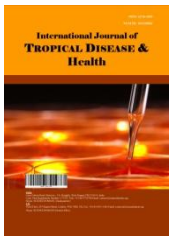

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Effect of HB Cleanser® Bitters on Haematological Parameters in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author SOA designed, supervised and wrote the protocol while author OSO carried out the study. Both authors read and approved the final manuscript.

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ABSTRACT

The public advertisement with indiscriminate sale of herbal bitters has led to increase in consumption without adequate information on the safety profile of the product. Thus, this study aimed to investigate the effects of HB cleanser® bitters (a polyherbal mixture composing of *Aloe vera, Acinos arvensis, Moringa oleifera, Chenopodium murale* and *Cinnamomum aromaticum*, *Allium sativum* on haematological parameters in Wistar rats.

Twenty-eight animals were randomly allotted into 4 groups comprising 7 animals each. HB cleanser® bitters was administered at doses of 1 ml/kg, 1.03 ml/kg and 1.29 ml/kg according to the manufacturers recommendation while the fourth group was administered with normal saline orally for 28days consecutively. Feed and fluid intake were measured daily. Haematological parameters (PCV, RBC, WBC, HB, platelet, neutrophil, lymphocyte, monocyte, eosinophil, and basophil) were evaluated. Flavonoids, saponins and cardiac glycosides were revealed as the phytoconstituents. The results showed that the HB cleanser bitters at 1.03 ml/kg significantly ($P<0.05$) increased the WBC ($17.00 \pm 3.22 \times 10^9$/L), while there was a non-significant (($P>0.05$) decrease in RBC, PCV and Hb ($4.88 \pm 0.14 \times 10^{12}$/L, 44.40 ± 1.17%, 14.54 ± 0.55 g/dl) when compared to control. A non-significant increase in platelets occurred at all doses. The findings of this study have shown that HB cleanser® bitters caused an increase in white blood cell count with a reduction in haemoglobin concentration and red blood cell count hence, it should be taken with caution.

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1. INTRODUCTION

There has been a constant and substantial growth in global interest in the use of herbal medicines in treatment of ailment for more than three decades [1]. Herbal medicines could be classified as finished herbal product, herbs and herbal medicinal preparations and are now being exported to foreign countries. In addition, it's estimated that 80% of the world population use some form of herbal medicine [2]. This increase in patronage and consumption might be due to the claim of the manufacturers of these herbal products that these products are effective against several pathological conditions, intense advertisements and the fact that people believe that herbal medicines are safe and devoid of adverse effect because they are made from natural products which in most cases may not be completely true. This is because some of the components of this products may likely be toxic on their own not to talk of interactions that may arise when combined with other plants. Poor adherence to good manufacturing practice (GMP) may also contribute to increased toxicity, as the dosages and formulations are not standardized [3]. Furthermore, herbs have been reported to produce a range of adverse effect such as necrosis, life threatening conditions and even death. Several cases of poisoning due to plants have also been reported [4]. Consumption of herbal medicines have also been associated with incidence of liver toxicity [5]. The blood of an animal is able to reflect the pathological state of an animal after exposure to a toxic substance [6]. Alterations in haematological indices helps in the assessment of the health status and evaluation of stresses due to environmental, nutritional with or without pathological factors [7]. Thus, herbal medicines need to be subjected to standardization requirements and tests so as to ensure strict adherence to the regulatory standards on quality, efficacy and safety and to protect the health and general wellbeing of the public. HB cleanser® bitters is an herbal mixture manufactured by Luckystar global natural health care company, Nigeria Limited. It has been claimed to promote detoxification of toxins, improve digestion and treatment of constipation, malaria, typhoid, rheumatism, arthritis, painful menstruations, waist pain, joint pain, general body weakness and reduction of blood sugar.

HB cleanser® bitters is composed of *Aloe vera* (25%), *Acinos arvensis* (20%), *Moringa oleifera* (10%), *Allium sativum* (15%), *Chenoppodium* murale (15%), *Cinnamomum aromaticum* (15%). *Aloe vera* is used for treatment of digestive system ailments such as constipation, irritable bowel syndrome and ulcerative colitis [8]. *Acinos arvenisis* commonly known as Basil thyme is reported to improve digestion with good antioxidant activity and anti-bacterial activity [9]. *Moringa oleifera* is used as an antioxidant due to its polyphenolic constituent [10]. *Allium sativum* has anti-bacterial activities [11]. *Chenopodium murale* (nettle leaf goosefoot) has been reported to possess anti- bacterial activities [12]. *Cinnamomum Aromaticum* is used in the treatment of type 2 diabetes [13]. Hence, this study investigated the effect of HB cleanser bitters on haematological parameters in Wistar rats.

2. MATERIALS AND METHODS

2.1 Herbal Bitters Sample

HB cleanser® bitters was purchased from the Luckystar global sales office at Mile 3, Port Harcourt, Rivers State.

2.2 Experimental Animals

Adult Wistar rats (46) of both sexes were purchased from the animal house of the Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria. The rats were placed in cages in a well-ventilated room and were fed ad-libitum with standard feed and water for two weeks while under acclimatization and throughout the study period.

2.3 Phytochemical Screening

Phytochemical evaluation was conducted on HB cleanser® bitters to determine the phytochemical constituents of its components [14].

2.4 Experimental Protocol

Acute toxicity:

Eighteen albino rats of both sexes were used to assess the acute toxicity of HB cleanser bitters in accordance with Lorkes method [15]. The animals were grouped into two groups of 9

animals each. The 9 animals in the first group were subdivided into 3 groups of 3 animals each and were administered with 10, 100 and 100 mg/kg of HB cleanser bitters through the oral route after an overnight fasting. The animals were observed closely for twenty-four hours for any signs of toxicity or death, and the results obtained were recorded. The 9 animals for the second phase were grouped into three groups of 3 animals each also and treated with 1600, 2900 and 5000 mg/kg of HB cleanser bitters respectively.

Calculation of dose:

Density of HB cleanser bitters were determined at (20^0C) using 25ml pycnometer.

Density of product =weight of the HB cleanser bitters/volume.

Density of HB cleanser bitters= 26.3535g/25ml HB cleanser bitters.

Density of HB cleanser bitters=1.05415g/ml.

The above calculation was used to determine the required volume of the HB cleanser bitters to be administered.

2.5 Sub-acute Toxicity Study

Twenty -eight male Wistar rats were weighed and divided into 4 groups of seven animals. Group A received normal saline while groups B, C and D were administered with 1, 1.03 ml/kg and 1.29 ml/kg of HB cleanser® bitters calculated according to the manufacturer's dosage recommendation. The body weights of the animals were assessed using a sensitive weighing balance before the commencement of dosing and then weekly throughout the duration of the experiment. The feed and fluid consumption of the animals were also monitored and recorded during the study period. The animals were observed for clinical signs of toxicity such as weakness or aggressiveness, food refusal, loss of weight, diarrhea, discharges from the eyes and ears, noisy breathing and mortality [16].

2.6 Blood Sample Collection and Preparation

The animals were weighed and sacrificed under diethyl ether anaesthesia at the end of the study. Blood sample was collected through cardiac puncture into anti-coagulant bottles containing EDTA for haematological assay vis: red blood cell, platelet, packed cell volume, haemoglobin, white blood cell and the differentials count.

Red blood cell (RBC):

A 1:200 dilution of blood was made in red blood cell dilution fluid, mixed and allowed to stand for 5minutes. The suspension was mixed and put into the counting chamber filled with mixed cover slip which was then read and counted with a magnification of ×10 [17].

Packed cell volume (PCV):

The uncoagulated blood was put into a capillary tube and the end was sealed with plasticine, centrifuged in an haematocrit for five minutes at 1000rpm and then read with a haematocrit reader in percentage [17].

Reading =Cell×10^{12}/L.

Haemoglobin (Hb): Cyanomethaemoglobin Method:

0.02 ml of blood was added to 5ml of Drabkins solution, mixed and allowed to stand for 10 minutes for full color development. The haemoglobin level was read in a calorimeter at 546 nm wavelength against a blank Drabkins solution.

Calculation =Absorbance of test /Absorbance of standard ×Concentration of the standard.

White blood cell:

A 1:20 dilution of blood in WBC fluid was made (Turks solution), mixed and allowed to stand for five minutes. The solution was mixed to homogeneity and put into the counting chamber. The white blood cells were read in the four big squares while the number of cells was calculated as N/20×10^9cell /per.

Where

N =number of cells in four square [17].

Red blood cell indices:

Mean corpuscular haemoglobin was calculated as: MCH=Hb (g/L)/RBC (million /ul).

Mean corpuscular haemoglobin concentration was calculated using: MCHC=Hb (g/dl)/Hct (%).

Mean corpuscular volume was calculated by: MCV (fl) = Hct (L/L)/RBC($\times 10^{12}$/L) $\times$1000.

Platelet count:

A 50 ul EDTA blood is mixed in 950ul of 1% ammonium oxalate dilution solution (1:20) in platelet unopette system. The mixture was allowed to stand for 5minutes for the erythrocytes to be completely lysed. Then the suspension was thoroughly mixed and put into a counting chamber. The chamber was then left in a moist environment for 20 to 30 minutes, after which, 80 small squares were counted, and the number of platelets calculated per ml ($\times 10^9$/L of blood [17].

2.7 Statistical Analysis

Values were expressed as Mean ±SEM, Data was statistically analyzed using one- way analysis of variance (ANOVA) followed by Post hoc test with SPSS version 15.0. P-values were considered statistically significant at a value of P<0.05.

3. RESULTS

3.1 Phytochemical Screening

Preliminary phytochemical screening of the HB cleanser® bitters revealed the presence of saponin, flavonoid, cardiac glycoside and carbohydrate. However, free and combined anthraquinone, alkaloid, purine alkaloid, tannin and phlobatanin were absent.

3.2 Acute Toxicity Test

The acute toxicity test result of the HB cleanser® bitters showed that the mean lethal dose LD_{50} is greater than 5000 mg/kg as no death was recorded above this dose.

3.3 Effects of HB Cleanser® Bitters on Haematological Parameters

Effects of HB cleanser bitters on body weight:

There was a significant increase in the body weight of all treated groups when compared to the control in Table 1.

Effects of HB cleanser® bitters on fluid intake:

There was a statistically significant difference (P<0.05) in fluid intake in all the HB cleanser bitters groups even though the pattern was not definite (Table 2).

Effects of HB cleanser bitters on feed intake:

There was a significant difference (P>0.05) in feed intake in groups fed HB cleanser bitters when compared to the control. However, a decrease in feed intake was observed in the group treated with 1.03 ml/kg HB bitters on the 21st and 28th day of treatment.

Effects of HB on haematological parameters:

The results showed that the bitters significantly (P<0.05) increased the white blood cells at all the examined doses (1, 1.03 and 1.29 ml/kg), while there was a non-significant ((P>0.05) decrease in red blood cells, packed cell volume and haemoglobin when compared to the control (Table 4). The neutrophils count showed an increase at doses of 1.03 and 1.29 ml/kg, while the level of lymphocytes increased at 1 and 1.29 ml/kg. A significant (P<0.05) decrease was observed in the level of the monocytes while basophils was completely absent as shown in Table 5. A non-significant increase occurred in the levels of platelets, mean corpuscular haemoglobin (MCH), mean cell volume (MCV) and mean corpuscular haemoglobin concentration. When compared to the control (Table 6).

Table 1. Effects of HB cleanser bitters on body weight

Group	Dose (ml/kg)	Days				
		0	7	14	21	28
Control	5	179.70 ±2.05	188.40 ±3.51	192.00 ±3.01	183.30 ± 3.41	197.20 ± 4.01
HB cleanser	1	198.60 ±1.07*	212.00 ±3.06**	223.70 ±3.71**	222.40 ± 4.01**	233.10 ± 4.61**
HB cleanser	1.03	203.10 ±2.34*	197.90 ±4.00**	207.70 ±4.32*	219.90 ± 3.87**	208.40 ± 5.38**
HB cleanser	1.29	215.60 ±3.21*	209.50 ±2.13**	219.70 ±4.52*	224.10 ± 4.01**	225.30 ± 4.21**

*Values are expressed as Mean±SEM, n=5, Significance=*P<0.05, **P<0.01*

Table 2. Effects of HB cleanser® bitters on fluid intake

Group	Dose (ml/kg)	Days			
		7	14	21	28
Control	5	250.00 ± 3.51	117.10 ± 3.72	88.00 ± 3.08	80.00 ± 3.98
HB cleanser	1	221.40 ± 4.31**	224.30 ± 3.21**	215.70 ± 3.61**	224.30 ± 3.21**
HB cleanser	1.03	218.60± 4.32**	167.10 ± 4.02**	210.00 ± 4.01**	211.40 ± 3.81**
HB cleanser	1.29	241.40 ± 4.51*	179.60 ± 3.09**	195.70 ± 4.32**	202.90 ± 3.42**

*Values are expressed in Mean±S.E.M, n=5, Significance= *P<0.05, **P<0.01*

Table 3. Effects of HB cleanser® bitters on feed intake

Group	Dose (ml/kg)	Days			
		7	14	21	28
Control	5	196.30 ± 4.21	194.90 ± 3.80	168.00 ± 4.05	231.00 ± 3.05
HB cleanser	1	172.00 ± 3.10*	221.20 ± 4.50*	158.80 ± 3.47*	223.40 ± 4.01*
HB cleanser	1.03	194.00 ± 2.07*	208.80 ± 3.09*	180.00 ± 4.57*	167.80 ± 2.98*
HB cleanser	1.29	175.00 ± 3.01*	219.20 ± 3.41*	194.50 ± 4.09*	225.50 ± 3.07*

*Values are expressed in Mean± S.E.M, n=5, Significance= *P<0.05*

Table 4. Effect of Hb cleanser bitters on haematological parameters

Group	Dose (ml/kg)	RBC ($x10^{12}$/L)	Hb (g/dL)	PCV (%)	WBC ($x10^{9}$/L)
Control	5	5.22 ± 0.13	15.20 ± 0.53	46.40 ± 0.92	11.98 ± 0.92
HB cleanser	1	4.92 ± 0.07*	14.96 ± 0.26 *	44.80 ± 0.58 *	13.58 ± 2.38 *
HB cleanser	1.03	4.88 ± 0.14 *	14.54 ± 0.55 *	44.40 ± 1.17 *	17.00 ± 3.22 *
HB cleanser	1.29	4.78 ± 0.02*	14.64 ± 0.08 *	43.80 ± 0.20 *	13.04 ± 1.52*

*Values are expressed as Mean ± SEM, n=5, Significance = *P < 0.05, Red blood cell (RBC), Hb (Haemoglobin), Packed cell volume (PCV), White blood cell (WBC)*

Table 5. Effect of HB cleanser bitters on haematological parameters

Group	Dose (ml/kg)	%				
		N	L	M	E	B
Control	5	12.40 ± 1.47	83.20 ± 2.06	4.00 ± 0.63	2 ± 0.00	-
HB cleanser	1	9.60 ± 1.94 *	88.40 ± 1.07 *	2.40 ± 0.40 *	-	-
HB cleanser	1.03	13.60 ± 2.79 *	81.20 ± 4.04*	4.00 ± 1.04 *	2.50 ± 0.45 *	-
HB cleanser	1.29	14.00 ± 2.29 *	84.4 ± 1.73 *	2.67 ± 0.52 *	-	-

Values are expressed as Mean ± SEM, n=5, significance= P< 0.05, Neutrophil (N), Lymphocyte (L), Monocyte (M), Eosinophil (E), Basophil (B)*

Table 6. Effect of HB cleanser bitters on haematological parameters

Group	Dose (ml/kg)	Platelets	MCH (pg)	MCV (fl)	MCHC(g/dl)
Control	5	305.80 ± 32.38	29.44 ± 1.21	89.98 ± 1.18	32.56 ± 1.42
HB cleanser	1	419.00 ± 27.7**	30.42 ± 0.87 *	91.08 ± 1.68 *	33.38 ± 0.68 *
HB cleanser	1.03	360.20 ± 18.88 *	29.78 ± 1.31 *	91.02 ± 0.61*	32.70 ± 1.46 *
HB cleanser	1.29	334.60 ± 21.04 *	30.62 ± 0.45 *	91.66 ± 1.42*	33.44 ± 0.29 *

*Values are expressed as Mean± SEM, n=5, significance = *P < 0.05, Mean corpuscular haemoglobin (MCH), Mean cell volume (MCV) and Mean corpuscular haemoglobin concentration (MCHC)*

4. DISCUSSION

Toxicity studies of medicinal plants products are usually evaluated in animals and the findings provides information on what may likely happen in humans due to close similarity in physiological system [18].

There was no uncoordinated muscle movement, sedation or excessive salivation during the acute toxicity study except for a slight increase in watery stool at a dose of 5000 mg/kg and no mortality was recorded. There was a significant increase in feed consumption in animals administered with HB cleanser ®bitters which led to a corresponding increase in the body weight of the animals. This corroborates the reports of Anionye et al. [19], which stated that rats fed with super bitters consumed a higher amount of feed than the control. The variation in feed

consumption is a reflection of the appetite stimulating activity of HB cleanser bitters which may also likely be responsible for the increase in body weight of the animals.

Blood components such as red blood cells, white blood cells, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are essential in toxicity evaluation or health status determination of living organisms [20].

The toxicity of herbal bitters mixture naturally leads to a mild or severe reduction in haematological parameter either through direct effect or lysis of blood cells. However, if not toxic, the haematological indices will either be maintained or increased especially for those implicated in boosting immunity [21].

There was a reduction in the levels of red blood cell, haemoglobin and packed cell volume.

The reduction in the levels of red blood cell and haemoglobin would reduce the oxygen content of the blood resulting in decreased oxygenation of the tissues, cells and organs which may ultimately affect several physiological functions [22].

The increase in the white blood cell counts implied that the HB cleanser bitters may enhance immunity against diseases [23]. Packed cell volume is the percentage of red blood cells in blood [24] and is also involved in the transportation of oxygen and absorbed nutrients [22]. The observed decrease in packed cell volume may lead to anaemia due to reduced transportation of oxygen and absorbed nutrients.

The increase in lymphocytes can be linked to the observed increase in white blood cells count. Hemolytic anemia results in thrombocytosis. Therefore, the increase in blood platelets (thrombocytosis) may likely be due to iron deficiency [25]. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are all red blood indices used in the classification of anaemia [26].

The slight increase in mean corpuscular haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration is an indication of the haemoglobin content and size of a single red blood cell. Akinwumi et al, [27] stated that decreased levels of PCV and haemoglobin might be associated with oxidative stress. The findings of this study correspond to that of Patrick-Iwuanyanwu and Nkpaa [28], which reported that the prolonged use of Classic bitters (a polyherbal formular) may have adverse effects on haematological indices and Odangowei et al. [29], which stated that Ruzu herbal bitters at higher therapeutic dosages and prolong administration may result in renal, hepatic, cardiac and hepatobiliary challenges. The findings of this study have shown that HB cleanser bitters may adversely affect hematopoiesis which may predispose to anaemia.

5. CONCLUSION

This study suggests that HB cleanser bitters produced a reduction in haematological indices and care should be exercised with its consumption.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

A written approval was obtained from the Research ethics committee of the University of Port Harcourt according to international ethics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kokwaro JO. Medicinal plants of East Africa. 2nd ed. Nairobi: Kenya Literature Bureau; 1993.
2. Mukherjee PW. Quality control of herbal drugs: An approach to evaluation of

botanicals. New Delhi, India: Business Horizons Publishers; 2002.

3. Barnes J, Mills SY, Abbot NC, Willoughby M, Ernst E. Different standards for reporting ADRs to herbal remedies and conventional OTC medicines: face to face interviews with 515 users of herbal remedies. British Journal of Clinical Pharmacology. 1998;45:496-500.
4. Cosyns JP, Jadoul M, Squifflet JP, De Plaen JF, Ferluga D, Van Ypersele de Strihou C. Chinese herbs nephropathy: a clue to Balkan endemic nephropathy? Kidney Int. 1994;45:1680–1688. DOI: 10.1038/ki.1994.220
5. Sunday OO, Uguru MO, Ochigbo EA. The effect of aqueous extracts on *Momordica balsamina* on haematological and biochemical parameters in rats. Asian Journal of Pharmaceutical and Clinical Research. 2009;2:21-25.
6. Olafedehan CO, Obun AM, Yusuf MK, Adewumi OO, Oladefedehan AO, Awofolaji AO et al. Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits. Proceedings of 35th Annual Conference of Nigerian Society for Animal Production. 2010;212.
7. Afolabi KD, Akinsoyinu AO, Olajide R, Akinleye SB. Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake. Proceedings of 35th Annual Conference of Nigerian Society for Animal Production. 2010;247.
8. Seung Wook H, Jaeyoung C, Sunmin P, Hyun Jung L, Jong PI, Joo SK. Aloe vera is effective and safe in short-term treatment of irritable bowel syndrome: a systematic review and meta-analysis. Journal of Neurogastroenterology Motility. 2018; 24(4):1-8. Available:https://doi.org/10.5056/jnm18077
9. Khan N, Afaq F, Mukhtar H. Cancer chemoprevention through dietary antioxidants: progress and promise. Antioxidants and Redox Signaling. 2008; 10:475–510.
10. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. Antioxidants. 2017;6(91): 1-13. DOI:10.3390/antiox6040091
11. Karuppiah P, Rajaram SK. Antibacterial effect *of Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pacific Journal of Tropical Biomedicine. 2012;2(8):597-601. DOI:10.1016/S2221-1691(12)60104-X.
12. Al Marby A, Ejike CECC, Nasim MJ, Awadh Ali NA, Albadani RA, Alghamdi GMA et al. Nematicidal and antimicrobial activities of methanol extracts of 17 plants of importance in ethnopharmacology, obtained from the Arabian Peninsula. Journal of Intercultural Ethnopharmacology. 2016;5(2):114-121. DOI: 10.5455/jice.20160217040157.
13. Ranasinghe P, Galappaththy P, Constantine GR, Jayawardena R, Dhananjaya H, W Premakumara WS et al. *Cinnamomum zeylanicum* (Ceylon cinnamon) as a potential pharmaceutical agent for type-2 diabetes mellitus: study protocol for a randomized controlled trial. 2017;18 (446):1-8. DOI: 10.5455/jice.20160217040157
14. Sofowora A. A medicinal plants and traditional medicine in West Africa, 3rd ed. John Wiley and Sons Ltd. New York. 2008;200-203.
15. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54(4):275-287.
16. Aniagu SO, Nwinyi FI, Akumka DD, Ajoku GA, Dzarma S , Kazeem S et al. Toxicity studies in rats fed nature cure bitters. African Journal of Biotechnology. 2005; 4(1):72-78.
17. Baker FJ, Silverton RE .Introduction to medical laboratory technology. 6th Ed. 1985;320-330.
18. Ashafa AT, Olunu OO. Toxicological evaluation of ethanolic root extract of *Morinda lucida* (L.) Benth. (Rubiaceae) in male Wistar rats. Journal of Natural Pharmaceuticals. 2011;2:108-114.
19. Anionye JC, Onyeneke EC, Eze GI, Edosa RO, Agu KC, Omorowa EF et al. Evaluation of the effect of super bitters on Albino rats. International Digital Organization for Scientific Research Journal of Applied Sciences. 2017;2(1): 1-24.
20. Oyawoye BM, Ogunkunle HN. Biochemical and haematological reference values in normal experimental animals. New York: Masson. 2004;212-218.
21. Mendie UE. Yoyo bitters clinical report. Abllat Nigeria Company Limited. Lagos, Nigeria. (Ed.). 2009;1-5.

22. Isaac LJ, Abah G, Akpan B, Ekaette IU. Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria. 2013;24-27.
23. Soetan KO, Akinrinde AS, Ajibade TO. Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (*Sorghum bicolor*) Proceedings of 38th. Annual Conference of Nigerian Society for Animal Production. 2013;49-52.
24. Purves WK, Sadava D, Orians GH, Heller HC. Life: The Science of Biology (7th ed). Sinauer Associates and W. H. Freeman. 2003;954.
25. Meena AK, Naidu KS, Murthy JM. Cortical sinovenous thrombosis in a child with nephrotic syndrome and iron deficiency anaemia. Neurology India. 2000;48:292.
26. Ashafa AT, Sunmonu TO, Afolayan AJ. Effects of leaf and berry extracts of *Phytolacca dioica* L. on haematological and weight parameters of Wistar rats. African Journal of Pharmacy and Pharmacology. 2011;5:150-154.
27. Akinwumi KA, Osifeso OO, Jubril AJ, Omirinde J, Aboyewaa JA. Alteration of hematological profile and nephro-testicular damage in rats exposed to coatings of mobile charge cards. Scientific African. 2021;13:1-9 e00954.
28. Patrick-Iwuanyanwu KC, Nkpaa KW. Toxicity effect of sub-chronic oral administration of Class bitters® - A polyherbal formula on serum electrolytes and hematological indices in male Wistar Albino rats. Journal of Xenobiotics. 2015; 5(5369):20-23. DOI:10.4081/xeno.2015.5369.
29. Odangowei IO, Frank-Oputu A, Shonubi OO, Anani RO. Biochemical study on the effects of Ruzu herbal bitters formulation on Wistar Albino rats. Biomedical Journal of Scientific and Technical Research. 2022;41:32434-32439. DOI:10.26717/BJSTR.2022.41.006558.

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