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Erythropoietic and Hepatoprotective Potential of Ethanolic Extract of *Nauclea latifolia* in Mice Infected with *Plasmodium berghei berghei*

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Abstract *Nauclea latifolia* (NL) is widely used as decoctions or concoctions by indigenous tribes in Africa, particularly in Nigeria where it has been reported for its antiplasmodial activities among other properties. This study was carried out to investigate the effect of ethanolic extracts of the leaves on the haematological parameters and histomorphology of the liver of male Swiss albino mice infected with *Plasmodium berghei berghei (Pbb).* Thirty five mice weighing about 28g were divided into seven groups of five mice each. Group 1(control) was given normal saline 0.3 ml, Group 2 was passaged with *Pbb*, Group 3 was passaged with *Pbb* and treated with Coartem®, Group 4 was administered NL500mg/kg only, Group 5 was administered NL1000mg/kg only, Group 6 was passaged with *Pbb* then treated with NL500mg/kg, while Group 7 was passaged with *Pbb* then treated with NL1000mg/kg. *Pbb* was passaged intraperitoneally, while the test drug and extracts was given via orogavage once daily. On the 12th day, animals were humanely sacrificed; whole blood collected for haematological investigation, while the liver was processed for light microscopy. The result revealed that the extract exhibited a hepatoprotective and reversibility effects at a dose dependent level on the histological architecture of treated groups administered compared with the control, and also caused a significant ($P < 0.001$) reduction in the RBC parameters in a dose dependent manner especially in non-parasitized mice. In conclusion, acute toxicity test of ethanolic extract of *Nauclea latifolia* up to 5000mg/kg may be considered as relatively safe if mortality alone is the yardstick. However, at a dose of 1000mg/kg, it is severely hepatoxic in non-parasitized mice, yet the extract at 500mg/kg had beneficial effects in both the haematological indices and liver cytoarchitecture of parasitized host via a possible synergistic mechanism of its rich bioactive ingredients comparable with Coartem®.

Keywords: *malaria, Nauclea latifolia, Plasmodium berghei, liver, haematology*

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1. Introduction

Globally, an estimated 3.3 billion people were at risk of malaria in 2011, with populations living in sub-Saharan Africa having the highest risk of acquiring malaria: approximately 80% of cases and 90% of deaths are estimated to occur in the WHO African Region, with children under five years of age and pregnant women most severely affected [1]. Plants from different botanical sources have been used by many traditional medical practitioners in Nigeria for the treatment and cure of numerous diseases that are locally endemic [2]. The dangers associated with the potential toxicity of herbal therapies used over long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs [3]. *Nauclea latifolia* have been reported for its antiplasmodial activities [4,5,6,7,8,9,10] and a root method and composition for treating malaria under the US patent application [11]. Anti- inflammatory and analgesic activities in the leaves aqueous extract [12]; wound healing activity in stem bark with methanolic extract [13]; antimicrobial activities [14,15,16,17]; and then antidepressant/myorelaxant and anti-anxiety activities [18]. There has been no report on the effect of *Nauclea latifolia* on haematological indices as well as histomorphology of the liver in a parasitized mice model; hence this study was designed to contribute information in this area.

The determination of hematological indices provides physiological information on a proper blood assessment. Accurate laboratory determination of blood parameters remains the only sensitive and reliable foundation for ethical and rational research, diagnosis, treatment and

prevention of anemia. The major concern of the scientific communities with regard to medicinal plants and hematological studies focuses on the measures that can maintain a normal hematological state of being and reverse any negative hematological status associated with various anemic conditions [19].

2. Materials and Methods

2.1. Experimental Animals

Thirty five male Swiss mice were obtained from the animal holding facility of Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria and acclimatized for two weeks before the start of the experiment. They were allowed access to water and feed *ad libitum*. All procedures involving animals in this study conformed to the guiding principles in the care and use of animals [20] and the institution's code of ethics for the use of laboratory animals.

2.2. Plant Collection

Fresh leaves of *Nauclea latifolia* was obtained at the medicinal farm of the Department of Pharmacology and Toxicology, University of Uyo during the August to October period of 2012. They were identified and authenticated by the Curator at the Herbarium with voucher numbers UUH/67 (g) for *Nauclea latifolia* deposited.

2.3. Plant Extraction

Fresh leaves of *Nauclea latifolia* 1100g, was macerated in 96% ethanol (Sigma Aldrich, Germany) in a flat bottom flask in a Soxhlet extractor for 72 hours at room temperature. The cooled liquid extract was concentrated by evaporating its liquid contents in rotary evaporator. The dried ethanol extract was suspended in distilled water.

2.4. Parasite Inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.3 ml of infected blood containing about 1×10^7 *Plasmodium berghei berghei* parasitized erythrocytes. The inoculums consisted of 5×10^7 Plasmodium berghei berghei erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations [21].

2.5. Dosage

All extracts dosage was determined after toxicity test (LD50) Median lethal dose using the modified Lorke's method [22]. The 10% and 20% of the LD50 of the extracts was administered as low and medium doses.

Table 1. Experimental Design

Treatment Groups & Dosage (n=5)	Duration
1. Control (0.3ml) normal saline	11 days
2. PBB	11 days
3. PBB + Coartem®	6+5 days
4. NL500mg/kg	11 days
5. NL1000mg/kg	11 days
6. PBB + NL500mg/kg	6+5 days
7. PBB + NL1000mg/kg	6+5 days

Legend: NL – Nauclea latifolia, PBB – Plasmodium berghei berghei

2.6. Determination of Haematological Parameters

Blood was collected from the left ventricle of each animal in a vial containing 0.5M EDTA. Haematological indices were determined after Day 11 of treatment using an Automated Mindray BC-5300 Haematolog Analyzer Made in China at the University Of Uyo Teaching Hospital.

2.6.1. Tissue Collection

Each mouse was humanely sacrificed by chloroform inhalation and the liver was dissected, immediately weighed and rinsed with normal saline and fixed in 10% neutral buffered formaldehyde for light microscopy investigation [23].

2.6.2. Statistical Analysis

One way analysis of variance (ANOVA) was applied to compare the relationship of the groups, and Dunnett post-hoc test was used to compare the experimental groups and the control. All values were presented as mean ± standard error of mean (SEM), and values were considered significant at $p < 0.05$.

3. Results

Table 2. Phytochemical Constituents of *Nauclea latifolia*

Phytochemical	*Nauclea latifolia*
Alkaloids	++
Flavonoids	+++
Phlobatannins	++
Saponin (Frothings' test)	+++
Saponins (Fehlings' solution + NA_2CO_3)	++
Saponins (Fehlings' solution)	++
Tannins	++
Terpenes	+
Anthraquinones	-
Deoxy-sugar	-
Cardiac glycosides	-

Method: Trease and Evans, 1989 [24]
Key: + = lowly present, ++ = moderately present, +++ = highly Present, - = absent

3.1. Acute Toxicity Test (LD_{50})

Using the formula: $LD_{50} = \sqrt{ab}$; Where a = maximum dosage that results in 0% mortality, b = minimum dosage that results in 100% mortality.

Table 3. LD_{50} for *Nauclea latifolia*

Groups (n = 3)	Dosage (mg/kg)	Mortality	% Mortality
Group 1	1000	0/3	0
Group 2	3000	0/3	0
Group 3	3500	0/3	0
Group 4	4000	0/3	0
Group 5	4500	0/3	0
Group 6	5000	0/3	0

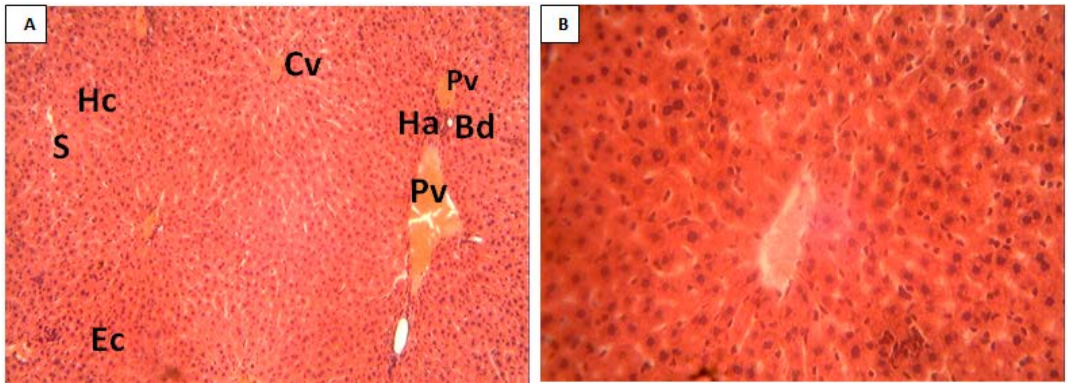
No mortality was recorded even as at 5000mg/kg, as such 10% and 20% of 5000mg/kg was adopted as effective dose according to Lorke's method [22]. 10% of 5000mg/kg = 500mg/kg = Low dose and 20% of 5000mg/kg = 1000mg/kg = Medium dose.

Table 4. Effect of the ethanolic extract of *Nauclea latifolia* on haematological parameters in male mice infected with Plasmodium berghei berghei

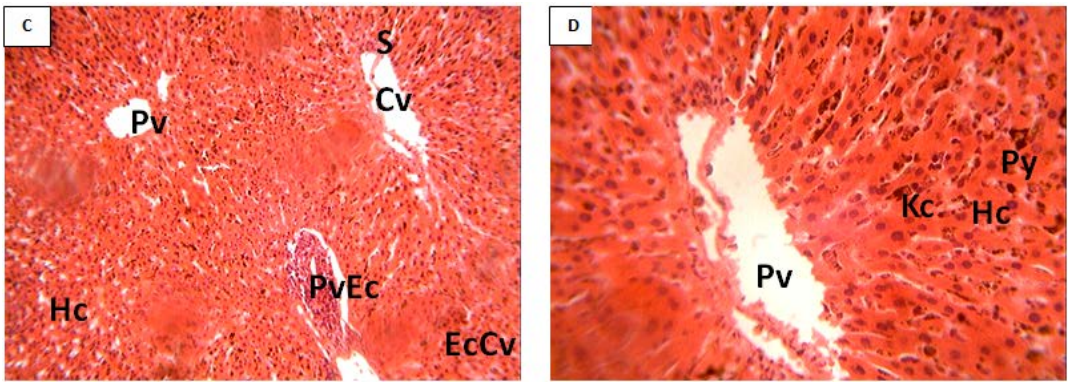
Groups	RBC	HB	PCV	MCV	MCH	MCHC	WBC	NEU	LYM	PLT
Group 1	7.21±	111.00±	40.10±	55.28±	15.40±	277.00±	7.27±	33.24±	61.32±	1086.28±
Normal	0.35	2.49	1.20	0.51	0.12	2.24	0.58	3.98	4.52	30.70
Saline 0.3ml										
Group 2	2.39±	41.00±	17.26±	73.50±	18.04±	243.40±	36.37±	48.06±	50.56±	441.00±
PBB only	0.73***	8.79***	4.11***	2.43***	0.99**	5.06**	11.32**	6.07*	6.66	106.07***
Group 3	6.72±	98.20±	35.12±	54.14±	14.36±	275.40±	28.16±	38.64±	60.00±	955.00±
PBB+	0.86	10.15	2.23	5.03	0.65	14.15	13.48*	8.78	9.36	93.24
Coartem®										
Group 4	3.85±	63.20±	22.50±	61.22±	16.32±	268.52±	24.62±	31.00±	69.00±	463.40±
NL500mg/kg	1.12**	16.08**	5.10**	2.56	0.33	8.40	2.62	4.57	4.57	78.85***
Group 5	1.85±	32.40±	12.62±	71.62±	18.28±	255.80±	45.49±	39.20±	60.00±	381.20±
NL1000mg/kg	0.09***	1.25***	0.64***	3.20**	0.69***	3.06	9.30***	2.24	1.92	55.78***
Group 6	5.28±	87.40±	29.12±	55.48±	16.50±	297.40±	12.49±	29.06±	70.32±	535.00±
PBB+NL 500mg/kg	1.15	18.51	6.03*	1.13	0.28	3.54	1.00	2.86	2.88	44.22***
Group 7	4.24±	68.40±	23.74±	62.36±	16.62±	274.20±	33.63±	17.20±	82.40±	766.20±
PBB+NL	1.23**	17.28**	5.05**	4.82	0.58	12.81	8.99**	3.26**	3.26**	31.50**
1000mg/kg										

Values in means + S.E. (Standard error), n = 5,*P < 0.05, **P < 0.01, ***P < 0.001, when compared with control.

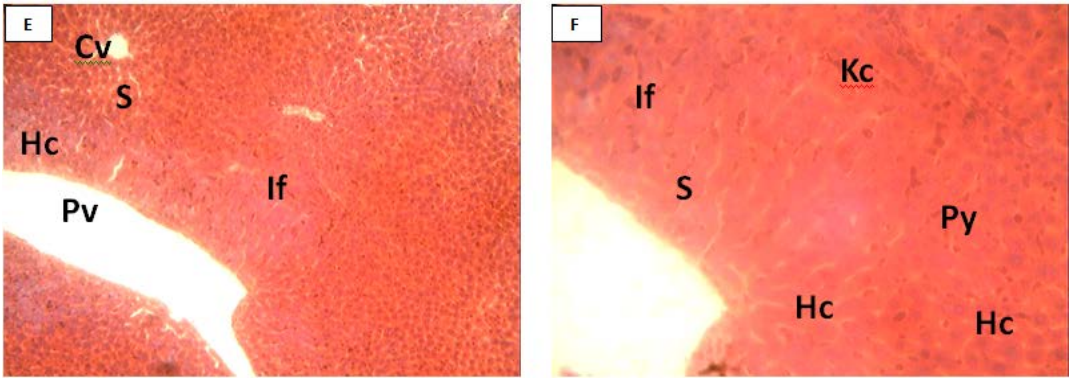
3.2. Photomicrographs of the Liver



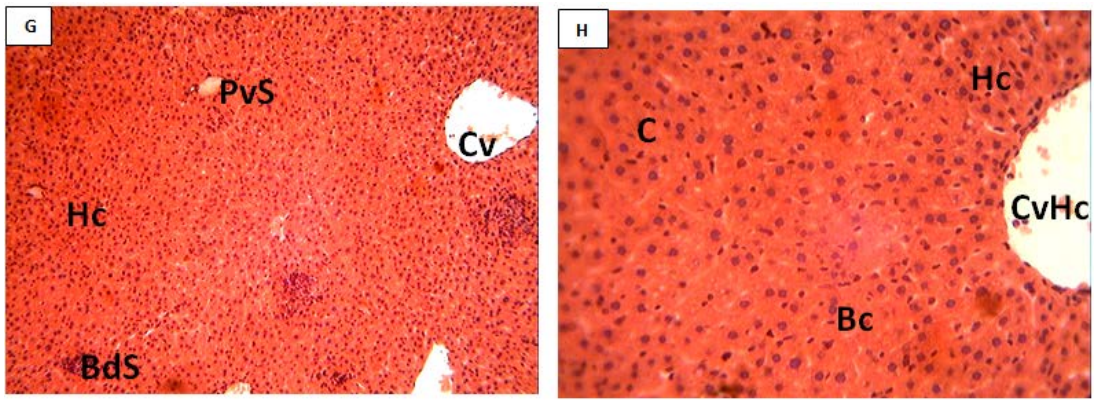
Group 1. A. Control – Liver X100 (H&E); B. Control – Liver X400 (H&E) (The micrographs show the Central vein (Cv), the plates of hepatic cells (Hc), Portal vein (Pv), Endothelial cells (Ec), Sinusoids (S). No abnormality seen)



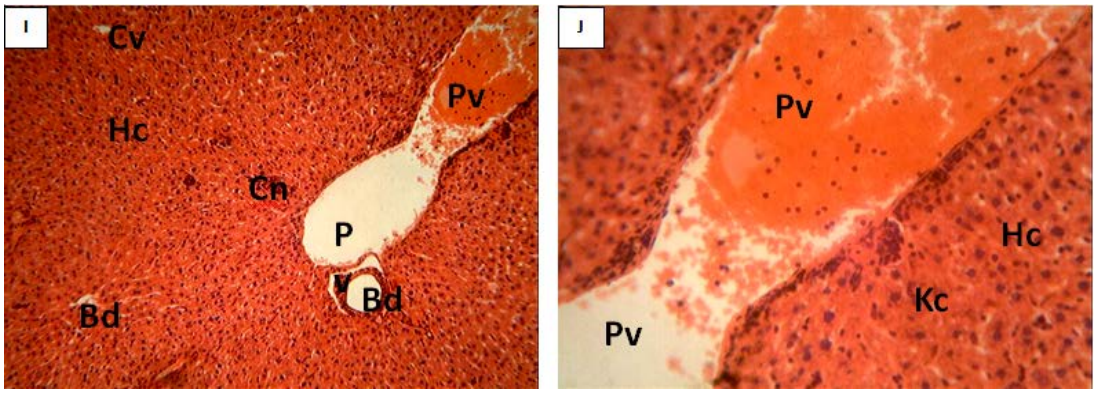
Group 2. C. Pbb Only – Liver X100 (H&E); D. Pbb Only – Liver X400 (H&E). (The micrographs show the Central vein (Cv), the Portal vein (Pv), plates of hepatic cells (Hc), sinusoids (S), Hypertrophy, Inflammation, Numerous Karyorrhectic hepatic cells (Kc) and Necrosis (N). Strongly affected)



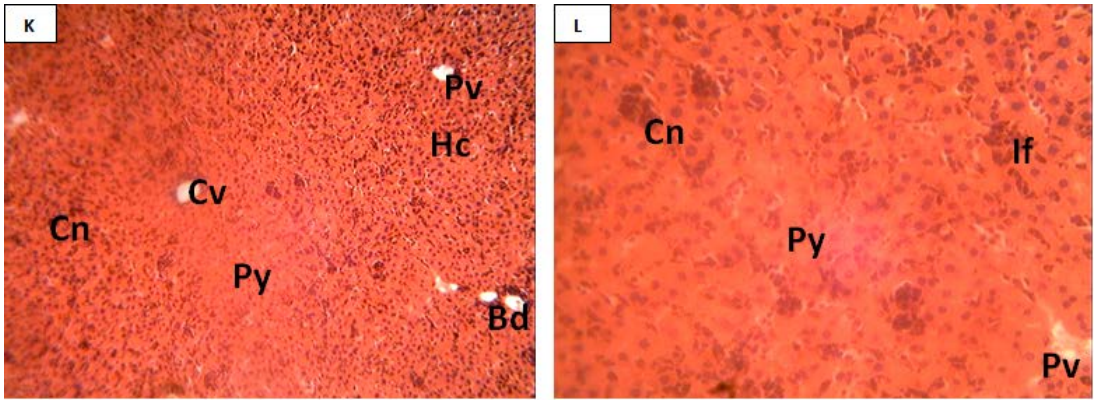
Group 3. E. Pbb + Coartem Liver X100(H &E), F. Pbb + Coartem Liver X400(H &E) (The micrographs show the Portal vein (Pv), Central vein (Cv), plates of hepatic cells (Hc), Sinusoids (S), sparse karyorrhectic cells (Kc) and Necrosis (N), few inflamed areas (If). Strongly affected)



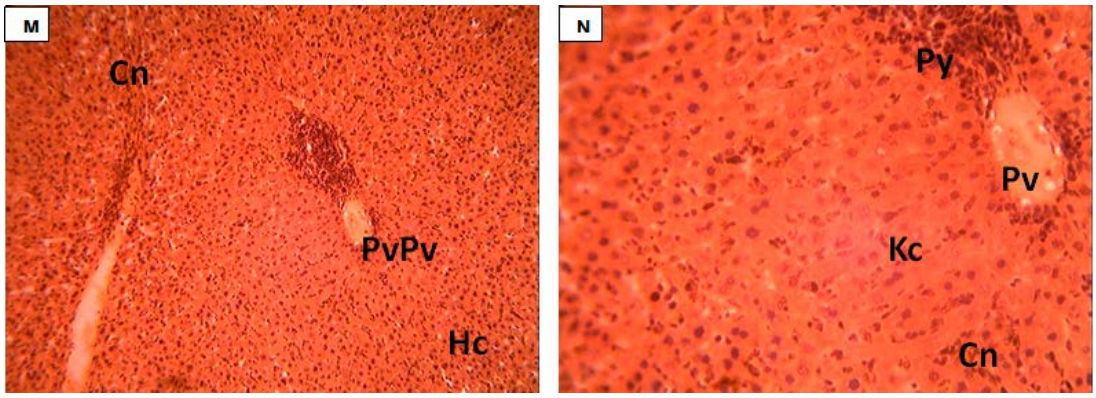
Group 4. G. *NL* 500mg/kg Liver X100(H &E), H. *NL* 500mg/kg Liver X400(H &E) (The micrographs show Central vein (Cv), plate of hepatic cells (Hc), Portal vein (Pv), few Pyknosis present. Slightly affected)



Group 5. I. *NL* 1000mg/kg Liver X100 (H&E), J. *NL* 1000mg/kg Liver X400 (H&E) (The micrographs show Portal vein (Pvs), Bile duct (Bd), sparse clumping nuclei (Cn), numerous pyknosis (Py), numerous nuclei within lumen of Portal vein. Slightly affected)



Group 6. K. Pbb+NL 500mg/kg Liver X100 (H&E), L. Pbb+NL 500mg/kg Liver X400 (H&E) (The micrographs show Central Vein (Cv), Portal vein (Pv), Bile duct (Bd), plate of hepatic cells (Hc), widely interspersed clumping nuclei (Cn) and sparse pyknosis (Py). Strongly affected)



Group 7. M. *Pbb+NL* 1000mg/kg Liver X100 (H&E), N. *Pbb+NL* 1000mg/kg Liver X400(H&E) (The micrographs show numerous plates of hepatic cells (Hc), Portal vein (Pv), sparse pyknotic cell (Py), sparse clumping nuclei (Cn). Strongly affected)

4. Discussion

This study was designed to examine the effect of *Nauclea latifolia* on the haematological system and liver histomorphology of Plasmodium berghei berghei infected Swiss male mice. The result from the haematological indices in Table 4 indicates that the RBC, Hb and PCV in group 2 (Pbb only) was significantly reduced ($P < 0.001$) compared to the control group and the other treatment groups, except in group 5 (NL1000mg/kg). This reduction may be attributed to the mechanism of action of plasmodium parasite on the RBC counts and its cells [25,26,27], whereas the values indicating a haemolytic activity obtained in group 5 may be as a result of the actions of saponins, which is highly present in the *Nauclea latifolia* leaves as reported in Table 2. In particular, steroid and triterpene saponins with a single sugar chain at C3 have strong hemolytic activity [28]. Saponins promote hemolysis of RBC by increasing the water transport by the water channel aquaporin rather than by acting on the lipid phase [29]. Cholesterol does not serve as the specific binding site for saponins in erythrocytes, but rather a decrease in its level affects the susceptibility through structural changes in the membrane [30]. However, the extract at a dose of NL500mg/kg in group 4, had a significantly increased RBC values compared with group 5 (NL1000mg/kg) suggesting a dose-dependent effect.

The low erythropoietic values in group 2 also indicates an alteration in the incorporation of Hb into RBC, also in the morphology and osmotic fragility of the RBCs [31]. RBCs can be lysed by drugs and infections, a susceptibility that increases the deficiency of the enzyme glucose 6-phosphate dehydrogenase (G6PD), which catalyzes the initial step in the oxidation of glucose via the hexose monophosphate pathway, which generates NADPH needed for the maintenance of normal red cell fragility. Severe G6PD deficiency also inhibits the killing of bacteria by granulocytes and predisposes the individual to severe infections [31].

The WBC and its differentials in groups 2, 3, 5 and 7 showed significantly ($P < 0.01$) increased values compared to control group except among lymphocytes which was not significant. WBCs are the mobile units of the body's protective system, usually transported to sites of serious infection and inflammation. This is observed in the photomicrographs of group 2 (plates C and D). Neutrophils significantly increased ($P < 0.1$) in group 2 and group 7, compared to the control, and the most important function of neutrophils is phagocytosis (cellular of an offending agent) by the process of opsonization [32], which may validate the presence of these agents compared to the control group. The platelets were significantly ($P < 0.001$) reduced in all groups compared to the control, perhaps released into circulation after the needle prick for the collection of a blood drop for a smear to perform parasite count (data not presented here), the platelets levels increased most especially in group 1, to prevent further bleeding from the lateral tail vein of the mice. But this was not significantly high in other treatment groups, may be due to the burden of their treatment. When platelet is low, clot retraction is deficient and there is poor constriction of ruptured vessels, resulting in a clinical syndrome; thrombocytopenic purpura, characterized by easy bruisability and multiple subcutaneous hemorrhages [31].

The phytochemical report in Table 2 indicates that *Nauclea latifolia* leaves has high presence of flavonoids, and preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers". In vitro studies show that flavonoids also have anti-allergic, antiflammatory [33], antimicrobial (antibacterial) [34,35,36], antifungal [34,36], antiviral [34,36,37], anti-cancer [38], antidiarrheal and antioxidant activity [39], while some studies have suggested that flavonoids may have a role in cancer prevention, others have been inconclusive [40]. Thus, the rich presence of flavonoid in this extract might have helped to ameliorate the negative impact of saponins on the blood cells.

The histomorphology of the liver in the treated groups showed varying severity of adaptive responses which consisted of inflammation, hyperplasia, hypertrophy of the hepatocytes with reduced sinusoidal sizes, and pyknotic nuclei, especially in the parasitized groups, Plates M and N. The alteration appeared to be dose-dependent across the groups, but less severe in parasitized groups compared to non-parasitized groups Plates I and J. These changes may be indicative of an underlying cellular trauma and morphological change in the tissue cytoarchitecture, a normal reaction of the liver tissue to insults [41]. Splenotoxicity from the ethanolic extract has been reported in a dose-dependent manner [42].

5. Conclusion

After an acute toxicity test of 5000mg/kg, Nauclea latifolia may be considered as relatively safe, if mortality is the only factor considered, but at a dose of 1000mg/kg it is severely hepatoxic and caused significant haemolysis in a dose-dependent manner especially in non-parasitized mice. However, the extract exerts erythropoietic and hepatoprotective effects in parasitized host via a possible synergistic activity of its rich bioactive ingredients.

Conflict of Interest

None declared.

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