



Antiplasmodial, Hepatic and Nephritic Effects of Fractions of Methanol Leaf Extract of *Glyphaea brevis* in *Plasmodium berghei*-Infected Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Author TMA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OAA managed the analyses of the study. Author OAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the antiplasmodial, hepatic and nephritic effects of fractions of *Glyphaea brevis* methanol leaf extract in *P. berghei* infected mice. Mice weighing between 15-30 g were infested intraperitoneally with 0.2ml plasmodium infected blood and left for 3 hours before treatment. Infected test groups were treated via oral route of administration with varying doses (200, 300 and 400 mg/kg body weight) of ethylacetate, *N*-butanol and residual aqueous portion fractions of the *Glyphaea brevis* methanol extract and Artemisinin (5 mg/kg b.wt) for four days. *N*-butanol fraction showed the highest antiplasmodial activity (76.64%), followed by residual aqueous portion (73.25%) and ethylacetate (72.99%); Artemisinin has 86.13%. Serum bilirubin (total and conjugated) concentrations of the untreated group (0.82 ± 0.20 , 0.51 ± 0.12) were significantly lower ($P < 0.05$) than those in the infected group treated with 300 mg/kg of the residual aqueous portion (1.36 ± 0.20 , 0.76 ± 0.05) respectively. Serum albumin levels showed significant ($P < 0.05$) increase in all the groups treated compared to the positive control. Serum total protein, urea and

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creatinine levels of test groups were not significantly ($P>0.05$) different from the positive control group. Conclusively, *Glyphaea brevis* has substantial antiplasmodial activity and could provide a lead for new antimalarial drug development.

Keywords: Antimalarial; *glyphaea brevis*; *plasmodium berghei*; hepatic; nephritic; parasitaemia.

1. INTRODUCTION

Malaria is a disease caused by protistan (sporozoans) parasites of genus *Plasmodium*. It is endemic in ninety-one countries and territories in 2016. Analysis showed that in 2016 that there were 216 million malaria cases, an increase of about 5 million cases over 2015; deaths reached 445 000, a similar number to the previous year [1], including perinatal and infant mortality resulting from complications such as maternal anaemia and low birth weight [2]. Malaria is commonly associated with poverty, and can indeed be a cause of poverty and a major hindrance to economic development [3].

Plasmodium berghei has extreme similarity in its life cycle, infectious behavior, and genetic map to mammals' malaria parasites, making it an ideal model for research into treatments and investigations into the more refined aspects of infection and thus huge hope for possible understanding of all malarias [4].

Potentially, plants are essential sources of new anti-malarial treatments with over 1,200 plant species reportedly used for fever and malaria treatment worldwide [5]. *Glyphaea brevis*, known as masquerade stick (common name), "Aloanyansi" (Ibo) or *Atori* (Yoruba) and *Dorina* (Hausa) have multiple physiological and pharmacological effects when used; some of these effects include antitrypanosomal, aphrodisiac, antibacterial, anti-inflammatory and antioxidant properties [6].

The most effective treatment of *P. falciparum* malaria infection based on therapeutic combinations with artemisinin and derivatives (ACTs) recommended by the World Health Organization (WHO) has suffered setback due to clinical resistance reported to these combinations [7], hence a need for further research in search for new antimalarials.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of sixty mice purchased from Nigeria Veterinary Research Institute, (VOM), Jos,

Plateau State, weighing between 15-30 g were used for this experiment. The animals were housed in well-ventilated cages and allowed to adapt for 2 weeks. They were fed on commercial laboratory diet and water *ad libitum*. To conform with internationally accepted laboratory animal use and care guidelines, study protocols and ethical approval were obtained from the University Research and Ethical Committee of Ahmadu Bello University (A.B.U), Zaria [8].

2.2 Plant Collection and Identification

Glyphaea brevis leaves were harvested from Irun Akoko, Ondo State, Nigeria and identified at the herbarium unit, Botany Department, A.B.U Zaria, Nigeria by a Taxonomist, Gallah U.J, and a voucher specimen number (2634) was obtained.

2.3 Parasites

Plasmodium berghei was obtained from National Institute of Medical Research (NIMR), Lagos, Nigeria.

2.4 Preliminary Plant Extraction and Fractionation of Extract

The air dried *G. brevis* leaves were reduced to coarse powder by means of wooden mortar and pestle. Then, 100 g of the powdered leaves were subjected to 48 hours (cold maceration) extraction with 500 ml of methanol. It was filtered and evaporated to near dryness using rotary evaporator. The methanol extract was resuspended in 1L of distilled water for partitioning using separating funnel with 750 ml of ethyl acetate. The ethyl acetate fraction was concentrated on a water bath. The aqueous portion was further partitioned with 750 ml *N*-butanol to get the *N*-butanol fraction after concentration [9]. Obtained fractions were kept in sealed containers until ready for use.

2.5 Animal Groupings and Treatments for *in vivo* Antiplasmodial Test

The *in vivo* suppressive antiplasmodial test was used to evaluate the antimalarial property of the plant [10].

Sixty (60) mice divided into 12 groups of 5 mice each were used for this study. Infection was achieved by inoculating mice with 0.2 ml of parasite-infected blood solution. Group I was normal control, not infected and not treated; Group II was infected and not treated (Positive control); Group III was infected and treated with 5 mg/kg standard artemisinin (standard control); Groups IV, V, X: were infected and treated with 200 mg/kg b. wt ethylacetate, *N*-butanol, residual aqueous fractions respectively; Groups VI, VII, XI: were infected and treated with 300 mg/kg b. wt ethylacetate, *N*-butanol, residual aqueous fractions respectively; Groups VIII, IX, XII: were infected and treated with 400 mg/kg b. wt ethylacetate, *N*-butanol, residual aqueous fractions respectively. First treatment began 3 hours after inoculation of parasite while others were administered at the same time on the following days. Treatment continued daily for four consecutive days. On the fifth day, samples of blood were taken from the caudal vein of each mouse to clean slides and stained with Giemsa stain to determine the number of the parasitized cells microscopically.

2.6 Determination of Biochemical Parameters

At the end of the experiments, animals were sacrificed and blood samples were collected. The blood was centrifuged and the serum was used for further analysis.

Serum total protein was evaluated by the protocol described by Fine et al. [11]; the serum albumin concentration was determined by the method described by Doumas et al. [12]; the serum total and conjugated bilirubin were determined by the method described by Jendrassik and Grof [13] and Sherlock [14]; the serum creatinine concentration was determined by the method described by Bartels and Bohmer [15]; the serum urea concentration was determined by the method of Fawcett and Scout [16].

2.7 Statistical Analysis

Data obtained were expressed as mean \pm SD (standard deviation). Data were analyzed by one way analysis of variance (ANOVA) using IBM SPSS Statistics Version 20. The differences in mean were compared using Duncan Multiple Range Test. $P < 0.05$ value was considered to indicate a significant difference between groups.

3. RESULTS AND DISCUSSIONS

Medicinal plants form the basic foundation of traditional medical practice worldwide [17]. Even though about 80% of the African populace relied on plant for the management of diseases including malaria, yet plants are not yet fully explored [18]. In contrast, plants still remain potential target for research and development of alternative malarial drugs, with new modes of action [19].

The rodent model (albino mice) of malaria has been employed in this study for prediction of efficacy of antimalarial effect of *Glyphaea brevis* leaf extract. Several conventional antimalarial agents have been identified using rodent malaria model [20]. *P. berghei* are used in the forecast of treatment outcomes, hence it was suitable parasite for the study. The sensitivity of *berghei* parasite to Artemisinin, its solubility and action on all the stages of malaria parasite life cycle, influenced its choice to be used as the standard drug in this study.

The *in vivo* model was adopted for this research work due to its ability of taking into account potential prodrug effect and promising involvement of immune system in obliteration of infection [21]. The oral median lethal dose for the fractions of *G. brevis* was found to be over 5000 mg/kg body weight [22], making it a good candidate for further studies.

The results obtained for the antimalarial screening from the 4-day suppressive test showed significant ($P < 0.05$) decrease in parasitaemia of *berghei* parasite infected mice treated with the methanol fractions of *Glyphaea brevis*. The suppression observed was dose dependent (Fig. 1). However, this effect was lower in the groups that received 200 mg/kg bw; which might be due to little period of action of the extract occasioned by rapid metabolism, therefore parasite clearance could not be entire.

The antiplasmodial effect seen in this study is consistent with the ethnomedicinal use of *Glyphaea brevis* as an antimalarial in some parts of Nigeria [22, 23]. Antiplasmodial effects of natural plant products have been ascribed to some active phytochemical components in them since the mechanisms of antiplasmodial action of these extracts have not been known. Alkaloids, terpenes and flavonoids are phytochemicals that have been detected in *Glyphaea brevis* which have also been proposed to be responsible for antiplasmodial activities. The antiplasmodial

effect of *Glyphaea brevis* plant might therefore be due to the phytochemical components (alkaloids, flavonoids and terpenes) present in the plant; these components have been implicated to have antiplasmodial effect [22, 24].

Prompt detection of complication linked with acute malaria infection that may require most urgent intensive care necessitate inclusion of biochemical investigation in malaria infection in order to avoid mortality due to complications; and so many research findings have shown significant biochemical alteration in malaria infection [25, 26].

The ethylacetate fraction presented a non-significant ($P>0.05$) difference in the concentration of serum total protein in the positive control and treated groups (Table 1). For serum albumin, only the treated group with 400 mg/kg b. wt dosage showed significant increase ($P<0.05$) (3.75 g/dl) compared with the positive control group (3.05 g/dl). This result agrees with a previous report [27]. A detectable change in the total protein concentration may not be noticed since most proteins except albumin contribute little to the total protein concentration, quite a large percentage change in the concentration of one may not cause significant effect. The liver is the major site infected by the malaria parasite and as well the site for synthesis of serum proteins. Infection of the liver may lead to

decrease in plasma albumin concentration due to diminished synthesis of albumin. Also illnesses such as malaria may result in increased catabolism of albumin and therefore leads to nitrogen loss [27, 28]. Concentration of total bilirubin also showed a statistical ($P<0.05$) increase in the untreated group in comparison with normal and standard control groups; but no significant differences ($P>0.05$) with the treated groups (as shown in Table 1). However, conjugated bilirubin showed no significant difference ($P>0.05$) between the positive control and the treated groups. This observation may be due to the short period of parasitaemia and minimal degree of liver damage by the parasites.

The result on kidney function also for ethylacetate fraction (Table 1) showed significant ($P<0.05$) increase in serum urea level of the positive control group compared with the standard and normal control groups; this is in conformity with the findings of Nwodo et al. [29] who reported similar trend of findings; however non-significant ($P>0.05$) difference was observed between positive and extract treated control groups. The serum creatinine levels are not significantly different ($p<0.05$) in all the groupings. This non-significant difference may depict ameliorative effect of the ethylacetate fraction of methanol extract of *G. brevis* leaves on kidney damage.

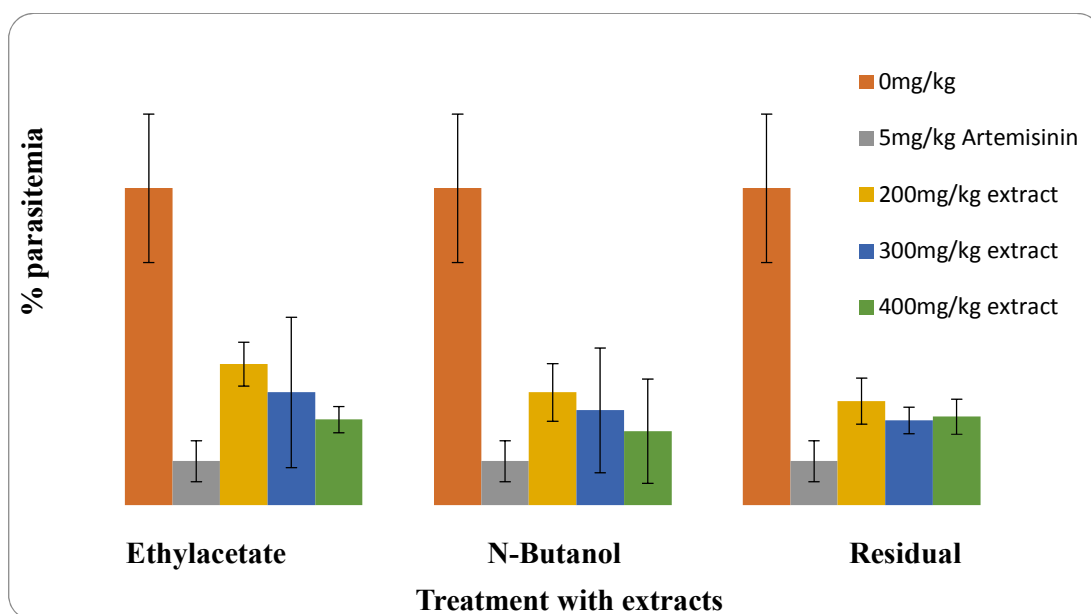


Fig. 1. Average Parasitaemia of Various Fractions of Methanol Extract of *Glyphaea brevis* on *Plasmodium berghei* Infected Mice

Table 1. Effect of ethylacetate fraction of *Glyphaea brevis* on serum concentrations of liver and kidney functions markers

Parameters	Normal Control	Positive Control	Standard Control	200mg/kg dosage	300mg/kg dosage	400mg/kg dosage
Total Protein (g/dL)	6.77 ±0.29 ^a	6.30 ± 0.26 ^a	6.87 ±0.21 ^a	6.60 ±0.20 ^a	6.60 ±0.20 ^a	6.87 ±0.21 ^a
Total bilirubin (mg/dL)	0.75±0.06 ^a	0.82±0.20 ^b	0.42±0.16 ^a	0.65±0.03 ^b	0.65±0.06 ^b	0.66±0.05 ^b
Conjugated bilirubin(mg/dL)	0.33±0.09 ^{ab}	0.51±0.12 ^b	0.26±0.16 ^a	0.47±0.03 ^b	0.42±0.05 ^{ab}	0.50±0.09 ^b
Albumin(g/dL)	3.70±0.14 ^{ab}	3.05±0.07 ^a	3.65±0.21 ^{ab}	3.55±0.21 ^{ab}	3.50±0.14 ^{ab}	3.75±0.07 ^b
Urea(mg/dL)	0.90±0.07 ^a	1.36±0.27 ^{bc}	0.92±0.13 ^a	1.04±0.04 ^{ab}	1.35±0.27 ^{bc}	1.17±0.32 ^{abc}
Creatinine(mg/dL)	0.49±0.02 ^a	0.53±0.01 ^a	0.48±0.04 ^a	0.50±0.07 ^a	0.53±0.05 ^a	0.52±0.05 ^a

Values are expressed as mean ± SD (standard deviation) Values with different superscripts across the rows are significantly different (P<0.05)

Table 2. Effect of N-butanol fraction of *Glyphaea brevis* on serum concentrations of liver and kidney functions markers

Parameters	Normal Control	Positive Control	Standard Control	200mg/kg dosage	300mg/kg dosage	400mg/kg dosage
Total Protein (g/dL)	6.77 ±0.29 ^{ab}	6.30 ± 0.26 ^a	6.87 ±0.21 ^b	6.80 ± 0.24 ^{ab}	6.63 ±0.23 ^{ab}	6.63 ± 0.15 ^{ab}
Total bilirubin (mg/dL)	0.75±0.06 ^{ab}	0.82±0.20 ^b	0.43±0.16 ^a	0.53±0.08 ^{ab}	0.75±0.03 ^{ab}	0.56±0.06 ^{ab}
Conjugated bilirubin(mg/dL)	0.33±0.09 ^{abc}	0.51±0.12 ^b	0.261±0.16 ^a	0.31±0.09 ^{ab}	0.48±0.03 ^{bc}	0.32±0.07 ^{abc}
Albumin(g/dL)	3.70±0.14 ^b	3.05±0.07 ^a	3.65±0.21 ^{ab}	3.60±0.14 ^{ab}	3.40±0.14 ^{ab}	3.50±0.00 ^{ab}
Urea(mg/dL)	0.90±0.07 ^a	1.36±0.27 ^b	0.92±0.13 ^a	1.57±0.04 ^b	1.50±0.09 ^b	1.35±0.08 ^b
Creatinine(mg/dL)	0.49±0.02 ^a	0.53±0.01 ^{ab}	0.48±0.04 ^a	0.51±0.04 ^{ab}	0.49±0.02 ^a	0.51±0.02 ^{ab}

Values are expressed as mean ± SD (standard deviation) Values with different superscripts across the rows are significantly different (P<0.05)

Table 3. Effect of the residual aqueous fraction of *Glyphaea brevis* on serum concentrations of liver and kidney functions markers

Parameters	Normal Control	Positive Control	Standard Control	200mg/kg dosage	300mg/kg dosage	400mg/kg dosage
Total Protein (g/dL)	6.77 ±0.29 ^a	6.30 ± 0.26 ^a	6.87 ±0.21 ^a	6.60 ±0.20 ^a	6.60 ±0.27 ^a	6.60 ±0.20 ^a
Total bilirubin (mg/dL)	0.75±0.06 ^b	0.82±0.20 ^{bc}	0.43±0.16 ^a	0.77±0.18 ^b	1.36±0.20 ^d	1.12±0.17 ^{cd}
Conjugated bilirubin (mg/dL)	0.33±0.09 ^{ab}	0.51±0.12 ^b	0.26±0.16 ^a	0.43±0.05 ^{abc}	0.76±0.05 ^e	0.63±0.03 ^{de}
Albumin(g/dL)	3.70±0.14 ^{ab}	3.05±0.07 ^a	3.65±0.21 ^{ab}	3.80±0.42 ^b	3.55±0.21 ^{ab}	3.63±0.21 ^{ab}
Urea (mg/dL)	0.90±0.07 ^a	1.36±0.27 ^{bc}	0.92±0.13 ^a	1.33±0.14 ^{ab}	1.45±0.09 ^b	1.238±0.48 ^{ab}
Creatinine (mg/dL)	0.49±0.02 ^a	0.53±0.01 ^a	0.48±0.04 ^a	0.55±0.03 ^{ab}	0.52±0.01 ^{ab}	0.52±0.06 ^{ab}

Values are expressed as mean ± SD (standard deviation) Values with different superscripts across the rows are significantly different (P<0.05) 4.0

From the results obtained and presented in Table 2, the positive control group and the various *N*-butanol treated groups showed non-significant difference for total protein, total and conjugated bilirubins, albumin, urea and creatinine. However, positive control group had significant ($P < 0.05$) decreased Total protein compared to the standard control group; Total and conjugated bilirubins and urea were significantly increased ($P < 0.05$) in the positive control group compared to standard group. This is in agreement with the work of Adekunle et al. [27] where they reported a decrease in the total protein level in infected group when compared to their uninfected groups. Treatment with the extract helped in ameliorating the plasmodial effect of the *berghei* parasites on the liver as shown by the differences in the levels of total and conjugated bilirubin of positive (untreated) group and treated (standard) group.

Liver and kidney function indicators for mice treated with the residual aqueous fraction (Table 3) revealed that there was non-significant ($P > 0.05$) difference in serum total protein and creatinine in all the groups; there was significant ($P < 0.05$) decrease in concentration of serum albumin of the positive control group (3.05 g/dl) compared to 200 mg/kg extract dosage group (3.80 g/dl). Serum albumin level has been suggested to be a dependable biochemical marker for establishing severe pathologic conditions such as malnutrition and infectious diseases. Infections of malaria go along with noticeable decrease in plasma albumin concentration as well as in malnutrition and pregnancy [30]. This significant reduction in the concentration of albumin is a possible indication of severe liver injury.

For the total and conjugated bilirubin, there was a significant increase ($P < 0.05$) in the 300 mg/kg administered with residual aqueous portion test group when compared to the positive control group. For the urea, non-significant difference ($P > 0.05$) was observed between the positive control and extract treated groups. This may be attributed to the ameliorative effect of the extract fraction of *Glyphaea brevis* on the tissue damage by the malaria parasites. Unusual unconjugated bilirubin observed in malaria patients does not conclusively imply liver disease, this could be as a result of *Plasmodium* parasite induced intravascular haemolysis and it is not all the cases of malaria infections that are associated unfairness of liver functions [31].

The concentration of serum urea significantly increased ($P > 0.05$) in the positive control group

compared to the normal and standard control groups. A similar reported work of Nwodo et al. [29] depicted this same outcome. Creatinine and urea are nitrogenous low edge substances with vast clinical application in setting up renal capacity or function. Impairment of renal capacity during serious falciparum malaria fever is normal and common [32]. An observable clue that moderately malaria infection exhibited change in nitrogen metabolism with fundamental compromised renal function. Increased blood urea concentration reveal steady progression in the direction of renal dysfunction. Precisely, serum levels of urea had been observed to increase more rapidly than serum creatinine concentration in individuals with renal dysfunction [33].

4. CONCLUSION

Extracts of the leaves of *Glyphaea brevis* exhibited dose-dependent antiplasmodial activity on parasitaemia for the various methanol fractions. *N*-butanol fraction has the highest activity (over ethylacetate and aqueous fractions) on the parasites. Thus, it can be said that *Glyphaea brevis* has antimalarial activities as a phytomedicine and this study has provided a scientific basis for its continuous use in traditional medicine for the management of malaria.

ETHICAL APPROVAL

Ethical approval were obtained from the University Research and Ethical Committee of Ahmadu Bello University (A.B.U), Zaria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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