In vivo Antimalarial Activity and Toxicological Effects of Ethanolic and Hot Water Extracts of *Rauwolfia serpentina* leaf in mice infected with Chloroquine-sensitive *Plasmodium berghei*

FUNMILOLA OLUYEMI OMOYA[1], OLATUNDE ADEMOYE FALUSI [1], AYODELE OLUYEMISI OGUNDARE[1]

[1]*Department of Microbiology, The Federal University of Technology, Akure*

Received 27 April 2019
Accepted 28 July 2019

ABSTRACT

Objective: Antiplasmodial activities of *Rauwolfia serpentina* and its impact on the haematological and organ function indices in mice were evaluated in this study.
Methods: The dried powdered leaf was exhaustively extracted with hot water and ethanol successively and extracts were screened for phytochemicals. Prophylactic and curative antimalarial test were carried out using *Plasmodium berghei* NK65 infected mice, to which the extract was administered at doses of 100, 200 and 400 mg/kg body weight (BW). Toxicity of the extract was evaluated in rats using selected hematological parameters and organ function indices after prophylactic and curative test.
Results: In 5 days curative tests, hot water and ethanol extract suppressed parasitaemia by 82.53 and 88.12% at 400 mg/kg dose level, respectively however in prophylactic test, water extract has chemo-suppression of 97.72% while ethanol extract had 99.83%. All parasitaemia suppressions were statistically significant at $P < 0.05$ and are dose dependent. All hematological parameters evaluated were not significantly altered ($P>0.05$) at all doses of the extract, except packed cell volume which was significantly higher ($P<0.05$) at 200 mg/kg BW. The extract significantly increased ($P<0.05$) bicarbonate, urea, creatinine, uric acid and calcium in the serum at higher doses compared to the controls. Alkaloid, anthraquinone, cardenoloides, glycosides, phenol, phlobatanin, resin and saponin were present in the extract.
Conclusions: The results support the traditional use of the herbal combination in the treatment of malaria.

KEY WORDS: Prophylactic
Antiplasmodial
Phytochemicals.

Introduction

Traditional medicines have played an important role in provision of healing as well as to the discovery of most pharmacologically active substances in plant such as tannin, saponnins, alkaloids, steroids, terpenoids, flavonoid which have been exploited in the commercial production of drugs worldwide [1]. The relationship between the indigenous people and their plants forms the subject of ethno botany.

Rauwolfia (Rauvolfia) plant belongs to the family apocynaceae and occurs in terrestrial habitats of tropical and subtropical regions with over 50 species that are distributed worldwide. In Nigerian, the plant is commonly referred to as Asofeyeje (Yoruba), Akanta (Igbo) and Aada (Hausa) [2]. *Rauwolfia serpentina* (L). Benth. ex Kurz is an important medicinal plant of Indian subcontinent and South East Asian countries [3]. It is evergreen perennial, glabrous and erect under-shrub plant. The main alkaloid present in the root, stem and leaves of *Rauwolfia serpentina* which is called reserpine has long been widely used as antihypertensive drug South East Asian countries [4]. Leaf paste of this plant made into pill, sun dried has been used in treatment of malarial fever by the Mandi ethnic community in Bangladesh [5]. Malaria is a mosquito-borne infectious disease affecting humans and other animals, it is caused by haemoparasitic protozoans [6]. There are five species of *Plasmodium* that may infest man, namely; *P. falciparum, P. vivax , P. ovale , P. malariae* and *P. knowlesi* [7]. Most deaths are caused by *P. falciparum* because *P. vivax , P. ovale ,* and *P. malariae* generally cause a milder form of malaria [8] while *P. knowlesi* rarely causes disease in humans [9]. Malaria is a major en-

Correspondence to: Dr. Funmilola Oluyemi Omoya

Email: fomoya@yahoo.com

demic disease with high mortality rate in many tropical and subtropical countries. Malaria is one of the most prevalent, devastating parasitic infectious diseases in the world. Forty percent of the world's population is at risk of infection in about 106 countries and in year 2016 there were an estimated 216 million cases of malaria reported worldwide and an estimated 445,000 deaths worldwide [10]. Globally, the two regions with the highest malaria transmission rate are Oceania and sub Saharan Africa. The greatest brunt of the disease is felt in sub-Saharan Africa where more than 90% of morbidity and deaths occurs in Nigeria and Democratic Republic of Congo and accounted for more than 35% of global malaria death in 2015 [10]. Between 2000 and 2015, malaria incidence rate fell by 37% globally and by 42% in Africa. During this same period, malaria mortality rate fell by 60% globally and 66% in the African region [10].

The burden of this disease is on the rise partly due to the increasing resistance of *Plasmodium falciparum* against the widely available antimalarial drugs. Chloroquine (CQ) was the mainstay of malaria treatment for many decades, but development of drug resistance by the parasite led to therapeutic failure [11].

Materials and Methods

Source and Identification of Plant Leaves

The leaves of *R. serpetina* was harvested from Owo metropolis, Ondo North Senatorial District, Ondo State before the sunrise to prevent plant photo-oxidation, the leaf that has neither injury nor chlorosis were sorted out and kept in a clean sack for further work. The plant was authenticated by plant scientist at the Federal University of Technology, Department of Crop Science and Pest management.

Processing and Extraction of *Rauwolfia Serpentina* Leaves

The method of [12] modified was used. The fresh leaves were washed with sterile distilled water and air dried on a mat until they turned brittle and fully crispy. The dry leaves were crushed manually using clean mortar and pestle, then pulverized into fine powder by a blending machine (Philips HR2001). They were separately kept in an airtight container to avoid the absorption of moisture. The powdered samples were soaked for 72 hours (3 days) in 70% ethanol and hot water (100 °C) in the ratio 1:10 each (500 g of the powdered sample in 5000 ml of 70% ethanol and hot water separately) for the extraction of the bioactive compounds from the plants. After 72 hours it was sieved using muslin cloth and then filtered using Millipore filter paper. The filtrates were vaporized to dryness using rotary evaporator (Union Laboratories England). The extracts were preserved in a sterile bottle at 4 °C ready for use.

Source of Experimental Mice

Experimental mice were obtained from school of Biological Science, Afe Babalola University, Ado-Ekiti and kept in a wooded cage for 14 days for acclimatization under standard environmental conditions with a 12 hour light/dark cycle and were maintained on standard feed (vital feed) and water *ad libitum.* The laboratory animals were used in accordance with laboratory practice regulation and the principle of humane laboratory animal care as documented by [13]. They weigh between 19-23g and were all male. During the acclimatization period, all mice were well fed with water and commercial feed.

Parasite inoculum preparation

The inoculum consisted of 1.2×10^6 *Plasmodium berghei* parasitized red blood cells per millilitres. This was done by determining both the percentage of parasitaemia and the red blood cells count of the donor mice (this was obtained from Nigeria Institute of Medical Research (NIMR), Yaba, Lagos) using an improved neubear haemocytometer and diluting the blood with normal saline.

Infection of Experimental Mice

The mice were carefully infected intraperitonially with 0.2 ml of 1.67×10^6 parasitised red blood cell of *Plasmodium berghei* NK 65.

Making of Blood Films

The parasitemia levels of the mice were observed after thin smears was prepared from tail blood on microscopic slides, dried and fixed with methanol. The blood films were stained with 10% Giemsa for 15 minutes and allowed to air-dry before it was examined under the microscope. The parasitemia were determined by counting minimum of three fields per slide with 100 RBC per field [14]. The percentage suppression of parasitaemia was calculated for each test concentration by comparing the para-

sitaemia in infected controls with those that received different concentrations of the test extract [15].

Parasitemia Count

The parasitemia count was carried out daily starting from after the first oral treatment. This was carried out by a little cut on their tail and making a smear of the blood on the slide.

$$\%\text{Parasitaemia} = \frac{\text{total number of Parasitized red blood cells}}{\text{total number of red blood cells}} X\ 100$$

$$\%\text{Chemo} - \text{suppression} = \frac{(\text{Parasitaemia in control group} - \text{parasitaemia in study group})\ \text{X}\ 100}{\text{Parasitaemia in control group}}$$

Standard Drug

Chloroquine was obtained from a registered Pharmacy in Akure, Ondo State and prepared to 5 mg/ml for the group that served as the positive control. They were given 0.2 ml of the drug for the treatments (prophylactic and curative test).

Reconstitution of the Ethanolic and Hot Water Extract

Plant extracts were reconstituted separately in distilled water to give the required doses of 100, 200 and 400 mg/kg body weight (BW) used in this study. The reconstituted crude extracts were given to mice oralgastrically.

Experimental Design

Mice were grouped into 8 groups of 3 mice each labeled A-H as follows;

Group A- Infected and treated with 100mg/kg *R. serpentina* of ethanol extract.

Group B- Infected and treated with 200mg/kg *R. serpentina* of ethanol extract.

Group C- Infected and treated with 400mg/kg *R. serpentina* of ethanol extract.

Group D- Infected and treated with 100mg/kg *R. serpentina* of hot water extract.

Group E- Infected and treated with 200mg/kg *R. serpentina* of hot water extract.

Group F- Infected and treated with 400mg/kg *R. serpentina* of hot water extract.

Group G- Infected and treated with 5mg/kg chloroquine.

Group H- Infected and treated with 0.2mls of distilled water.

Antiplasmodial Curative Activity of *R. serpentina* Leaf Extracts on Mice Infected with *Plasmodium berghei*

Curative assay was carried out by inoculating each mouse in the 8 groups with the parasite intraperitonally and left for 72 hours (3 days) to achieve parasitemia count of 100,000 parasites/µl. Mice in group A-F were treated orogastrically with plant extracts at different concentrations (100, 200 and 400 mg/kg) while mice in the positive (Group G) and negative control (H) group were treated with 5 mg/kg chloroquine and 0.2mls of distilled water respectively for 5 days. The blood samples of the mice were collected daily from the tail of the mice for the 5 days of treatment to check the effect of the extract on the parasite level.

Prophylactic Activity of *R. serpentina* Leaf Extracts of Mice Infected with *Plasmodium berghei*

Prophylactic activity was tested. Mice in group A-F were treated with graded dose of extracts, group G and H received 5 mg/kg of chloroquine (positive control) and 0.2 ml of normal saline (negative control) for 3 days after which the mice were inoculated with *Plasmodium berghei*. After 72 hours of inoculation, the parasitemia level of each mouse was evaluated.

Weight Evaluation

Weights of the mice were taken daily using a digital weighing machine. The mice were demobilized and fixed in a container while taking note of the weight of the container.

Temperature Evaluation

Temperatures of the mice were checked daily using a clinical thermometer fixed into their anus for 30 seconds after demobilizing them in a fixed container. The readings were taken daily for the period of the experiment.

Phytochemical Screening

The phytochemical analysis was carried out according to the standard methods of analysis by [16].

Collection of Blood from Experimental mice

Collection of blood samples from the mice was carried out by Anaesthetization method. Cardivascular puncture was used to withdraw blood from mice. The blood collected

was carefully transferred into EDTA bottles for haematological assays [17].

Haematological Tests

Haematological tests such as Packed Cell Volume (PCV), Haemoglobin Concentration (HB), Red Blood Cell Count (RBC), Erythrocyte Sedimentation Rate (ESR), White Blood Cell Count (WBC) and White Blood Cell differential count were done according to [17].

Biochemical Tests for Organ function indices

Biochemical tests for determination of bicarbonate, creatinine, calcium, uric acid and urea level were done according to [18]. The general procedures involve the pipetting of the standardized amount (0.5 ml) of sample that was applied on the test zone of the appropriate test strip. The strips were inserted into the test chamber of Reflotron M06-02<06.00 (produced by Boehringer Mannheim Company, Germany) and the flap closed. The tests were carried out at 25 ^{o}C. The results obtained were displayed on the computer monitor connected to the machine after few seconds. All assays were carried out in triplicates.

Statistical Analysis

Result obtained were subjected to descriptive statistics and one way Analysis of Variance (ANOVA), means were separated by new Duncan Multiple Range and significant level was taken at ($p<0.05$) using SPSS version 20 Microsoft windows 8.

Results

Parasiemia Count and Chemo-suppression

It was observed that the parasitemia count and chemo-suppressive activity result of curative and prophylactic treatments at 100, 200 and 400 mg/kg body weight as shown in Table 1 was dose dependent and also had better suppression activity with ethanol extract than the hot water extract. For the curative treatment, chloroquine had the highest chemo-suppression activity (88.98%) while the 400 mg/kg BW of *R. serpentina* ethanol extract had chemo-suppression activity of 88.12%. However, the group treated with 100 mg/kg body weight water extract had the least chemo-suppression activity of 70.46%. Comparatively, for the prophylactic treatment, it was observed that plant extracts had higher chemo-suppression activity than the chloroquine treated mice. The 400 mg/kg body weight of ethanol extract had the highest suppression activity of 99.83% while the chloroquine treated group had the least chemo-suppression activity of 81.88%.

Table 1. Curative and Prophylactic effects of *R. serpentina* ethanol and hot water leaf extract on *P. berghei* infected mice

Group	Doses mg/kg	Average Parasitemia (parasite/µl)	% chemo suppression
		Curative	
A	100	19364.20±2186.03^{bc}	77.21
B	200	13479.80±4557.52^{ab}	84.14
C	400	10095.00±3717.28^{a}	88.12
D	100	25098.60±2150.48^{c}	70.46
E	200	18985.60±1383.18^{bc}	77.66
F	400	14847.60±1927.09^{ab}	82.53
G	5	9366.75±424.46^{a}	88.98
H	5	84971.50±1906.80^{d}	
		Prophylactic	
A	100	2056.00±139.31^{a}	95.93
B	200	919.00±43.84^{a}	98.18
C	400	383.50±44.35^{a}	99.83
D	100	4687.25±302.05^{ab}	90.72
E	200	2238.50±161.54^{a}	95.57
F	400	1150.75±150.99^{a}	97.72
G	5	9150.00±1621.47^{b}	81.88
H	5	50500.00±5484.83^{c}	

Values are presented as Mean ± SEM, Values in the same column followed by the same letter do not differ significantly ($p \geq 0.05$) according to New Duncan's Multiple Range test.

Effect of Plant Extracts on body weight of Mice Infected with *Plasmodium berghei* in curative and prophylactic treatments

The result of curative and prophylactic treatment on the mice showed (Table 2) that the extract treated group increased the body weight of the mice. The 200 mg/kg body weight of the plant extract treatments (curative and prophylactic) had the highest increase in weight of mice from an initial weight of 18.21±0.37 and 18.50±0.50 kg to 19.96±0.40 and 20.25±0.50 kg after 5 days of treatment respectively. Comparatively, untreated infected mice in group H (negative control groups) had significant reduction in the body weight of the mice from 19.45±1.23 and 18.20±0.10 kg (initial body weight) to 16.21±0.97 and 16.50±0.05 kg (after 5 days) respectively while the chloroquine treated group had no significant difference after 5 days of treatment.

Effect of Plant Extracts on body temperature of Mice Infected with *Plasmodium berghei* in curative and prophylactic treatment

The effect of curative treatment on the temperature of infected mice showed (Table 3) there was increase in the body temperature of mice after 5 days of treatment as

against the untreated group which had reduction in the temperature.

Table 2. Effect of Plant Extract on body Weight of Mice Infected with *Plasmodium berghei* in curative and prophylactic treatment.

Groups	Initial body weight (g)	Days		
		D_1	D_3	D_5
Curative				
A	19.67±1.04^{a}	23.66±0.01^{c}	24.65±0.31^{d}	20.27±0.21^{b}
B	18.21±0.37^{a}	18.27±0.63^{a}	19.22±0.84^{b}	19.96±0.40^{c}
C	20.58±0.56^{b}	18.98±0.21^{a}	18.44±0.34^{a}	20.62±0.34^{b}
D	19.94±0.62^{a}	24.42±0.25^{c}	24.51±0.32^{c}	19.98±0.61^{a}
E	19.21±0.23^{b}	18.67±0.33^{b}	18.25±0.75^{a}	19.43±0.73^{b}
F	20.62±0.22^{b}	19.00±0.67^{a}	18.80±0.41^{a}	20.78±1.62^{b}
G	20.66±0.36^{b}	20.21±0.44^{b}	20.52±0.21^{b}	20.49±0.55^{b}
H	19.45±1.23^{c}	19.02±1.57^{c}	17.15±0.75^{b}	16.21±0.97^{a}
Prophylactic				
A	20.51±0.43^{c}	19.77±0.11^{b}	19.86±1.04^{a}	19.94±0.32^{a}
B	18.50±0.50^{a}	18.60±0.04^{a}	19.50±0.05^{b}	20.25±0.50^{b}
C	22.61±0.71^{b}	22.59±0.08^{b}	22.55±0.16^{b}	22.72±0.31^{b}
D	24.20±0.32^{c}	24.12±0.41^{c}	23.78±0.22^{b}	24.72±0.06^{b}
E	18.70±0.30^{b}	18.71±0.03^{b}	18.30±0.60^{a}	18.50±0.05^{b}
F	23.52±0.53^{b}	23.34±0.32^{a}	23.27±0.44^{a}	23.42±0.06^{a}
G	24.04±0.06^{a}	23.89±0.01^{a}	23.99±0.03^{a}	24.01±0.52^{a}
H	18.20±0.10^{c}	17.82±0.12^{bc}	16.60±0.02^{a}	16.50±0.05^{a}

Values are presented as Mean ± SEM, Values in the same column followed by the same letter do not differ significantly ($p \geq 0.05$) according to New Duncan's Multiple Range test.

Table 3. Effect Plant Extracts on body temperature of Mice Infected with *Plasmodium berghei* in prophylactic and curative treatment

Groups	Initial body temperature (ºC)	Days		
		D_1	D_3	D_5
Prophylactic				
A	34.72±0.33^{c}	34.17±0.31^{a}	34.03±0.51^{a}	34.17±0.61^{a}
B	36.60±0.50^{a}	36.60±0.20^{a}	36.60±0.20^{a}	36.80±0.20^{a}
C	34.74±0.33^{c}	34.86±0.45^{d}	34.45±0.22^{a}	34.54±0.40^{b}
D	34.67±0.21^{b}	34.48±0.42^{a}	34.77±0.55^{c}	34.68±0.22^{b}
E	36.70±0.30^{a}	36.80±0.20^{a}	36.70±0.20^{a}	36.80±0.10^{a}
F	34.69±0.01^{b}	34.87±0.17^{c}	34.76±0.32^{c}	34.98±0.04^{d}
G	34.42±0.41^{a}	34.32±0.11^{a}	34.40±0.02^{a}	34.40±0.01^{a}
H	35.90±0.10^{c}	35.60±0.20^{c}	35.20±0.10^{b}	34.80±0.05^{a}
Curative				
A	36.52±0.01^{c}	35.78±0.52^{b}	34.62±0.51^{a}	35.61±0.21^{b}
B	36.70±0.03^{b}	36.51±0.50^{a}	36.80±0.40^{b}	36.80±0.50^{b}
C	35.78±0.01^{c}	35.85±0.52^{c}	35.38±0.34^{b}	36.65±0.13^{d}
D	36.44±0.44^{e}	36.21±0.32^{d}	34.81±0.41^{b}	35.33±0.05^{c}
E	36.50±0.10^{c}	36.40±0.60^{b}	36.00±0.11^{a}	36.20±0.50^{b}
F	36.10±0.11^{c}	35.78±0.41^{b}	35.70±0.22^{b}	36.63±0.32^{d}
G	35.89±1.24^{b}	35.61±0.02^{b}	35.00±0.13^{a}	35.67±0.43^{b}
H	36.80±0.04^{c}	36.20±0.40^{b}	35.11±0.97^{a}	34.60±0.40^{a}

Values are presented as Mean ± SEM, Values in the same column followed by the same letter do not differ significantly ($p \geq 0.05$) according to New Duncan's Multiple Range test.

The mice in all the groups had an initial average body temperature of 36.28±0.24 ºC. After 5 days of treatment, the body temperature of mice in the negative control group reduced significantly to 34.60±0.40 ºC, however the highest significant increase (36.80±0.50 ºC) in the extract treated groups was observed in the ethanol extract of 200 mg/kg body weight while there was no significant difference in the choroquine treated mice. The prophylactic treatment result as showed (Table 3) , there was no significant difference in the initial body temperature of mice after 5 days in the treated mice with plant extracts and choroquine respectively, however there was significant decrease in the body temperature of mice observed in untreated group from 35.90±0.10 ºC (initial body temperature) to 34.80±0.05 ºC.

Haematological parameters

Figure 1 and 2 showed the effect of curative and prophylactic treatment on haematological parameters and differential White Blood Cell counts (Figure 3 and 4) of treated and the untreated group. There was significant ($p<0.05$) increase in the Packed Cell Volume (PCV) and decrease in the Erythrocyte Sedimentation Rate (ESR) of the treated group compared to untreated group. For the curative treatment, mice treated with 200 mg/kg of *R. serpentina* ethanol extract had highest increase in PCV from 28.33±1.45% (negative control) to 43.33±1.45% and the least decrease in ESR from 6.50±.29 mm/hr to 1.17±1.67 mm/hr at the same concentration and no significant difference with the chloroquine treated mice except the WBC which had 9.59±0.21 $mm^{-3}\times10^{6}$ (chloroquine treated mice) and 14.97±0.18 $mm^{-3}\times10^{6}$ (400 mg/kg hot water extract). The WBC differential count showed that parasite caused reduction to the lymphocytes (48.33±1.86%) as against the plant extract (69.00±0.58%) and chloroquine (62.67±0.33%) treated mice respectively. The prophylactic treatment showed that chloroquine and plant extract had PCV values of 41.00±1.00 and 46.67±0.33 % (400 mg/kg ethanol extract) respectively.

Organ function indices (Biochemical parameters)

The curative and prophylactic result of the biochemical assay as showed in Table 4 revealed that the positive control group, 100 and 200 mg/kg treated group significantly ($p<0.05$) decreased the biochemical parameters and at a

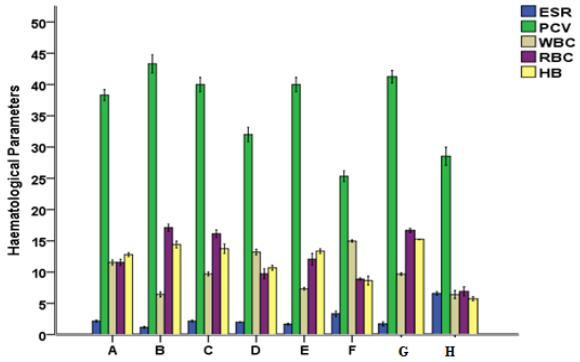
Table 4. Result of Biochemical Assay of Curative Treatment

Groups	BC (mMol/L)	CR (mMol/L)	UA (mMol/L)	U (mMol/L)	CA (mMol/L)
			Curative		
A	21.33±2.40^{bc}	0.06±0.01^{a}	0.33±0.02^{ab}	3.60±0.23^{a}	3.50±0.32^{c}
B	31.33±2.40^{e}	0.05±0.00^{a}	0.46±0.02^{abc}	3.90±0.15^{abc}	2.53±0.18^{a}
C	28.67±1.76^{de}	0.17±0.01^{cde}	0.55±0.04^{cd}	3.80±0.23^{ab}	2.43±0.12^{a}
D	16.00±1.15^{ab}	0.14±0.01^{bcd}	0.48±0.03^{bcd}	5.27±0.23^{ef}	5.37±0.15^{f}
E	26.00±1.15^{cde}	0.08±0.01^{a}	0.36±0.03^{ab}	4.57±0.26^{cd}	3.17±0.14^{bc}
F	41.33±1.86^{f}	0.47±0.04^{f}	0.48±0.05^{bcd}	4.80±0.12^{de}	4.47±0.20^{d}
G	31.33±1.45^{e}	0.06±0.00^{a}	0.49±0.01^{bcd}	4.23±0.02^{abcd}	2.53±0.12^{a}
H	51.33±1.20^{g}	1.19±0.05^{g}	0.64±0.03^{d}	5.93±0.04^{g}	5.37±0.15^{f}
			Prophylactic		
A	16.00±1.15^{a}	0.08±0.01^{a}	0.23±0.02^{a}	3.20±0.15^{a}	3.67±0.28^{bc}
B	27.00±1.17^{cd}	0.12±0.01^{ab}	1.07±0.09^{h}	4.40±0.35^{bcd}	4.40±0.23^{d}
C	32.00±1.15^{d}	0.25±0.03^{c}	0.50±0.05^{cdef}	4.17±0.08^{bc}	3.03±0.12^{ab}
D	28.00±2.08^{cd}	0.17±0.03^{b}	0.38±0.03^{bcd}	4.57±0.24^{bcd}	4.50±0.21^{d}
E	19.00±0.58^{ab}	0.08±0.01^{a}	0.90±0.06^{g}	4.50±0.17^{bcd}	3.10±0.17^{ab}
F	53.67±0.48^{f}	0.52±0.02^{e}	0.62±0.04^{ef}	6.33±0.24^{f}	4.70±0.12^{de}
G	31.33±1.45^{d}	0.06±0.00^{a}	0.49±0.01^{bcde}	4.23±0.02^{bc}	2.53±0.12^{a}
H	51.33±1.20^{f}	1.19±0.05^{f}	0.64±0.03^{f}	5.93±0.03^{f}	5.37±0.15^{f}
Standard value	10-35	0.05-0.15	0.2-0.5	2.5-5.0	2.5-4.0

Values are presented as Mean ± SEM, Values in the same column followed by the same letter do not differ significantly ($p \geq 0.05$) according to New Duncan's Multiple Range test.

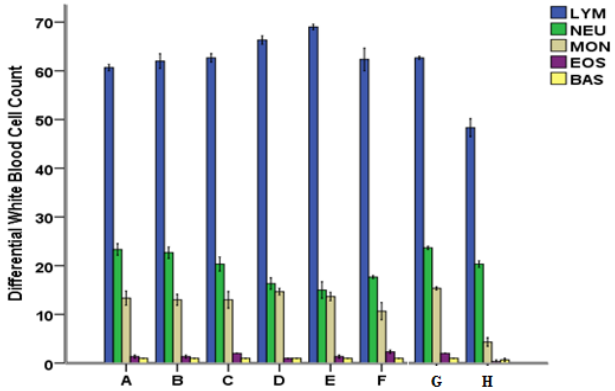
higher concentration (400 mg/kg) increased some parameters compared with the standard range of organ function indices. Water extract of 400 mg/kg (prophylactic treatment) had the highest increase in biocarbonate (53.67±0.48 mMol/L), creatinine (0.52±0.02 mMol/L), uric acid (0.62±0.04 mMol/L), urea (0.62±0.04 mMol/L) and calcium (4.70±0.12 mMol/L).

Figure 1. Haematological assay curative treatment.



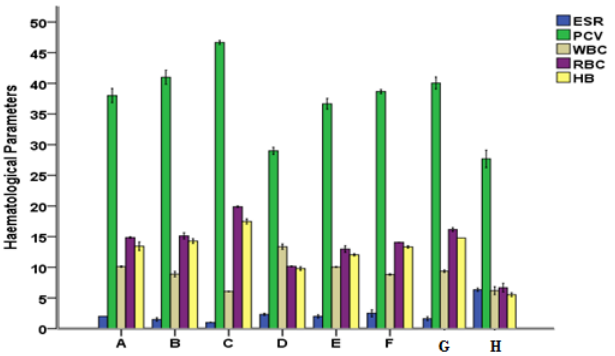
Keys: ESR= Erythrocyte Sedimentation Rate, PCV= Packed Cell Volume, WBC= White Blood Cells, RBC= Red Bloood Cells, HB= Haemoglobin.

Figure 2. Differential White Blood Cell count of curative treatment



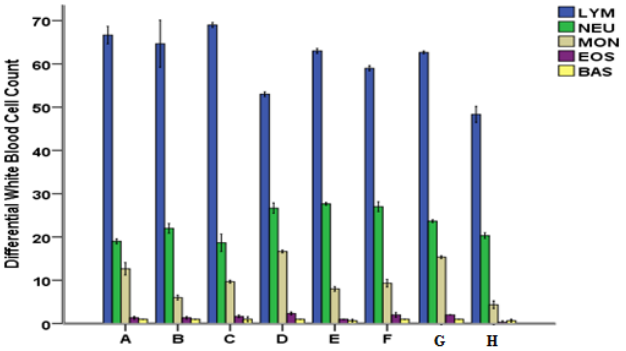
Keys: LYM= Lymphocytes, NEU= Neutrophils, MON= Monocytes, EOS= Eosinophils, BAS= Basophils

Figure 3. Haematological assay of prophylactic treatment.



Keys: ESR= Erythrocyte Sedimentation Rate, PCV= Packed Cell Volume, WBC= White Blood Cells, RBC= Red Bloood Cells, HB= Haemoglobin.

Figure 4. Differential White Blood Cell count of Prophylactic treatment



Keys: LYM= Lymphocytes, NEU= Neutrophils, MON= Monocytes, EOS= Eosinophils, BAS= Basophils

Qualitative Phytochemical Compositions of *R. Serpetina* and *R. Vomitoria* Extracts

The result of *R. Serpetina* extracts qualitative phytochemical compositions revealed that alkaloid, anthraquinones, cardenoloides, glycosides, phenol, phlobatanin, resin and saponin were present in hot water and ethanol extract of both plants, however, ajimaline and reserpine was absent in the water extract.

Discussion

The results of this study indicated that the water and ethanol extracts of *R. serpentina* produced a dose dependent chemo-suppressive antimalarial activity. The ethanol extract of *R. serpentina* had higher chemo-suppression activity than the water extract, this could be that ethanol extract contained more bioactive compounds which act as antiplasmodial agents. This corroborates the work of [19], the concentrations and proportions of the active compounds in plant extracts components depend on the solvent used in processing the plant. This could also be due to the presence of serpentine in *R. serpentina* which acts as a type II topoisomerase inhibitor [20]. This justifies the work of [21] which stated that drugs that target the type II topoisomerase could stop the proliferation of malaria parasite by inhibiting DNA transcription, DNA replication, DNA repair, and overall cell division of the parasite. This result also showed that the plant extracts had a better chemo-suppressive activity for the prophylactic assay than the curative assay; this indicates that the plant extracts provide a better antiplasmodial efficacy when used as prophylaxis antiplasmodial drug.

The extracts produce notable increase on the body tem-

perature of the treated groups; this is may be due to the antiplasmodial effect of the plant extracts as there was significant decrease in the body temperature of mice in the untreated group. According to [22] this may be due to the high level of parasitemia which tends to disrupt the body's temperature-regulating center.

Comparatively the result indicated that the extracts significantly caused body weight increase in treated mice and the increase is dose dependent compared to the untreated group. The result is in accordance with the study of [23, 24] who stated that the physical status of experimental animals were better and this may be due to ameliorating effect of the plant extracts on acute fluid loss, proteolysis and lipolysis which are usually associated with weight loss in malaria infection.

According to [25], malaria infection is associated with increased risk of severe anaemia. The observed low Heamoglobin and Pack Cell Volume (PCV) in the untreated group may be due to RBC destruction caused either by parasite multiplication or by spleen reticuloendotelial cell action [26]. In this study, the extracts produced a significant positive effect on the Packed Cell Volume (PCV), Red Blood Cells (RBC), hemoglobin (HB) and White Blood Cells (WBC) values after treatment except the Erythrocyte Sedimentation Rate (ESR) when compared with the untreated group. This corroborate the study of [23], who stated that plant extracts used in the treatment of malaria increased the PCV, HB and WBC of experimental mice significantly. Similar to this present study, [27] reported that there was a general increase in the WBC count following treatment, which also lends support to the study of [28], who reported that malaria infection may contribute to the localization of leukocytes away from the peripheral circulation, the spleen and the other marginal pools rather than the actual depletion and stasis. The ESR after treatment with plant extracts reduced significantly against the untreated group, this study justifies the work of [26] who reported that the ESR depends on the density of RBC, that is, when the RBC increases the ESR reduces and when the RBC reduces the ESR increases. Eosinophil was higher in the treated groups, this conform to the report of [29], who stated that eosinophila occur after initiation of anti-malaria treatment and do not conform to the report of [30] that increase in parasitemia is accompanied by increase in eosinophil count. Although [30] further reported that increase in eosinophil count in relation to the level of parasiteamia depend upon duration of exposure to the parasite or an individual difference in the strength of the immune system to mount an effective immune response against the parasite and that there are other underline factors that can induced the production of eosinophil other than malaria infection. The basophils had no significant increase or decrease with the positive control. Neutrophils count did not correlate with the parasitemia density following treatment as there was no consistency in the increase or decrease of the neutrophil count, this report is in accordance with the report of [31] who reported that neutrophil counts were not raised by hyperparasitemia and that the positive association of neutrophil with parasitemia may have been influenced by the presence of bacterial infection or toxic fluids in the system, this also agreed with the work of [32]. The monocyte count of the mice increased significantly after treatment this justifies the study of [33] who reported that low monocyte count is associated with severe malaria infection.

The result of the biochemical assay that is, organ function indices showed that there was significant decrease in the level of biochemical parameters at lower concentration of extracts in the treated group compared to the untreated group, however, there was increase in the biochemical parameters at higher concentration as against the standard range of the parameters. This justify the study of [34, 35 36, 37, 38] that the sudden elevation in the urea level and imbalance in the electrolytes level may be due to high concentration of plant extracts and malaria infection which could serve as indicators for kidney dysfunction.

The percentage yield of the plant extracts using different solvents revealed that ethanol solvent gave the highest yield. [39] reported that alcohol is a better solvent for more consistent extraction of active substances against microbes from medicinal plants compared to other solvents. This may be due to the fact that ethanol is polar solvent and may be able to extract the bioactive ingredients without denaturing them. This agreed with the findings of [40], who reported that ethanol extract of some certain plants have higher phenolic and flavonoids when compared with the percentage yield of other solvents. This justifies the traditional use of alcohol in extracting the components of medicinal plants for application against pathogens [41]. The presence of phytochemicals is an indication that the plants contain bioactive components that might be responsible for the antiplasmodial properties

of the extracts. This result is in accordance with the report of [20, 42] who reported the presence of some phytochemical constituents in these plants.

Conflict of Interest

We declare that we have no conflict of interest.

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