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Biochemical and Haematological Effects of Aqueous Leaf Extract of *Sida cordifolia* in *Plasmodium beighei* Infected Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Assessment of haematological and biochemical parameters can be predictive of the adverse effects resulting from ingesting foreign substances. Thus, the aim of this study was to evaluate the biochemical and haematological indices in *Plasmodium beighei* infected wistar rats. Freshly harvested leaves of *Sida cordifolia* were washed and dried at room temperature, after which they were ground to fine powder and subsequently extracted. Twenty-five adult Wistar rats were divided into five groups of five rats each. Group I was the normal control and was administered 2 ml of

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distilled of water. Group II was infected without treatment, while Group III and IV were infected and afterwards administered 200 and 400 mg/kg of aqueous extract of *Sida cordifolia* respectively. Group V was administered the standard drug. Biochemical and haematological indices were determined using standard procedures. The Packed Cell Volume (PCV) reported for group II (negative) control was significantly (P<0.05) lower than that reported for the normal control. However, oral administration of 200 and 400 mg/kg of *Sida cordifolia* leaf extract significantly (P<0.05) increased it, though to a level which was significantly (P<0.05) lower than that reported for the normal control. Similar observation was made on Haemoglobin Concentration (Hb) and Red Blood Cell (RBC). However, a contrary observation was made on the white blood cell. Urea and creatinine reported for the negative control were significantly (P<0.05) higher than those reported for the normal control. However, the aforementioned parameters were significantly (P<0.05) reduced following oral administration of *Sida cordifolia*. In conclusion, it can be deduced from this study that *Sida cordifolia* leaf extract has the ability to restore distorted haematological and biochemical status resulting from *P. berghei* infection.

Keywords: Sida cordifolia leaf; blood; cell; haemoglobin.

1. INTRODUCTION

Sida cordifolia a notorious specie in the genus (Malvaceae) is commonly recognized for its therapeutic potentials [1]. It is commonly known in India as Bala and one of the ingredients used in making Ayurvedic formulations [2]. *Sida cordifolia* leaf known to habour certain therapeutic substances such as ephedrine, pseudoephedrine, betaphenethylamine, hypaphorine and indole alkaloides is reportedly used as antioxidant, anticancer, and antidiabetic [3]. Other activities of *S cordifolia* include analgesic and anti-inflammatory activities among others [3].

The wrongly held impression that plant based medicinal preparations wield minimal or no side effects has undermined approach to cautious application of this category of therapy in the treatment of diverse human diseases and its attendant consequences [4,10]. Medicinal plants being endowed with diverse arrays of biological compounds with great complexity tend to have several broad actions on the physiological and biochemical systems [5].

Assessing the haematological parameters can be relied upon to diagnose unpleasant effects resulting from the usage of foreign compounds on the blood constituents [6]. Furthermore, ingestion of chemical compounds at toxic doses can orchestrate alterations in blood parameters that are suggestive of haematological disorders [7]. The role of biochemical markers in performing accurate diagnosis as well as in the assessment of risk and adoption of therapy that improve clinical outcomes [8,11] cannot be overemphasized thereby informing the imperativeness of this study.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Mature leaves of *Sida cordifolia* were harvested from a bush in Uturu, Isikwuato Local Government Area of Abia State. The leaves were conveyed in a dark polythene bag to the herbarium unit of the Department of Forestry, Michael Okpara University of Agriculture Umudike, South Eastern Nigeria.

2.2 Experimental Animals

Twenty-five (25) adult male Wistar rats were procured from the animal House of the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Uwana Afikpo. The animals were handled in accordance with ethics governing the handling and use of laboratory animals for research purposes. They were kept in transparent plastic cages and were fed guinea grower feed. They were allowed two weeks to acclimatize before the commencement of the experiment.

2.3 Malaria parasite

Mice passaged with *Plasmodium berghei* were bought from the Department of Parasitology, University of Nigeria Nsukka.

2.4 Extraction

Freshly harvested leaves of *Sida cordifolia* were washed with clean tap water to get rid of dirts. The leaves were subsequently dried at room temperature after which they were ground into

fine powder. Exactly 500 g of powdered plant sample was subsequently soaked in 3 L of distilled water for 24 h. The mixture was filtered with a clean sieve and was concentrated to dryness in a water bath for 3 days at 40 ^{o}C.

2.5 Acute Oral Toxicity Study

The method of the Organization for Economic Cooperation and Development (OECD 425, 2008) was used to determine the acute toxicity of extract using limit test dose of 2 g/kg. Five apparently healthy Wistar rats which had been starved of food for 4 h were dosed. No mortality was recorded on the first animal administered a limit dose of 2000 mg/kg. The remaining four male Wistar rats were dosed and observed for signs of toxicity [9].

2.6 Parasite passaging

The approach of Peter and Anatoli (1998) was relied upon to inoculate *Plasmodium berghei* (NK 65) into the mice by intraperitoneal route (Anatoli, 1998). Administration of extract began three (3) days after inoculation.

2.7 Biochemical analysis

Sample preparation: Exactly 2 mL of blood sample was introduced into the EDTA tube prior to centrifugation at 4,000 rpm for 15 min and the plasma obtained was stored for biochemical analysis.

2.8 Serum Urea Determination

Exactly 10 µL of sample was placed into a tube containing 1000 µL of the working reagent. The contents of the tube were thoroughly mixed, incubated for 5 minutes at 37^{o}C (Kaplan, 1982). Blood urea concentration was determined using the formula below:

$$Urea\ conc\ (mg/dl) = (A\ Sample)/(Acal/STD) \times conc.cal/STD(mg/dl)$$

2.9 Haematological Evaluation

Haematological parameters (Red Blood Cells, Haemoglobin concentration, packed cell volume and white blood cell) were determined with the aid of an automatic hematological analyzer (Coulter STKS, Beckman) (Yang et al., 2019).

2.10 Data Analysis

Data obtained were expressed as Mean ± Standard Deviation using SPSS (Ver. 23). Data were analysed using one way Analysis of Variance (ANOVA). Differences in mean were compared using Turkey Test. *p-values* less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Assessment of haematological parameters can be diagnostic of adverse effects of foreign compounds on blood constituents of animals" [6]. Table 1 shows the effect of oral administration of aqueous extract of *Sida cordifolia* on the haematological indices of *Plasmodium berghei* infected rats indicating that the packed cell volume (PCV) reported for the negative control was significantly ($p<0.05$) lower than that reported for the normal control. However, oral administration of aqueous extract of *S. cordifolia* significantly ($p<0.05$) increased it in a dose dependent manner. It was also observed that there was no significant ($P>0.05$) difference in the PCV reported for the normal control and the standard control. The haemoglobin concentration (Hb) reported for

Table 1. Haematological Indices of *Plasmodium berghei* Infected Rats administered Aqueous Extract of *Sida cordifolia*

TREATMENT	PCV	Hb (g/dl)	WBC (×10^9/l)	RBC(×10^6/l)
Group I (Normal Ctrl)	48.05±3.73^{d}	18.09±2.42^{e}	6.24±0.24^{b}	7.20±2.59^{d}
Group II (Negative Ctrl)	28.09±1.05^{a}	8.40±1.98^{a}	9.82±0.82^{e}	3.70±3.05^{a}
Group III: INF+200 mg/kg Extract	35.02±4.54^{b}	12.09±2.33^{b}	8.21±0.20^{d}	4.20±3.22^{ab}
Group IV: INF+400 mg/kg Extract	39.09±5.08^{c}	13.07±1.82^{bc}	7.08±2.31^{c}	5.20±2.30^{c}
Group V: INF+STD	47.08±3.42^{d}	15.07±0.92^{d}	4.70±5.30^{a}	7.30±3.52^{d}

Values are expressed as mean ± standard deviation of three determinations. Values with different superscript in a column are significantly (p<0.05) different

Table 2. Renal function markers in rats administered aqueous extract of *Sida cordifolia*

Treatment	Urea (mg/dl)	Creatinine (mg/dl)
Group I (Normal CTRL)	14.40±5.87[a]	0.81±0.98[a]
Group II (Negative CTRL)	31.50±4.32[c]	3.10±0.19[c]
Group III: INF+200 mg/kg Extract	20.12±2.02[b]	1.02±0.32[b]
Group IV: INF+400 mg/kg Extract	19.14±0.34[b]	0.96±0.23[c]
Group V: INF+STD (chloroquine)	14.98±0.98[a]	0.89±0.23[a]

Values are expressed as mean ± standard deviation of three determinations. Values with different superscript in a column are significantly (p<0.05) different

the negative control was significantly (p<0.05) lower than that reported for other groups. However, following oral administration of extract, there was a significant increase in the Hb reported for groups II and III which though were not significantly (p>0.05) different from each other but lower than that reported for the normal control. The red blood cell (RBC) reported for the negative control was significantly (p<0.05) lower than those reported for other groups. The RBC reported for group III was not significantly (p>0.05) different from that reported for the negative control. Similar observation was made between the normal control and the standard control. The anti-anemic effect of *S. cordifolia* extract could be attributed to antioxidant activity of the plant extract which had been reported in previous study. This is consistent with the finding of Ukpanukpong et al. [12] which showed that "ethanol leaf extract of S. *rhobofolia*, a member of the *malvaceae* family to which *S. cordifolia* belongs orally administered on rats induced with artificial infertility significantly (P<0.05) increased the red blood cell". The White Blood Cell (WBC) reported for the negative control was significantly (p<0.05) higher than that reported for the normal control which in turn was significantly (p<0.05) lower than those reported for groups III and IV which were infected and treated with extract. This is contrary to the finding made by Ukpanukpong et al. [12] which showed that oral administration of 200 and 400 mg/kg of *S. rhombifolia* extract significantly increased the white blood cell in rats induced with artificial infertility. Biochemical markers play an important role in accurate diagnosis and also for assessing risk and adopting therapy that improve clinical outcome [8]. Furthermore, creatinine is commonly used as a measure of kidney function [13]. In addition, the most frequently determined clinical indices for estimating renal function depends upon the concentration of urea in the serum [14]. The kidney is one the organs that receive the bulk of the toxic impacts of toxic substances. Table 2 shows the effect of oral administration of aqueous extract of *Sida cordifolia* on the renal health of rats infected with *Plasmodium berghei* indicating that serum creatinine and urea were significantly (p<0.05) higher in the negative control group which were infected without treatment than those reported for the normal control group. These were significantly (p<0.05) reduced in a dose dependent manner. This is consistent with the finding of Ukpanukpong et al. (2019) which showed that ethanol leaf extract of *Sida cordifolia* was not toxic.

4. CONCLUSIONS

It has been reveled through this study that aqueous leaf extract of *Sida cordifolia* wield the potential to reverse distorted haematological and biochemical status of rats previously infected with *P. berghei*.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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