In vivo ANTIPLASMODIAL EFFECT OF ETHANOLIC LEAF EXTRACTS OF *Senna occidentalis* ON RODENTS MALARIA (*Plasmodium berghei*) INFECTION

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ABSTRACT
Background: Malaria has been the major cause of morbidity and mortality in Nigeria despite numerous attempts to control it and huge amount of fund spent on its eradication programs for decades. Up to now, there are no standard treatment modalities and most of the anti-malarial drugs are expensive and confer side-effects. Besides these, the emerging resistance trend of Plasmodium species to available antimalarial drugs poses another problem of clinical concerned. There is therefore the need for new effective, safe and cheaper drugs. *Senna occidentalis* has been used in traditional African pharmacopoeia. However, little information has been documented on its use in the treatment of malaria.
Aim: To determine the anti-plasmodial activity of ethanolic leaf extracts of *Senna occidentalis* on *Plasmodium berghei* in vivo using Swiss albino mice.
Methods: Fresh leaves of *Senna occidentalis* were collected and authenticated at the Herbarium of Ahmadu Bello University, Zaria. Phytochemical screening and composition analyses of the leaf were carried out. The ethanolic extracts of *S. occidentalis* leaves were prepared using 95% ethanol. Three different concentrations of 10, 100 and 1000mg/kg were prepared from the leaves extracts while normal saline and 10 mg/kg chloroquine were used as negative and positive controls respectively. Fifteen (15) Swiss albino mice were inoculated intraperitonealy with infected blood suspension (0.2 mL) containing $1x10^7$ *P. berghei* parasitized red blood cells. The data obtained were analyzed using Analysis of Variance with Duncan's Multiple Range Test used to separate the means.
Results: The results obtained revealed the presence of alkaloids, saponins, cardiac glycosides, diterpenoids, flavonoids, steroids, tannins, Triterpenoids, carbohydrates and proteins. The ethanolic extracts significantly ($P \leq 0.05$) reduce the number of parasitized erythrocytes among infected mice treated with the extracts. The effect of the extract is concentration dependent, increase with increase in concentration. Similarly, significant decreased ($P \leq 0.05$) in percentage change in body weight of malaria-infected mice treated with different concentrations of the extracts.
Conclusion and Recommendation: It was concluded that, the leaf of *S. occidentalis* is rich in phytochemicals that are highly active against *P. berghei*. The effect of the leaf ethanolic extracts of *S. occidentalis* increase with increase in concentration. We therefore recommended the use of 1000 mg/kg of the extract in the treatment of malaria fever in rodents.
Key Words: Albino mice, Ethanolic Extracts, *Plasmodium berghei*, *Senna occidentalis*.

INTRODUCTION

Malaria has become a euphemism in the African continent especially in countries south of the Sahara. It is an endemic disease of public health concern common in the tropics. Malaria is a life-threatening ailment (WHO, 2005) and is the major cause of morbidity and mortality especially among the expecting mothers and the under-ages. It is responsible for one to three million deaths and 300 to 500 million infections annually (Krishna *et al*., 2009; Verma *et al*., 2011).

Although, efforts were made for decades to over-come the burden of malaria in Africa and Nigeria in particular, the prevalence of the disease is continuously escalating at an alarming rate. Up to now, malaria treatment relies on certain drugs that easily confer side effects and easily become resistant by the parasites to the extent that, there is no single antimalarial drug in clinical use to which the parasite has not yet developed resistance (Adebayo and Krettli, 2011; Abdillah *et al*., 2015; Chandra and Arbor, 2016; Alebie *et al*., 2017). Furthermore, the most successful malaria vaccine was only partially efficient and short lived (Kayano *et al*., 2011). There is therefore the need for alternative therapy in the treatment of malaria in Nigeria.

The use of plant preparations for the treatment of diseases still holds a strong potential since the influence of natural products upon drug discovery is impressive and a number of clinically active drugs are either natural products or have a natural product pharmacophore (Koehn and Carter, 2005). Ethnopharmacological reports revealed a number of tropical plants with certain antimalarial potentials (Onaku *et al*., 2011; Rasoanaivo *et al*., 2011).

Senna occidentalis is one of the most widely used herbal plants among people of tropical and sub-tropical regions of the world (Veronique and Gabriel, 2013). It is used for various therapeutic purposes in traditional medicine (Yadav *et al*., 2010, Silva *et al*., 2011). In Nigeria, this plant is locally called 'Sanga-sanga' or 'Rai dore' in Hausa language (Nuhu and Aliyu, 2008; Sadiq *et al*., 2012); 'Akidi agbara' in Igbo language and 'Abo rere' in Yoruba language (Egharevba *et al*., 2010). Roots, leaves, flowers and seeds of *Senna occidentalis* are the different parts of the plant used in medication (Shafeen *et al*., 2012; Tanimu and Wudil, 2012; Veronique and Gabriel, 2013). The need to search for and develop more effective antimalarial drugs, that are safer and inexpensive available to people in the developing countries like Nigeria has necessitated this study. *Plasmodium berghei* is one of the species of intracellular protozoan parasites from the genus Plasmodium which are responsible for causing malaria. It provides a well established experimental model of malaria infection (Margarida *et al*., 2006) because of its critical mimicry with the species that infect human (Van der Heyde *et al*., 2006). The aim of the study is to determine the anti-plasmodial activity of leaf ethanolic extracts of *Senna occidentalis* on *Plasmodium berghei* in experimental Swiss albino mice with the view of finding lasting solution to the treatment and control of Plasodium parasites.

MATERIALS AND METHODS

Plant Collection

Fresh leaves of *Senna occidentalis* were collected from the field in Kano State. The collected sample*s* were carried to the Herbarium unit of the Department of Botany, Ahmadu Bello University Zaria for authentication. A herbarium voucher number 900078 was assigned to the specimen and deposited in the Department.

Extraction of Plant Ethanolic Extracts

The extraction methodology followed the protocols of Sasidharan *et al*. (2011). The collected samples were thoroughly washed and air-dried under shade at room temperature for 2 weeks. The dried leaves were then ground into fine powder with mortar and pestle, and were stored in dry containers until needed. The ethanolic extracts of *S. occidentalis* leaves were prepared by soaking 100 g of each powder in 150 ml of 95% ethanol and shaken in orbital shaker at 120 rpm. The preparations were left to stand for another 24 hours and then filtered through a gauze and then Whatman No 1 filter paper. The filtrates were concentrated to dryness at 40°C under reduced pressure on a rotary evaporator and were stored in a refrigerator at −20°C until use. Different concentrations of 100, 200 and 400mg/kg were prepared from the leaves extracts.

Phytochemical Analysis

The phytochemical screening of the ethanolic extract of *S. occidentalis* was carried out according to the methods described by Sofowara (1993), Mukherjee (2006) and Adegoke *et al*. (2010) to determine the presence of active constituents in the plant leaves and their composition. The aqueous extract of *S. occidentalis* was subjected to qualitative test for the presence of bioactive components that include Molisch's test for detection of Carbohydrates, Meyer's test for detection of Alkaloids, Wagner's test for detection of Alkaloids, Lead subacetate test for detection of Tannins, Keller- Killiani's test for detection of Cardiac Glycosides, Frothing/Foaming test for detection of Saponins, Libermann-Burchard's test for detection of Steroids and Triterpenoids, Copper Acetate's test for detection of Diterpenoids, Alkaline test for the detection of Flavonoids, Xanthoproteic test for the detection of Protein and Borntrager's test for the detection of Anthraquinones.

Experimental Animals

Ethical clearance was obtained from Ahmadu Bello University committee on Animal Use and Care (ABUCAUC). The protocol employed met the guidelines of the Good Laboratory Practice (GLP) regulations of World Health Organization. Apparently fifteen (15) healthy white Swiss albino mice of both sexes weighing between 140 and 260 g were used for the work and were obtained from the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The mice were housed in metal cages and kept in a well ventilated room with 12 hours dark/light cycle. They were fed with standard feed pellets and tap water *ad libitum*. The animals were acclimatized for 2 weeks before proceeding with the experiment.

Animals Inoculation

P. berghei infected blood was obtained from the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Parasites were harvested from the blood of a donor rat at peak parasitemia (10^9 parasites/ml) and was diluted with normal saline and then used for infection of experimental animals. The parasitized erythrocytes were diluted with normal saline in proportion indicated by Akuodor (2010). Each mouse was inoculated intraperitonealy with infected blood suspension (0.2 mL) containing $1x10^7$ *P. berghei* parasitized red blood cells.

Suppressive Tests

A 4-day suppressive test was conducted according to the method described by Mbah *et al*. (2012). Fifteen (15) albino mice of both sexes weigthing (140-260g) were injected intraperitonealy with standard inocula of *P. berghei* containing $1x10^7$ infected erythrocytes. After four hours of inoculation, the infected mice were randomly divided into 5 groups of 3 mice per cage and were treated for four consecutive days. Group 1 received 0.2 mL of normal saline (drug free control). Group 2, 3, 4 received 100, 200, and 400mg/kg of the ethanol leaf extract respectively while group 5 received 10mg/kg of chloroquine phosphate. All doses were administered orally. On the fifth day, thin blood film was made from the tail blood of each mouse. Thin blood films were made on a slide and air dried, and then fixed in absolute methanol and stained with 3% Giemsa solution at pH 7.2. Slides were viewed using light microscopy (Vickers Instruments) with oil immersion (X1000 magnification). The percentage of infected RBCs was determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs. A minimum of 500 RBCs total was counted.

$$\text{Percentage of Infected RBCs} = \frac{\text{Number of infected RBCs}}{\text{Total Number of RBCs Counted}} \times 100$$

Average chemo suppression was calculated as

$$\%\ \text{Suppression} = \frac{\text{Average parasitemia in Control} - \text{Average parasitemia in Treated}}{\text{Average parasitemia in Control}} \times 100$$

The body weights of mice were measured throughout the study using a weighting balance to the nearest 0.1g. All control and malaria-infected mice were observed visually throughout the experiment for behavioral changes and signs of illness.

Statistical Analyses

The data obtained were analyzed using one-way Analysis of Variance (ANOVA) with Duncan's Multiple Range Test (DMRT) used to separate the means.

RESULTS

The result for the phytochemical screening of the ethanolic leaf extracts of *S. occidentalis* is presented in Table 1. The result revealed the presence of ten active constituents in the form of alkaloids, carbohydrates, cardiac glycosides, diterpenoids, flavonoids, proteins, saponins, steroids, tannins and Triterpenoids. However, the composition of such constituents varies as shown in Table 2. Carbohydrates (63.48%), proteins (18.91%), tannins (11.42%), alkaloids (1.97%) and flavonoids (1.83%) were the major phytochemicals while glycosides, terpenoids, steroids and saponins were detected at minute concentrations (Table 2).

Table 1: Phytochemical Constituents of the Ethanolic Leaf Extracts of *S. occidentalis*

S/N	Phytochemical Compound	Test	Result
1	Alkaloids	Wagner's test	+
2	Anthraquinones	Bornfrager's	-
3	Carbohydrate	Molisch	+
4	Cardiac glycosides	Keller-Killani	+
5	Diterpenoids	Copper acetate	+
6	Flavonoids	Alkaline reagent test	+
7	Protein	Xanthoproteic	+
8	Saponins	Frothing test	+
9	Steroids	LibermannBuchard's test	+
10	Tannins	Lead subacetate test	+
11	Triterpenoids	LibermannBuchard's tes	+

Key: (+) = Present (-) = Absent

Table 2: Phytochemical Composition of the Ethanolic Leaf Extracts of *S. occidentalis*

S/N	Phytochemical Compound	Composition (mg/g)	Percentage (%)
1	Alkaloids	7.20	1.97
2	Anthraquinones	2.10	0.57
3	Carbohydrates	232.31	63.48
4	Cardiac glycosides	0.88	0.24
5	Diterpenoids	0.25	0.07
6	Flavonoids	6.70	1.83
7	Proteins	69.23	18.91
8	Saponins	2.20	0.60
9	Steroids	2.90	0.79
10	Tannins	41.80	11.42
11	Triterpenoids	0.45	0.12
	TOTAL	**366.02**	**100**

However, the result of the parasitemia profile of infected groups of mice treated with different concentrations of the extract and controls is presented in Table 3. The result shows that, *P. berghei* parasites were first detected in all groups on Day 5 and progressively decreased with increase in the concentrations of the extracts. All infected treated groups demonstrated a significantly ($P \leq 0.05$) lowered number of parasitized erythrocytes than the infected untreated group throughout the experimental period. More so, the lowest number of *P. berghei* infected red blood cells is found among 400mg/kg extracts treated mice but higher among the normal saline and chloroquine treated groups.

Table 3: Effect of Leaf Ethanolic Extracts of *S. occidentalis* against *P .berghei* infection in Swiss Albino Mice

Treatments	Dose (mg/kg)	Parasitemia count	% suppression
Normal saline	0.2ml	$4.20\pm0.21^{a*1}$	-
Chloroquine	10	4.10 ± 0.40^{a}	2
Leaf Extract	100	2.70 ± 0.1^{b}	35
Leaf Extract	200	1.83 ± 0.55^{bc}	56
Leaf Extract	400	1.03 ± 0.03^{c}	75

N.B: $*^{1}$ Mean(s) with the same superscripts down a column are not significantly different

Furthermore, the results for the percentage changed in body weights of experimental mice before and after administration are presented in Table 4. The result indicated significant decreased ($P \leq 0.05$) in percentage change in body weight in mice on day 3 and 5. There was also significant decrease ($P \leq 0.05$) in body weights of malaria-infected mice treated with 200 mg/kg, 400 mg/kg and 100 mg/kg on day 3 before treatments. But, daily treatments of malaria from day 3–5 with difference in doses of ethanolic extract from leaves of *S. occidentalis* increased significantly.

Table 4: Effect of ethanolic leaf extracts of *S. occidentalis* on body weights of malaria-infected mice

	Dose (mg/kg/day)	Weight (g/day)					
Weight before Administration (g)		Wt on D1 (g)	% change in wt on D1	Wt(g)on D3	% Change in wt on D3	Wt on D5	% Change in wt on D5
248.30	Saline (0.2ml)	248.32	0.00	246.97	-0.54	259.31	4.99
255.30	Chloroquine (10)	255.31	0.00	253.68	-0.64	257.96	1.69
256.14	100	256.14	0.00	252.78	-1.31	254.94	0.85
252.63	200	252.65	0.00	245.56	-2.81	249.95	1.79
258.14	400	258.14	0.00	251.33	-2.64	257.23	2.35

DISCUSSION

Phytochemical analysis is very useful in the evaluation of some active biological components of some plants as reported by Anwar *et al*. (2007). Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract which may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs as stressed by Sibanda and Okoh (2008). The presence of high concentrations of active constituents in the leaves of *S. occidentalis* revealed in this study is in conformity with the findings of Aja *et al*. (2010) who reported similar compounds in the leaves of *Talinum triangulare* and Aja *et al.* (2017) who reported similar compounds in the leaves of *S. occidentalis*. Similar finding was also found in the work of Egharevba *et al*. (2010). This result is also in line with similar studies conducted by Nuhu and Aliyu (2008) and Shafeen *et al*. (2012) who reported the absence of anthraquinones in the leaves of *S. occidentalis* but was in contrast to the work of Taiwo *et al*. (2013) and Garba *et al*. (2015) who individually reported presence of anthraquinones in their phytochemical analysis of ethanolic extract of *Senna occidentalis* leaf. More so, these differences can possibly be attributed to different methods of extraction employed or probably due to environmental variations. However, the presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines as reported by Egharevba *et al*. (2010).

The reduction of parasitemias in *Plasmodium berghei*-infected mice with increased in the concentration of the ethanolic leaf extracts of *S. occidentalis* indicated the significant role played by the extracts in controlling *P. berghei* infection. This is in conformity with the work of Franssen *et al*. (1997) who reported similar finding. The antiplasmodial effect of *S. occidentalis* leaves extracts suggest that the extracts contain some phytochemicals that could interfere with the survival of the parasites in vivo. The ability of the extract in the higher dose of the leaf extracts of *S. occidentalis* (1000 mg/kg) indicated that the active compounds are concentrated in the higher doses. More so, the presence of flavonoids, tannins, saponins, steroids, triterpenes and dihydrostilbenes nucleus as reported by Ene *et al*. (2009), Musa *et al*. (2010) and Kayano *et al*. (2011) has attributed the antimalarial activities of plants to the presence of flavonoids, tannins and triterpenes. Similar finding was also reported by the work of Ibrahim *et al*. (2011) from *Indigofera pulchra* leaves. Thus, treatment with the ethanolic extracts of *S. occidentalis* leaves significantly inhibited parasitemia compared to the controls indicating their efficacy in malaria treatment. This finding is in conformity with that of Francois *et al*. (1996) who reported similar finding from the leaves extracts of *Neutolaena lobata.* Thus, the efficacy of *S. occidentalis* extracts even at lower concentrations on *P. berghei* shows the potentiality of such plant as excellent antimalarial agent in the prophylactic model of malaria treatment. This finding is in line with the previous report by Calabrese and Baldwin (2003) who reported similar finding.

The over-whelming weight loss observed in this study indicates the effect of malaria in causing weight loss. This result is in agreement with that of Dikasso *et al*. (2006) who observed weight loss in *P. berghei*–infected animals after infection, with the initial weight loss recorded reversed after 5 days of treatment with the extracts in the prophylactic group, but not in the curative and suppressive groups, which showed progressive weight loss. The reduction in body weight gain is a simple and sensitive index of toxicity after exposure to toxic substances as stressed by Pillai *et al*. (2011). The reduction in weight might probably be due to anorexia for food and cachexia as reported by Dondorp *et al*. (2005) and Arora and Arora (2008). This loss of appetite is one of the major symptoms of malaria as reported by Perlmann and Troye-Blomberg (2007). Treatments of rodent malaria with the ethanolic extract increased body weights of malaria-infected mice after certain period of treatment.

This shows that the ethanolic leaf extract of *S. occidentalis* promotes good health, which might be due to the phytochemicals contained in the leaves and that the crude extracts may not contain appetite stimulant compounds as reported by Chinchilla *et al*. (1998).

CONCLUSION

It was concluded that, the leaf of *S. occidentalis* is rich in phytochemicals that are highly active against *P. berghei*. The effect of the leaf ethanolic extracts of *S. occidentalis* increase with increase in concentration. It is therefore recommended that, 400 mg/kg of the ethanolic leaf extract of *S. occidentalis* could be effective in the treatment of rodents' malaria.

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