GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Evaluation of varying doses of ethanol leave extract of *senna occidentalis, annona muricata* and *aju-mbaise* on liver biochemical parameters and hematological indices in Wistar rats

Esther Ifeyinwa Etu [1], Nyejirime Young Wike [2,*], Kenneth Eze Etu [3], Ekoh Adaorah Jennifer [4], Uche Bethel Mmesomachi [5], Maria Chinedu Ugwuodo [6], Ezeobiora John Ozioma [5] and Achi Franklyn Ugochukwu [7]

[1] *Department of Anatomic Pathology, Faculty of Basic Clinical Sciences, Alex Ekwueme Federal University, Ndufu Alike, Ebonyi State, Nigeria.*
[2] *Department of Human Physiology, Faculty of Basic Medical Science, State University of Medical and Applied Science, Igbo-eno, Enugu State, Nigeria.*
[3] *Department of Pharmacology, Faculty of Basic Clinical Sciences, Ebonyi State University, Ebonyi State, Nigeria.*
[4] *Department of Histopathology, Enugu State Teaching Hospital, Parklane, Enugu, Enugu State, Nigeria*
[5] *Department of Medical Laboratory Science, Faculty of Health Science and Technology, University of Nigeria, Enugu, Enugu State, Nigeria.*
[6] *Department of Histopathology, Enugu State University of Science and Technology, Enugu State, Nigeria.*
[7] *Department of Morbid Anatomy, Faculty of Medical Laboratory Science, University of Nigeria Teaching Hospital Enugu.*

GSC Biological and Pharmaceutical Sciences, 2024, 29(03), 311-321

Publication history: Received on 03 November 2024; revised on 27 December 2024; accepted on 30 December 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.29.3.0471

Abstract

Purpose of the Research: This research evaluated the effect of varying doses of ethanol extract leaves of *Senna occidentalis*, *Annona muricata,* and *Aju-mbaise* on the liver biochemical parameters and hematological indices in Wistar rats.

Scope of the experiment: Twenty Wistar rats weighing 120 g to 150 g were used. They were distributed into four groups, with five rats each. Group one served as a control and was given water and normal rat chow. Groups 2, 3, and 4 were given *S. occidentalis, A. muricata,* and *A. mbaise* extracts for 30 days, respectively. At the end of the experiment, blood samples were collected for hematological and biochemical analysis. Liver tissue was also harvested for histopathological examination.

Result: There was a significant increase ($p < 0.05$) in the hematological parameters of rats that were administered *A. muricata and A. mbaise*. For the liver enzymes (ALT, AST, and ALP), there was no significant ($p < 0.05$) increase across the test groups when compared with the control. However, *A. mbaise* administration resulted in alterations in serum concentration of conjugated bilirubin and serum albumin when compared with other test groups and with the control. Histological examination of the liver tissue revealed that the extract of *A. muricata* resulted in liver toxicity.

Conclusion: The result implies that *A. mbaise* may lead to hemolysis, which can result in anemia as opposed to *S. occidentalis* and *A. muricata,* while *A. muricata* can result in liver toxicity.

Keywords: Hematological indices; Hemolysis, biochemical; *Senna occidentalis*; *Annona muricata; Aju-mbaise*

* Corresponding author: Nyejirime Young Wike

1. Introduction

The liver is known to be the major organ responsible for the metabolism of foreign chemical substances in the body. Most toxicity effect(s) associated with exposure to chemical agents and their metabolites are known to be indications of tissue, or tissue component-reactive metabolite species interactions in the body. However, the presence of antioxidants has been reported to provide a protective mechanism against different toxicity effects associated with the reactive species generated by the chemical substances in the body tissues [1]. While some antioxidants are endogenously generated within the body, others (including antioxidant vitamins) may be provided as micronutrients in the diets. According to literature reports, such antioxidant vitamins as ascorbate (vitamin C) and α-tocopherol (vitamin E) have been demonstrated to provide protection against chemicals-induced oxidative stress in different tissues via several mechanisms [2].

Plants are a source of natural ingredients that are widely used as medicines. The use of natural plants as treatments for a lot of diseases is on the increase. The phytochemical constituents of plants are responsible for their activities against several diseases; a lot of research has been carried out to identify the active compounds in plants and determine their pharmacological activities against diseases [3].

Annona muricata Lin., commonly called soursop is widely used as a traditional medicine for skin disease, respiratory disease, fever, bacterial infections, diabetes, hypertension, and cancer [3,4]. Different parts of *A. muricata* have different activities. The seeds combat parasitic infections; the fruit is used for the treatment of arthritis, nervous disorders, and diarrhea; and the leaves are used to treat cystitis, headaches, insomnia, and cancer [5]. The main active components of *A. muricata* are acetogenin, alkaloids, and flavonoids [6]. Analysis of the compounds in *A. muricata* leaf extract revealed secondary metabolites such as flavonoids, terpenoids, saponins, coumarins, lactones, anthraquinones, glycosides, tannins, and phytosterols [7].

Aju Mbaise is a polyherbal formulation used by many women to enhance labor, remove retained placenta after delivery, and manage pains from postnatal and menstrual cramps [8]. *A. Mbaise* got its name from Mbaise, a large community in Imo State, southeast Nigeria [9]. It is a herbal mixture that combines ginger roots, traditional leaves, uziza seeds, uda, and the bark of a special medicinal tree found in Mbaise. The plant is used for the treatment of diarrhea, dysentery, gonorrhea, wound healing, and respiratory tract infections [8; 10]. It has antimalarial, uterotonic, anti-inflammatory, and anti-anemic effects [11,12].

Senna occidentalis L. is a widely used plant in traditional medicine throughout the world [13]. This plant is frequently used to treat a variety of infections and other conditions like snake and insect bites [13–16]. In traditional medicine, all parts of the plant are used [14]. The plant possesses antioxidant, nephroprotective, and hepatoprotective activity; immunomodulatory activity; anti-diabetic activity; analgesic and antipyretic activity; anti-anxiety, antidepressant, and anti-mutagenic activity; as well as antibacterial and anti-fungal activity [17–20].

Despite their traditional use and anecdotal reports of therapeutic benefits, comprehensive scientific investigations comparing the effects of *Senna occidentalis*, *Annona muricata*, and *Aju-Mbaise* on the liver and hematological indices are lacking. Given the increasing interest in herbal remedies and the need for evidence-based healthcare practices, there is a growing imperative to evaluate the potential hepatoprotective and pancreatic effects of these herbal preparations using rigorous scientific methodologies.

2. Materials and method

2.1. Collection of plant material

The plant materials, *Senna occidentalis*, and *Annona muricata* were obtained from the botanical garden of the University of Nigeria Nsukka Enugu State. The leaves were identified and authenticated by a Taxonomist in Botany Department, University of Nigeria Nsukka while *Aju-mbaise* was purchased at international market Ebonyi State and was properly identified before use.

Experimental animals: Twenty (20) male and female Albino rats weighing between 120g and 150g were used for this study. They were purchased from animal house of the Department of medical laboratory science, Faculty of Basic medical Science, University of Nigeria Nsukka, Enugu State. The rats were weighed and acclimatized for seven days after which, they were distributed into four groups, with five rats per group. All the animals were allowed free access to water and feeds *ad libitum*.

Table 1 Distribution of rats into experimental groups

Group	Number of rats	Treatment
1	5	Control Group (No exposure) water + feed only
2	5	*Senna occidentalis* + water and feed
3	5	*Annona muricata* + water and feed
4	5	*Aju-mbaise* + water and feed

The extracts were administered orally for 30 days.

- **Preparation of Plant Extract:** The leaves of *S. occidentalis*, *A. muricata* and *A. mbaise* were properly dried at room temperature for two weeks and grounded into powdered form, using a grinding machine. It was soaked in ethanol for twenty-four (24) hours to obtain the active ingredients. After twenty-four hours, they were filtered, and the extracts were kept in the laboratory at room temperature for proper and easy evaporation of the ethanol. After the total evaporation of the ethanol, active ingredients were collected using spatula and after which, they were kept in a refrigerator for further use.
- ***Sample Collection****: At the end of the experimental period, the animals were starved for 24 hours after the last experimental treatment and were anaesthetized with chloroform in an anesthetic chamber. The animals were sacrificed using the cervical dislocation method. Blood samples were obtained by cardiac puncture from each rat by means of a 5 ml syringe and needle. Liver samples were collected and preserved in 10% formal saline for histological analysis.*
- **Hematological Assay:** The Red blood cell (RBC), Packed cell volume (PCV), White blood cell (WBC) count and Hemoglobin (HB) concentration were determined by the method of [22].

2.2. Liver Function Test Activity:

The activity of aspartate aminotransferase ((AST) and alanine aminotransferase (ALT) were assayed by the method of [23] as outlined in the Randox kit used while alkaline phosphatase (ALP) was assayed by the method of [24] as outlined in Randox kit, used.

2.3. Determination of Total Protein

The total protein content was determined using the method of [25].

Histological Analysis: The liver tissues were harvested from the *sacrificed animals. The harvested tissues were immediately fixed in 10% formal saline for twenty-four (24) hours. Automatic tissue processor was used to process the tissues.* The histology of the Liver was analyzed using Hematoxylin and Eosin staining method.

3. Result

Table 2 Comparative Effect of *Senna Occidentalis*, *Annona Muricata* and *Aju-Mbaise* on Hematological Parameters

GROUPS	RBC(µL)	WBC(µL)	HB (µL)	PCV (%)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group A (Control)	7.40±0.15	436.00±230.2	10.86±0.61	34.00±1.6
Group B *(Senna Occidentalis)* 200 mg/kg	7.18±0.35	432.00±363.3	10.48±0.41	34.4±3.3
Group C *(Annona Muricata)* 400 mg/Kg	7.00±0.16*A	430.00±158.1	11.76±0.47*B	35.8±0.8
(Group D *Aju-Mbaise*) 600 mg/kg	7.04±0.11	438.00±402.5	12.02±0.05*AB	36.4±1.1*AB

Results are expressed in Means ± SD; Level of significance for P value was set at $P < 0.05$

Table 3 Comparative Effect of *Senna Occidentalis, Annona Muricata* and *Aju-Mbaise* on Liver Enzymes

GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L) Mean	CREATININE (Mg/d L)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group A (Control)	37.600±5.18	31.600±2.07	37.690±10.074	0.312±0.091
Group B *(Senna Occidentalis)* 200 mg/kg	34.800±11.80	29.401±2.70	33.549±8.523	0.463±0.070*A
Group C *(Annona Muricata)* 400 mg/Kg	43.80O±3.13	32.200±2.17	38.885±5.699	0.489±0.809*A
(Group D *Aju-Mbaise*)600 mg/kg	34.200±8.22	30.400±8.22	32.329±3.251	0.489±0.809*A

Results are expressed in Means ± SD; Level of significance for P value was set at P < 0.05

Table 4 Comparative Effect of *Senna Occidentalis*, *Annona Muricata* and *Aju-Mbaise* on Differential White Count

GROUPS	Neutrophil (µL)	Lymphocyte (µL)	Monocyte (µL)	Eosinophil (µL)	Platelets (µL)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group A (Control)	55.8±2.8	33.2±2.4	2.4±0.5	2.4±0.5	96.4±1.5
Group B *(Senna Occidentalis)* 200 mg/kg	58.2±1.5	36.4±1.5*C	2.8±0.8	2.6±0.8	90.8±7.0
Group C *(Annona Muricata)* 400 mg/Kg	60.8±0.8*A	32.6±1.1	2.4±0.6	2.4±0.5	87±1.6*A
(Group D *Aju-Mbaise*)600 mg/kg	59.6±1.1*A	33.0±2.8	2.0±1.0	2.4±0.5	94.2±4.7

Results are expressed in Means ± SD; Level of significance for P value was set at P < 0.05

Table 5 Comparative Effect of *Senna Occidentalis*, *Annona Muricata* and *Aju-Mbaise* on Serum levels of bilirubin and protein

GROUPS	Conj. Bil. (mg/d L)	Total Bil. (mg/d L)	Total Protein (g/d L)	Albumin (g/d L)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group A (Control)	0.211±0.08	0.589±0.091	5.489±1.55	3.224±0.863
Group B *(Senna Occidentalis)* 200 mg/kg	0.291±0.08	0.562±0.206	6.045±0.607	3.422±0.660
Group C *(Annona Muricata)* 400 mg/Kg	0.540±0.08*ABD	0.557±0.716	5.670±0.671	1.857±0.464*ABD
(Group D *Aju-Mbaise*) 600 mg/kg	0.258±0.06	1.169±0.900	5.391±O.878	3.090±0.464

Results are expressed in Means ± SD; Level of significance for P value was set at P < 0.05

3.1. Histological effects of *Senna occidentalis, Annona muricata* and *Aju-mbaise* on the liver tissue of the Albino rats

3.1.1. LIVER

Group 1

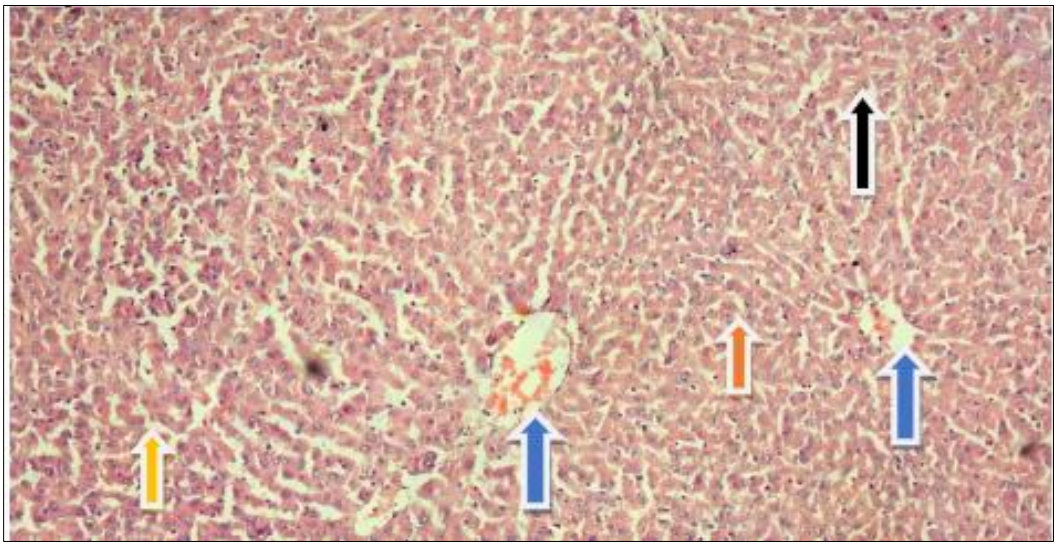

Figure 1 Plate IA: photomicrograph of the liver section from the control rat, stained with H and E showing normal hepatocytes (Magnification of x10)

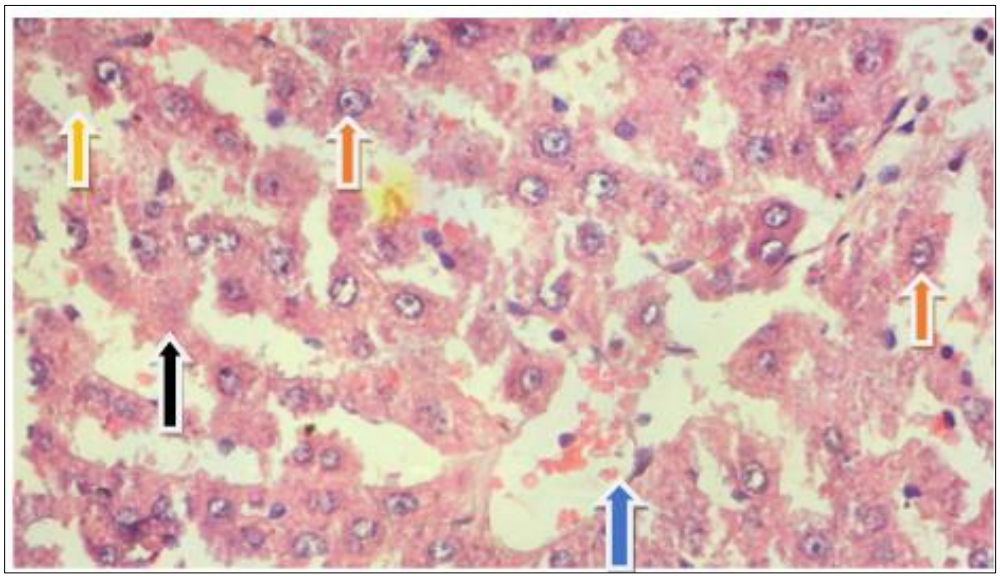

Figure 2 Plate IB: photomicrograph of the liver section from the control rat, stained with H and E showing normal hepatocytes (Magnification of x40)

Group 2

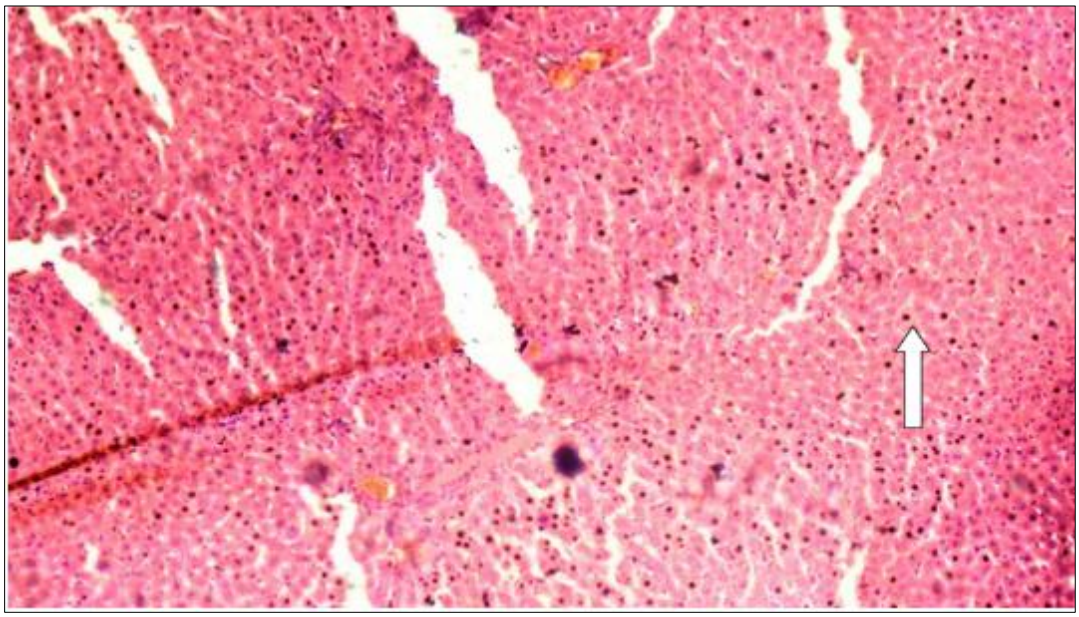

Figure 3 Plate 2A: Photomicrograph of a section of liver of Albino rat, using H and E stain; and a magnification of x10. This result shows normal liver

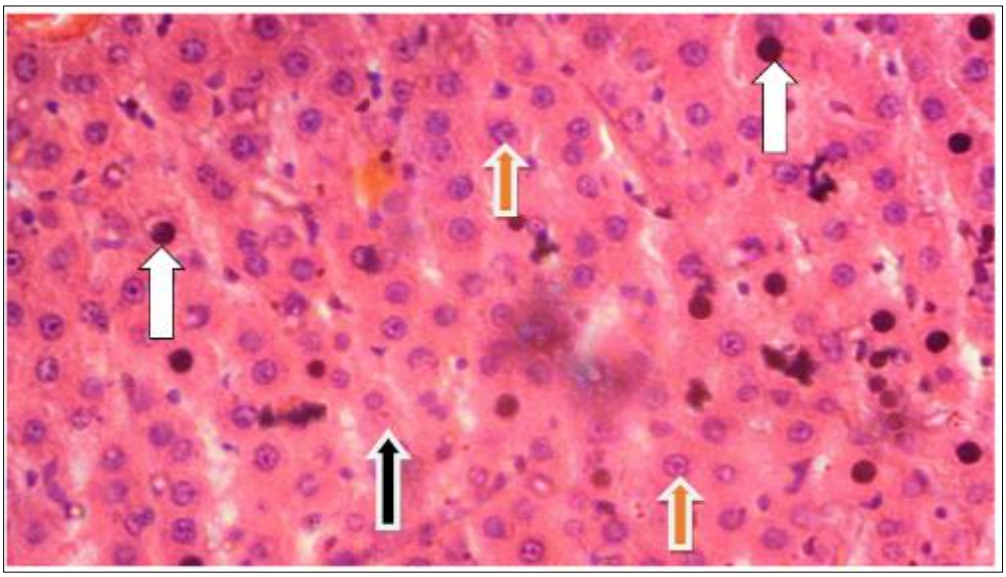

Figure 4 Plate 2B: Photomicrograph of a section of liver of Albino rat, using H and E stain; and a magnification of x40. This result shows normal hepatocytes

Group 3

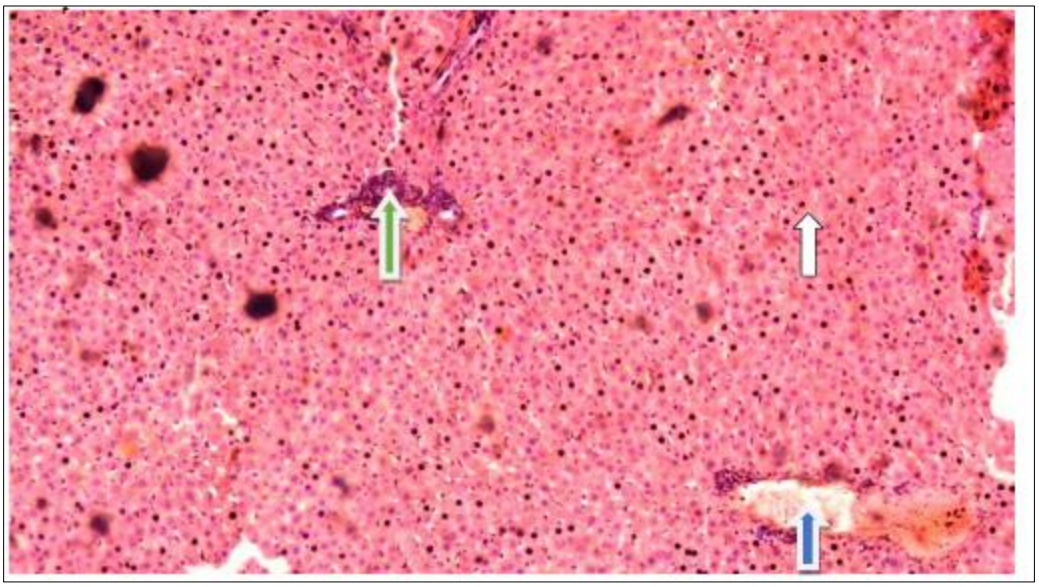

Figure 5 Plate 3A: Photomicrograph of a section of liver of Albino rat, using H and E stain; and a magnification of x10. This result shows hepatitis

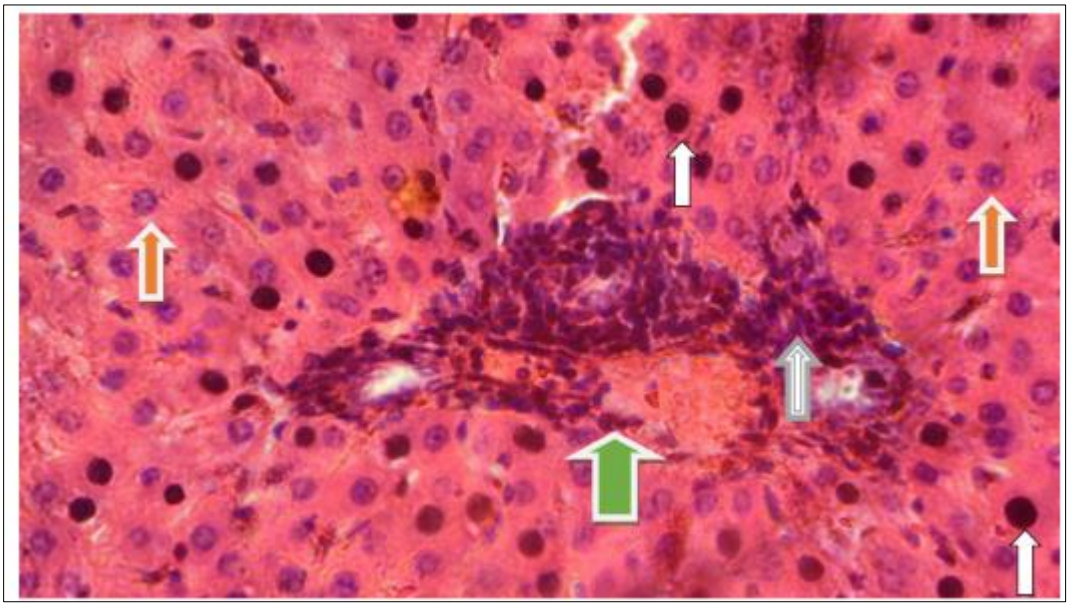

Figure 6 Plate 3B; Photomicrograph of a section of liver of Albino Rat, using H and E stain; and a magnification of x40. This result shows hepatitis

Group 4

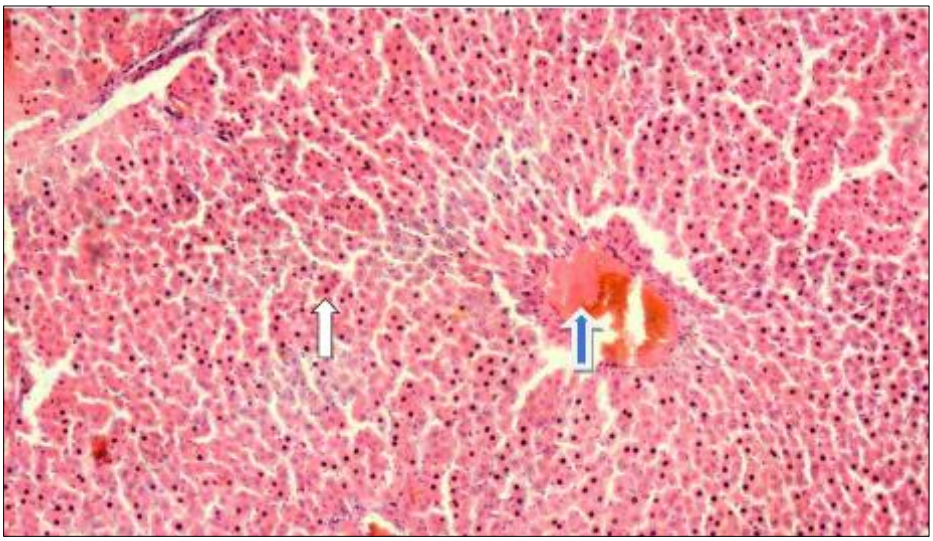

Figure 7 Plate 4A: Photomicrograph of a section of liver of Albino Rats, using H and E stain; and a magnification of x10. This result shows normal liver

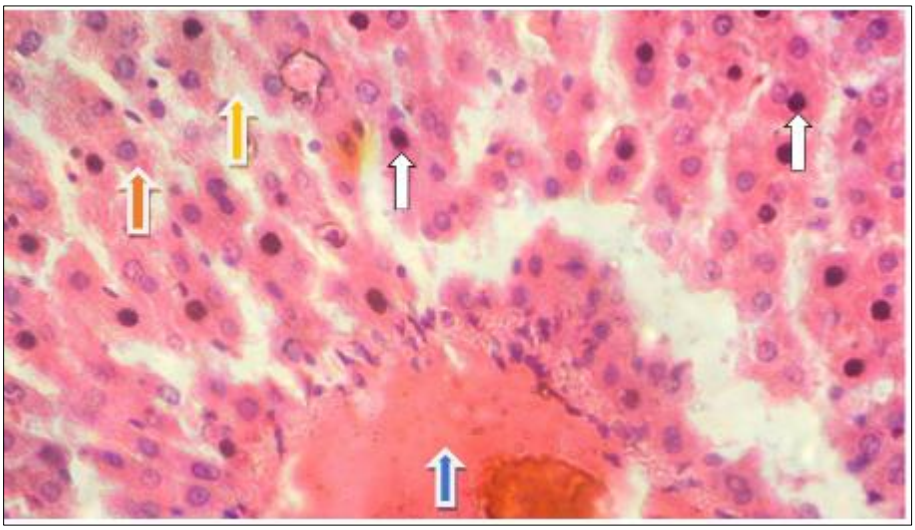

Figure 8 Plate 4B; Photomicrograph of a section of liver of Albino Rats, using H and E stain; and a magnification of x40. This result shows normal liver

	Normal hepatocyte
	Hepatocyte with mitotic figure
	Hepatic plate
	Central vein
	Lymphocyte
	Portal tract
	Hepatic sinusoid

4. Discussion

The comparative effects of *Senna occidentalis, Annona muricata* and *Aju-mbaise* on hematological indices, biochemical status and the liver of albino rats were assessed in this study. There was no statistically significant difference ($P < 0.05$) in the WBC and PCV of all the test groups when compared with the control. This suggests that the extract of the plants in this study has the capacity of maintaining the integrity of the immune system. The RBC count in test Group C revealed a significant reduction ($P < 0.05$) which indicates that the plant extract was able to hemolyze RBCs which can result to anemia. This implies that caution should be taken on consumption of *A. muricata* especially with respect to the quantity. However, the result obtained from this study on the effect of *A. muricata* on RBCs contradicts the research carried out in a documented literature on the effect of *A. muricata* on RBC. Their result proved that the extract of the plant was able to maintain the concentration of RBCs [26].

On the other hand, test Group D was able to cause a significant increase ($P < 0.05$) in hemoglobin concentration and also the PCV. This implies that extract of *A. mbaise* can counter the hemolytic effect caused by *A. muricata* on the RBCs. This supports the finding that *A. mbaise* attenuates changes in hematological indices against dutasteride-induced biochemical and hematological changes in rats [27]. Another study also confirmed that *A. mbaise* is a hematopoietic agent since it has the tendency to synthesize blood cells in high-fat diet/streptozotocin-induced diabetic Wistar rats treated with Ethanol extract of herbal mixture of *A*. *mbaise* [28]. There was no significant difference ($P < 0.05$) observed with respect to the extract of *S. occidentalis.* This confirms the ability of *S. occidentalis* to protect against anemia by maintaining the concentration of RBCs [29].

The mean serum concentration of AST, ALT and ALP showed a non-statistically significant ($p>0.05$) decrease among all the test groups (Groups B, C and D) when compared with the control (Group A). This implies that the extract of the plants *S. occidentalis, A. muricata* and *A. mbaise* may likely not cause liver damage suggesting its potential against liver toxicity. The result agrees with the study done by Hauwa et al., **[**30]. In their study, it was observed that there was no significant ($P>0.05$) increase or change occurred on the serum liver enzyme, and kidney function when compared with control. Meanwhile, salient mild effect on the liver and kidney was observed from the histopathological examination. However, the result obtained from this study opposes the findings that administration of *A. mbaise* elevated the serum levels of ALT and ALP in dyslipidemic female wistar rats [31].

The reduction noticed in the lymphocyte concentration of the group administered *A. muricata* is an indication that the plant extract may be suppressing the immune system, leading to a decrease in lymphocyte production or increased lymphocyte destruction. It also suggests that the administration of *A. muricata* extracts could be toxic to the lymphocytes, causing cell death and a subsequent decrease in lymphocyte count. The significant increase in the neutrophil level may indicate that the extracts of *A. muricata* and *A. mbaise* triggered an inflammatory response owing to the fact that neutrophils are the first line of defense against infection and inflammation. A significant decrease ($P < 0.05$) was noticed in the test group that received *A. muricata* extract indicating that the extract may be suppressing bone marrow function, leading to a decrease in platelet production.

The statistically significant increase in the serum concentration of conjugated bilirubin observed in test Group C is an indication that the extract of *A. muricata* may be inhibiting the enzymes responsible for breaking down bilirubin, leading to an accumulation of conjugated bilirubin. Also, it could indicate liver toxicity. The statistically significant decrease also observed in serum albumin level of test Group C indicates that *A. muricata* is toxic to the liver [32].

5. Conclusion

The result obtained from this study revealed that *A. Mbaise* had a more detrimental effect on hematological indices as well as the biochemical status of the rats when compared to *A. muricata* and *S. Occidentalis*. It also resulted to hemolysis of the red blood cells and also caused liver toxicity.

Compliance with ethical standards

Disclosure of conflict of interest

There is no competing interest

Statement of ethical approval

Ethical clearance for this study was obtained from the research and ethics committee of the college of Medicine, University of Nigeria, Enugu.

Authors' Contribution

EIE wrote the original draft. NYW conceptualized the methodology and formal analysis of the data. KEE collected the data. EAJ reviewed and edited the literature. UBM was involved in project administration. MCU carried out the LD_{50}. EJO validated the results. AFU was involved in the field work. All the authors read and approved the manuscript.

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