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Original article

Haemato-biochemical alterations in *Plasmodium-berghei*-infected mice treated with methanol extact of *Sterculia setigera* leaves

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ABSTRACT

The effect of methanol extact of *Sterculia setigera* on some serum and liver enzymes, as well as, haematological parameters in *Plasmodium bergei*-infected mice was investigated. Fifteen mice were randomly allocated into six groups of three animals each. Group I and II were given normal saline (2 ml/kg) and chloroquine (5 mg/kg) to serve as untreated and treated controls, respectively. Groups III-V received the extract at 150, 300 and 600 mg/kg body weight, respectively. All treatments were administered through the oral route. Haematological and Biochemical parameters including alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities and total protein concentration in serum and liver of mice were evaluated. Results showed that the liver ALP activities significantly ($p<0.05$) increased in infected untreated mice. There were also increases in serum ALT and AST activities with concomitant decrease in liver activity of infected untreated group when compared with control mice and infected treated with extract. Treatment with methanol extract of *S. setigera*, however enhanced the situation as observed in the serum and liver enzymes activities in the treated mice. Total white blood cell was significantly lowered in untreated mice. There were also significant increases in the red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV) and mean cell volume (MCV) of infected extract (only at 600 mg/kg) treated mice when compared with the infected untreated. The results suggest that *S. setigera* probably has the ability to reduce *Plasmodium berghei* induce alteration in biochemical and hematological parameters. It could therefore be a useful cheap agent for the management of malaria

Keywords: Biochemical, Haematological, *S. setiger*, *Plasmodium berghei*, malaria

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INTRODUCTION

Malaria is a mosquito-borne infectious disease caused by a protozoan parasite of the genus *Plasmodium.* The disease is widely distributed in tropical and sub-tropical Africa and Southwest Asia [1]. The species capable of infecting human includes; *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax and Plasmodium malariae,* and *Plasmodium knowelsi* [2]. Malaria is a leading killer disease in the world, with about 3.2 billion people – almost half of the world's population at risk of malaria [3]. In 2013, there were about 198 million malaria cases with an uncertainty Kotepuirange of 124 million to 283 million and an estimated 584 000 malaria deaths with an uncertainty range of 367 000 to 755 000 [3]. Developing countries, where malaria is endemic, still depend on traditional medicine as a source for the treatment of the disease [4]. However, few scientific data are available to assess the efficacy of these herbal remedies [5]. Therefore, it is important that medicinal plants which have folklore reputation for antimalarial properties are investigated, in order to establish their efficacy and determine their potentials as sources of new antimalarial drugs [6].

Sterculia setigera Del. (Family; Sterculiaceae) is widespread in tropical Africa and is common locally, the natural distribution range stretches from Senegal to Cameroon in West Africa, Eastwards to Eritrea, and Southwards to Angola [7]. It is used in trado-medicine by various indigenous communities. The leaves are used to treat malaria, and the stem bark decoction is used for the treatment of asthma, bronchitis, wound, fever, toothache, gingivitis sore, abscess, and diarrhoea. The paucity of information on efficacy of *S. setigera* as a promising antimalarial agent informed this study to access the anti-plasmodial efficacy of methanol extract of *S. setigera*.

Blood is the most easily accessible diagnostic tissue. Changes in biochemical and haematological parameters are likely to be influenced by malaria, affecting health of mankind with various clinical presentations [8]. Malaria patients exhibit important changes in biochemical, including liver based enzyme and body proteins, as well as, haematological parameter including packed cell volume, low platelet, white blood cells and lymphocyte counts [8]. The haematological abnormalities that have been reported in malaria include anaemia, thrombocytopenia, lymphocytosis and rarely, disseminated intravascular coagulation [9].

MATERIAL AND METHODS

Plant Material

Leaves of *Sterculia setigera* were collected between May and June 2018 from Gidan mangoro, Minna, Niger State Nigeria. The plant was authenticated by a botanist from the Department of Animal Biology, Federal University of Technology, Minna. Voucher specimen of the plant sample was deposited in the Departmental Herbarium. Plant material were washed, immediately after collection and there after air-dried under shade in the Biology Laboratory for two weeks, pulverized to powdered form and stored in paper containers prior to extraction.

Extraction

The air-dried leaves (50 g) were extracted with 200 mL of methanol using soxhlet apparatus. The extract was filtered through a B¨uchner funnel using Whatman no. 1 filter paper. The extraction and filtration was

subsequently repeated three times with 200 m L of methanol and the combined solvent evaporated under reduced pressure at 30^{o}C.

Experimental Animals

Healthy albino mice of average weight 22-25g were purchased from small animal holding unit of School of Life Sciences, Federal University of Technology, Minna, Nigeria. The rodents were housed in standard environmental conditions of 70% relative humidity, (27 ± 20) °C of temperature, 12 h night/day light cycles, free water access and pellets. All experimental procedures involving animals were conducted in accordance to Canadian Council on Animal Care Guidelines and Protocol Review and approved by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research [10].

Reagent and Assay kits

The assay kits for T. protein, AST, ALT and ALP were products of Randox Laboratories Ltd, United Kingdom. All other reagents used were of analytical grade and were prepared in distilled water.

Parasites

Plasmodium berghei NK65 chloroquine sensitive strain was obtained from NIPRD Abuja, Nigeria and maintained in our laboratory by serial passage in mice.

Acute Toxicological Studies

Acute toxicological study was carried out to determine the median lethal dose (LD50) in accordance with Lorke's method [11].

***In vivo antiplasmodial* study**

Standard inoculums of 1 x 10^{7}*P. berghei* infected red blood cells were injected into mice intra-peritoneally. Seventy-two hours later, the mice were divided into five groups of three mice each. The negative control received distilled water and the positive control received chloroquine (5 mg/kg) and the test groups received 150 mg/kg, 300 mg/kg and 600 mg/ kg body weight of *S. setigera*. All treatment was administered intra-peritoneally for 5 days.

Determination of Haematological Parameters

The haematological components including haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), Granulocyte count (GRA) lymphocytes (LY), platelate count (PLT) *and* differential count were determined using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by [12].

Serum and Liver Collection

Mice were anaesthetized in slight chloroform through cardiac puncture. The blood was collected into a centrifuge tube and allowed to stand for 10 minutes and then centrifuged at 1000 rpm for 15minutes. The supernatant (serum) was carefully removed and stored until when needed for further analysis. The liver was check and transferred into ice-cold 0.25 M sucrose solution. This was later homogenized in ice-cold 0.25 M sucrose solution [1:5w/v] and the supernatant were used for further studied.

Serum and Liver Biochemical Assay

The enzymes: alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were assayed using standard procedures [13]. Total protein concentration was determined using Biuret method described by [14]. All measurements were done using UV Spectrophotometer.

Data analysis

Results were expressed as multiple comparison of Mean ± SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Turkey-Kramer multiple comparisons post-test. A probability level of less than 5% was considered significant [15].

RESULTS

Acute oral toxicity

The Acute oral toxicity profile of *S. setigera* is shown in Table 1. With the acute toxicity test up to the limit test dose of 5,000 mg/kg b.wt, neither mortality nor changes related to behavioural, autonomic and neurological profile were observed within the first 24 h.

The physical signs of toxicity observed at doses of 2900 and 5000 mg/kg b.wt included excitation, paw licking and increased respiratory responses; however, they disappears within the 24 h of extract administration.

Table 1: Acute Oral Toxicity profile of methanol extract of *S. setigera* in mice

Group	Dosage (mg/kg b.wt)	No of animals	Mortality
Group 1	10	3	0/3
Group 2	100	3	0/3
Group 3	1000	3	0/3
Group 4	1600	3	0/3
Group 5	2900	3	0/3
Group 6	5000	3	0/3

Biochemical parameters

Effects of methanol extract of *S. setigera* on serum and liver biochemical parameters are represented in Figure 1 to 4. There were significant ($p < 0.05$) decrease in liver AST activities with concomitant increase activities in serum of infected untreated mice when compared with normal control and extract treated group. However, no significant ($p > 0.05$) differences in serum liver AST activities of mice treated with the extract when compared with untreated mice (Figure 1). There were significant ($p < 0.05$) decrease in liver ALT activities with increase activities in serum of infected untreated mice when compared with normal control and extract treated group (Figure 2). There were significant ($p < 0.05$) increase in liver ALP activities (Figure 3) and total protein concentration (Figure 4) of infected untreated mice when compared with normal control and extract treated group.

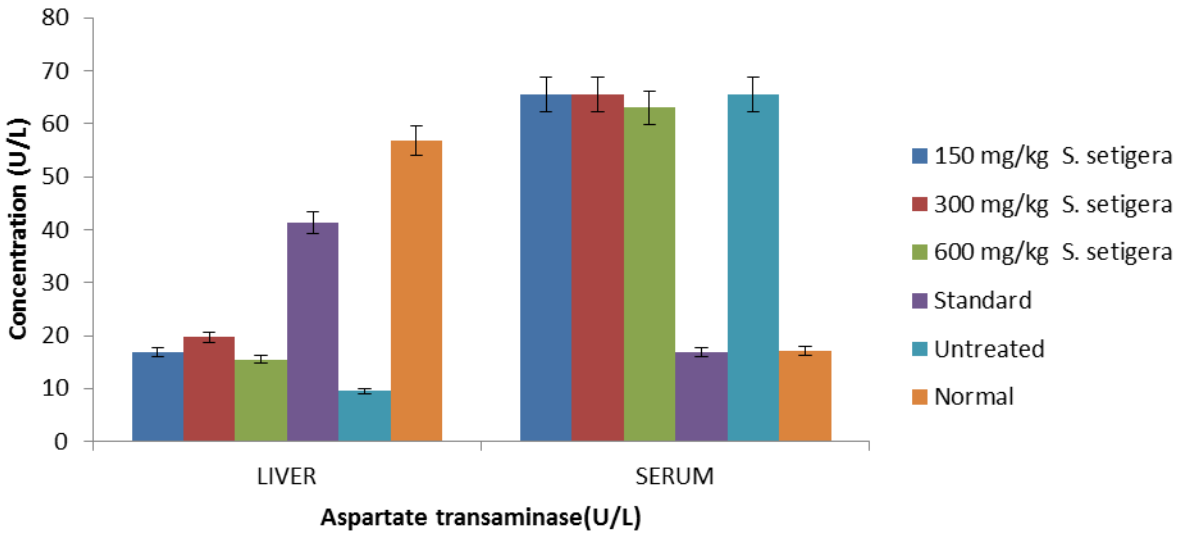

Figure 1: Effect of methanol extract of *S. setigera* on serum and liver AST in *P. bergei* infected mice

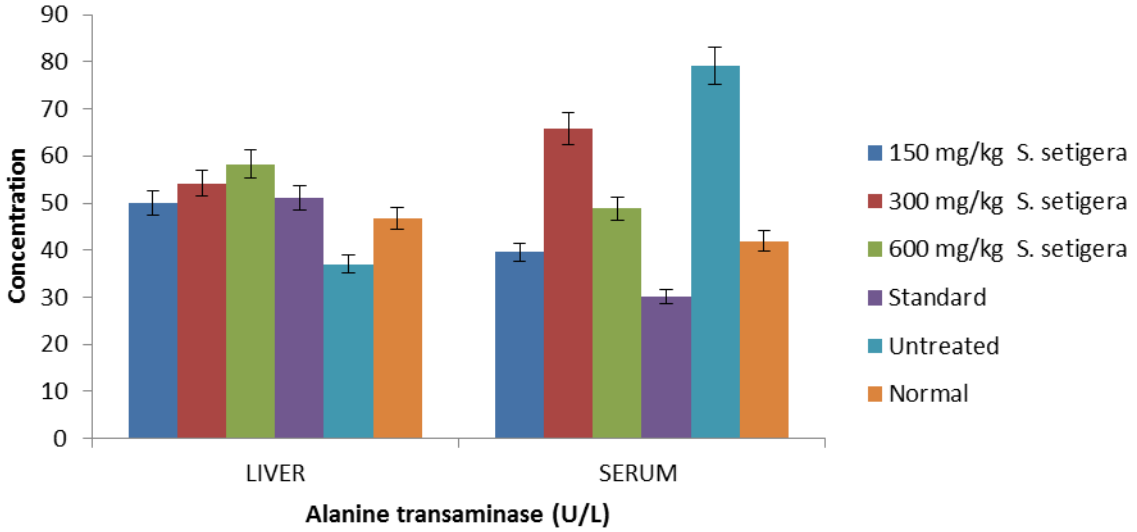

Figure 2: Effect of methanol extract of *S. setigera* on serum and liver ALT in *P. bergei* infected mice

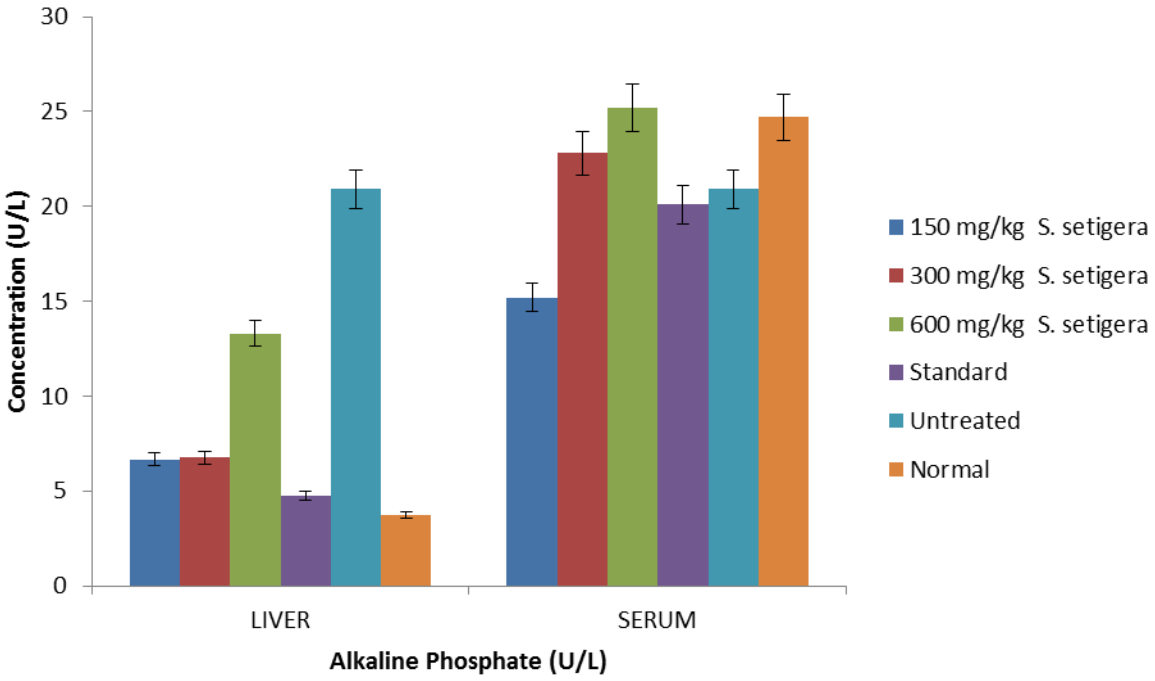

Figure 3: Effect of methanol extract of *S. setigera* on serum and liver ALP in *P. bergei* infected mice

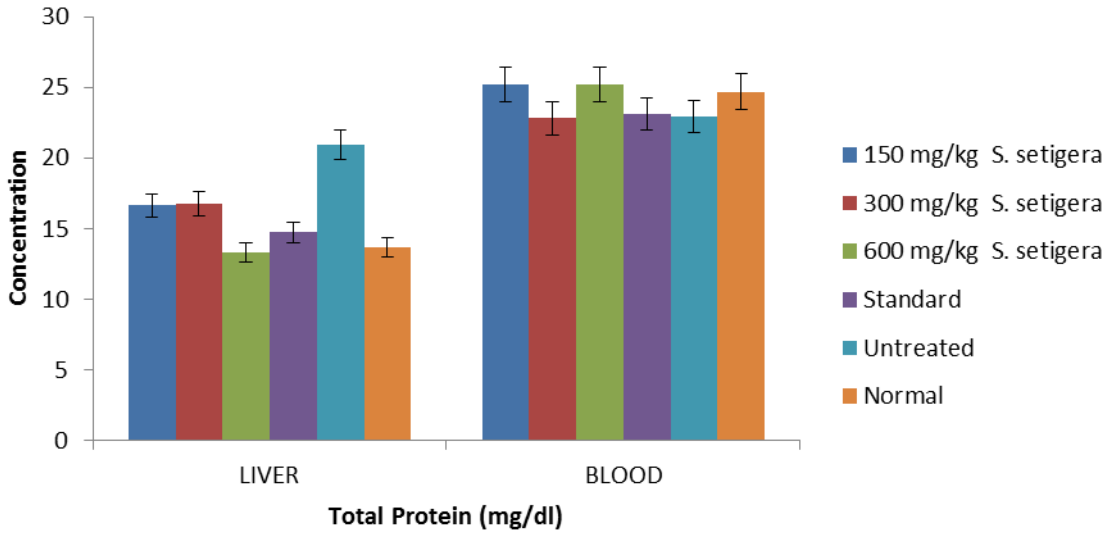

Figure 4: Effect of methanol extract of *S. setigera* on serum and liver total protein in *P. bergei* infected mice

Hematological parameters

Effect of methanol extract of *S. setigera* on haematological parameters in *P. bergei* infected mice is shown in Table 2. Infected untreated mice showed significant ($p< 0.05$) decrease in red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV)and mean cell volume (MCV) compared with the normal control and mice treated with 600 mg/kg of extract. However, mice treated with 150 and 300 mg/kg extract compared none significantly ($p>0.05$) with untreated mice. No significant ($p>0.05$) difference in mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) among all the experimental groups. TWBC were significantly lowered in untreated mice when compared with other experimental groups.

Table 2: Effect of methanol extract of *S. setigera*on hematological parameters in *P. bergei* infected mice

Treatments	HB	PCV	MCV	MCH	MCHC	RBC	PLC	TWBC
150 mg/kg *S. setigera*	10.10±0.10^{a}	29.45±0.10a	48.00±1.00^{b}	20.50±0.50^{a}	32.50±1.50^{a}	6.90±0.10^{ab}	731.00±3.00^{d}	4.00±0.00^{b}
300 mg/kg *S. setigera*	9.60±0.10^{a}	27.76±0.35^{a}	39.00±1.00^{a}	14.00±0.00^{a}	33.00±1.00^{a}	6.00±0.00^{ab}	356.10±0.10^{a}	3.00±0.00^{a}
600 mg/kg *S. setigera*	13.70±0.10^{b}	38.95±0.44^{b}	44.00±0.90^{b}	15.00±0.40^{a}	34.00±0.20^{a}	7.50±0.40^{b}	873.00±2.00^{e}	6.01±0.01^{c}
Standard	13.81±0.01^{b}	39.28±0.56 ^{b}	44.00±1.00^{b}	15.02±0.01^{a}	35.00±0.00^{a}	7.71±0.01^{b}	479.00±4.00^{b}	8.10±0.20^{e}
Untreated	9.00±0.00^{a}	29.76±0.52^{a}	34.45±0.21^{a}	15.16±0.21^{a}	35.50±0.40^{a}	5.35±0.20^{a}	541.04±3.67c	3.27±0.29^{a}
Normal	14.90±0.12^{d}	39.76±0.87 ^{b}	43.00±0.00^{b}	16.01±0.01^{a}	32.00±0.50^{a}	9.35±0.07^{c}	558.00±2.00^{c}	7.10±0.10^{d}

Values are expressed as means ± Standard error of three replicates

Values followed with the same superscript alphabets on the same column are not significantly different at p >0.05

n= 5

Keys: HB = Haemoglobin Count; PCV = Packed Cell Volume; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration; RBC = Red blood cell count; TWBC = Total White blood cell count; PLC = Platelet Count

DISCUSSION

Malaria is a global leading cause of morbidity and mortality due to frequent haemato-biochemical alteration that occur during the infections [3].Therefore evaluation of hematological and biochemical parameters during malaria infection is a valuable tools for monitoring pathological effect of the infection as well as treatment outcome of a test substances. It has been established that malaria infection can alter the normal values of hematological indices [16]. However, efficacy of folklore herbal remedies in ameliorating parasite induce haemato-biochemical alterations has been well documented [5]. Many studies have been carried out in recent years on the pharmacological effects of *S. setigera* [7]. In the present study, significant protective effects of *S. setigera* against *P. bergei* induce hemato-biochemical alteration was recorded.

Erythrocytes, leucocytes and thrombocytic indices provide valuable information on the adverse effects of malarial infections on the blood and also explain blood-related functions of chemical compounds [17].The increases in red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV) and mean cell volume (MCV) in mice treated with 600 mg/kg of extract in comparison with infected untreated suggest that methanol extract of *S. setigera* reduces the severity of *P. bergei* infection in mice. This is an indication that methanol extract of *S. setigera* has beneficial effects in stimulating the erythropoietin release in the kidney, which is the humoral regulator of RBC production during malarial infection [18]. The observed increases in Hb and RBC concentrations are probably as a result of reduced severity of the infection and possible increase in the synthesis of the protein in line with observations. White blood cell and its differentials are known for their defensive role against foreign body and infectious agents through the production, transportation and distribution of antibodies in immune response [19]. The increased WBC and platelet counts are also indicative of the increased host action in the presence of *S. setigera* against the infection as these will contribute to the development of phagocytes and antibodies against the recognizable antigens of parasite origin.

Evaluation of serum biochemical indices in animals has become the most valuable tools for assessing the integrity and functionality of organs as well as risk assessment, pathological condition [20] during malarial infection. AST and ALT are biomarkers of hepatic integrity and to a certain level can be used to assess the extent of hepatocellular damage, The ALT activities however, give more valuable information relevant to the integrity of the hepatocyte than AST [18]. In the present study, the liver ALP activities are increased in infected untreated mice when compared with those of the treated and normal control. This suggests and probably confirms earlier results [21] that infection could lead to gradual tissue especially liver destruction. Similarly, the increase in serum ALT and AST activities with concomitant decrease in liver activity of infected untreated group when compared with infected treated with methanol extract of *S. setigera*, suggested possible leakage of the enzyme from tissues as a result of damage to the cell membrane [22]. Treatment with methanol extract of *S. setigera* however enhanced the situation as observed in the serum and liver enzymes activities in the treated mice

CONCLUSION

The results suggest that *S. setigera* probably has antiplasmodial ability to reduce parasitaemia induce alteration in biochemical and hematological parameters. It is however possible, at this point, that *S.*

setigera could be a useful cheap agent for the management of malaria infection as its benefits outweigh any possible side effects it may possess

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