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# Anti-malarial and Histopathological Effect of *Plasmodium berghei* Infected Mice Treated with Extracts of *Sarcocephalus latifolius* and *Pterocarpus osun*

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author LEU designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MIA managed the analyses of the study. Author UEW performed the statistical analysis. Author LEU managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** The study addressed anti-malarial activity, haematological, biochemical and histopathological changes due to the administration of ethanol extract of *Sarcocephalus latifolius* (leaf), *Pterocarpus osun* (stem bark) and the combined extract of the two plants in *Plasmodium berghei* parasitised mice.
**Study Design:** Fifty-six mice were weighed and divided into 14 groups of 4 mice each. Infected mice in groups 1-9 were treated with *P. osun* extract (PO1, PO2, PO3), *S. latifolius* extract (SL1, SL2, SL3), combined extracts of *P. osun* and *S. latifolius* (POSL1, POSL2, POSL3); all at doses of 100, 200 and 400 mg/kg body weight respectively. Group 10 received chloroquine, Group 11 were

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infected without treatment. Group 12 and 13 respectively received *P. osun* and *S. latifolius* extract (200 mg/kg body) only. Animals in group 14 serve as normal control.
**Place and Duration of Study:** Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria July-November, 2017.
**Methodology:** Extracts obtained from powdered plant materials were screened for phytochemical constituents and used in the treatment of mice infected with *Plasmodium berghei*. Average percentage parasitaemia was calculated by dividing the number of erythrocytes by number of parasitised erythrocytes multiplied by one hundred. Toxicity and histopathological studies were carried out on the typical spleen and liver sections of the experimental mice. One way Analysis of variance (ANOVA) was used for statistical analysis.
**Results:** Qualitative phytochemical screening indicated that alkaloids, flavonoids, saponins and tannins were present in the plant samples. The highest percent reduction in parasitaemia was observed in *S. latifolius* treatment group (400 mg/kg body weight) which also compared favourably with Chloroquine treatment group (control). *S. latifolius* treatment group (400 mg/kg body weight) and the combined extract at 100 mg/kg body weight could be considered as effective doses considering the observable changes noted in the hematological indices. Histopathological examination revealed hemosiderosis and hepatic necrosis.
**Conclusions:** The result showed that *S. latifolius* extract at 400 mg/kg body weight reduced parasitaemia significantly while *P. osun* boosted the haematological indices without parasite reduction.

*Keywords: Anti-malarial, biochemical indices; phytochemical; histopathology; Plasmodium berghei; Sarcocephalus latifolius; Pterocarpus osun*

## 1. INTRODUCTION

The World Health Organization in 2016 reported a total of 216 million cases of malaria from 91 countries, a total of 5 million cases over the year 2015 [1]. The African region continues to account for about 90% of malaria cases and deaths worldwide with 15 countries carrying 80% of the global malaria burden [1]. Although the burden of malaria is declining globally, there is the need for a robust large scale surveillance mechanisms especially in the African region where malaria is endemic [1,2]. In Nigeria, malaria is caused mostly by *P. falciparum* and *P. malariae* [3,4] and is transmitted by *An. gambiae*, *An. funestus* and *An. arabiensis* [5]. In Nigeria malaria is responsible for economic loss of 132 billion Naira annually to treatment, prevention and loss of man-hours [6,7].

Malaria parasite resistance to the anti-malarial drug is highly on the increase [8]. This is as a result of the malaria parasite's ability to increase its capacity in repairing damages caused by anti-malarial drugs and also because most anti-malarial drugs are more effective against the parasite at its later stage of development, so the parasite slows down its growth as a mechanism for survival [9]. This could also be as a result gene mutation [10,11], biological influences such as HIV/AIDS [12], malnourishment [13] and human behavior such as the use of presumptive treatment [14].

About eighty per cent (80%) of humans depend on locally prepared medicine for their primary health care in Africa [15]. Medicinal plants have been documented for the treatment of diverse diseases in Nigeria [16,17]. The main alternative to anti-malarial resistant drugs has always been plants. Many plants serve as beneficial source of malaria treatment as most commercially available anti-malarial drugs are derivative of plants [18,19,20]. The use of monotherapy is strongly discouraged in combating drug resistance for most parasitic diseases [21], hence the different combinations of medicinal plants in malaria treatment and research [22,23].

This study aimed to investigate anti-malarial activity of *S. latifolius* and *P. osun*, and also to evaluate the effects of extracts of these plants on some haematological, biochemical parameters and histopathological leisons on the liver and spleen of *Plasmodium berghei* infected mice.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Experimental Plant Materials

The samples of *S. latifolius* leaves and stem bark of *P. osun* were collected from Awi forest, Akampa local government area of Cross River State and were authenticated by a botanist and

deposited in the Department of Botany, University of Calabar, Cross River state, Nigeria.

## 2.2 Preparation of Plant Samples

Fresh leaves of *S. latifolius* and stem bark of *P. osun* were washed with water, air-dried and ground separately with an electric blender. Five hundred grams of each sample were respectively soaked in 80% ethanol at room temperature for 72 hours. The ethanol extracts were filtered using whatman No.1 filter paper. The filtrate was then concentrated in open water bath at 45˚C and the dry extracts refrigerated at 4˚C until it was needed.

## 2.3 Phytochemical Analysis

The powdered samples were screened for alkaloids, Saponins, cardiac glycosides, Phlobotanins, Tannins, Flavonoids, Anthra-quinones, Terpenes, De-oxy sugars and steroids.

## 2.4 Experimental Animals

Swiss albino mice weighing between 20 - 34 g were obtained the animal house of the College of Health Sciences, University of Uyo, Nigeria and were acclimatized for 3 days in the experimental section of the animal house. The mice were kept in airy cages under 12 hour light/dark cycle. The animals were fed with growers Mash and tap water given *ad libitum*.

## 2.5 Determination of Lethal Dosage

The crude extracts of *S. latifolius* and *P. osun* were assessed for toxicity using albino mice according to the modified Lorke's method [24]. Lethal dosage ($LD_{50}$) was calculated as the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded.

## 2.6 Experimental Design

Fifty-six (56) mice were weighed and divided into 14 groups of four (4) mice each. Table 1 shows the experimental design. Six (6) days after passage of malaria parasite, the respective extracts, and Chloroquine were administered daily for 6 days by oral gastric intubation. All procedures involving animals were carried out according to accepted regulations for laboratory animal use and care as documented in the care and use of animals [25] and the institution's ethics code for the use of laboratory animals.

## 2.7 Innoculation of Mice with Experimental Malaria Parasites

*Plasmodium berghei* (NK 65) parasitised mice were obtained from National Institute of Medical Research (NIMR), Lagos, Nigeria. The malaria parasite, *P. berghei* was subsequently passaged from mouse to mouse every 5 - 7 days. The confirmed parasitised mice were euthanised and dissected. Blood was collected by cardiac puncture and mixed with normal saline; 0.3 ml of the diluted blood was passaged intraperitoneally to the experimental mice (about $1 \times 10^7$ parasitised cells/mice). Six days after inoculation of parasite, blood was collected from the tail of each mouse in the various groups to make thin and thick blood smear, for the baseline parasitaemia determination.

## 2.8 Treatment of Mice with Extracts

Extracts were prepared daily and administered according to the dosage shown in Table 1. Each extract was reconstituted with tween 80 (20% v/v). Control groups (Groups 10 – 14) were treated accordingly. Treatment was administered using oral gastric intubation for 6 days.

## 2.9 Toxicity Analysis

Six days after treatment, the mice were euthanised under chloroform vapour and dissected. Whole blood was collected via cardiac puncture with the aid of sterile syringes and needles. Blood sample for haematological, biochemical and malaria parasite analysis was collected using a precision variable volume pipette into separate sample bottles and labelled appropriately.

## 2.10 Determination of Parasitaemia

Blood smear from each blood sample was used for a thick and thin smear on a slide. The smears were stained with Giemsa. The thin smear was fixed with methanol. Each slide with the entire smear was screened to find appropriate fields with an even distribution of blood cells [1]. Smears were critically analyzed using X 100 oil immersion lens. The number of parasitised cells and the total number of cells in the magnification

field were recorded. This was used in deriving the percentage parasitaemia.

$$\text{Percent (\%) Parasitaemia} = \frac{\text{Number of parasitised erythrocytes}}{\text{Number of erythrocytes}} \times 100$$

## 2.11 Histopathology Analysis

The tissues (liver and spleen) specimens from the mice were fixed in Bouin's solution. The liver and spleen tissues were dehydrated in ascending series of alcohol, cleared in two changes of xylene and embedded in two changes of paraffin wax melted at 56˚C and then allowed to solidify. Paraffin-embedded tissues were serially sectioned using rotary microtome into 5 µm thickness. The sections were de-paraffined, hydrated and stained in Mayer's Haematoxylin. The slides were washed and allowed to absorb alcohol in ascending grades, cleared in xylene and mounted in DPX, cover slipped, allowed to dry and observed under digital imaging microscope. Histopathological changes were recorded.

## 2.12 Data Analysis

All analysis were performed using statistical package for social sciences (SPSS) version 18.0 software package and values were expressed as mean ± SEM (standard error of the mean), and comparisons were made using one-way ANOVA. Values were considered significant at $P < 0.05$.

# 3. RESULTS

## 3.1 Plant Profile

The profile of the plants used is shown in Table 2.

## 3.2 Phytochemical Constituents of Plant Extracts

Presence of alkaloids, saponin, cardiac glycosides, terpenes and flavonoids was confirmed in *S. latifolius* while *P. osun* contained tannins, phlobotanin, saponin, cardiac glycosides, terpenes and alkaloids (Table 3).

**Table 1. Experimental design**

| Group | Infection status | Treatment | Dosage (mg/kg/ body weight) |
|---|---|---|---|
| 1(PO1) | Infected | *P. osun* | 100 |
| 2(PO2) | Infected | *P. osun* | 200 |
| 3(PO3) | Infected | *P. osun* | 400 |
| 4(SL1) | Infected | *S. latifolius* | 100 |
| 5(SL2) | Infected | *S. latifolius* | 200 |
| 6(SL3) | Infected | *S. latifolius* | 400 |
| 7(POSL1) | Infected | *P. osun* + *S. latifolius* | 100 |
| 8(POSL2) | Infected | *P. osun* + *S. latifolius* | 200 |
| 9(POSL3) | Infected | *P. osun* + *S. latifolius* | 400 |
| 10(CQ) | Infected | Chloroquine | 0.4 |
| 11(NTPO) | Infected | Distilled water | - |
| 12(PONP) | Not infected | *P. osun* | 200 |
| 13(SLNP) | Not infected | *S. latifolius* | 200 |
| 14(NTNP) | Not infected | Distilled water | - |

*PO = Pterocarpus osun, SL = Sarcocephalus latifolius, NTPO = not treated parasite only, PONP = P. osun not infected, SLNP = S. latifolius not infected, NTNP = not infected or treated*

**Table 2. Profile of plants**

| Botanical name | Family | Local name (Efik) | Common name | Habit | Part used |
|---|---|---|---|---|---|
| *Sarcocephalus latifolius* | Rubiaceae | Mbom Ibon | African Peach | Tree | Leaf |
| *Pterocarpus osun* | Papiloniaceae | Ukpa | Bloodwood | Tree | Bark |

### 3.3 Anti-malarial Activity of Plant Extracts

A decrease was observed in the parasite level in mice that were treated with SL3 compared to the NTPO control. There were decreases in parasitaemia level in POSL3 and POSL2 groups compared to POSL1 group. The chloroquine control (CQ) showed a total clearance of the parasite (Fig. 1). SL3 group compared favourably with CQ, showing there was no meaningful difference observed between both groups.

### 3.4 Serum Biochemical Changes in Mice

There was a significant reduction ($P < 0.05$) in serum cholesterol and alkaline phosphatase (ALP) level. A significant increase was observed in serum Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) (Table 4).

### 3.5 Changes in Hematological Indices of Mice

The increase in white blood cells (WBC) for PO3 and SL3 was significant in comparison with the control groups except for SLNP group. A significant reduction ($P < 0.05$) in red blood cell (RBC), haemoglobin (HB), packed cell volume (PVC) and platelets (PLT) was observed in most groups (Table 5).

**Table 3. Phytochemical characteristics of ethanolic extract of *S. latifolius* and *P. osun***

| Phytochemicals | *S. latifolius* | *P. osun* |
|---|---|---|
| Tanin | - | + |
| Phlobotanin | - | + |
| Saponin | + | + |
| Anthraquinone | - | + |
| Cardiac Glycoside | + | + |
| Flavonoids | + | - |
| Deoxy sugar | + | + |
| Terpene | + | + |
| Alkaloids | + | + |

*+ indicates present; - indicates absent*

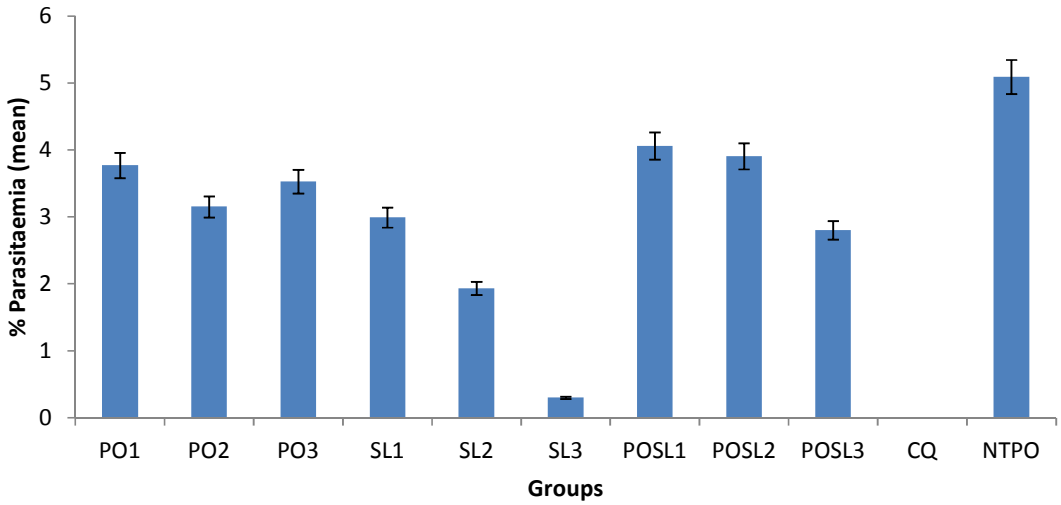



**Fig. 1. Percentage parasitaemia in mice showing total clearance in the CQ group and reduction in SL3 group compared to the negative control, NTPO. PO1-PO3 (*Pterocarpus osun* groups 1 - 3), SL1-SL3 (*Sarcocephalus latifolius* groups 1 - 3), POSL1-POSL3 (combined extract of *P. osun* & *S. latifolius* groups 1 - 3), CQ (chloroquine group) and NTPO (parasite control group). P = 0.06**

**Table 4. Some biochemical changes in the serum of experimental mice**

| Groups | AST(µl) | ALT(µl) | ALP(µl) | Triglyceride (g/L) | Cholesterol (mg/dL) |
|---|---|---|---|---|---|
| PO1 | 165.75 ± 24.04 | 40.66±10.1$^{de}$ | 108.8±16.11$^{cd}$ | 233.06 ± 16.28$^{abc}$ | 178.22 ± 29.11 |
| PO2 | 335.92 ± 255.52 | 36.69 ± 16.42 | 107.89 ± 31.03$^{cd}$ | 231.43 ± 11.93$^{abc}$ | 163.86 ± 21 |
| PO3 | 151.61 ± 29.17$^{cd}$ | 49.5 ± 10.1 | 100.06 ± 16.69$^{cd}$ | 255.24 ± 3.3$^{bcde}$ | 161.88 ± 53.31 |
| SL1 | 195.81 ± 27.89 | 36.69 ± 19.06 | 134.5 ± 42.46$^{cd}$ | 140.94 ± 31.03 | 158.14 ± 25.92$^{c}$ |
| SL2 | 220.56 ± 113.16$^{cd}$ | 41.99 ± 14.32 | 112.08 ± 29.69$^{cd}$ | 266.94 ±17.47$^{bcde}$ | 180.45 ± 21.5 |
| SL3 | 163.1 ± 17.64 | 23.57 ± 2.04 | 105.52 ± 15.91$^{cd}$ | 215.51 ± 26.05$^{abd}$ | 192.33 ± 28.88 |
| POSL1 | 194.48 ± 25 | 35.95 ± 12.79 | 143.61 ± 39.39$^{cd}$ | 203.54 ± 55.39$^{abd}$ | 160.4 ± 29.75 |
| POSL2 | 224.541 ± 82.75$^{cd}$ | 38.31 ± 16.04 | 314.93±121.47$^{ad}$ | 193.38 ± 31.92$^{b}$ | 152.15 ± 29.14$^{c}$ |
| POSL3 | 181.22 ± 52.58 | 37.57 ± 3.64 | 99.87 ± 36.09$^{cd}$ | 209.78 ± 20.24$^{abd}$ | 172.24 ± 5.23 |
| CQ | 182.99 ± 97.27 | 24.75 ± 15.11 | 152 ± 36.09 | 135.31 ± 38.45 | 206.93 ± 38.29 |
| NTPO | 123.76 ± 10 | 45.97 ± 4.68 | 196.1 ± 76.05 | 124.26 ± 49.14 | 165.89 ± 5.02 |
| PONP | 36.24 ± 9.84 | 22.98 ± 3.23 | 400.22 ± 50.76 | 169.3 ± 17.63 | 217.77 ± 18.34 |
| SLNP | 37.57 ± 8.83 | 19.89 ± 5.38 | 455.44 ± 33.23 | 138.6 ± 35.66 | 171.09 ± 26.59 |
| NTNP | 154.7 ± 5.3 | 20.33 ± 5.5 | 159.1 ± 16.11 | 163 ± 24.81 | 199.22 ± 6.96 |

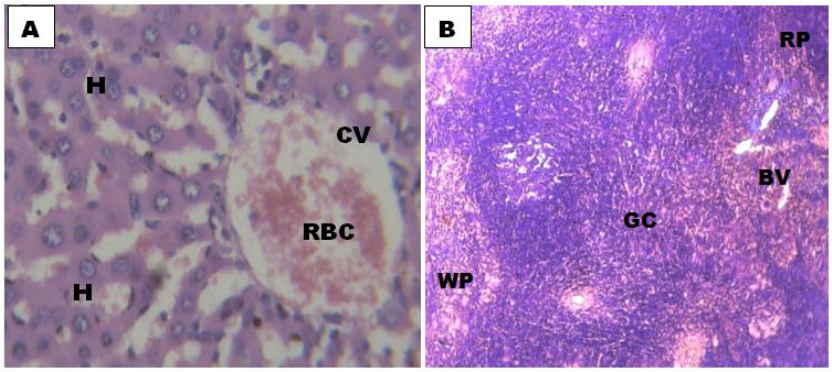
*Values are represented as Mean ± S.D, n = 4; values are compared down the group; a = p < 0.05 (all test groups in comparison with CQ); b= p < 0.05 (all test groups in comparison with NTPO); c= p < 0.05 (all test groups in comparison with PONP); d= p < 0.05 (all test groups in comparison with SLNP); e= p < 0.05 (all test groups in comparison with NTNP)*

**Table 5. Changes in some hematological indices of mice**

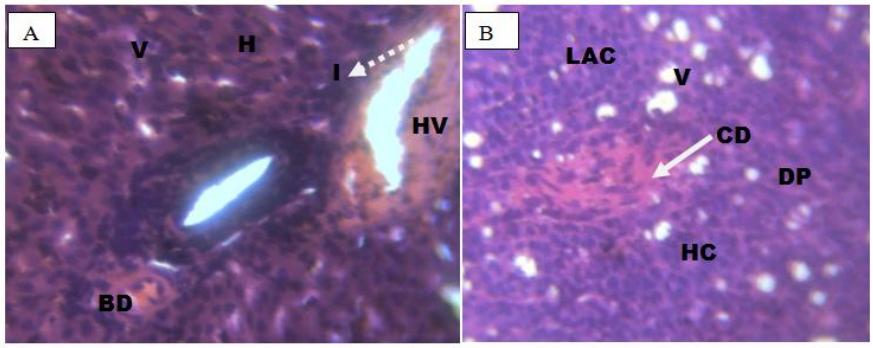
| Groups | WBC x $10^3$(µl) | RBCx$10^6$(µl) | HB (g/dl) | PCV (%) | PLTx$10^3$(µl) | LYM (%) |
|---|---|---|---|---|---|---|
| PO1 | 14.27 ± 4.14 | 4.48 ± 1.01$^{abce}$ | 6.03 ± 1.1$^{abc}$ | 23.3 ± 3.91$^{bce}$ | 773.00 ± 262.31 | 51.8 ± 6.36 |
| PO2 | 18.30 ± 3.31 | 3.25 ± 1.49$^{abce}$ | 4.4 ± 1.77$^{abce}$ | 17.4 ± 7.59$^{abce}$ | 707.25 ± 213.48 | 31.15 ± 44.05 |
| PO3 | 31.75± 19.13$^{abce}$ | 5.97 ± 0.62 | 8.28 ± 0.92$^{b}$ | 31 ± 2.87 | 675.25 ± 265.63 | 40.02 ± 6.2 |
| SL1 | 22.65 ± 9.97 | 3.14 ± 1.92$^{abce}$ | 4.65 ± 2.47$^{abce}$ | 18.43 ± 8.5$^{abce}$ | 285.75 ± 145.85$^{a}$ | 39.95 ± 56.6 |
| SL2 | 14.15 ±5.16 | 5.50 ± 0.64$^{bc}$ | 7.95 ± 1.04$^{b}$ | 23 ± 14.98$^{bce}$ | 595.75 ± 211.14 | 19.93 ± 39.85 |
| SL3 | 25.20 ± 8.78$^{ac}$ | 5.33 ± 1.19$^{bc}$ | 8.18 ± 1.8$^{b}$ | 30.63 ± 6.76$^{b}$ | 568.25 ± 187.12 | 70.38 ± 21.36 |
| POSL1 | 25.77 ± 3.35 | 6.28 ± 1.99$^{b}$ | 9.03 ± 2.64 | 34.5 ±7.78 | 498.33 ± 387.70 | 68.03 ± 3.3 |
| POSL2 | 11.45 ± 11.38 | 3.68 ± 1.88$^{abce}$ | 3.93 ± 2.21$^{abce}$ | 13.9 ± 11.93$^{abce}$ | 295.33 ± 297.38$^{a}$ | 44.7 ± 63.22 |
| POSL3 | 19.00 ± 1.30 | 3.23 ± 1.15$^{abce}$ | 5.28 ± 1.7$^{abce}$ | 19.78 ± 6.38$^{abce}$ | 721.50 ± 575.52 | 45.05 ± 52.15 |
| CQ | 5.17 ±1.87 | 7.70 ± 0.38 | 10.97 ± 1.1 | 40.67 ± 3.59 | 1138.00 ± 72.75 | 89.87 ± 6.67 |
| NTPO | 3.90 ± 2.55 | 2.19 ± 0.85 | 5.83 ± 1.25 | 21.47 ± 5.11 | 703.33 ± 90.69 | 53.24 ± 6.2 |
| PONP | 5.47 ± 1.16 | 8.68 ± 0.37 | 12.8 ± 0.82 | 48.77 ± 2.3 | 654.67 ± 294.78 | 90.37 ± 1.17 |
| SLNP | 8.40 ± 1.93 | 7.80 ± 1.43 | 11.23 ± 1.61 | 43.13 ± 7.5 | 514.67 ± 404.27 | 80.1 ± 12.95 |
| NTNP | 9.43 ± 0.14 | 8.45 ± 0.36 | 10.2 ± 2.4 | 46.8 ± 3.11 | 937.08 ± 212.10 | 65.9 ± 13.9 |

*Values are represented as Mean ± S.D, n = 4; values are compared down the group; a= p < 0.05 (all test groups in comparison with CQ); b= p < 0.05 (all test groups in comparison with NTPO); c= p < 0.05 (all test groups in comparison with PONP); d= p < 0.05 (all test groups in comparison with SLNP); e= p < 0.05 (all test groups in comparison with NTNP)*

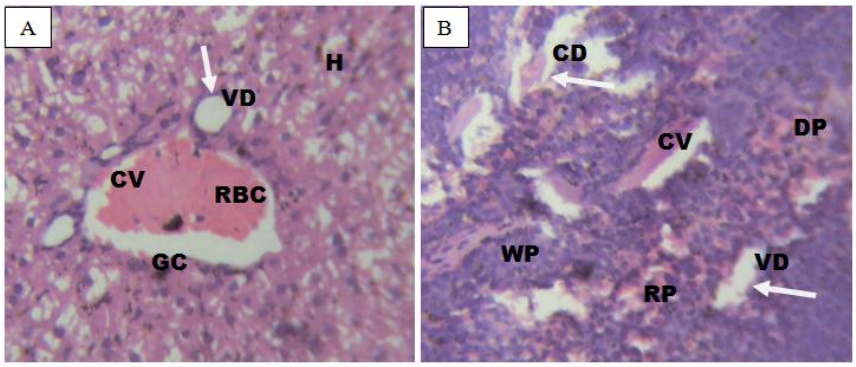
### 3.6 Photomicrograph of Typical Sections of the Liver and Spleen of Mice in All Experimental Groups. Max X 400



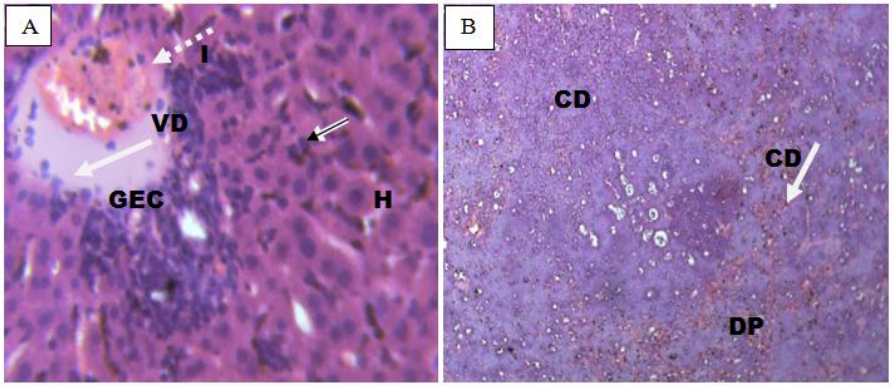

**Group 1A. NTNP Liver (H&E); B. NTNP Spleen (H&E). The photomicrographs show Central vein (Cv), hepatocytes (H), red blood cell (RBC), germinal centre (GC), blood vessel (BV), red pulp (RP). No abnormality**



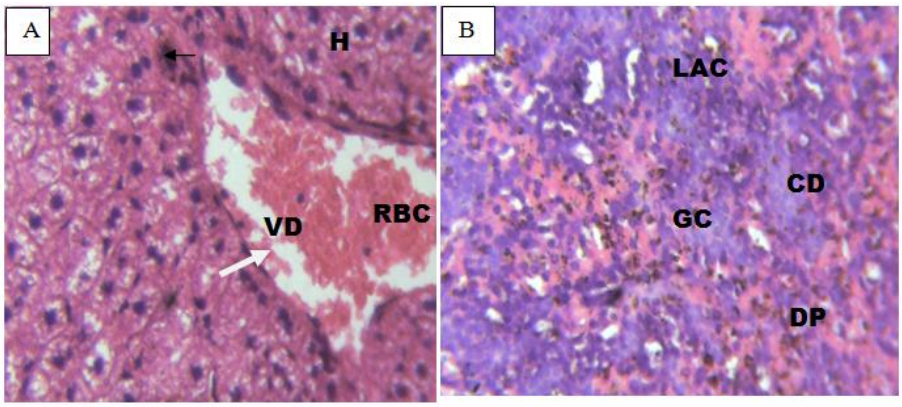

**Group 2A. NTPO Liver X400 (H&E); B. NTPO Spleen X400 (H&E). The photomicrographs show vacuolization (V), bile duct (BD), cellular degeneration (CD), diminished pulp (DP), inflammation (I) shown by arrow, vascular degeneration (VD), Strongly affected**



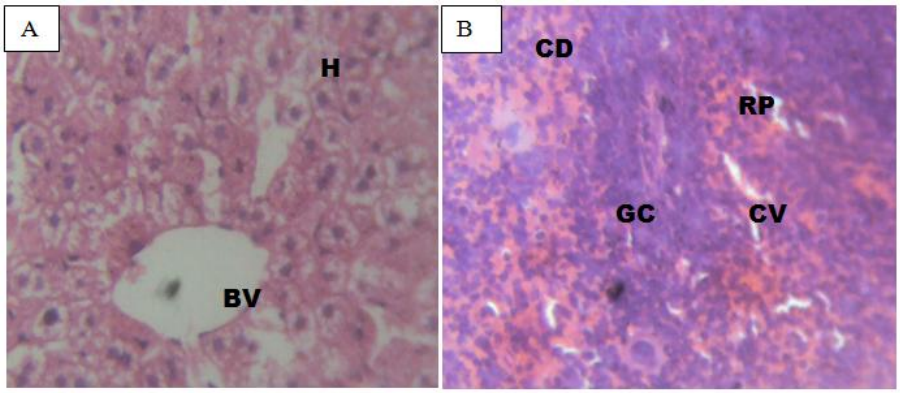

**Group 3A. CQ Liver (H&E); B. CQ Spleen (H&E). The photomicrographs show vascular degeneration (VD) indicated by arrow, cellular degeneration (CD), central vein (Cv), hepatocytes (H), red blood cell (RBC), germinal centre (GC), white pulp (WP), red pulp (RP), bile duct (BD), diminished pulp (DP),Slightly affected**



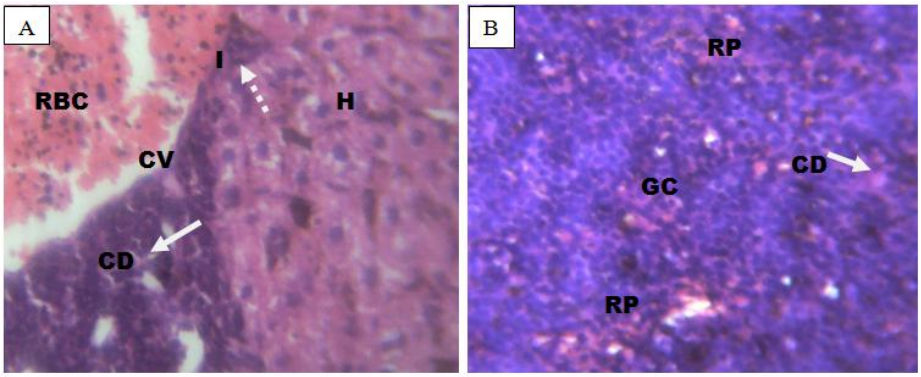

**Group 4A. POSL Liver (H&E); B. POSL Spleen (H&E). The photomicrographs show hepatocytes (H), granulated eosinophilic cells (GEC), vascular degeneration (VD) and cellular degeneration (CD) of severely affected liver and spleen, inflammation (I).→ show numerous dark oval structures likely to be Plasmodium gametocytes/extra erythrocytic stage. Strongly affected**



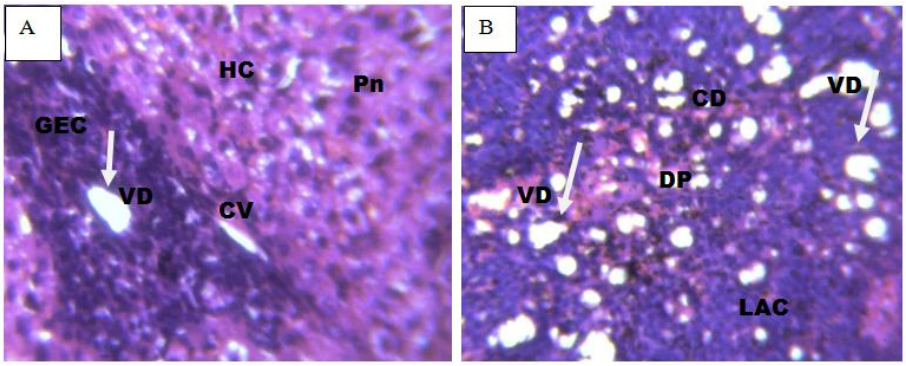

**Group 5A. SLNP Liver (H&E); B. SLNP Spleen (H&E). The photomicrograph shows, hepatocytes (H), red blood cell (RBC), germinal centre (GC), cellular degeneration (CD), diminished pulp (DP), inflammation (I) → shown by; Liver was strongly affected, spleen was slightly affected**




**Group 6A. PONP Liver (H&E); B. PONP Spleen (H&E). The photomicrographs show Central vein (Cv), hepatocytes (H), germinal centre (GC), bile duct (BV), cellular degeneration (CD), red pulp (RP). Slightly affected**




**Group 7A. PO2 Liver (H&E); B. PO1 Spleen (H&E). The photomicrographs shows Central vein (Cv), hepatocytes (H), red blood cell (RBC), germinal centre (GC), arrow shows cellular degeneration (CD) and inflammation (I), red pulp, (RP). Strongly affected**




**Group 8A. SLI Liver (H&E); B. SL1 Spleen (H&E). The photomicrographs show Central vein (Cv), hepatocytes (H), red blood cell (RBC), vascular degeneration (VD), germinal centre (GC), blood vessel (BV), vacuolization (V), bile duct (BD), cellular degeneration (CD), diminished pulp (DP), inflammation (I). Strongly affected**

## 4. DISCUSSION

Combined effects of crude extract of medicinal plants tend to be more effective than isolated compounds [26]. Combined extracts of *S. latifolius* (formerly known as *Nauclea latifolia*) in addition with nine other plants have been reported [22] for its anti-malarial property. A combination of *S. latifolius* with *Enanthia cholorantha* had a positive effect in the prophylactic and suppressive activities of the individual plant [27]. *S. latifolius* has been reported for use in treating various diseases like fever [28,29,30,31], diarrhoea and dysentery [32,33] without harmful effects. *P. osun* is used in the treatment of surface skin problems e.g Eczema [34]. The bark of this plant is used in soup preparation due to its erythropoietic properties. The popular antiseptic black soap known as 'Dudu osun' in Nigeria is said to contain *P. osun* [34]. *P. osun* contains plant chemicals such as saponins, tannins, phenols and alkaloids [35,36].

Flavonoids found in *S. latifolius* in combination with other chemical compounds in plants have been confirmed to have antiplasmodial properties [34,35,36]. It has been reported that various antibiotics which contain phytochemicals like alkaloids, saponins and tannins as found in these plants serve the purpose of treating infections [37,38]. The indole alkaloid strictosamide located in the root, stem bark and leaf of *S. latifolius* have been shown to display antiplasmodial activity which can combat *P. falciparum* in humans [30].

Enzyme assays are routinely carried out to determine the functional status, assess the integrity of specific organs or cellular lesion.

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) are biomarkers for liver health. The effects of the extract control (PONP & SLNP) on AST and ALT generally lowered the levels of these enzymes which is a good indicator since an increase in these enzymes' activities indicates an increase in liver inflammation. This study supports previous findings [39] showing that liver injury due to malaria is accompanied by increase hepatomegaly, elevated liver enzymes such as AST and ALT.

Noticeable changes were observed in the hematological indices of the combined extract POSL1 at 100 mg/kg body weight. *P. osun* extract control (PONP) boosted the red blood cells, haemoglobin, haematocrit and lymphocytes when compared to the normal control mice (NTNP). This is indicative of the erythropoietic properties of *P. osun,* one of four herbs used in the formation of Niprisan, a drug used in treating sickle cell disease in Nigeria [40,43]. 100 mg/kg body weight may be considered an effective dose for the treatment of malaria.

Histological studies showed damages that occurred as a result of malaria parasite and the effect of the extract treatment which include iron build up in the liver and spleen known as haemosiderosis and dead liver cells (hepatic necrosis). The combined extract had severe effects of these damages (Group 4). The liver and spleen of chloroquine and *P. osun* treatment groups (Groups 3 & 7) were moderately affected. The liver and spleen *of S. latifolius* treatment group were severely affected (Group 8). This observation is in line with earlier report [41,42,44]. These pathological changes indicate that caution must be exercised in the use of these plants.

## 5. CONCLUSIONS

The findings of this study support the claimed traditional use of *S. latifolius* in treating malaria and the beneficial erythropoietic effects of *P. osun* and also the side effects of these extracts on the liver and spleen.

## ETHICAL APPROVAL

All procedures involving animals were carried out according to accepted regulations for laboratory animal use and care as documented in the care and use of animals [25] and the institution's ethics code for the use of laboratory animals.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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# APPENDIX

## Pictures of Plants

***S. latifolius* leaves**

**Dried leaves of *S. latifolius***

**Stem bark of *P. osun***

**Dried stem bark of *P. osun***



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