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Ethnobotanical Survey and *In vivo* Assessment of the Antimalarial Activities of a Locally Used Medicinal Plant (*Senna occidentalis*) for "Malaria Suspected" Fever in Potiskum and Nangere Local Government Areas of Yobe State

Abdullahi Muhammad Daskum[1*]

[1]Department of Biological Sciences, Yobe State University, P.M.B 1144, Damaturu, Nigeria.

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Malaria is a life threatening infectious disease that has affected economic development in many parts of the world. Although preventable, malaria has claimed the lives of thousands of individuals in endemic African countries. Antimalarial drug resistance, lack of vaccines in clinical use as well as complexities of malaria parasite genomes remains a serious threat to malaria eradication efforts. The search for antimalarials from plant sources has yield significant success in drug discovery approaches. The specific objective of this study is to establish the acute toxic effect and antiplasmodial efficacy of crude methanolic leaf extract of *Senna occidentalis* in an *in vivo* assay. The four (4) days suppressive test was used in Swiss mice experimentally infected with chloroquine sensitive (CQS) *Plasmodium berghei* (ANKA). Results obtained revealed no lethality nor any sign of acute toxic reactions following the administration of 2000 mg/kg body weight of the extract. Percent reduction of parasite growth obtained was observed to be dose dependent in all groups treated with the herbal extract and ranges between 66% and 73%. Relative to the negative and positive control groups, a significant reduction in parasitaemia (P≤ 0.01) was observed in all groups treated with the plant extracts. A gradual increase in body weight was observed in extract treated

**Corresponding author: E-mail: daskum341@gmail.com;*

groups throughout the period of the investigation. The antiplasmodial efficacy observed may well be attributed to the presence of alkaloids, flavonoids and other important phytochemicals present. *S. occidentalis* is therefore, considered a good candidate source for development of novel antimalarial drugs.

Keywords: Malaria; Plasmodium berghei (ANKA); swiss mice; Senna occidentalis; Yobe State.

1. INTRODUCTION

Malaria is an infectious disease that has claimed many lives and affected economic development in many parts of the world. Pregnant women, children under five years of age and immunocompromised individuals are worst hit by the parasite [1]. One of four (4) species of Plasmodium parasites (*P. falciparum, P. vivax, P. ovale* and *P. malariae*) in addition to the zoonotic species *P. knowlesi* known to infect non-human primates in the Asian sub-continent are responsible for the disease. The infective stages (Sporozoites) are injected into a potential definitive host (Human) during blood meal by female *Anopheles* mosquitoes [2]. In endemic areas, malaria remains the most common cause of high fever, associated with chills, rigors, loss of appetite, convulsions, low birth weight (in the case of placental malaria), vague absence of wellbeing, headache, fatigue, muscle aches, neurological disorders such as brain damage and coma, in the case of cerebral malaria [3]. Although preventable, an estimated 228 million cases of malaria was reported globally, with approximately 405,000 deaths in 2018 alone [1]. It is worth mentioning that most deaths due to malaria occurred in rural areas, where standard health facilities or health attendants to report cases or deaths are lacking, hence posing a serious set back to malaria case reports and management. In Nigeria health centers, 63.4% of all diagnosed symptoms of fever, chills, malaise and fatigue are confirmed to be malaria [4]. Although, there are indications that malaria mortality had reduced from 533, 000 to 380, 000 between 2010 and 2018, the mortality reduction had slowed since 2016 [1].

In developing African countries, an estimated US$10 billion to US$12 billion is attributed to malaria annually on the continents Gross Domestic Product [5]. Approximately 13% annual reduction in economic growth due to malaria was predicted for malaria endemic African countries, including Nigeria [5]. This economic burden may not be unconnected to loss of man hours, cost of treatment and transport to health facilities, school absenteeism as well as other indirect cost. In farming communities, low productivity was also attributed to malaria [6].

Resistance to commonly available antimalarial drugs [7], lack of vaccines in clinical use and cost of malaria treatment has necessitated developing countries to rely heavily on traditional herbalists and medicinal plants for treatment of the disease [8,9]. Despite the availability of orthodox medicines, 1 out of 5 malaria patients in many developing countries were thought to use indeginous herbal remedies to treat the disease [9]. This is an indication that traditional herbal medicines maintain their popularity because of their availability, historical and cultural reasons [10].

Any plant in which one or more of its many organs contains compounds or substances used to manage or treat diseases is regarded as medicinal plant [11]. For this and many other reasons, *Senna occidentalis* generally referred to as coffee senna in English or Majamfari /Bazamfari or Tafasar Masar by locals in Hausa language may be regarded as a medicinal plant. *S. occidentalis* is a sparsely branching perrenial shrub characterized by foul odour, bearing reddish purple stem which is erect and four angled (when young), becoming rounded with age. The plant bears pale green leaves on reddish stalks and pale to bright yellow flowers. In Nigeria, *S. occidentalis* is found in water canals, open dumping sites and/or by roadsides [12].

In traditional Hausa medicine, *S. occidentalis* is used to treat diseases including but not limited to; Typhoid fever, malaria and hepatitis in Northern Nigeria [12,13]. Similarly, the plant was reported to possess larvicidal and mosquitocidal activity [14], antitrypanosomal [15], antioxidant and antimicrobial [16], anti-inflammatory, immunosupressive, antianxiety, antidepressant, analgesic, antidiabetic and antipyretic activities [17] as well as antiplasmodial activities [18,19,20,21].

In This study, the acute toxic effect and antimalarial activities of crude methanolic leaf

extract of *Senna occidentalis* was established in Swiss mice experimentally infected with *Plasmodium berghei* ANKA.

2. MATERIALS AND METHODS

2.1 Ethnobotanical Survey

Ethnobotanical survey was conducted between the months of February and March, 2020. Prior to data collection, familiarization visits to traditional herbalists were conducted to build their trust and confidence as well as obtain their consent to participate in the research. The survey (semi-structured questionnaire) was carried out to assess the indigenous knowledge of traditional medicinal practice and how to improve health-care situation in Nigeria and by extension, Yobe State was equally conducted. Following the survey, *Senna occidentalis* was identified as the most frequently used medicinal plant for malaria among locals, hence the reason for its selection in this study.

2.2 Phytochemical Extraction

Fresh leaves of *Senna occidentalis* was handpicked, washed in tap water, dried under shade and ground to powder [22]. Extraction of secondary metabolites from powdered plant sample was performed by cold extraction procedures with Methanol [23]. Samples were weighed as previously described [24,25,26,27]. Powdered plant material, 100 g was transferred into screw cap, wide mouth, clear sample bottles and macerated in 500 ml each of methanol (i.e. 1:5 w/v). The suspension was stirred, screws capped and shake for 24 hours in a shaker (IKA WERKE, HS 501) at room temperature. Mixture was filtered using muslin cloth. Consequent to filtration, the methanolic filtrates was concentrated at 40°C using a rotary evaporator (Stuart, RE300DB) according to standard procedures [28] and solvents recovered.

2.3 Phytochemical Screening

Crude extracts were subjected to qualitative phytochemical screening as per the protocol previously described [29]. Analysis for the following active metabolites: Phenols, Anthraquinones, Saponins, Alkaloids and Flavonoids was performed.

2.4 Ethical Clearance

Ethical clearance was sought and obtained from the Institutional Animal Care and Use Committee of Yobe State University, Damaturu. The OECD 423 guideline [30] was adopted and all protocols are in accordance with the committees' approvals.

2.5 Experimental Animals

Swiss mice (age 8-12 weeks), weighing 25-30 g were purchased from a local supplier in Damaturu and acclimatized for two weeks in the Biology Laboratory, Department of Biological Sciences, Yobe State University. Animals were maintained under standard 12 hour dark and 12 hour light cycles, fed with poultry diets (Rico Gado Nutrition, Yola, Nigeria), and served *water ad libitum*.

2.6 Acute Toxicity Test

The OECD 423 [30] guideline for testing of chemicals in experimental animals was adopted. In accordance with paragraphs 18 and 19 of the guideline, the limit test involving three animals per step were used, with a 2000 mg/kg body weight starting dose selected.

Animals were grouped and Group 1 (n=3) were fasted for 4 hours, with only water *ad libitum* but not food served, during which they were weighed and served 0.2 ml of 2000 mg/kg body weight of the plant extract in 5% dimethylsulfoxide (DMSO) by oral gavage [31,32]. Following administration of extract, food was still withheld for another 1 hour [30]. All animals were monitored individually for signs of toxic reactions during the first 30 minutes after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours.

The procedure was repeated with Group 2 (n=3) and monitored as described. All groups were then monitored for 14 days with normal diet and water served.

2.7 Infection of Experimental Animals

The classical four days suppressive test of Peter et al. [33] and modified [32,34] was adopted. *Plasmodium berghei* (ANKA) parasitized erythrocyte was obtained from a donor rat by cardiac puncture and diluted with 0.9% Phosphate Buffered Saline (PBS) in proportion of 1:4 as previously described [32]. Healthy female Swiss mice (n=25; weighing 25-30 g; age 8-12 weeks) were experimentally infected with blood suspension (0.2 mL i.e. $1x10^7$) *P. berghei* parasitized erythrocytes by intraperitoneal

injection as previously described [32,35,36,37,38].

2.8 Grouping and Treatment of Experimental Animals

Following infection, animals were randomly grouped into 5. Groups 1-3 had 5 mice each and serves as the test groups while groups 4 and 5 also having 5 mice each, serves as positive and negative controls respectively. The test groups (1-3) were served 0.2 mL (200 µl) of the extracts at (50, 100 and 200 mg/kg/body weight) respectively by oral gavage, 2 hours post infection on day 0. Treatment continued once daily for four (4) days (Day 0, 1, 2 and 3) [39,40] while group 4 (n=5) were served 0.2 mL of the vehicle (5% DMSO) and group 5 (n=5) were served 0.2 mL of 25 mg/kg chloroquine phosphate by oral gavage to serve as negative and positive controls respectively [38,41]. The highest dose served (200 mg/kg/body weight) was capped at 1/10 of the maximum tolerant dose based on the outcome of acute toxicity study [38].

2.9 Determination of Body Weight

The body weights of individual rat was taken daily in the morning before feeding and administration of crude extract or drug (day 0 to day 4 post infection). Subsequently, the change in body weight was determined by taking the mean weight of mice in respective groups and the weight gain or loss was obtained by subtracting the initial weight (Weight in day 0) from the final weight (weight in day 4) post infection.

2.10 Blood Collection for Determination of Percent Parasitaemia

On the 4th day post infection, an alcohol treated swab was used to disinfect the tail of individual rat and tip end of tail of individual rat was cut, following which, two drops of blood was collected directly on a clean, grease free glass slide to make thin films (smear). Following blood collection, a finger pressure was applied to the site of collection to stop blood flow before placing the mice back in the cage. Smears were air dried, fixed with absolute methanol, stained with 10% giemsa in PBS for 20 minutes and examined microscopically (Olympus CX22LED) at 100x magnification under oil immersion [42]. Blood collection for thin smears and determination of percent parasitaemia was performed in alternate days (48 hour intervals) for 6 days from the 4th day post infection until the 14th day post infection. Later, all mice were euthanized by cervical dislocation. Percentage parasitaemia and antimalarial suppressive effect of extract was calculated as previously described [43].

2.11 Statistical Analysis

Data obtained was analysed using Graphpad prism 5.0. One-way analysis of variance (ANOVA) followed by Tukey's (post-hoc test) was used to determine statistical significance for comparison of percent parasitaemia in different concentrations (groups) of extracts, standard drug (chloroquine phosphate) and negative control, *in vivo*. P-values ≤ 0.05 was considered statistically significant.

3. RESULTS

3.1 Phytochemical Analysis

Results of the phytochemical screening revealed the presence of phenols, terpenoids, tanins, cardiac glycosides, Xanthoproteins, Alkaloids, flavonoids as well as Anthraquinones but not saponins in the methanolic extract of *S. occidentalis*.

3.2 Acute Toxicity

To determine the safety of the crude methanolic extracts, a limit test was conducted with six female Swiss mice as described. Results obtained revealed no lethality nor any sign of acute toxic effects following the admininstration of 2000 mg/kg of the plant extract. This implies that the acute lethal dose (LD_{50}) of the extract was above 2000 mg/kg body weight.

3.3 *In vivo* Antimalarial Tests (4-Days Suppressive Test)

Result of the four-days suppressive test are expressed as percent parasitaemia (Mean ± SEM) and percent supression of parasite growth in relation to the control groups (Table 1). Significant ($P < 0.05$) reduction of parasitaemia was observed in all groups of mice treated with the plant extracts when compared to the negative control. Furthermore, no statistical significance ($P \geq 0.05$) was observed when test groups, treated with the methanol extract were compared. A

significant chemo suppression (P≤ 0.01) was however, observed at all dose levels (50 mg/kg, 100 mg/kg and 200 mg/kg respectively), relative to the negative and positive control groups. Percent reduction in parasite growth was in the range of 66% to 73% and Chemosupression of parasitaemia was observed to be dose dependent.

3.4 Effect of Crude Methanol Extacts on Body Weight

Body weight of experimental animals treated with plant extracts were monitored as described. Results obtained (Table 2) revealed a decrease in body weight (-34.30%) of animals in the negative control group. This was expected since parasitaemia was allowed to build up, treatment was not served and mortality was observed on day 4 post infection. In the extract treated group however, a gradual increase in body weight was observed throughout the period of the investigation. Specifically, in the 100 mg/kg body weight treated group (Group 2), the percentage change in body weight is clearly pronounced (4.44%).

4. DISCUSSION

The presence of important secondary metabolites observed in the methanolic extract of *S. occidentalis* may be an indication for its use in the management of certain ailments. The secondary metabolites identified in this study corroborate with the results of Ibrahim et al. [44]; Nuhu and Aliyu, [12] who also reported tannins, alkaloids, glycoside, flavonoids, steroids, in addition to anthraquinones in methanol leaves extract of *S. occidentalis.* However, saponins identified in those studies were not observed here. This variation may be attributed to the purity of extraction solvent used and protocols of phytochemical analysis adopted. The presence of these bioactive compounds may likely be responsible for some of the pharmacological properties of the plant. Most of these metabolites were reported for their biological activities: Flavonoids and Alkaloids for example, exert their effects by way of scavenging free radicals during infection or by activating a series of self-protective proteins [45]. In reaction to malaria infection, activation and up regulation of immune response induce the generation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Intermediates (RNIs) respectively, to fight infection, thus causing an imbalance between the formation of oxidizing species and the activity of antioxidants. This imbalance has been reported to be responsible for oxidative stress, an important mechanism of human hosts in response to infections [46], thereby resulting in parasite death.

Table 1. Antimalarial suppressive effect of crude methanolic extract

Plant Name	Type of extract	Groups	Dose (mg/kg)	Parasitaemia (%) Mean ± SEM	Supression (%)
S. occidentalis	Methanol	1	50	4.23 ± 0.60 ^{ansb***c***}	66.02
		2	100	3.75 ± 0.58 ^{ansb***c**}	69.92
		3	200	3.32 ± 0.66 ^{ansb***c**}	73.37
Negative control	-	4	0.2mL	12.46 ± 0.00 d^{***}	0
Positive control	-	5	25	0.58 ± 0.00 d^{***}	95.35

*Percent parasitaemia are expressed as Mean ± SEM; n = 5: a, between groups; b, compared to negative control; c, compared to Positive control; d, between controls (Positive and Negative controls, respectively). *p≤0.05, **p≤0.01, ***p≤ 0.001 and ns= P≥ 0.05.*

Table 2. Effect of crude extracts on body weight

Plant Name	Type of extract	Groups	Dose (mg/kg)	Body weight		Change in Body weight (%)
				Day 0 Mean ± SEM	Day 4 Mean ± SEM	
S. occidentalis	Methanol	1	50	26.24±0.65	26.72±1.18	1.83
		2	100	25.70±0.62	26.84±0.96	4.44
		3	200	26.86±0.48	27.33±0.47	1.76
5% DMSO (NC)	**-**	4	0.2mL	27.84±0.62	18.29±4.62	-34.30
Chloroquine phosphate (PC)	**-**	5	25	28.64±0.39	30.23±0.62	5.55

Values are presented as mean±SEM; n=5 NC= Negative control; PC= Positive control

The absence of mortality or sign of acute toxic reactions (pilo erection, moribund behaviour, tremors, convulsions, salivation, diarrhoea, lethargy and coma) in all experimetal animals dosed with 2000 mg/kg body weight of the extracts, throughout the follow-up period suggests the extracts is safe for use in experimental animal models and that the LD_{50} is > 2000 mg/kg body weight. This finding conform with the results of Nwaehujor et al. [47]; Silva et al. [48] who reported that the LD_{50} of *S. occidentalis* is > 2000 mg/kg body weight.

In accordance with Mulisa et al. [49], a compound or extract may be considered active in an experimental animal when such a compound is capable of supressing parasiteamia by 30%. Despite the availability of relevant literature [19,20,21,22] on the antimalarial activity of *S. occidentalis,* Tona et al. [21] reported almost, for the first time, the antimalarial activities of *S. occidentalis* (root bark). Although, this research focused primarily on the antiplasmodial activity of the leaves of *S. occidentalis*, results obtained substantiate the findings of Tona et al. [21] and Tona et al. [20], who reported good *in vivo* antimalarial propoerties of ethanolic, dichloromethane and lyophilized aqueous (root bark) extract of *S. occidentalis*. However, findings also validate the *in vitro* antimalarial activities of *S. occidentalis* (leaves) reported in a recent study [22]. This antiplasmodial efficacy observed may well be attributed to the presence of alkaloids, flavonoids and other important phytochemicals. Specifically, suppression of parasitaemia by the methanol extract may not be unconnected to the presence of alkaloids as this metabolite identified in other plants [40,50,51,52] was thought to be responsible for the antimalarial activities exerted.

The effect of crude methanolic *S. occidentalis* extract on body weight could be related to the initial body weight and survival of experimental animals until 4th day post infection. As reported, a gradual change in body weight was observed in Group 3 served with 200 mg/kg body weight of the extract. Although, all animals in group 2 administered with 100 mg/kg body weight of the plant extract showed relatively appreciable change in body weight, the exact cause of the change may be attributed to the absence of mortality in the experimental groups.

Even though, the crude plant extract studied was less active when compared to the reference antimalarial (chloroquine phosphate), it is important to bear in mind that chloroquine was officially banned in Nigeria since 2004 for factors related to resistance and safety associated side effects such as cinchonism [53]. In agreement with Mulisa et al. [49], the methanol extracts in the present study may serve as a good candidate source for development of novel antimalarial drugs. This study also validates the traditional use of *S. occidentalis* in the treatment of malaria "suspected" fever.

5. CONCLUSION

The crude methanolic leaves extract of *Senna occidentalis* used in this study was proved to be safe in experimental animals. At various dose levels, the crude extract demonstrated a significant (P≤ 0.01) reduction in parasitaemia in Swiss mice experimentally infected with *P. berghei* (ANKA). This activity was attributed to the presence of bioactive metabolites especially flavonoids, alkaloids and anthraquinones. Findings of this study validates traditional claim for the use of *S. occidentalis* leaves by locals to treat malaria. This plant could serve as a good candidate source for development of novel antimalarial drugs. Work to compile the data obtained during ethnobotanical survey is still ongoing.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance was sought and obtained from the Institutional Animal Care and Use Committee of Yobe State University, Damaturu. The OECD 423 guideline [30] was adopted and all protocols are in accordance with the committees' approvals.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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