 5 µ thickness. They were further deparaffined with xylol, and histologic observations were performed after staining with haematoxylin and eosin. The slides were examined under a light microscope and the magnified images of the tissues structure were captured.

2.8 Statistical Analysis

Mean values of replicates were reported with their standard deviations using SPSS 16.0. Analyses of Variance (ANOVA) were achieved to calculate significant differences in the treatment means, and the mean separations were achieved by Duncan's Multiple Range Test ($p \leq 0.05$).

3. RESULTS

3.1 Infectious Dose (ID) of *Salmonella typhi* on Experimental Rats

The dose of *Salmonella typhi* that produced infection signs like unformed stool, weakness, scattered fur, falling hairs, stool with mucous and weight loss in the experimental rats was 6.8 x 10^6 cfu/mL.

3.2 Effect of Treatment on Relative Organ Weights (ROW)

The effect of treatment on Relative Organ Weight (g/100 g) of the rats is shown in Table 1. The weights of liver, heart and spleen in all groups under observation were not significantly different from the control. However, a significant increase in the heart was observed in group IV and V given the extract and conventional antibiotics (ciprofloxacin).

3.3 Haematological Analysis

The results of the haematological analysis of the blood samples collected from the rats after treatment are shown in Table 2. In this study, there was no significant difference in the Erythrocyte Sedimentation Rate (ESR) level after treatment. The PCV of rats given extract (group IV) and ciprofloxacin (group V) increased significantly ($p<0.05$) compared to the control. Similarly, infected/treated group showed a significant increase in the PCV. The RBC of the rats administered with only extract and ciprofloxacin experienced an insignificant decrease. However, a significant increase ($p<0.05$) was reported for the infected/treated group. Also, the Hb significantly increased ($p<0.05$) in groups given only extract and ciprofloxacin as well as infected/treated group when compared to control after treatment.

The WBC of the group of rats given with extract (group IV) and ciprofloxacin (group V) significantly reduced ($p<0.05$) after treatment while the infected/treated group was not significantly different from the control. However, a significant increase ($p<0.05$) was observed in the group infected only with *S. typhi*.

When compared to control after treatment, the lymphocyte of rats given only extract (group IV) and infected-treated group (group V) were not significantly different while the neutrophils of rats in all groups after treatment significantly increased ($p<0.05$) when compared to the control. But the monocyte and eosinophil level were not significantly different from the control after treatment (Table 2). Basophil level significantly increased ($p<0.05$) in group administered only the extract of *A. muricata* while for other groups, the differences were not significant compared to the control.

3.4 Histological Analysis

The result of the histopathological examination carried out on the liver, kidney and spleen of the experimental rats is indicated on Plates 1A-3B. Less congested parenchyma, more distinct nuclei, improving polyhedral shape of the hepatocyte and the Kuffer's cell were observed in the infected/ treated group of rats.

Similarly, a more distinct glomerulus as well as the proximal and distal tubules filling the parenchyma, odematous space and disappearance of inflammatory cells in the glomerulus was observed in the histopathological examination of the kidney of infected/treated rats (Plate 2B).

The photomicrograph of the spleen revealed that the white and red pulp were visible. The white pulp (WP) has a centrally placed blood vessel called central arteriole while the red pulp (RP) consist of mild population of red blood cell (Plate 3A) while the white and red pulp of the infected/treated group of rats showed restoration in nuclear material and marginal zones respectively (Plate 3B).

4. DISCUSSION

Relative Organ Weight (ROW) is an important indicator of physiological and pathological status in animals. It is instrumental when diagnosing whether the organs was exposed to injury or not. In addition, heart, liver, kidney and spleen have been reported to be primary organs affected by metabolic reactions caused by toxicants [15]. The non-significant change observed in the liver, kidney and spleen weights in this study is similar to the works of [16] who reported that there was no significant change in the relative organ weights of liver, kidney and spleen of Swiss albino mice treated with hydro-methanolic extract of *Coriandrum sativum*. Similarly, [14] reported that differences in the relative weights of lungs, kidney, spleen and liver were not significant in rats administered with the extract of *Moringa oleifera* bark. Therefore, the non-significant change observed in most of these organs suggests that the extract may be virtually non-toxic. However, the slight increment observed in the relative weight of the heart in group IV and V compared to control is worthy of note. Cardiac glycosides which is the highest occurring phytochemical in the extract under study are known to improve cardiac output and reduces distention of the heart. Hence, their reported use in the treatment of congestive heart failure and cardiac arrhythmia. However, [17] reported the phytochemical to possess narrow therapeutic index and may cause intoxication at higher dose.

Table 1. Effect of treatment on relative weight of the organs

Groups	Relative organ weight (g/100 g)			
	Liver	Spleen	Heart	Kidney
Group 1	2.93^{a}±0.07	0.84^{ab}±0.27	0.48^{ab}±0.05	1.23^{bc}±0.02
Group 2	2.79^{a}±0.05	0.64^{ab}±0.12	0.25^{a}±0.04	1.07^{b}±0.33
Group 3	2.97^{a}±0.08	0.67^{ab}±0.07	0.23^{a}±0.07	1.54^{c}±0.07
Group 4	2.79^{a}±0.12	1.02^{b}±0.09	0.58^{b}±0.16	1.15^{bc}±0.25
Group 5	3.08^{a}±0.08	0.50^{a}±0.17	0.99^{c}±0.02	0.60^{a}±0.09
Group 6	3.03^{a}±0.18	0.56^{ab}±0.85	0.28^{a}±0.07	0.99^{ab}±0.16

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Group 1 = Infected & untreated, Group 2 = Infected & treated with extract, Group 3= Given extract before infection, Group 4 = Given extract only, Group 5 = Infected & treated with Ciprofloxacin, Group 6 = Basal diet and water (Control)

Therefore, the increment in the relative weight of the heart reported in this study might be due to high presence of cardiac glycoside in the extract of *A. muricata*. Hence, its use should be controlled. The increased weight of the heart could also be an indication of cardiomegaly. Cardiomegaly is simply the enlargement of heart. A condition which results from stress such as high blood pressure, anaemia, cardiomyopathy and heart attack or underlying diseases suffered by the host.

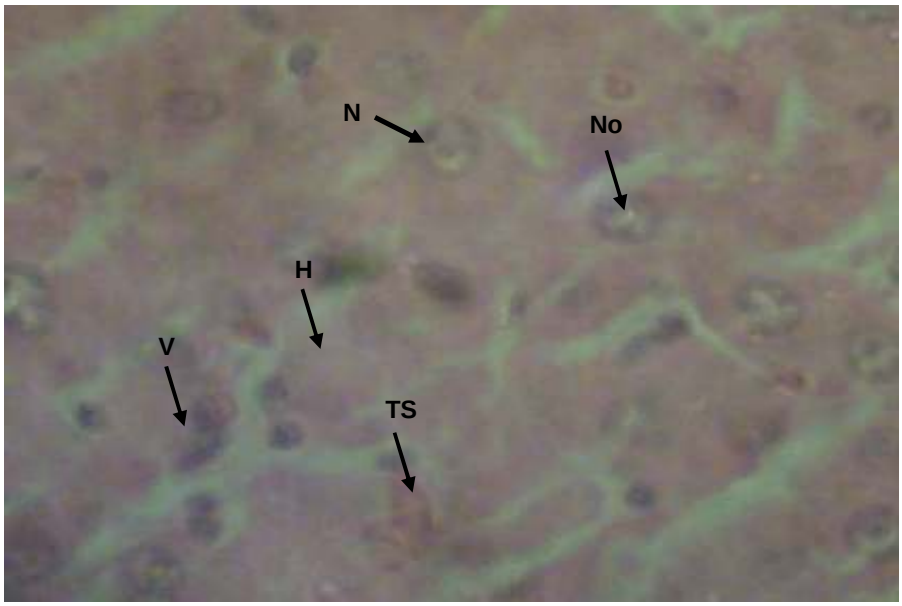

Plate 1a. Photomicrograph of a liver section of non-infected rats showing (a) polyhedral hepatocyte – h, (b) blurred trabecular structure – ts, (c) nucleus and the nucleolus -n & no, and (d) a clear intracytoplasmic lipid vacuoles in hepatocytes - v. H&e x400

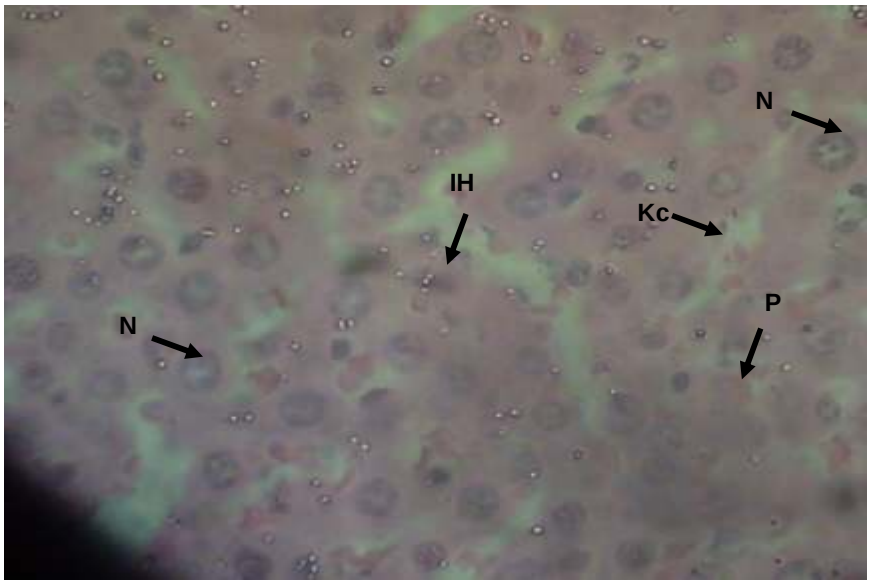

Plate 1b. Photomicrograph of a Section Liver of Rats Infected and treated with the extract of *A. muricata* showing (a) less congested parenchyma – P, (b) more distinct nucleus – N, (c) improving polyhedral shape of the hepatocyte – IH and the (d) Kuffer's cell – Kc. H&E X400

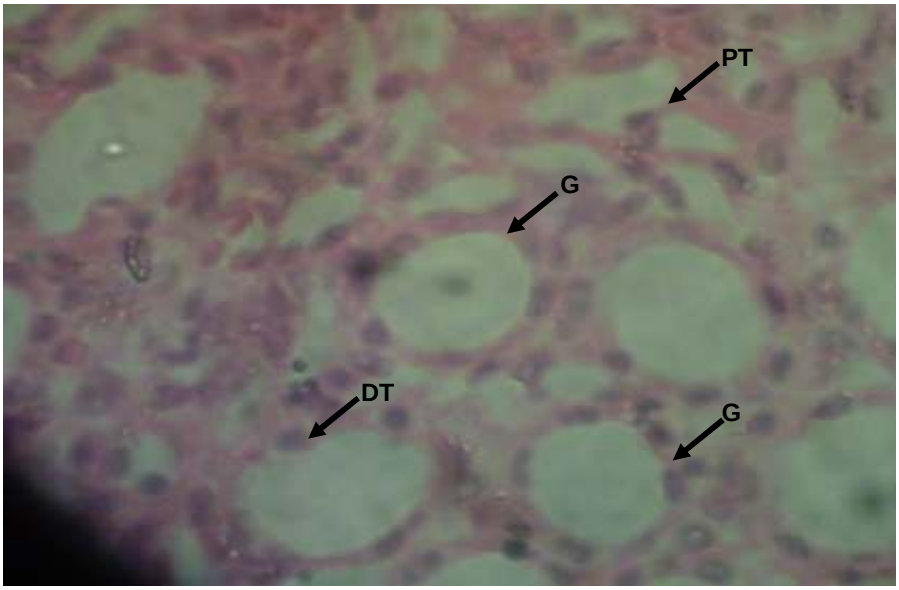

Plate 2a. Photomicrograph of a section kidney of the non-infected rats showing (a) normal glomerulus capsule - G, (b) distal tubule – DT and (c) proximal tubule. H&E x400

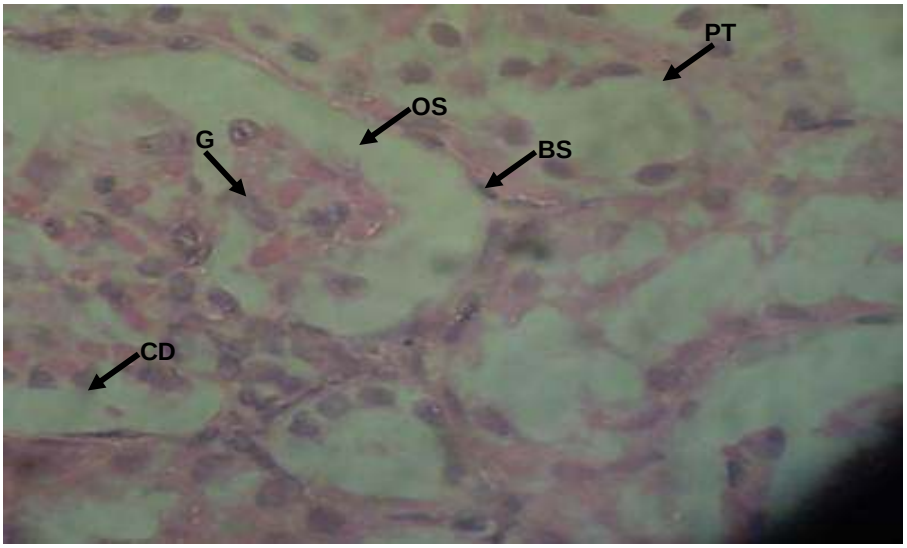

Plate 2b. Photomicrograph of a section kidney of rats infected and treated with the extract of *A. muricata* showing the (a) glomerulus – G, (b) Odematous space of the Glomerulus – OS, (c) Bowman's space – BS, (d) congested distal tubules – CD, and (e) proximal tubule – PT. H&E x 400

In this present study, the significant elevation ($p<0.05$) in the PCV and Hb of rat given only extract and the infected/treated group after the treatment corroborated the works of [18] who reported an increase in the PCV and Hb of rats treated with the leaf extract of *Voacanga africana*. However, the result differed from the findings of [19] who reported a non-significant difference in the PCV levels in the Wistar albino rats administered the ethanolic leaf extract of *Petroselinum crispum.* Odesanmi et al. [20] reported that the PCV and Hb levels of Dutch white rabbits administered the extract of *Tetrapleura tetraptera* remain unaffected. Packed Cell Volume is a measure of the volume of blood consisting of solid cells. Therefore, the increase

in PCV and Hb in this study clearly indicates that the extract of *A. muricata* has a stimulatory property which ultimately results in increased blood volume. The extract possess the potential to stimulate the release of erythropoietin which is the hormonal regulator of RBC production from the kidney [21]. The stimulatory activity might be attributed to the presence of some active agents in the fruit extract of *A. muricata.* In addition, the increase in haemoglobin level observed implied an enhancement of the oxygen carrying capacity of the blood in the rats given the fruit extract of unripe *A. muricata*.

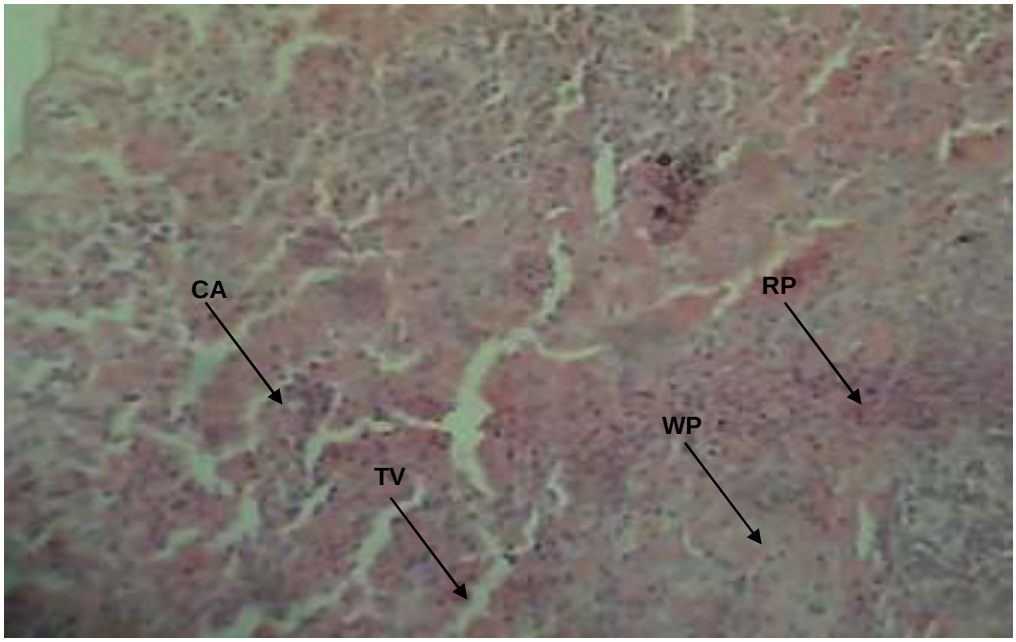

Plate 3a. Photomicrograph of a spleen section of non-infected rats showing (a) the trabecular vessel - TV, (b) white pulp - WP, (c) red pulp - RP and (d) a central arteriole - CA. H&E x400

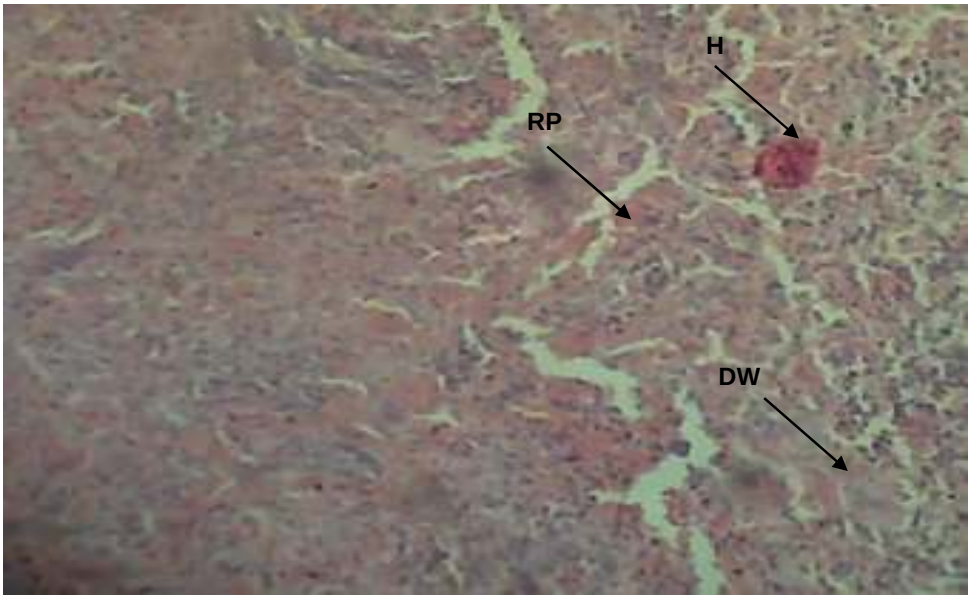

Plate 3b. Photomicrograph of a spleen section of rat infected and treated with the extract showing (a) normal structure with improvement in the red (RP) and white pulp (WP), (b) haemorrhage – H. H&E x400

Table 2. Haematological analysis after treatment

Groups	ERS (mm/hr)	PCV (%)	RBC (x10mc^3)	WBC (x10mc^3)	Hb (g/100mL)	Lymph (%)	Neutro (%)	Mono (%)	Eosin (%)	Baso (%)
Group 1	4.00^{b}±0.58	40.00^{ab}±0.00	10.07^{c}±0.10	11.13^{e}±0.15	13.30^{ab}±0.17	66.00^{ab}±0.58	23.33^{cd}±0.88	7.33^{a}±0.88	2.33^{b}±0.33	0.35^{ab}±0.33
Group 2	1.50^{a}±0.28	42.00^{bc}±2.31	9.53^{c}±0.38	9.53^{cd}±0.26	13.88^{b}±0.70	69.00^{bcd}±0.58	21.50^{bc}±0.87	7.50^{a}±0.28	1.67^{ab}±0.33	0.67^{ab}±0.33
Group 3	2.00^{a}±0.00	40.00^{ab}±1.73	11.20^{d}±0.57	9.20^{c}±0.17	13.49^{ab}±0.69	67.00^{abc}±1.15	23.00^{bcd}±0.58	7.67^{a}±1.45	1.67^{ab}±0.33	0.67^{ab}±0.33
Group 4	2.00^{a}±0.00	43.00^{bc}±0.58	7.80^{ab}±0.17	8.10^{b}±0.06	14.86^{bc}±0.13	70.33^{cd}±0.33	19.33^{b}±0.33	8.00^{a}±0.58	1.00^{a}±0.00	1.33^{b}±0.33
Group 5	1.33^{a}±0.33	45.33^{c}±1.45	7.05^{a}±0.20	6.90^{a}±0.06	15.65^{c}±0.61	64.67^{a}±2.6	26.00^{d}±2.30	8.67^{a}±0.88	1.33^{ab}±0.33	0.33^{a}±0.33
Group 6	1.33^{a}±0.33	36.00^{a}±0.58	8.33^{b}±0.88	10.03^{d}±0.20	12.15^{a}±0.09	72.33^{d}±0.33	8.67^{a}±0.88	7.00^{a}±0.00	1.33^{ab}±0.33	0.00^{a}±0.00

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Group 1 = Infected & untreated, Group 2 = Infected & treated with extract,
Group 3 = Given extract before infection, Group 4 = Given extract only,
Group 5 = Infected & treated with Ciprofloxacin,
Group 6 = Basal diet and water (Control)

The insignificant decrease observed in the RBCs of rats administered only the extract of *A. muricata* after treatment in this study corroborated the earlier findings of [21] who reported that the aqueous flower extract of *Hibiscus sabdariffa* had a non-significant decrease in level of RBC of rats at 400 mg/ml. However, the result is in contrast to the findings of [22] reported a significant increase in the RBC of albino rats treated with the leaf extract of *Mangifera indica*. This non-significant decrease might suggest a gradual tendency of the extract of *A. muricata* to possess an adverse effect on the bone marrow or haemoglobin metabolism in such group of rats. Similarly, saponin reported to be present in the extract [23] under study has been demonstrated to be haemolytic [24]. However, the significant increase ($p<0.05$) in RBC observed in the infected/treated group might be due to the ability of the extract to slow down the processes involved in oxidative breakdown of RBC membrane by the microorganism.

The significant increase ($p<0.05$) observed in the neutrophil level across all groups in this study is similar to the findings of [13] who reported a significant increase in all groups of rats treated with the leaf extract of *O. gratissimum*. However, this result differed from previous findings of [19] who reported a significant decrease in the level of neutrophils in Wistar albino rats when treated with leave extract of *P. crispum*. The neutrophils possess inconspicuous organelles known as primary and secondary granules which contain lytic enzymes and bactericidal substances. These granules help digest foreign materials after it is phagocytosed. They also use oxygen dependent and oxygen independent pathways that generate additional antimicrobial substances to kill ingested microorganisms [25]. Therefore, the increase observed in the neutrophil level was justified since it responds primarily to infection caused by bacteria.

Also, the insignificant change observed in the level of eosinophil and monocyte in this study agreed with the findings of [19] who reported a non-significant difference in the eosinophil levels in the Wistar albino rats administered the ethanolic leaf extract of *Petroselinum crispum.* Eosinophils are important in the defense against protozoan and helminth parasites mainly by releasing cationic peptides and reactive oxygen immediately into the extracellular fluid [25]. Hence, its non-significant change in infection involving bacteria as obtained in this study. In sub-acute oral toxicity study, non-toxic nature of extract is indicated by lack of significant changes in the blood parameters because it is one of the most sensitive targets of toxic substances [14]. Hence, this result suggests that the extract of unripe *A. muricata* did not interfere with the activities eosinophil and monocytes.

The significantly increase ($p<0.05$) in basophil level of rats administered only the extract of *A. muricata* is in agreement with the findings of [26] who reported that the basophil level of group of rats treated with aqueous fruit extract of *Solanum macrocarpum* increased significantly ($p<0.05$). Basophils are non-phagocytic cells that release specific compounds called vasoactive mediators from their cytoplasmic granules in response to different stimulations. They possess high affinity receptors for the type of antibody associated with allergic response [25]. Hence, the observed increase in basophil in this study is indicative of the likelihood of the fruit extract of *A. muricata* to possess allergic properties.

The WBC of the group of rats given the extract (group IV) and ciprofloxacin (group V) significantly reduced ($p<0.05$) after treatment. This result agreed with the works of [20] who reported a decrease in the WBC of Dutch white rabbits treated with ethanolic fruit extract of *Tetrapleura tetraptera.* However, this result differed from the findings of [18] who reported an increase in the level of WBC in rats given the extracts of *Voacanga africana*. Granulocytes regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBC [25]. Thus, some components of the extract might have interfered with the sensitivity of the stem cells responsible for the production of WBCs. The decrease in the WBC suggests an impairment in the ability of the extract of unripe *A. muricata* fruits to stimulate production of WBCs. However, the significant increase ($p<0.05$) in the group infected only with *S. typhi* was expected as literatures have shown that WBCs are produced rapidly in response to infection [25]. The insignificant difference observed in lymphocytes of rats given only extract (group IV) and infected/treated group (group V) could suggest the beneficial effect of the extract in improving the immunity and general well-being of the animals [2].

The result of the histopathological examination carried out on the liver, kidney and spleen of the experimental rats is indicated on Plates 1A-3B. The less congested parenchyma, more distinct

nuclei, improving polyhedral shape of the hepatocyte and the Kuffer's cell observed in the group of rats infected/ treated suggests that the extract had a restorative effect on the liver architecture. This result is in agreement with the findings of [20] who reported that the extract of *Tetrapleura tetraptera* did not reveal any gross damage to tissue of the liver in experimental animals that received the dose of the extract.

The more distinct glomerulus as well as the proximal and distal tubules filling the parenchyma observed in the histopathological examination of the kidney of infected/treated rat suggests that the extract of *A. muricata* partially improved the architecture of the kidney. The improved structure of the glomerula is in agreement with a previous report that the leaf extract of *Moringa oleifera* did not produce adverse effect on the kidney of experimental animals [27]. However, odematous space observed is indicative of adverse activity. This result is comparable with the findings of [28] who reported that standardized methanolic extract of *Mitragyna speciosa* revealed some abnormal morphological characteristics in all treated rats. The partial improvement in the kidney structure after treatment may indicate that the period of treatment was not sufficient for good protection/restoration against the adverse effect of infection. It is therefore suggested that a varied period of treatment should be employed in further studies. In addition, the gradual disappearance of inflammatory cells in the glomerulus in the treated rats as against the inflammed glomerulus observed in the rats infected with *S. typhi* may be due to the anti-inflammatory activity of terpenoids detected in the extract [29].

In this study, there was not much difference in the spleen architecture of rats in the control group compared to the rats infected/treated with the extract. This suggests that the extract has a protective effect on the largest lymphoid organ of the body. This result is in agreement with works of [30] who reported that the extract of *Moringa oleifera* leaves maintained the structural integrity of the spleen tissue of murine models used. This confirmed previous reports that *A. muricata* promotes immune responses [5]. However, the haemorrhage observed on the spleen of the rats infected/treated (Plate 3B) may be due to the mechanical injury done to the tissue during excision of the organ for histopathological examination. Therefore, it is opinioned that the protective effects of detoxification carried out by the liver may explain the preserved architecture spleen in this study.

5. CONCLUSION

This present study showed that the extract of unripe *Annona muricata* fruit has stimulatory effect on the blood production and possesses restorative effect on various organs examined in this study. However, excessive use might be toxic to heart. Therefore, it is recommended that further research should be carried out using varying concentrations of the extract in order to determine its dose dependent effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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