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Original Research Article

Evaluation of *In-vivo* Antimalarial Activity of Methanol Leaf Extract of *Glyphaea brevis* in *Plasmodium berghei*-Infected Mice

Tayo Micheal Anjuwon *, Sani Ibrahim and Andrew Jonathan Nok

Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria

For correspondence:* ***Email:* *tayoanjuwon@gmail.com;* ***Tel:*** *+2348066917667*

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Abstract

Purpose: *To evaluate the in-vivo antimalarial activity of the methanol extract of the leaves of Glyphaea brevis in Plasmodium berghei infected mice.*

Methods: *The phytochemical profile of ethylacetate, n-butanol, and residual aqueous fractions of the methanol extract of G. brevis were determined using standard procedures. Mice, weighing between 15 - 30 g, were used for this study. Plasmodium berghei infected blood (0.2 ml) was used to infect each of 55 mice (5 in 11 groups) intraperitoneally. Animals in the infected groups were treated orally with varying doses (200, 300 and 400 mg/kg body weight) of the ethylacetate, n-butanol and residual aqueous fractions daily, using artemisinin (5 mg/kg body weight per day) as standard drug, over a period of four days. The non-infected (normal control, n = 5) received distilled water (0.2 ml) while the infected control group (n = 5) was administered 0.2 ml normal saline. The suppressive antiplasmodial properties of the fractions as well as the serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined.*

Results: *Alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins, steroids and triterpenes were present in the extract fractions. The suppressive antiplasmodial activity of n-butanol, residual aqueous portion and ethylacetate fractions was 76.64, 73.25 and 72.99 %, respectively, while that of artemisinin was 86.13 %. The serum concentrations of AST, ALT, and ALP in the infected control group were significantly higher ($p < 0.05$) than those of the treated malaria-infected groups treated with the three fractions.*

Conclusion: *Glyphaea brevis possesses significant antiplasmodial properties and could be a source of lead molecules for the development of new antimalarial agents.*

Keywords: *Antimalarial, Glyphaea brevis, Plasmodium berghei, Artemisinin*

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INTRODUCTION

Malaria, a disease caused by protistan parasites belonging to the genus *Plasmodium*, results from the multiplication of the parasite within red blood cells of the host, causing symptoms that typically include fever and headache, nausea and vomiting. In severe cases, this symptoms progress to anemia, coma or death, particularly among children and the elderly [1]. Like all malaria parasites of mammals, *Plasmodium berghei* is transmitted by Anopheles mosquitoes. The parasite has been an important element in the attempts to learn how to manage and eradicate malaria because of its extreme similarity in life cycle and infectious behavior, to malaria infections in humans. *P. berghei*'s genetic map has been completed, bringing hope

that further understanding of all malarias will be possible, and the elements that cause *P. berghei* to be noninfectious to humans can be brought into play to deal with the malaria affecting humans [2].

Glyphaea brevis also called masquerade stick (common name), Aloanyansi (Ibo) or Atori (Yoruba) and Dorina (Hausa) has been reported to be used in the treatment of sleeping sickness and as an aphrodisiac. It possesses antibacterial, antioxidant and anti-inflammatory properties and beneficial effect in wound healing [3]. It is undoubtable that medicinal plants form the basic foundation of traditional medical practice worldwide; hence, the World Health Organization (WHO) encouraged the inclusion of herbal medicine of proven safety and efficacy in health care delivery programme in developing countries. In this respect, plant resources are potential targets for research and development of alternative malarial drugs, with novel modes of action [4]. Although the combinations of artemisinin and derivatives (ACTs) has been recommended for the treatment of malaria by the World Health Organization (WHO), resistance of malaria parasites to the drugs is now commonly reported [5]. This creates and urgent need for further research to develop new antimalarial drugs. The use of *Glyphaea brevis* in traditional medicine in the world to prevent or to cure malaria [6] lacks scientific evidence, hence the need for this study.

EXPERIMENTAL

Chemicals and reagents

N-butanol, ethylacetate and methanol were purchased from Sigma-Aldrich (Jos, Nigeria) while Giemsa stain powder and artemisinin (dihydroartemisinin tablets) were products of Bliss GVS Pharma (India) purchased from Okey Medicus (Kaduna, Nigeria).

Equipment

This included light microscope (Olympus CH; magnification X 100/125160/0.17), glass slides, Sherwood Colorimeter 257, Grant JB Series Water Bath, Heraeus Labofuge 300 centrifuge, RS-232C weighing balance, Sysmex haematological auto analyzer and haematocrit reader.

Experimental animals

A total of eighty mice (60 males and 20 females) weighing between 15 - 30 g which were purchased from Nigeria Veterinary Research Institute, (VOM), Jos, Plateau State were used for this experiment. Twenty (20) of these mice were infected with *Plasmodium berghei* using blood samples of mice (obtained from National Institute of Medical Research (NIMR), Lagos, Nigeria) newly infected with *P. berghei*. All animals were housed in well-ventilated cages, allowed to acclimatize for two weeks, and were fed on commercial laboratory diet and water *ad libitum*. Study protocols and ethical issues were approved by the University Research and Ethical Committee (number: sci10-11/10604) of Ahmadu Bello University, Zaria, and animals were handled in conformity with internationally accepted laboratory animal use and care guidelines [7].

Plant collection and identification

Leaves of *Glyphaea brevis* were collected from farmland in Irun Akoko, Ondo State, Nigeria and the plant was identified by a taxonomist, Gallah UJ, at the herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria where a voucher specimen number 2634 was deposited for future reference.

Preliminary extraction of plant material and fractionation of plant extract

Leaves of *G. brevis* were air dried, reduced to coarse powder using wooden mortar and pestle. Coarsely powdered leaves (100 g) were extracted with 500 ml of methanol by cold maceration and left for 48 h. The extract was filtered and evaporated to dryness using rotary evaporator. The methanol extract was then suspended in 1 L of distilled water and the filtrate was partitioned in separating funnel with 750 ml of ethyl acetate. Resultant ethyl acetate fraction was concentrated on a water bath and the aqueous portion was further partitioned with 750 ml of n-butanol to get the n-butanol fraction after concentration [8]. The fractions were stored in suitable containers until ready for use.

Preliminary phytochemical screening

Phytochemical screening to determine the constituent of the plant extract was carried out by the method described by Sofowora [9] and Trease and Evans [10].

Acute toxicity (LD_{50}) test

The mean lethal dose of the three fractions (ethylacetate, n-butanol, residual aqueous fractions) of the extract of *G. brevis* were

determined in mice (weighing between 15-30 g) using the method described by Lorke [11].

In vivo antiplasmodial determination

The antimalarial property of the plant was evaluated by determining its suppressive antiplasmodial properties *in-vivo* using the method of Bulus *et al* [12]. For this purpose the 60 mice were divided into 12 different groups of five mice each. The mice in 11 of the groups were infected with the parasites by inoculating them with 0.2 ml of blood sample obtained from the mice infected with *P. berghei*. Mice in group I served as normal control and were not infected with *P. berghei* or treated with the plant extract but all other mice were infected with *P. berghei*. The mice in group II served as positive control and were not treated. Animals in group III were treated with 5 mg/kg standard artemisinin (standard control) while those in groups IV, V, X were treated with 200 mg/kg body weight of the ethylacetate, n-butanol and residual aqueous fractions. The mice in groups VI, VII, XI and those in groups VIII, IX, XII were treated with 300 mg/kg and 400 mg/kg body weight of the various extracts, respectively. The first treatment started 3 h after parasite inoculation, and the others were given at the same time on the following days for four consecutive days. On the fifth day, samples of blood was collected from the caudal vein of each mouse on to a clean glass slide and stained with Giemsa stain after which the number of the parasitized cells was determined microscopically and the percentage suppression determined using Eq 1.

$$\text{Mean suppression (\%)} = \{(APC - APT)/APC\}100 \quad \ldots(1)$$

where APC is the average number of parasitaemia in the positive control and APT is the average number of parasitaemia in the test group [12]. At the end of the experiments, the animals were sacrificed and blood samples were collected, centrifuged and used for further analysis.

Determination of biochemical parameters

The estimation of packed cell volume (PCV) was carried by microhaematocrit method of Alexander and Grifiths [13]. Serum concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel [14] while alkaline phosphatase (ALP) was determined using assay kits described by Haussament [15].

Statistical analysis

Data obtained were expressed as means ± SD except where otherwise stated. Statistical comparison was carried out using one way analysis of variance (ANOVA) and Duncan Multiple Range Test. $P < 0.05$ was considered to indicate a significant difference between groups.

RESULTS

Phytochemical screening of the different fractions of *G. brevis* showed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins, steroids and triterpenes. Saponin was absent in the ethylacetate fraction and steroids were absent in both n-butanol and residual aqueous fractions.

Antiplasmodial activity of *Glyphaea brevis*

The fractions exhibited significant dose-dependent ($p < 0.05$) parasitaemia suppressive activity for the various fractions with n-butanol fraction having the highest suppressive activity on the parasites (Figure 1). The ethylacetate, n-butanol, and residual aqueous fractions caused 72.99 %, 76.64 % and 73.25 % suppression, respectively, in parasitaemia of *P. berghei* infected mice with n-butanol having the highest suppressive activity while Artemisinin (a standard antimalaria drug) exerted 86.13 % suppression.

Table 1: Phytochemical profile for methanol fractions of *Glyphaea brevis* leaf extract

Class	*EtAcFx	*N-ButFx	*ReAqFx
Alkaloids	+	+	+
Carbohydrates	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	+	+
Saponins	-	+	+
Tannins	+	+	+
Steroids	+	-	-
Triterpenes	+	+	+

**EtAcFx: Ethylacetate Fraction, *N-ButFx: N-Butanol Fraction, *ReAqFx : Residual aqueous Fraction; where + represent positive; - represent negative*

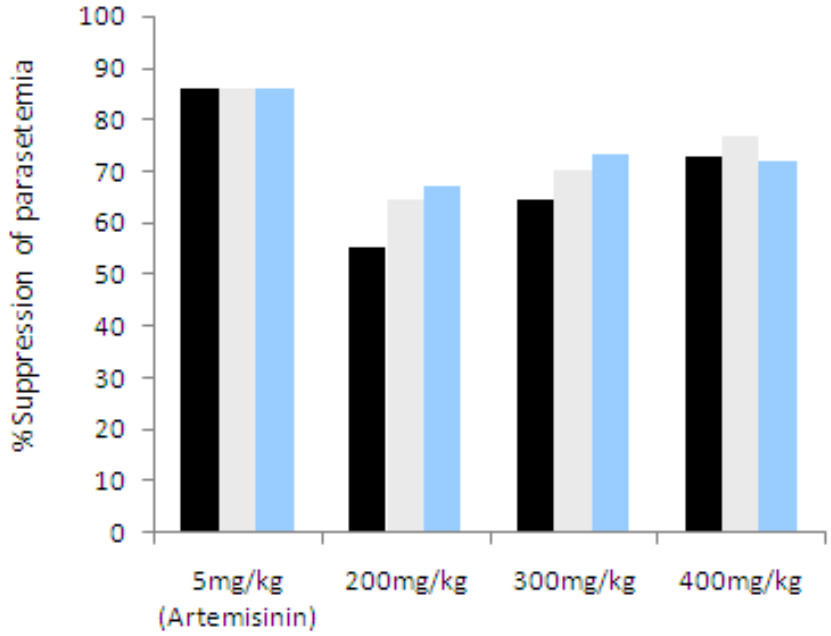

Figure 1: Percentage suppression of various fractions of methanolic extract of *Glyphaea brevis* on *Plasmodium berghei* infected mice (ethyl acetate fraction, *n*-buyanol fraction, aqueous fraction)

Effects of *Glyphaea brevis* leaf fractions on serum liver function

The serum levels of AST, ALT and ALP were significantly higher in the positive controls than the enzyme levels in the *P. berghei* infected mice treated with the fractions of the extracts (Tables 2-3). Table 4, however, indicated significant decrease ($p < 0.05$) in the level of the liver enzymes (ALT, AST and ALP) in the positive control group when compared with those from the animals treated with the fractions of the extracts.

The residual-aqueous fraction treated group of mice showed a higher increase in the serum concentration of these liver enzymes than the positive control group. However, the toxicological profiling of the fractions of the extract indicated that oral median lethal dose was above 5000 mg/kg body weight.

DISCUSSION

The methanol extract of the leaves of *Glyphaea brevis* has demonstrated significant dose dependent suppression of parasitaemia in *P. berghei* infected mice. Alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins, steroids and triterpenes were found to be present in the plant extract. The oral median lethal dose of the extract was above 5000 mg/kg body weight indicating that the plant extract is relatively safe and a good candidate for further studies for antimalarial medicine.

Although the mechanism of antiplasmodial action of this extract has not been elucidated, antiplas-

Table 2: Effect of ethylacetate fraction of *Glyphaea brevis* extract on the activities of hepatic marker enzymes in *Plasmodium berghei* infected mice

Treatment (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Control (Distilled H_2O)	41.00 ± 3.03^{a}	48.33 ± 11.06^{a}	64.00 ± 6.56^{a}
Positive Control (0.2 ml Normal saline)	96.67 ± 63.61^{b}	157.33 ± 124.60^{b}	88.33 ± 11.02^{c}
Standard Control (5 mg/kg Artemisinin)	54.00 ± 6.08^{ab}	62.33 ± 10.21^{a}	74.33 ± 4.62^{abc}
200 mg/kg (Ethylacetate fraction)	44.25 ± 8.54^{a}	60.00 ± 7.55^{a}	71.33 ± 8.39^{ab}
300 mg/kg (Ethylacetate fraction)	57.33 ± 25.15^{ab}	68.33 ± 23.03^{ab}	85.33 ± 5.13^{bc}
400 mg/kg (Ethylacetate fraction)	66.00 ± 16.52^{ab}	81.33 ± 11.93^{ab}	74.67 ± 6.35^{abc}

Values are expressed as mean ± SD (standard deviation).Values with different superscripts down the columns are significantly different ($p < 0.05$); where AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase

Table 3: Effect of n-butanol fraction of *Glyphaea brevis* extract on the level of hepatic marker enzymes in *Plasmodium berghei*-infected mice

Treatment (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Control (Distilled H_2O)	41.00 ± 6.06^{a}	48.33 ± 11.06^{a}	64.00 ± 6.56^{a}
Positive Control (0.2 ml normal saline)	96.67 ± 63.61^{b}	157.33 ± 124.60^{b}	88.33 ± 11.02^{c}
Standard Control (5 mg/kg artemisinin)	54.00 ± 6.08^{a}	62.33 ± 10.21^{a}	74.33 ± 4.62^{ab}
200 mg/kg (N-butanol fraction)	63.50 ± 16.05^{ab}	71.33 ± 12.66^{ab}	84.67 ± 4.04^{bc}
300 mg/kg (N-butanol fraction)	54.60 ± 15.21^{a}	58.00 ± 2.00^{a}	67.25 ± 1.26^{a}
400 mg/kg (N-butanol fraction)	59.75 ± 7.37^{ab}	69.33 ± 18.82^{ab}	83.25 ± 2.22^{bc}

Values are expressed as mean ± SD (standard deviation). Values with different superscripts down the columns are significantly different ($p < 0.05$); where AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase

Table 4: Effect of residual aqueous fraction of *Glyphaea brevis* Extract on the activities of hepatic marker enzymes in *Plasmodium berghei*-infected Mice

Treatment (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Control (Distilled H_2O)	41.00 ± 6.06^{a}	48.33 ± 11.06^{a}	64.00 ± 6.56^{a}
Positive Control (0.2 ml Normal saline)	96.67 ± 63.61^{a}	157.33 ± 124.60^{ab}	88.33 ± 11.01^{cd}
Standard Control (5 mg/kg Artemisinin)	54.00 ± 6.08^{a}	62.33 ± 10.21^{a}	74.33 ± 4.62^{abc}
200 mg/kg (Residual Aqueous fraction)	78.33 ± 8.33^{a}	81.67 ± 8.02^{a}	71.33 ± 4.93^{ab}
300 mg/kg (Residual Aqueous fraction)	198.33 ± 79.43^{b}	235.00 ± 86.26^{b}	102.00 ± 8.89^{de}
400 mg/kg (Residual Aqueous fraction)	191.33 ± 41.77^{b}	231.00 ± 45.83^{b}	110.00 ± 10.15^{e}

Values are expressed as mean ± SD (standard deviation). Values with different superscripts down the columns are significantly different ($p < 0.05$); where AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase

modial effects of natural plant products have previously been attributed to some of their active phytochemical components including alkaloids, flavonoids and terpenes. Some of the phytochemicals such as terpenes and flavonoids detected the extract have been earlier reported to have antiplasmodial activity [16].

Many published works [17-20] have investigated biochemical alteration in malaria infection and reported significant alteration ($p < 0.05$) in biochemical variables. The observed elevations of serum concentration of ALT, AST and ALP in the positive control group of the mice are indications of liver damage in the mice when compared to the animals treated with fractions of the extracts. These findings conform to the pathogenesis of *P. berghei* infection and correlates particularly with the work of Onyesom and Onyemakonor [21]. The increase in concentration of the liver enzymes in mice treated with the residual-aqueous fraction compared with the positive control group indicates that the fraction may have some hepatotoxic effect. However, the median lethal dose which was above 5000 mg/kg is indicates that the extract could be safe.

CONCLUSION

N-Butanol and ethylacetate fractions of the methanol extract of *G. brevis* possesses antiplasmodial activity against *P. berghei*-infected mice. Toxicological profile indicates that the fractions are safe. However, the mechanism of the antiplasmodial effect is yet to be determined.

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