ACUTE TOXICITY PROFILE AND PLASMODIUM BERGHEI INHIBITORY ACTIVITY OF OCHNA SCHWEINFURTHIANA (F. Hoffm) OCHNACEAE LEAF EXTRACT IN LABORATORY ANIMALS

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ABSTRACT

The resurgence of drug-resistant strains of *Plasmodium falciparum* to currently used chemotherapeutic agents necessitates the search for newer anti-malarial agents, natural products being the most promising. *Ochna schweinfurthiana* (Ochnaceae) is a plant used in traditional medicine for treating malaria and other infections. Acute toxicity (LD_{50}) of the methanol leaf extract of the plant was tested using Lorke's method, intra-peritoneally and orally. Antiplasmodial activity in early and established infections were tested using Peter's 4-day suppressive and Ryley and Peters' Curative tests respectively in *Plasmodium berghei* infected mice. From the suppressive test result, there was significant plasmodial growth inhibition at administered doses of 50 and 100 mg/kg, both higher than chloroquine (5 mg/kg); the standard drug. The 200 mg/kg dose resulted in parasitaemia suppression, though statistically non-significant. The curative test showed significant activity ($P<0.01$) at a dose of 50 mg/kg comparative with the standard chloroquine. Mortality was observed in group treated with 200 mg/kg of the extract. The LD_{50} of the extract was found to be 774.6 mg/kg intraperitoneal and > 5000 mg/kg orally. The present research revealed that the extract possesses antimalarial effect with relative intra-peritoneal toxicity but safe orally, thereby validating its traditional use in the management of plasmodial infection.

Keywords: Antiplasmodial, *O. Schweinfurthiana,* extract, curative, suppressive, toxicity

INTRODUCTION

Malaria is a major public health problem in developing countries including Nigeria. A total of 109 countries, 45 of which are within the World Health Organisation African region were reported as endemic to malaria in 2008 [1] ; [2]. Malaria is arguably the number one cause of death in Nigeria accounting for 15% of hospital admission and about 50% out-patient consultation [3]. It is estimated that malaria affects 350–500 million people worldwide and causes 1.1 million mortalities annually [4]. The development of drug resistance by *Plasmodium falciparum*, the malaria causative agent to the current clinically used anti-malarials has been partly attributed to the resurgence of malaria [5]. The emergence of drug resistance to monotherapy and combination therapy and absence of functional, safe and widely available vaccine therefore necessitate a continuous search and development of new anti-malarial drugs.

Nature, particularly plants' selection and study based on ethno-pharmacological data are a potential source of new antimalarial drugs as they contain molecules with a great variety of structures and pharmacological activities [4]. Furthermore, most prominent antimarial in current clinical use such as the quinines and artemisinins were obtained from plants. Traditional medicine still remains the means of disease management for up to 80 per cent of the African population due to its availability and affordability [6].

Ochna schweinfurthiana F Hoffm. (Ochnaceae) is a small tree or shrub that stands at about 4m. It grows in the savannah woodland from West to Central Africa and across Asia [7]. The plant has acclaimed efficacy in the treatment of several illnesses. The powdered bark is used as antihelmintic and antimalarial, its leaf or root decoction is used in wound dressing, treatment of measles, fungal infection and typhoid fever. The plant is also used as laxative, antiseptic, stimulant, febrifuge, among others [7]. Result of preliminary phytochemical

screening of the plant showed that it contains saponins, flavonoids, carbohydrates, steroids, glycosides and tannins [8]. Other species from the Ochnaceae have been reported to have potent anti-malarial activity [9]. Chemotherapeutic agents will continue to be in demand for the complete management of malaria [10]. The issue of resistance means that discovering new compounds with an original mode of action is an urgent priority. In addition, establishment of the safety and efficacy of traditional medicinal plants which are used to fight the disease is essential. On this account and the absence of scientific validation for the acclaimed usefulness of the plant in malaria management, the present study was aimed at establishing the *in-vivo* anti-plasmodial activity of the methanol leaf extract of *Ochna schweinfurthiana* in laboratory animals.

MATERIALS AND METHODS

Drugs

Standard chloroquine phosphate (Sigma Aldrich) and methanol leaf extract of *Ochna schweinfurthiana* (CME) (50, 100 and 200 mg/kg).

Experimental animals

Locally bred adult Swiss albino mice of either sex (15-30 g body weight) were acquired from Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. The animals were fed with laboratory diet and water *ad libitum* and maintained under standard conditions in clean cages under normal 12 hours light, 12 hours dark circle. All experimental procedures followed the 2010 revised ethical guideline for the care and use of laboratory animals as provided by the Ahmadu Bello University Research Policy and accepted internationally.

Malaria parasite

Mouse-infective chloroquine-sensitive strain of *Plasmodium berghei berghei* NK-65 was obtained from National Institute of Medical Research, Lagos. The parasites were kept alive by continuous intra-peritoneal passage in mice.

Collection, identification and preparation of plant material

The whole plant *Ochna schweinfurthiana* was collected from Samaru, Zaria, Nigeria in June 2013. It was identified and authenticated at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna State-Nigeria by comparing with existing specimen (number 900229). The leaves were removed, shade dried to constant weight, pulverized, labelled and stored for further use.

Extraction

300g of the plant sample was macerated twice (5 days each) with methanol. The resulting extract was concentrated *in-vacuo*. This afforded 36g (12%) of a greenish-brown concentrate and was subsequently referred to as crude methanol extract (CME).

Acute Toxicity Study

The method of Lorke [11] was employed for both intraperitoneal and oral LD_{50}. In the first phase, nine mice were divided randomly into 3 groups of 3 mice each. Varying doses of the extract (10, 100 and 1000 $mgkg^{-1}$) were administered to groups 1, 2 and 3 respectively and observed for 24 hours for any sign of toxicity and mortality. In the second phase, four more specific doses based on the result from the first phase were administered to 4 fresh mice each and observed also for 24 hours. The geometric mean of the lowest lethal dose and the highest dose survived by the animal was taken as the median lethal dose (LD_{50}). The procedure was conducted separately for the oral and intraperitoneal routes (Tables 1 and 2).

Antiplasmodial Screening

Parasite Induction

A donor mouse with about 20-30% parasitaemia was sacrificed with chloroform and the blood collected through cardiac puncture. The blood was diluted with normal saline such that the inoculums contained approximately 10^7 infected erythrocytes. Inoculated was done through the intra-peritoneal route using hypodermic needle fitted to 1-ml syringe.

Schizonticidal Effect in Early Infection (Suppressive Test)

This study was done according to the method of Peters [12]. Swiss Albino mice (30) were grouped into 5 each containing 6 mice. Each mouse was inoculated with 0.2ml of the infected blood and allowed for 2 hours. Doses of the extract at 50, 100 and 200 mg/kg were administered to groups 1, 2 and 3 respectively, once daily as treatment for 4 days (day 0 to day 3) intra-peritoneally. Parallel tests were conducted with 5 mg/kg standard chloroquine for reference purpose and 0.2 ml/kg normal saline as vehicle in groups 4 and 5 respectively. On day 4, thin blood smears were made from the tail. The slides were fixed with methanol, stained with 10% Giemsa for 15 minutes and examined under microscope at × 100 magnifications. The parasite count was recorded and

the suppression of parasitemia was expressed as per cent for each dose, by comparing the parasitemia in the control group with the treatment groups.

$$\% \text{ suppression} = \frac{\% \text{ parasitaemia in control - } \% \text{ parasitaemia in treated group}}{\% \text{ parasitaemia in control}} \times 100$$

Schizonticidal Effect in Established Infection (Curative Test)

The method of Ryley and Peters [13] was employed. Mice (30) were inoculated with 0.2ml standard inoculums. 72 hours post infection; the mice were grouped into 5 groups of 6 mice each. Graded doses of 50, 100 and 200 mg/kg of body weight of the extract were administered to groups 1, 2 and 3 respectively. Groups 4 and 5 were taken as positive and negative controls and administered 5 mg/kg standard chloroquine and 0.2 ml/kg of body weight normal saline respectively. Administrations were done intraperitoneally once daily for 4 days. On day 7 i.e. a day after last treatment, thin blood smears from the tail blood were taken and analyzed for parasite suppression after fixing and staining the slides.

Statistical Analysis

Statistical analysis of control and test data was based on simple one-way ANOVA and Dunnett's post hoc test were used for different doses within a group. All groups were compared against the negative control group.

RESULTS AND DISCUSSION

Acute toxicity studies

The extract was found to have LD_{50} values of 774.6 mg/kg and 5000 mg/kg intra-peritoneally and orally respectively suggesting that the plant is relatively toxic intraperitoneally and safe orally [11].

Schizonticidal effect in early infection

The crude methanol extract exerted a suppressive effect against *Plasmodium berghei*. There was 92.2% suppression at a lower dose of 50 mg/kg, 89.7 and 37.5% for the median (100 mg/kg) and high (200 mg/kg) doses respectively. The extract at the lowest dose had higher efficacy compared to the standard chloroquine (5 mg/kg) with 50% suppression (Table 3). The Peters' 4 day suppressive test is the most common standard test used for antimalarial screening of plants, and the most reliable parameter for assessment of this effect is the determination of percentage inhibition of parasitaemia [14] ; [15]. Despite researches, there is still controversy on the exact mechanism of action of most antimalarial drugs. Chloroquine is thought to exert its antimalarial effect by preventing the polymerisation of heme produced as a result of proteolysis of haemoglobin in the parasites' digestive vacuole or its cytosol [16]. Chloroquine is also known to exert chemo suppression through inhibition of Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) biosynthesis and induction of a rapid degradation of ribosomes and their RNA [17]. Both mentioned mechanisms show that chloroquine acts as a blood schizontocide. Peter and Anatoli (1998) reported that the antiplasmodial effect of plant-derived natural products may be through inhibition of protein synthesis [18]. This activity depends on their active phytochemical constituents [19]. The ability of the extract to suppress malaria at the early stage indicates that the extract possesses blood schizonticidal activity. This activity might also be through a completely unknown mechanism.

Schizonticidal activity in established infection

In the curative test, the extract caused a reduction in parasitaemia levels of groups treated with different doses of the extract. There was a 100% cure at an administered dose of 50 mg/kg similar to chloroquine treated group. A lower reduction (57.3%) was observed in the group treated with 100 mg/kg. Before day 7, there was 100% mortality in the group treated with 200 mg/kg of the extract (Table 4).

The effectiveness of any chemotherapeutic agent is dependent upon a favourable therapeutic ratio; either elimination or inhibition of the parasite with little or no toxicity to the host [10]. The percentage inhibition of parasitaemia level exerted by the extract at the lowest dose was higher than that in the suppressive test probably due to non-selectivity of the extract to the proliferative process of the parasite [18]. Chloroquine is a drug that combats established cases of malaria. It is known to be active only against the erythrocytic stage of the parasite [12] during which it actively degrades haemoglobin. This led to the assumption that chloroquine somehow interferes with the feeding process of the parasite [20]. Chloroquine concentrates up to several 1000 folds in the vacuole [21] through either ion trapping [22], active uptake of the drug by the parasite transporter [23] and/or binding of the drug to a specific receptor in the digestive vacuole [24], thereby interfering with heme biocrystallization. Accumulation of the metabolic waste (heme) eventually overwhelms the parasite [20]. The ability of the extract to cure plasmodial infection in the same manner with chloroquine suggests that it possesses a mechanism that involves the inhibition of food vacuole phospholipases. Moreover, researches have shown that the anti-malarial effect of any given drug is dependent on its interaction with the vertebrate immune response [25]; [26]; [27]; [28]. Furthermore, the counteractive effect of phytochemicals such as flavonoids, tannins, etc through cellular oxidative damage caused by parasites has been proposed as their probable mechanism of action against established plasmodial infection [29]. Phytochemical screening of this extract has shown it to contain

these constituents [8]. Therefore, the mechanism of action of the extract in established infection may be through oxidant action, direct killing of the parasite and action of one or more metabolites individually or in synergy.

The toxicity observed at an administered dose of 200 mg/kg of the extract in both suppressive and curative tests was probably due to *in-vivo* immuno-suppressive activity of the plant [30]; [28]. This toxicity may also not be unrelated to saturation of the active sites or activity on other body organs. Another factor that might have led to this toxicity is the route of administration, because the toxicity studies showed that the extract will be a lot safer when administered orally.

CONCLUSION

From the results of this study, it can be suggested that the methanol leaf extract of *Ochna schweinfurthiana* contain bioactive principles with anti-malarial activity, thus lending credence to the folkloric use of the plant in malaria management. Further study is however needed in order to determine the exact mechanism of action, isolate and characterise the bioactive principles responsible for the acclaimed activity. Chronic toxicity studies should also be carried out to clarify observed toxicity.

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Table 1: Determination of median lethal dose (LD_{50}) of Methanol Extract of *Ochna schweinfurthiana* via intra-peritoneal route

First Phase		
Dose ($mgkg^{-1}$)	Number of mice used	Mortality
10	3	0/3
100	3	0/3
1000	3	3/3
Second Phase		
140	1	0/1
225	1	0/1
370	1	0/1
600	1	0/1

Table 2: Determination of median lethal dose (LD_{50}) of Methanol Extract of *Ochna schweinfurthiana* via intra-peritoneal route

First Phase		
Dose ($mgkg^{-1}$)	Number of mice used	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3
Second Phase		
1200	1	0/1
1600	1	0/1
2900	1	0/1
5000	1	0/1

Table 3: Anti-plasmodial activity of crude methanol extract of *Ochna schweinfurthiana* in early infection (Suppressive Test)

Treatment (mg/kg)	**Average Parasitaemia ± SEM**	**Percentage Suppression**
Normal Saline	3.20±0.20	-
CME 50	$0.25\pm0.25^{**}$	92.2
CME 100	$0.33\pm0.33^{**}$	89.7
CME 200	2.00 ± 0.91^{ns}	37.5
Chloroquine 5	$1.60\pm0.24^{*}$	50.0

P>0.05 = ns, P<0.01 = **, P<0.05 = *, n=6

Table 4: Anti-plasmodial activity of crude methanol extract of *Ochna schweinfurthiana* in established infection (Curative Test)

Treatment (mg/kg)	**Average Parasitaemia ± SEM**	**Percentage Curation**
Normal Saline	1.17±0.31	-
CME 50	$0.00\pm0.00^{**}$	100
CME 100	0.50 ± 0.50^{ns}	57.3
CME 200	-	-
Chloroquine 5	$0.00\pm0.00^{**}$	100

P>0.05 = ns, P<0.01 = **, n=6