ANTIPLASMODIAL EFFECTS OF CRUDE ETHANOL AND ALKALOIDAL EXTRACTS OF *SIDA ACUTA* LEAF IN MICE

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Abstract
Antiplasmodial effect of extracts of crude ethanol and alkaloid of *Sida acuta* leaf were investigated in *Plasmodium berghei* infected mice. Acute toxicity and LD50 of the extracts of crude and alkaloid *Sida acuta* leaf were determined using the Lorkes method. Forty mice were intraperitoneally inoculated with blood of *Plasmodium bergei* infected mice and were divided into eight groups of five animals each. Groups 1-3 mice were treated with 200, 400 and 800 mg/kg body weight of crude ethanol leaf extract of *Sida acuta,* respectively. Groups 4 - 6 mice were treated with 200, 400 and 800 mg/kg body weight of crude alkaloid extract of *Sida acuta* leaf*,* respectively. Group 7 mice were treated with standard chloroquine at 5 mg/kg body weight, while group 8 served as control which were infected but not treated. *Plasmodium* parasite level was determined microscopically. Inhibition concentration, mean survival period, packed cell volume and body weights of the animals were determined. No death was recorded for the crude ethanol extract of *Sida acuta* leaf while the LD50 calculated for crude alkaloid extract of *Sida acuta* leaf was 2154 mg/kg body weight. By the 4th day of treatment the *Plasmodium* parasite level for the crude ethanol extract and extracted crude alkaloids from *Sida acuta* leaf at 800 mg/kg body weight were reduced to 18.0 and 5.0 parasite per field respectively compared to chloroquine (8.2 parasite level per field). The control untreated group showed increased parasitemia level of 110 parasites per field by the 4th day. The inhibition concentration was highest for chloroquine and comparable to the alkaloid extract which was not significantly different ($p>0.05$). The mean survival period was significantly higher for the chloroquine (30 days) compared to the 26.45 days for the animals that received 800 mg/kg body weight of crude alkaloid extract of *Sida acuta* leaf. The packed cell volume (PCV) significantly ($p<0.05$) decreased more for the control (17.7±1.8) compared to the treated groups (29.2±1.4 and 31.6±0.4), this was however comparable to the standard drug (31.5±1.0 %). There was no significant difference ($p>0.05$) between the decrease in weights of the animals treated with the plant extracts and the chloroquine drug. Based on the results obtained, it can be concluded that the crude ethanol and alkaloidal leaf extracts of *Sida acuta* leaf have a potency in mice comparable to chloroquine and this may be the rationale for its use in malaria treatment among the indigenous Nigerians.

Keywords: *Plasmodium berghei, Sida acuta*, alkaloid, chloroquine

Introduction
Malaria is a mosquito-borne infectious disease affecting humans and other animals. It is caused by parasitic protozoan belonging to the *Plasmodium* (*P.*) family (World Health Organization, 2014a). The disease is most commonly transmitted by an infected female *Anopheles* mosquito through bite (WHO, 2014a). The parasites from the mosquito saliva passes from the blood to the liver where they mature and reproduce. Five species of *Plasmodium* can infect and spread by humans; these are; *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* (Caraballo, 2014). The recommended treatment for malaria is a combination of antimalarial medications that includes an artemisinin components (Caraballo, 2014). The second medication may be either mefloquine, lumefantrine, sulfadoxine or pyrimethamine (WHO, 2010a). However, quinine, along with deoxycycline, may be used if an artemisinin is not available (WHO, 2010b). It is recommended that in areas where the disease is common, malaria is confirmed if possible

before treatment is started due to concerns of increasing drug resistance and similarities of some of the symptoms with other ailments. The malaria parasites has developed resistance to several antimalarial medications; for example, chloroquine-resistant *P. falciparum* has spread to most malarial areas, and resistance to artemisinin has become a problem in some parts of Southeast Asia (WHO, 2014b).

From the very beginning of human existence, man has familiarized himself with plants and used them in a variety of ways throughout the ages (Cragg and Newman, 2001). This relationship between plants and man has grown and many plants have been used as medicines. Medicinal plants have been recognized as potential drug candidates because they possess drug like properties (Owolabi *et al.,* 2007).

Sida, is one of such ethnomedicinally important genus of plants, and is a large genus with about 200 species distributed throughout the world (Sivarajan and Pradeep, 1996). The most medicinally important species of *Sida (S)* are; *S. acuta, S. cordifolia, S. rhombifolia, S. spinosa, S.carpenifolia, S.huilis, S. Veronicaefolia* (Kirtikar and Basu, 2010). These species are used for various purposes like neurological disorders, headache, leucorrhoea, tuberculosis, diabetics, fever, uterine disorders and as an antirheumatics and antipyretic agent (Kirtikar and Basu, 2010). Other uses of this ethnomedicinal plant are for treating dysentery, hemorrhoids, malaria, venereal diseases, ulcers, renal inflammations, fever and asthma (Dinda *et al*., 2015).

Sida acuta, (broom weed) is an annual herb that is commonly used in Nupe land in Nigeria in the treatment of fever attributed to malaria. It is a small and erect plant, with much branches. With a strong taproot, the stem and branches can be fibrous and woody at times. The weed is frequently found in pastures, wastelands, cultivated lands, roadsides, lawns, and in planted forests (Mann *et al.,* 2003). The aim of this study is therefore to screen *Sida acuta* leaf extract for anti-plasmodial activity in plasmodium berghei induced mice.

Materials and Methods

Plant Materials

Fresh leaves of *Sida. acuta* were collected in November, 2017 at Keteren Gwari, Minna, Niger State (N 9º 36'29.376" E 6º 32'18.6036"), Nigeria. It was identified and authenticated in the Department of Plant biology Biological Sciences, Federal University of Technology, Minna, Niger State.

Experimental animals

Healthy swiss albino mice of both sex, weighing between 20 to 30 g each were obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. The animals were conveniently housed in a plastic cage with free access to commercial feed pellets and water *ad-libitum*. Experiments were conducted in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care guidelines and protocol review (CCAC, 1997).

Malaria parasites

Plasmodium berghei NK65 chloroquine-sensitive strain was obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria and maintained in the laboratory by serial passage in normal mice.

Preparation and extraction of crude extract

The leaves of *Sida acuta* were air dried at room temperature (27 ^{o}C) and pulverized using mortar and pestle. The powdered sample was kept in a dry, clean container before extraction of the active constituents.

The weighed sample (100 g) was soaked in 70 % of ethanol in 1000 ml cornical flask. This was allowed to stand for 24 hours. The sample was filtered using Whatman filter paper No. 1 into a clean, dry beaker, which was then placed on a waterbath set at 60 ^{o}C to concentrate the filtrate until semi-dry to a constant weight. The crude extract was weighed and the percentage yield was calculated from the following equation (Dhawan & Gupta, 2017).

$$\% \text{ Yield (g/100 g)} = \frac{\text{Weight (g) of dried crude}}{\text{Weight (g) of finely ground plant material ~~used~~}} \times 100$$

Extraction of Crude alkaloid

The extraction of the alkaloid was done using the continuous extraction method with the soxhlet apparatus. Four hundred grams (400 g) of raw ground powdered sample of *S. acuta* leaves were weighed, divided into four fractions (100 g) each and packed in a cheesecloth bag which was placed in an extraction thimble. The thimble was then placed into a suitable jar with cover. The sample was moistened with 95 % ethanol (50 ml). This was made alkaline with ammonia (15 ml) and mixed thoroughly. The sample in the thimble was macerated overnight, and then placed in the soxhlet extractor the next day with 100 ml of 95 % ethanol placed in the solvent flask. The sample was extracted for about 5 hours.

The ethanol extract was filtered and the solvent was evaporated in water bath set at 45 ^{0}C. The crude extract was further treated with 1.0 N hydrochloric acid (HCl). This was filtered and the filtrate was collected. The filtrate was alkalinified with ammonia and placed in a separatory funnel. Then, 600 mls of chloroform was added gradually into the separatory funnel, mixed and shaken for five times and allowed to seperate into two layers. The lower layer of the chloroform fraction contained the crude alkaloids and the upper layer contained other constituents of the plant. The upper layer was extracted until the last chloroform was found negative to dragendoff's reagent (Mohammad *et al.,* 2016).

The combined chloroform fractions were concentrated in a water bath set at 60 ^{o}C until semi-dry. The extract was weighed and percentage yield was calculated as earlier reported (Dhawan & Gupta, 2017).

Acute Toxicity Studies of the Extracts

The acute toxicity studies were carried out using the method described by Lorke, (1984). The extract (0.5g) was dissolved in dimethylsulphoxide (DMSO) and made up to 10 ml with physiological saline at 0.4 % v/v), to serve as a bioactive compound vehicle (Kelava & Cavar 2011; Okonko *et al.*, 2017). Five groups of three mice each were used. The animals were administered intraperitoneally (i.p) with ethanol crude and crude alkaloid extracts of *S. acuta* respectively into two phases. Groups in phase 1 received ~~at~~ doses of 10, 100, and 1000 mg/kg bodyweight respectively while phase 2 animals were treated with 1600 2900, and 5000 mg/kg bodyweight. Animals were observed over 24 hours for physical signs and for mortality and subsequently for 14 days.

The LD_{50} was calculated by the formula below;

$LD_{50} = \sqrt{D_0} \times D_{100}$
D_0= Highest dose that gave no mortality
D_{100}= Lowest dose that produced mortality

In vivo antiplasmodial studies

Mice were pre-screened by microscopy of thin tail blood smears. This was done to exclude the possibility of test animals harboring rodent plasmodium specie.

Forty mice of mixed sex were divided into eight groups of five animals each. A donor mouse infected with *P. berghei* was anaesthetized with chloroform and its blood collected by cardiac puncture with a sterile syringe and needle earlier flushed with heparin. The blood was diluted with normal saline of 0.2 ml contained about 1 x 10?^{7} infected cells. Each of the forty mice was inoculated intraperitoneally with 0.2 ml diluted blood. The extracts were administered orally once daily for four days.

The method of Fidock *et al.* (2004) was adopted to test for antiplasmodial activity of the crude and alkaloid extract of the plant (*S. acuta*) in *Plasmodium* infected mice. Three days after the mice inoculation, mice in groups I, II and III were treated orally with 200, 400 and 800 mg/kg bodyweight of the crude ethanol extract of *Sida acuta* leaf respectively while groups IV - VI animals were also treated orally with the crude alkaloid extract of *Sida acuta* leaf at 200, 400 and 800 mg/kg bw doses, respectively. Groups VII and VIII animals served as positive and negative control and were administered with chloroquine phosphate (5 mg/kg bw) and physiological saline (2 ml). All treatment was once daily for four consecutive days. Parasitaemia was monitored and counted daily by collecting and fixing the blood on the slide and stained with 4 % Giemsa (pH 7.2) for 45 minutes before being examined under microscope. The percentage inhibition of parasites was calculated for each dose level by comparing the parasitemia in infected control with those of treated animals according to the method modified by Peters and Robinson (1992). Mean survival period (MSP) was determined as described by Chandel and Bagai, (2010)

Determination of packed cell volume (PCV)

The method employed was as described by Dacie and Lewis (1991). The tips of the tails of the mice were cut to obtain blood. The blood was collected in heparinised capillary tubes and sealed with sealing agent (plasticine). The capillary tubes were then centrifuged, using a micro-haemotocrit centrifuge, at 11,000 rpm (revolution per minute) for 5 minutes. The packed cell volume was read using the micro haemotocrit reader before and after treatment.

Determination of bodyweight changes

A sensitive digital weighing balance (Scientech balance, Model) was used to weigh each mouse in all the groups before infection, berore and after treatment.

Results

Percentage yield

The yield obtained from the extract of crude ethanol of *Sida acuta* leaf was 22.7 % and 0.43 % for the crude alkaloid of *Sida acuta* leaf.

Acute Toxicity of Crude Ethanol and Alkaloid Extract of *Sida acuta* Leaf in Mice

Table 1 showed the recorded mortality of the test animals, no mortality occured in all the animals that were dose with crude ethanol extract of *Sida acuta* leaf, therefore, LD_{50} could not be calculated for this group. The LD_{50} for the crude alkaloid extract of *Sida acuta* leaf that recorded death at 2900 mg/kg bodyweight was calculated to be 2154 mg/kg bodyweight (LD_{50} = 2154). ie $\sqrt{1600 \times 2900} = 2154$ mg/kg

Table 1: Acute Toxicity of Crude ethanol and alkaloid extracts of *Sida acuta* Leaf in mice

Extract of *Sida acuta* leaf	Phase 1 Dose mg/kg bw	Mortality	Phase 2 Dose mg/kg bw	Mortality
Crude Ethanol extract	10	0/3	1600	0/3
	100	0/3	2900	0/3
	1000	0/3	5000	0/3
Crude alkaloid	10	0/3	1600	0/3
	100	0/3	2900	1/3
	1000	0/3	5000	1/3

Antiplasmodial activity of crude ethanol extract and crude alkaloids extract of *Sida acuta* leaf in *Plasmodium berghei* induced mice

The result of antiplasmodia activity of the crude ethanol extract of *Sida acuta* leaf shown in Figure 1 indicated that the parasite level in infected mice treated with standard chloroquine drug decreased progressively from the 1st to the last day of experiment. Parasitemia level also decreased in mice treated with crude ethanol extract of *Sida acuta* leaf from the 1st day to 5th day of treatment. The induced untreated group that received normal saline (control) showed an increased parasitemia level from the 1st day of treatment to the last day of the experiment. The reduction in parasetemia level was dose dependent among the animals treated with crude ethanol extract of *Sida acuta* leaf, the 800 mg/kgbw dose showed a higher activity against *P. berghei* when compared with the lower doses (200 and 400 mg/kgbw). However, the animals treated with standard drug (chloroquine) had the highest activity against *P. berghei*.

Figure 2 showed the antiplasmodial effect of crude alkaloid extract of *Sida acuta* Leaf in *Plasmodium berghei* induced mice. There was a significant ($p<0.05$) reduction in parasite level in the animals treated with 200, 400 and 800 mg/kg body weight of crude alkaloid extracts of *Sida acuta* leaf, but treatment with the highest dose (800 mg/kg/bw) of alkaloid, showed a significant reduction in parasite level compared to animals treated with 200 and 400 mg/kg bw of alkaloid of the *Sida acuta* leaf and the standard respectively.

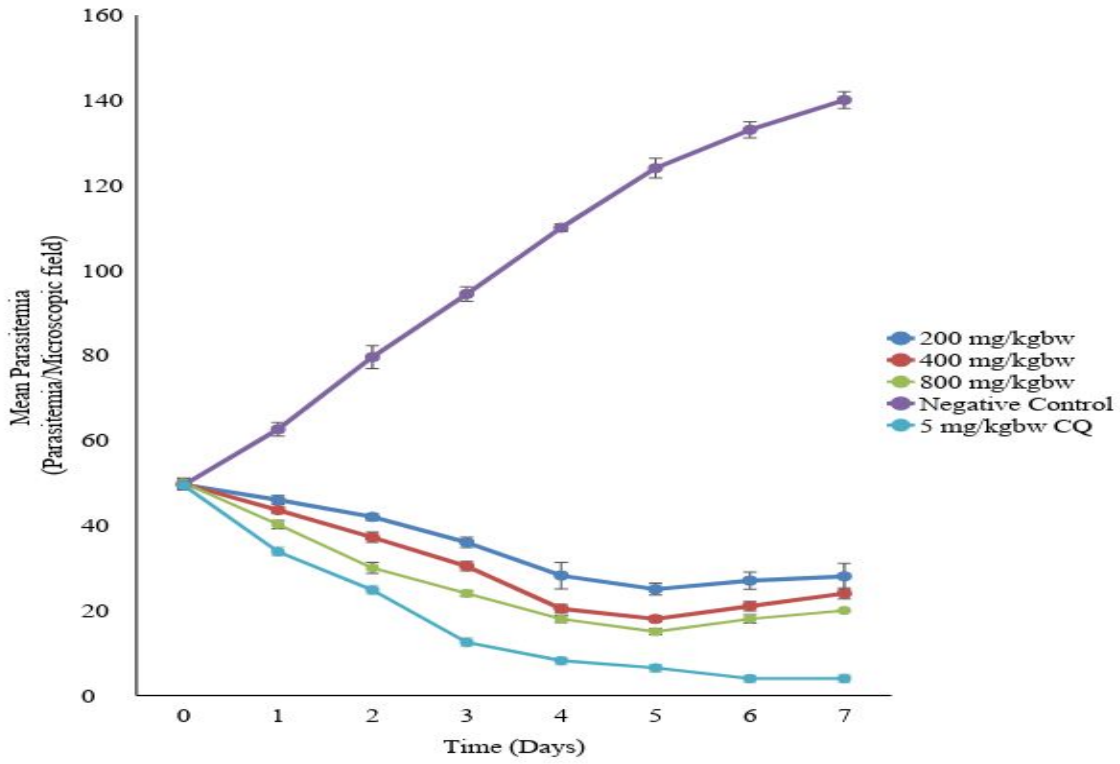

Figure 1: Antiplasmodial Effect of Crude Ethanol Leaf Extract of *Sida acuta* in *Plasmodium berghei* induced Mice

Results are expressed as mean ± standard error of mean (SEM).
Key: CQ = Chloroquine (standard drug)

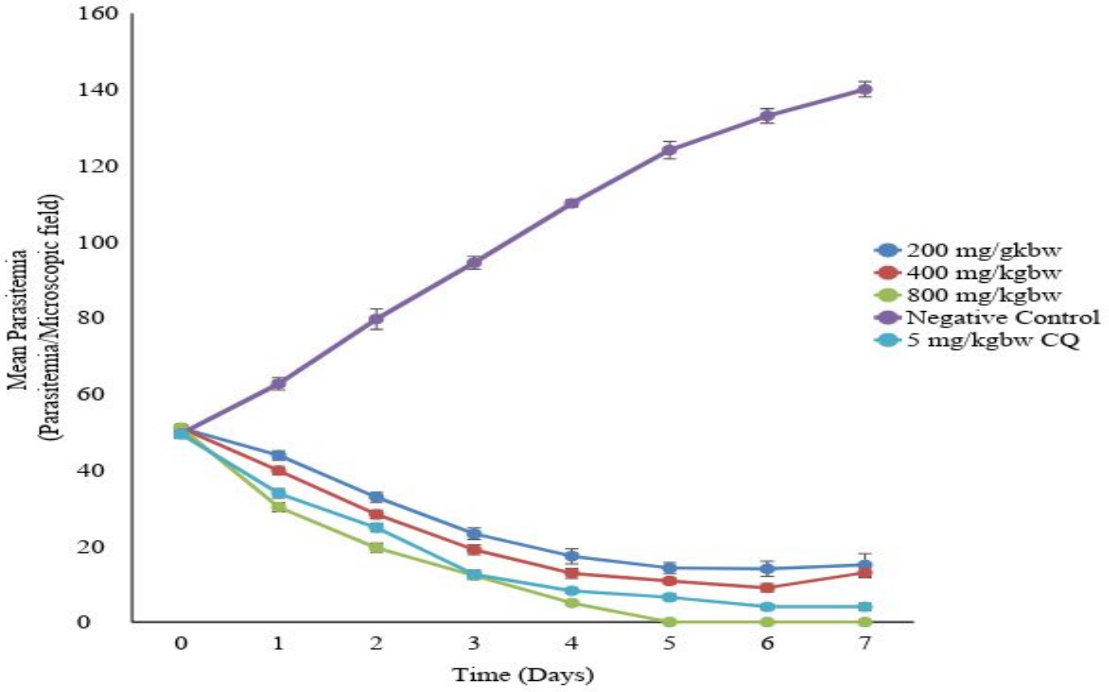

Fig. 2: Antiplasmodial Effect of Crude Alkaloid Extract of *Sida acuta* Leaf in *Plasmodium berghei* induced Mice

Results are expressed as mean ± standard error of mean (SEM).
Key: CQ = Chloroquine (standard drug)

Percentage inhibition of *plasmodium* parasites of crude ethanol extract and crude alkaloid extract of *Sida acuta* leaf in mice

Table 2 showed percentage *Plasmodium* inhibition by crude ethanol extract and crude alkaloid extract of *Sida acuta* leaf against *P. berghei* infected mice. The mean parasitemia inhibition for the standard drug (72.90±1.28 %) was significantly ($p<0.05$) different from that of the crude ethanol leaf extract of *Sida acuta* 200, 400 and 800 mg/kg bw (29.12±1.41, 30.60±2.05 and 31.42±0.86 % respectively). The parasitemia inhibition (%) of crude alkaloid extract of *Sida acuta* leaf were 64.10±1.33 and 67.67±1.06 for 200 and 400 mg/kg bw and were lower and significantly ($p<0.05$) different from that of the 800 mg/kg bw of the extract and standard drug (72.90±1.28 and 75.85±0.54 % respectively).

Table 2: Mean (%) Inhibition of *Plasmodium* of Crude Ethanol extract *Sida acuta* Leaf in Mice

Treatments	Dose (mg/kg bw)	Parasitemia inhibition (%) crude ethanol extract	crude alkaloid
SDE	200	29.12±1.41[a]	64.10±1.33[a]
SDE	400	30.60±2.05[a]	67.67±1.06[a]
SDE	800	31.42±0.86[a]	75.85±0.54[b]
Chloroquine phosphate	5	72.90±1.28[b]	72.90±1.28[b]
Control (Normal saline)	20 ml	-	-

Values are Mean ±standard error of mean (SEM). Values along the same column with different superscript alphabets are significantly different ($p<0.05$)
SDE: *Sida acuta* leaf Extract

3.5 Mean Survival Period (MSP) in *P. berghei* infected Mice treated with crude ethanol extract and crude Alkaloid extract of *Sida acuta* leaf

Table 3 shows that the infected untreated mice (control) had the least mean survival time of 11.25 days. The mice treated with crude extracts of ethanol *Sida acuta* leaf at 200, 400 and 800 mg/kg body weight had the mean survival periods of 12.05, 14.53 and 16.42 days respectively. Mice treated with crude alkaloid extract of *Sida acuta* leaf had the mean survival periods of 14.85, 19.35 and 26.45 days at doses of 200, 400 and 800 mg/kg body weight respectively. However, *Plasmodium* infected mice treated with 5 mg/kg body weight chloroquine had the highest mean survival period of 30.05 days.

Table 3: Mean Survival Period (MSP) in *P. berghei* infected Mice treated with Crude Ethanol extract and Crude Alkaloid Extract of *Sida acuta* leaf.

Treatments	Dose (mg/kgbw)	Mean Survival (days) Ethanol crude extract	Crude alkaloid
SDE	200	12.05±1.15[a]	14.85±0.73[a]
SDE	400	14.53±1.05[a]	19.35±1.36[b]
SDE	800	16.42±2.18[b]	26.45±0.84[b]
Chloroquine phosphate	5	30.05±1.57[c]	30.05±1.57[c]
Normal saline (Control)	20 ml	11.25±1.40[a]	11.25±1.40[a]

Values are Mean ±standard error of mean (SEM). Values along the same column with different superscript alphabets are significantly different ($p<0.05$)

SDE: *Sida acuta* leaf Extract

Weight of *P. berghei* Infected Mice Treated with crude Ethanol Extract and Alkaloid Extract of *Sida acuta* leaf.

The average weight of infected mice before and after treatment with crude ethanol extract and alkaloid of *Sida acuta* leaf are presented in Table 4. After treatment with the extracts there was a weight decrease in all the *Plasmodium berghei* infected animals. The decrease in weight was not significantly difference ($p>0.05$) between the control and the *Plasmodium berghei* infected animals treated with lower doses of extracts of *Sida acuta* leaf but there was a significant difference ($p<0.05$) at higher doses of the extracts and chloroquine. However, the mean weight of *Plasmodium* infected mice after treatment with higher doses of crude ethanol extract and alkaloid extract of *Sida acuta* leaf were not significantly different when and compared with the chloroquine treated animals.

Table 4: Mean Weight of Plasmodium beighei Infected Mice Treated with Extracts of crude Ethanol and Alkaloid of *Sida acuta* leaf

Treatments	Dose (mg/kg bw)	Weight (g) Before treatment	After Treatment
ETN	200	25.95	18.53[a]
ETN	400	24.91	19.37[a]
ETN	800	26.05	20.86[b]
ALK	200	25.90	18.76[a]
ALK	400	26.27	19.88[b]
ALK	800	25.89	21.24[b]
Control	20 ml	27.05	17.40[a]
Chloroquine phosphate	5	26.53	21.50[b]

Values are Mean ±standard error of mean (SEM). Values along the same column with different superscript alphabet are significantly different ($p<0.05$)
ETN = Ethanol extract of *Sida acuta* leaf
ALK = Alkaloid extract of *Sida acuta* leaf

Table 5 presents the mean packed cell volume (PVC) of *Plasmodium berghei* infected mice treated with extracts of crude ethanol and crude alkaloid of *Sida acuta* leaf. There was a reduction in the PCV of all the plasmodium parasite infected animals, which was higher in the control than in the animals treated with the extracts of crude ethanol, alkaloid of *Sida acuta* leaf and 5 mg/kg bw Chloroquine (standard drug). The PCV of the animals treated with 800 mg/kg bw extracts of crude ethanol and alkaloid of *Sida acuta* leaf were not significantly different at $p>0.05$ when compared with the 5 mg/kg bw chloroquine standard.

Table 5: Mean Packed Cell Volume of *Plasmodium berghei* Infected Mice Treated with Extracts of crude Ethanol and Crude Alkaloid of *Sida acuta* leaf

Treatments	Dose (mg/kgbw)	PCV (%) Before inoculation And treatment	After inoculation and Treatment
ETN	200	44.2±1.7[a]	23.4±1.2[b]
ETN	400	45.2±0.9[a]	26.2±0.7[b]
ETN	800	44.8±1.8[a]	29.2±1.4[c]
ALK	200	50.0±0.5[b]	26.0±0.6[b]
ALK	400	49.8±0.9[b]	29.4±0.8[c]
ALK	800	48.6±0.5[b]	31.6±0.4[c]
Control	20 ml	45.4±1.0[a]	17.7±1.8[a]
Chloroquine phosphate	5	45.2±1.6[a]	31.5±1.0[c]

Values are Mean ±standard error of mean (SEM). Values along the same column with different superscript alphabets are significantly different ($p<0.05$) to the standard drug (Chloroquine phosphate)
ETN = Ethanol extract of *Sida acuta* leaf
ALK = Alkaloid extract of *Sida acuta* leaf

Discussion

Biologically active compounds usually occur in low concentration in plants, it is necessary to use extraction process with the appropriate solvent that the compounds can be soluble in to obtain extracts with high yield and with minimal changes to the functional properties of the extract. The higher yield of the extract of the crude ethanol of *Sida acuta* leaf as compared to the extract of crude alkaloid of *Sida acuta* leaf is due to the alkaloid being a fraction of the phytochemical contained in the whole leaf. To improve the percentage yield a more suitable solvent and extraction method may be adopted (Dhanani *et al*., 2017). The low percentage yield of alkaloid extract (0.43 %) from *Sida acuta* leaf may not be a hindrance to formulating a viable drug against malaria because the *Sida acuta* plants are readily available and are easily accessible (Mann, *et al*., 2003, Raymond, *et al*., 2010). Also the plant can also be genetically improved so as to increase the yield of the plant (Ravina, 2011). Alkaloids block protein synthesis in *Plasmodium falciparium* (Bagnarello *et al*., 2018).

The LD_{50} of 2154 mg/kg body weight obtained in the acute toxicity test for crude alkaloid extract (Table 1) suggest a moderate tolerance level by tissues when used for chemotherapy. Some plants are effective as medicaments but are seldom used due to their acute toxicity (Lorke, 1984).

The LD_{50} of crude alkaloid extract of *Sida acuta* (2154 mg/kgbw) in this study is in contrast to the findings of Mallikarjuna (2013), who reported that “there was no mortality in animals administered with extract of *Sida acuta* leaf, up to a dose level of 2000 mg/kg body weight”.

This also is similar to the result that was reported by Pieme *et al.* (2010). This suggest the extract have a moderate toxicity. LD_{50} three times (X3) the effective dose is safe as reported by Toma *et al.*, 2015). Chemicals compounds after absorption by the gastrointestinal tracts are probably being eliminated, distributed away from target and may be detoxified before it is subsequently metabolized to less toxic compounds thereby reducing toxicity of the extract (Cassarret and Doull, 2014). Dimethylsulphur oxide (DMSO) is used as a delivery vehicle because it is a non-toxic solvent with a median lethal dose higher than ethanol and have an oral LD_{50} of 14,500 mg/kg bodyweight in rat (Abrar, *et al.*, 2013).

The significant and greater inhibition of *P. berghei* in mice by crude alkaloid extract (75 %) of *S. acuta* (Table 3) compared to the crude ethanol extract (31 %) and standard (72 %) suggest that its anti-plasmodial activity could be related to the presence of bioactive compounds from the extract of *Sida acuta* leaf. A compound which causes reduction in parasitemia determined by % inhibition greater than 30 % has been considered to be active (Carvalho *et al.,* 1991). The curative effect of crude alkaloid extract of *S. acuta* leaf on *P. berghei* infected mice is comparable and not significantly ($p>0.05$) different from the standard chloroquine treatment and therefore can possibly serve as a good alternative source of active compounds against malaria. The outcome of the antimalarial activities correspond with the work of Karou *et al.* (2003), who investigated the antimalarial activity of five plants used in the traditional medicine of Burkina Faso to treat malaria, including *Sida acuta.* The result revealed *Sida acuta* to be the most active plant in the study. Banzouzi, *et al.* (2004), also reported that the *in-vitro* antiplasmodial activities of the ethanolic and decoction with water extracts of the aerial part of *Sida acuta* in Ivory Coast was tested on two strains of *Plasmodium falcifarum,* Cameroon (chloroquine-resistant strain) and a Nigerian chloroquine-sensitive strain. The ethanolic extract of *Sida acuta* exhibited a better antiplasmodial activity than the decoction.

The percentage inhibition of parasite by crude ethanol extract of *Sida acuta* leaf (31.42 %) is similar to the reports from previous studies of other plants such as on *Asparagus africana, Withania somnifera* (Dikasso *et al.,* 2006), *Amarantus spinosus* (Hilou *et al.,* 2006) and *Clerodendrum myricoides* (Muregi *et al.,* 2007) with percentage inhibition of 27.84, 30.94, 33.75 and 31.7 % respectively as compared to *Sida acuta leaf* (Table 2). However, the percentage inhibition of parasites by the extract of crude alkaloid of *Sida acuta* (75 %) was higher compared to those reported by previous works, *Nigella sativa* (Abdulelah and Zainal-Abidin, 2007), *Boscia angustifolia* (Muthaura *et al.,* 2007) and *Azadirachta indica* (Valecha *et al.,* 2001) reported 55.88, 60.12 and 52.32 % respectively.

The mean survival period presented in Table 4 showed that at the highest dose the crude extracts of both ethanol and alkaloids of *S. acuta* significantly prolonged the mean survival time of the infected mice compared to the control and the standard drug (Chloroquine), this indicates that the extracts of *S. acuta* have bioactive compounds that were able to suppressed *P. berghei* and reduced the overall pathologic effect of the parasite though not better than the standard drug. It has been reported that crude plant extracts tend to have better plasmodistatic effects than plasmodicidal effects because unpurified bioactive principles may require initial conversions with time lag that allows for parasite proliferation (Noedl *et al.,* 2003).

Plasmodium infection is correlated with the incidence of high destruction of red blood cells due to the invasion of the red blood cells by the rapid multiplication of the *Plasmodium* parasites. The reduced PCV of the *Plasmodium* infected animals that were treated with extracts of crude ethanol, alkaloid of *Sida acuta* leaf and the chloroquine were below the normal level, this suggest the *Plasmodium* parasite may have induced aneamia which can be life threatening (Abdulkareem *et al.,* 2017). This may explain why the negative control animals

that were administered normal saline only could not survive for more than eleven days because the packed cell volume (PCV) was significantly reduced. Also, neither the extracts nor the chloroquine standard drug cured the *Plasmodium* infection after 7 days, the *P. berghei* parasite probably due to the dose administered. This suggest the possibility of the previous reports of ensuing resistance of *Plasmodium* parasites to chloroquine as the parasitemia level rose again to a detectable level in the blood of the treated mice which is in agreement and line with the report of Berhan *et al.* (2012).

The loss in bodyweight is one of the major characteristic of malaria infected mice, as a result of loss of appetite and the posibility of the parasites using the host nutrient to survive (Perlmann and Troye-Blomberg, 2007). The observed effect of *Sida acuta* on body weight in this study correspond with the findings of Dikasso *et al.* (2006) who reported a decreased in bodyweight of *P. Berghei* infected rodents treated with crude extracts of *Asparagus africanus.*

Conclusion

The crude ethanol extract of *Sida acuta* leaf was safe and there was no obvious acute adverse effect observed. Crude alkaloid extract of *S. acuta* leaf with LD_{50} of 2154 mg/kg bodyweight demonstrated antiplasmodial activity against the *P. berghei* malaria parasite which was not significantly different when compared with the chloroquine (standard drug). Therefore, alkaloids from *S. acuta* leaf have potentials as an antiplasmodial agent. Consequently, the alkaloids from *S. acuta* could be explored as a lead for the development of novel alternative agents for antiplasmodial chemotherapy.

Declaration of Interest: None

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