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Evaluation of the Anti-malarial effect of the methanolic leaf extract of *Vernonia glaberrima* (Asteraceae)

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Abstract:*The emergence of drug-resistant strains has compromised the efficacy of several anti-malarial drugs, including the artemisinins. Many species of Vernonia, family Asteraceae, have been employed in traditional medicine for the management of diabetes mellitus, microbial infections and malaria. The anti-malarial effect of the methanolic leaf extract of Vernonia glaberrima was investigated using Chloroquine-sensitive Plasmodium berghei (NK65) infected mice, according to Riley and Peters curative test model. Three test groups of mice were orally administered with the suspension of the graded doses of the extract at 300, 150 and 75 mg/Kg body weight for four consecutive days; a reference drug, Chloroquine phosphate (5mg/Kg) group, and the negative control group (0.2ml distilled water) were similarly administered. The level of chemo-suppression obtained on the eighth day was a function of the reduction in parasitemia as estimated from the mice tail blood smear. The mean survival time (in days) for each group of mice was also determined over a period of 28 days post-infection. The results showed a dose-dependent chemo-suppression by the three extract groups 300, 150 and 75 mg/Kg at 62.1%, 54.3% and 32.2% respectively, while Chloroquine (5mg/Kg) exhibited a higher suppression at 73.4%. Only the Chloroquine and 300mg/kg groups produced statistically significant ($p<0.05$) mean survival time of 19 and 17 days when compared to the negative control. The study demonstrated the anti-malarial effect of the leaf extract of V. glaberrima and lends credence to its ethno-medicinal use in treating malaria infection.*

Keywords: *Vernonia glaberrima* extract, *Plasmodium berghei, anti-malarial, Curative test*

I. Introduction

Malaria is a major public health problem affecting not less than 40% of the world's population (Snow *et al*., 2005). An estimated 1.2 billion are at high risk of transmission (≥1 case per 1000 population), more than half of which live in the African regions and Nigeria alone accounts for a quarter of all malaria cases in Africa (WHO, 2008). Malaria is the most common disease in Nigeria affecting more than 100 million people annually (Reyburn, 2010). In 2010, about 216 million people were infected with 655,000 mortality, worldwide. African region accounts for 81% and 91% of the cases and deaths respectively, with 86% of the mortalities observed among the under-fives (WHO, 2014). The emergence of drug-resistant strains has compromised the efficacy of several anti-malarial drugs, including artemisinins, thus necessitating the need for the discovery of other novel anti-malarial agents (Phyo, 2012; Nkhoma *et al*., 2012). Many anti-malarials including artemisinins were discovered from plant sources (Klayman 1985; Cragg, Newman *et al*., 1997; Newman and Cragg 2007; Newman and Cragg, 2012). Ninety-nine plant species in Brazil were reportedly claimed to have antimalarial activity (Milliken, 1997). In Nigeria, over 100 plant species including *Vernonia cinera* and *Vernonia amygdalina* are used in herbal medicine as remedies for malaria (Adebayo and Krettli, 2010). The genus Vernonia, family Asteraceae, has been employed in traditional medicine for the management of varied diseases including malaria (Muregi *et al*., 2003); microbial infections (Al-Magboul *et al*., 1988; Erastor *et al*., 2006), as analgesics and anti-inflammatory (Njan *et al*., 2008), cytotoxic (Kuo *et al*., 2003; Williams *et al*., 2005) and, many species have been reported to amend the hyperketonaemia, hyperlipidaemia and hypercholesterolaemia associated with diabetes mellitus (Nimenibo-Uadia, 2005). Sesquiterpene lactones are the major bioactive constituents isolated from the Vernonia species. They have been reported to exhibit antimalaria, antileishmania, antischistosoma, cytotoxic, anti-microbial and anti-inflammatory effects (Toyang and Verpoorte, 2013).

Vernonia glaberrima Welw. Ex O. Hoffm (family Asteraceae), Shìwaákár-ján-gágári (Hausa language - N. Nigeria) is an erect shrub, 2 meters high, found on hillside grassland in Guinea to Northern Nigeria, Western Cameroon and Central Africa to Angola. It is reported to be used traditionally against malaria, migraine, psoric and dysmenorrhoea (Burkill, 1985); it is also employed for treating pain, inflammation, vertigo

and microbial infections (Abdullahi *et al*., 2015). This study was aimed at evaluating the anti-malarial properties of the crude methanol leaf extract of *V. glaberrima*.

II. Materials And Methods

Collection and Identification of the Plant Sample

The whole plant material of *Vernonia glaberrima* was collected in Nassarawa State, Northern-Nigeria in June 2012 during rainy season. It was authenticated by U.S. Gallah of the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen (No. 899) was deposited at the herbarium for future reference. The leaves were removed, air-dried, powdered, labeled and stored in air-tight container prior to extraction.

Preparation of extract

The leaves were removed, shade dried, pulverized, labelled and stored at room temperature in an air-tight container prior to extraction. The Powdered leaves (2500g) was extracted with 70% methanol using maceration method for 10 days with occasional shaking. The extract was evaporated in-vacuo using rotary evaporator at 40°C to obtain a gummy greenish product (400g) subsequently referred to as the crude methanol leaf extract VGLE.

Experimental Animals

Swiss albino mice of either sex weighing (15-38g) obtained from the Animal House Facility of the Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria, were used for the study. They were fed with commercial feeds and water *ad libitum* and maintained under standard conditions (12hr light and 12hr dark cycle) in propylene cages at 25^{o}C room temperature. All experimental procedures were performed in accordance with the guidelines of the Animal right Ethics Community of the university.

Acute toxicity study

The safety of the extract was evaluated as described by (Lorke, 1983). The route of administration was intra-peritoneal. In the first phase, nine mice of either sex were divided into three groups containing three mice each. The first, second and third groups received 10mg/kg, 100mg/kg and 1000mg/kg respectively. The rats were observed continuously for behavioral, neurological, autonomic and any lethality in first 24 hours. From the result of the first phase, three mice were used for the second phase. They were given different doses 1600mg/kg, 2900mg/kg and 5000mg/kg of the extract, and were observed for any sign of toxicity and possibly death during the 24 hours. The median lethal dose was calculated using the following formula;

$LD_{50} = \sqrt{\textbf{minimal lethal dose} \times \textbf{maximal survival dose}}$

Plasmodium berghei parasite

The *NK65* Chloroquine sensitive strain of *Plasmodium berghei* was purchased from National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. The parasite was conserved in the Department of Pharmacology and Toxicology Laboratory, Usmanu Danfodiyo University Sokoto via passage of blood from infected into healthy mice.

Parasite Induction

Blood containing infected red cells (from infected donor mice) were diluted with normal saline so that 0.2mls will contain $1*10^6$ parasitized red cells. The mice were inoculated with 0.2mls of the infected blood intraperitonealy, using hypodermic needle fitted to 1-ml syringe.

Evaluation of schizontocidal activity of *V. glabberima* on established infection (Curative or Rane test)

Riley and Peter method (Riley, Allen *et al*., 1991) was employed to evaluate the curative potential of *Vernonia glaberrima*. On day one (1), twenty five mice were inoculated with 0.2mls of blood containing 10^6 *Plasmodium berghei* infected red cells, intraperitoneally. Three (3) days later, the mice (with established infection) were randomly divided into 5 groups of 5 mice each and treated for four consecutive days (days 4, 5, 6 and 7). The first three groups were orally treated with 10ml/kg of the dissolved extract daily at 300, 150 and 75mg/Kg body weight respectively. Two control groups (positive and negative) were similarly dosed with 10 ml/kg of chloroquine phosphate (at 5mg/Kg) and distilled water respectively. On day 8 of the experiment (a day after completion of treatment), tail blood was collected from each mouse and thin films of the samples were stained with leishman. Buffered water (pH 6.8) was added to the film, which was kept for 8-10 minutes, cleaned with cotton wool and allowed to air dry, before it was viewed at × 100 magnifications on the microscope. The average percentage parasitaemia and percentage of parasite suppression were calculated in each of the groups as shown below (Penna-Coutinho, Cortopassi *et al*., 2011).

Percentage Parasitaemia= $\frac{\text{Number of infected RBCS}}{\text{Total no. of RBCS examined}}$ X100

% suppression = $\frac{\text{PC - PTG}}{\text{PC}}$

Where PC is the parasitaemia in the untreated group
PTG is the parasitaemia in the test group.
Drugs that reduced parasitaemia by 29-40% were considered as partially active antimalarials, while those that produced greater than 40% reduction in parasitaemia were considered active antimalarials.

Determination of Mean Survival Time

The duration of 28 days survival was recorded for each mouse. Mean survival time (MST) was calculated using the following formula (Penna-Coutinho, Cortopassi *et al*., 2011).

MST= $\frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total no. of mice in that group}}$

III. Results

Acute toxicity test

The LD_{50} was extrapolated to be 1265mg. This indicates that the experimental doses used are relatively safe.

Curative antiplasmodial activity and the mean survival time

A high suppression (>40%) of parasites, in animals with established infection was observed in the groups treated with chloroquine, graded doses of the extract (300 and 150 mg/Kg), but only mild (29-40%) in the group treated with 75mg/kg. There was no significant difference (P>0.05) in chemosuppression between mice administered with 300mg/Kg, 150 mg/Kg and chloroquine-treated group. On the other hand, mice treated with 75mg/Kg did not differ significantly (P>0.05) in chemosuppression from the vehicle-treated control group (Table 1). The groups of mice treated with chloroquine and 300mg/Kg produced a statistically significant increase (P<0.05) in mean survival time compared with vehicle-treated animals (Table 1). No significant difference (P>0.05) was observed in the mean survival time of the groups of mice treated with either 150 or 75 mg/Kg of the plant extract when compared to the negative control group (Table 1).

IV. Discussion

In this study, the anti-malaria traditional claim of *Vernonia glaberrima* was evaluated. The rodent model was employed in the investigation because it takes into account potential pro drug formation and immune system effects in combating infection (Fidock, Rosenthal *et al*., 2004). The study showed that *V. glaberrima* has a curative anti-malarial effect at a dose of 150mg/kg and 300mg/kg. The high percentage cure, similar to that of chloroquine was observed for the groups treated with 150 and 300mg/kg. Moreover, the positive control and the group treated with 300mg/kg showed statistically significant increase in mean survival time when compared with the negative control. The finding that the group treated with 150mg/kg produces significant curative antimalarial activity which did not translate into increased mean survival time compared to the negative control is a paradox suggesting caution and further evaluation in the interpretation of results. A possible explanation is that the extract was toxic to mice at the dose administered, thus reducing the survival period even at a low parasite load. This paradox may need to be explored in further studies.

The exact mechanism of antiplasmodium activity of the plant extract was not explicated, though antiplasmodial effect of extracts from plants have been ascribed to their phytochemical components like flavonoids, terpenes, alkaloids, saponins and glycosides (Iwu, Duncan *et al*., 1999; Abosi and Raseroka 2003; Ayoola, Coker *et al*., 2008; Kaur, Jain *et al*., 2009). Bioactive sesquiterpene lactones such as Vernodalin, Vernomygdin, Vernodalol and Epivernodalol have been isolated from other Vernonia species (Kupchan *et al*., 1969; Igile *et al*., 1995; Ganjian *et al*., 1983; Owoeye *et al*., 2010) and may have contributed to the antimalarial activity. The plant *V. glaberrima* has the potential to be further explored for its anti-malaria effect through the isolation and characterization of the active principles.

V. Figures and Tables

Table 1: Curative effect and Mean survival time (MST) of chloroquine and *Vernonia glaberima* against *P. berghei* infection in mice.

S/No	Treatment Groups (mg/kg)		Parasite count	% Cure	Mean Survival Time (MST)
1	Chloroquine	5	0.55±0.5*	73.4	19.20±2.69*
2	VGLE	300	0.78±0.11*	62.1	17.20±3.04*
3	VGLE	150	0.94±0.18*	54.3	16.00±3.49
4	VGLE	75	1.40±0.19**	32.2	14.20±2.75
5	Distilled water		2.06±0.69	-	10.80±2.03

*Values are expressed as mean ± SEM, n = 5; Values of the group with superscript *are statistically significant (p<0.05) compared to negative control group; Values with superscript ** are statistical significant (p<0.01) compared to negative and positive control groups.*

VI. Conclusion

The study demonstrated the potent anti-plasmodium activity of the leaf extract of *V. glaberrima* and validates its use in traditional medicine for treating fever and pain associated with malaria infection.

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