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Research Article

A Comparative Study of Antimalarial and Toxicological Effects of Aqueous and Methanol Extracts of *Glyphaea brevis* Leaves in Mice

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ABSTRACT

Malaria is the most important parasitic disease of subtropical and tropical countries and its control remains serious challenge, especially through the development of parasite resistance to standard antimalarial drugs. Aqueous and methanol leaves extract of *Glyphaea brevis* were investigated for their activities against malaria infections using albino mice infected with *Plasmodium berghei* at dose levels of 100mg/kg, 200mg/kg and 300mg/kg per day. Artemisinin at 5mg/kg /day was used as standard control. Dose dependent chemo-suppression of the parasites was obtained at different dosages of the tested extract. The methanol extract was found to be more active in parasite inhibition growth than its aqueous counterpart. In the established infected control group, aqueous and methanol extracts both showed significant dose dependent ($P < 0.05$) inhibitory and suppressive activities on the extracts treated animals when compared with the infected (positive) control groups. The percentage suppression revealed that 400 mg/kg methanol extract treated group had the highest efficacy (81.89%) amongst the various dosages administered. There was reduction in PCV levels in all the *Plasmodium berghei* infected mice when compared to the normal control group. Also, there were significant ($P < 0.05$) increase in the serum concentration of alanine aminotransferase (80.75 ± 7.04) and aspartate aminotransferase (68.50 ± 4.66) of the infected control group compared to normal, standard and the extract treated groups. There was significant difference in the serum concentration of alkaline phosphatase in the positive control group and aqueous extract treated groups. Conclusively, aqueous and methanol leaves extracts of *G. brevis* possess antiplasmodial activity.

Keywords: *Antiplasmodial, Plasmodium berghei; Glyphaea brevis; Malaria suppression, Tropical disease*

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INTRODUCTION

Malaria is a parasitic protozoan's disease belonging to the plasmodium species (WHO, 2014) (*Plasmodium falciparum*, *P. ovale*, *P. malariae*, *P. vivax*, and *P. knowlesi* parasites) with *P. falciparum* being the leading one responsible for human infection. About 3.3 billion people worldwide are at risk of malaria (Taek and Agil, 2018). Malaria arguably remains the world most important tropical disease with prevalence and endemicity in 91 countries (Kager, 2002). It remains a critical health difficulty in Sub-Saharan Africa despite the huge progress that has been made in treatment and or management of the disease. The number of persons that were infected in Sub-Saharan Africa was estimated to be 114 million in 2015; children who are mainly susceptible accounted for more than two-thirds of global malaria deaths (WHO, 2016). The main explanations for this worsening

situation are therapy hitches: resistance to the current antimalarial drugs, unaffordability and or unavailability of antimalarial drugs and lack of new therapeutic targets (Jeruto *et al.*, 2015).

Investigation of medicinal plants used traditionally for malaria treatment is on the increase (Philip *et al.*, 2017). Medicinal plants have undoubtedly been a rich source for new drugs and some antimalarials - quinine and artemisinin, that are been used today. An estimate of about 122 drugs from 94 plant species have been revealed via ethnobotanical leads. Plants commonly used in traditional medicine are assumed to be safe due to their long usage in the treatment of diseases according to knowledge accumulated over centuries. Therefore, the search for new drugs through the evaluation and validation of traditional medicines offers a good opportunity for the discovery and development of better medicines (Jeruto *et al.*, 2015). Therefore, medicinal plants have remained the

main focus for scientists and researchers in the development of new antimalarial agents (Zelege *et al.*, 2017).

Glyphaea brevis is a shrub belonging to Tiliaceae family. In Africa and South America, it is used traditionally in the treatment of various disease conditions like fevers, gonorrhoea, dysentery, stomach troubles, lung troubles, parasitic infections, convulsions and constipation. In recent years, it has come under the lime light of researchers in various parts of the world due to its broad ethno-medicinal uses. This has made this plant an integral part of folkloric medicine in most parts of these regions. Remarkable rise in research on *G. brevis* has led to the establishment of some of its secondary metabolites to possess anti-proliferative, antioxidant and anti-inflammatory activities in *in vivo* and *in vitro* assay procedures. However, to the best of the knowledge of the authors, there is no existing report on the comparative studies on the antiplasmodial activities of this plant on *P. berghei* infected mice. Hence, the present study was undertaken to evaluate the comparative antimalarial and toxicological effects of aqueous and methanol extracts of *Glyphaea brevis* in experimental malaria mice models (Osafo and Boakye, 2016).

MATERIALS AND METHODS

Ethical consideration: This work was performed according to the approved guidelines of animal experiments of the Ethical Committee, Ahmadu Bello University (ABU) Committee on Animal Use and Care (ABUCAUC), Zaria, Nigeria

Plant Sample: *Glyphaea brevis* leaves were gathered from Irun Akoko, Ondo State; identified at the herbarium unit of Botany Department, Ahmadu Bello University (ABU), Zaria with voucher specimen number 2634 obtained for future reference. The leaves were thoroughly washed in clean water, shade-dried and ground into powder.

Experimental animals: Forty-five mice weighing between 15-30g were used for the study. The animals were purchased from Department of Pharmacology, A.B.U, Zaria. The mice were housed in well-ventilated cages and fed on commercial laboratory diet and water *ad libitum*.

Parasites: Sample of *Plasmodium berghei* was obtained from the Department of Veterinary Parasitology and Entomology, A.B.U, Zaria.

Solvent Extraction of Plant Samples: Powdered leaves of *G. brevis* (100grams) each were extracted separately with 500mls of distilled water and 500mls of methanol using cold maceration and left for 48 hours. The aqueous extract was sieved and filtered, evaporated to dryness on a hot water bath at 45°C. The methanol extract was sieved and filtered, solvent were recovered using rotary evaporator and later evaporated to dryness. The extracts were kept in suitable containers until further use. The extracts were administered via oral route using a cannula curved in a manner that enables smooth administration and as well avoid wastage.

Lethal Dose Determination for both Aqueous and Methanol Extracts: The median lethal dose (LD₅₀) of the plant extracts were conducted using a standard method as previously described (Lorke, 1983).

Inoculation of mice and Monitoring of infection: The blood from the donor mice was diluted with the same amount of normal saline so that each 0.2ml contained approximately 10⁶ – 10⁷ infected red cells which is expected to produce a steadily rising infection in the test group mice (Ogbonna *et al.*, 2008). Thin blood smears were made, Giemsa stained and viewed under a light microscope. The parasitaemia level was estimated by counting infected erythrocytes against normal erythrocytes in random fields of the (x100) microscope with the formula:

$$\% \text{ parasitaemia} = \frac{\text{Number of infected Red Blood Cells}}{\text{Total Number of Red Blood Cells}} \times 100$$

The mean % parasitaemia recorded for each mouse and for each group was used to determine variations in parasitaemia level with time of infection (Okokon *et al.*, 2008, Oyewole *et al.*, 2008)

Comparison of the antiplasmodial efficacy of Aqueous and Methanol extracts: A total of 45 mice randomized into nine (9) groups of five mice each were used in this experiment.

Group I: Uninfected with parasite and untreated with extract (normal control)

Group II: Infected with parasite and treated with 0.2ml of normal saline (Positive control)

Group III: Infected with parasite and treated with 5 mg/kg Artemisinin (Standard control)

Group IV: Infected with parasite and treated with 200 mg/kg aqueous extract.

Group V: Infected with parasite and treated with 200 mg/kg methanol extract.

Group VI: Infected with parasite and treated with 300 mg/kg aqueous extract.

Group VII: Infected with parasite and treated with 300mg/kg methanol extract.

Group VIII: Infected with parasite and treated with 400 mg/kg aqueous extract.

Group IX: Infected with parasite and treated 400 mg/kg methanol extract.

***In vivo* antiplasmodial studies:** The antimalarial activity of the plant was evaluated by its suppressive antiplasmodial properties *in-vivo* using standard method as previously described (Adzu *et al.*, 2003)

The percentage suppression of the parasite was evaluated with the equation below:

$$\text{Average Suppression} = \frac{APC - APT}{APC} \times 100$$

APC = Average Parasitaemia in the Positive Control,

APT = Average Parasitaemia in the Test group

Blood Samples Collection and Preparation: At the end of the experiments, the mice were anaesthetized and sacrificed,

blood samples were collected, centrifuged and used for further analyses.

Determination of Biochemical Parameters: At the end of the experiments, animals were sacrificed and blood samples were collected. Blood plasma was used for PCV estimation using a microhaematocrit method (Alexander, 1993). The blood was centrifuged and the serum harvested was used for further analyses. Aspartate aminotransferase, (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using standard methods as previously described (Reitman and Frankel, 1957, Haussament, 1977)

Statistical analysis: Data obtained were expressed as mean \pm standard deviation (SD) and analyzed by one way analysis of variance (ANOVA). The difference between means were compared using Post-Hoc Duncan Multiple Range Test. Values with $P \leq 0.05$ was considered significant.

RESULTS

Parasitaemia and Suppression Activities of Aqueous and Methanol *Glyphaea brevis* extracts on *Plasmodium berghei* Parasites

The aqueous and methanol extracts of *Glyphaea brevis* leaves both showed significant dose dependent ($p < 0.05$)

parasitaemia and suppressive activities on treated animals when compared with the infected (positive) control groups (Table 1).

Packed Cell Volume (PCV) of Experimental Mice before and after Infection and Treatment

Before the commencement of the study, the initial packed cell volume (PCV) of the experimental animals were determined and this showed no significant ($P > 0.05$) difference in all the groupings. However when they were infested with *Plasmodium berghei* parasite and treated, normal control animals showed a significant difference ($P < 0.05$), having the highest PCV compared to the rest of the experimental mice.

Effect of the Aqueous and methanol Leaves Extracts of *G. brevis* on Body Weight of Mice before and after Infection and Treatment

The result for the average change in body weight of the experimental mice before and after infection and treatment is shown in Fig 1. The average body weight of the mice in the normal and standard control groups showed significant increase, whereas the positive control group, aqueous and methanol extracts treated groups showed non-significant difference ($P > 0.05$) between their weight before and after treatment

Table 1:

Parasitaemia and Suppression Activities of Aqueous and Methanol *Glyphaea brevis* extracts on *Plasmodium berghei* Parasites

TREATMENT	DOSE (mg/kg)	% Parasitaemia	% Suppression
Normal Control	-	0.00 \pm 0.00 ^a	-
Positive Control	-	3.07 \pm 0.23 ^e	-
Standard Control	5mg/kg (Artemisinin)	0.29 \pm 0.08 ^b	90.49
Aqueous Extract	200mg/kg	1.07 \pm 0.05 ^d	65.15
	300mg/kg	0.67 \pm 0.05 ^c	78.27
	400mg/kg	0.69 \pm 0.07 ^c	77.69
Methanol Extract	200mg/kg	0.67 \pm 0.07 ^c	78.05
	300mg/kg	0.66 \pm 0.04 ^c	78.50
	400mg/kg	0.56 \pm 0.64 ^{bc}	81.89

Values are expressed as Mean \pm SD; and in percentages.

Values with different superscript down the column are significant ($P < 0.05$)

Table 2:

Packed Cell Volume (PCV) of the Experimental Mice before and after Infection and Treatment

Groupings	DOSE (mg/kg)	Initial PCV	Final PCV
Normal Control	-	47.17 \pm 2.52 ^a	49.63 \pm 4.65 ^c
Positive Control	-	54.33 \pm 2.83 ^a	44.37 \pm 5.49 ^{abc}
Standard Control	5mg/kg (Artemisinin)	46.20 \pm 0.00 ^a	46.20 \pm 2.62 ^{bc}
Aqueous Extract	200mg/kg	50.43 \pm 6.98 ^a	38.07 \pm 6.06 ^{ab}
	300mg/kg	54.57 \pm 4.36 ^a	46.23 \pm 5.33 ^{bc}
	400mg/kg	48.53 \pm 7.39 ^a	35.63 \pm 7.62 ^a
Methanol Extract	200mg/kg	50.33 \pm 0.58 ^a	44.07 \pm 6.65 ^{abc}
	300mg/kg	47.67 \pm 6.81 ^a	39.40 \pm 2.26 ^{ab}
	400mg/kg	49.90 \pm 7.39 ^a	41.73 \pm 3.04 ^{abc}

Values are expressed as Mean \pm SD; and in percentages.

Values with different superscript down the column are significant ($P < 0.05$).

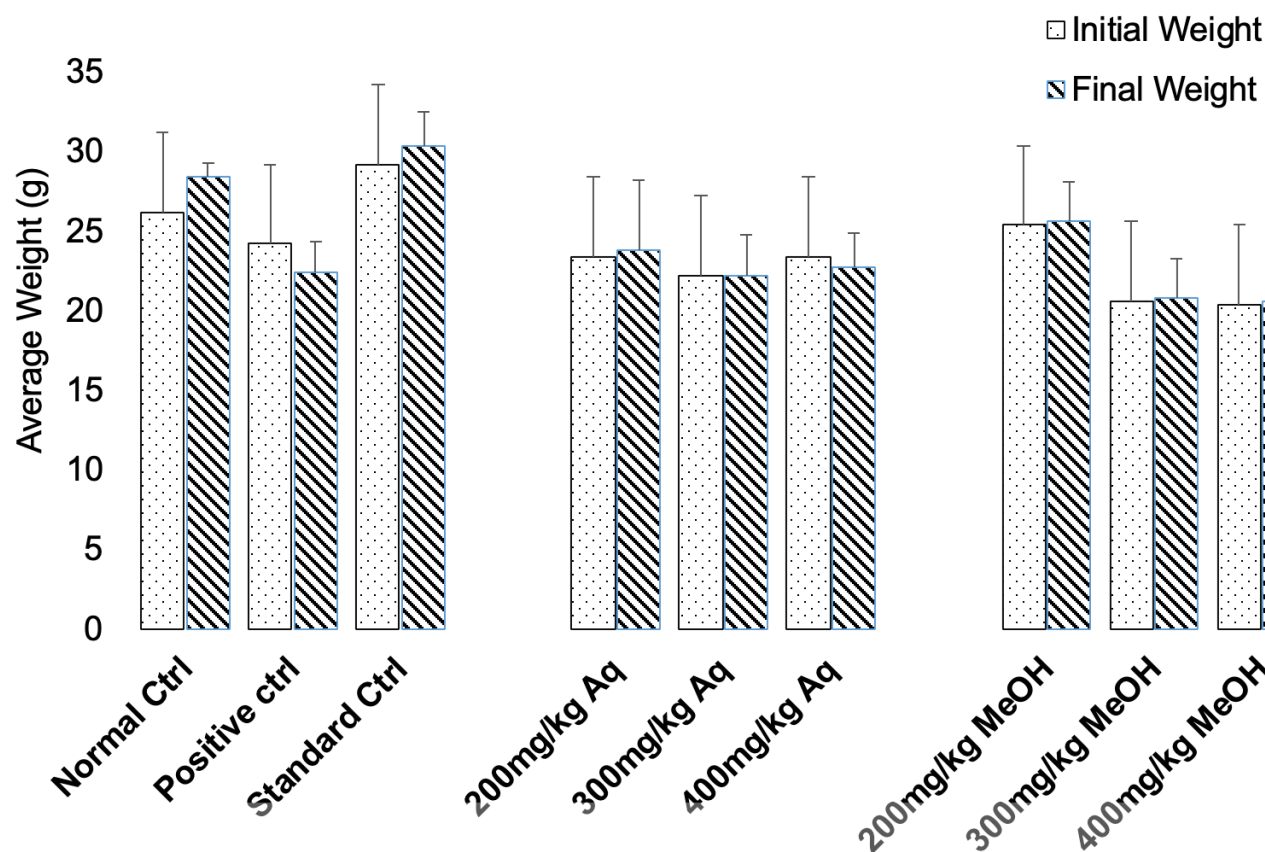


Figure 1: Average change in weight of the experimental animals before infections, and after infections and treatment

Table 3: Effect of Aqueous and Methanol Extracts of *Glyphaea brevis* Leaves on the Hepatic Marker Enzymes in *Plasmodium berghei* Infected Mice

TREATMENT	DOSE (mg/kg)	ALT (IU/L)	AST(IU/L)	ALP (IU/L)
Normal Control	-	56.20 ± 8.04 ^a	46.80 ± 6.18 ^{bc}	56.25 ± 6.60 ^a
Positive Control	-	80.75 ± 7.04 ^b	68.50 ± 4.66 ^d	72.75 ± 7.50 ^b
Standard Control	5mg/kg (Artemisinin)	64.25 ± 11.84 ^a	40.50 ± 3.11 ^{ab}	59.75 ± 12.68 ^{ab}
AQUEOUS EXTRACT	200mg/kg	60.75 ± 3.30 ^a	36.33 ± 3.06 ^a	63.00 ± 5.72 ^a
	300mg/kg	64.00 ± 5.57 ^a	50.00 ± 7.07 ^{bc}	52.67 ± 11.59 ^a
	400mg/kg	57.33 ± 9.07 ^a	52.00 ± 3.00 ^c	57.00 ± 9.17 ^a
METHANOL EXTRACT	200mg/kg	53.25 ± 5.74 ^a	36.50 ± 8.74 ^a	64.75 ± 5.25 ^{ab}
	300mg/kg	64.50 ± 6.86 ^a	47.00 ± 8.76 ^{bc}	61.25 ± 6.56 ^{ab}
	400mg/kg	62.67 ± 4.04 ^a	56.67 ± 4.16 ^c	65.67 ± 4.93 ^{ab}

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

Effect of Aqueous and Methanol Extracts of *Glyphaea brevis* Leaves on Hepatic Marker Enzymes in *Plasmodium berghei* Infected Mice

The result as presented in Table 3, revealed that there were significant ($P < 0.05$) increase in serum concentration of alanine aminotransferase (ALT) (80.75 ± 7.04) and serum aspartate aminotransferase (AST) (68.50 ± 4.66) compared to normal, standard and the extract treated groups. There was

significant difference in the serum concentration of Alkaline phosphatase in the positive control group and aqueous extract treated groups, however there was no distinct statistical difference ($P > 0.05$) between the positive control group (72.75 ± 7.50) and the standard control group (59.75 ± 12.68) as well as the various groups administered different dosages of methanol extract.

DISCUSSION

The positive control group (i.e. infected and left untreated) had highest level of parasitaemia (3.07 ± 0.23) while the Artemisinin treated group had the least (0.29 ± 0.08) parasitaemia. The percentage suppressive effect of the two extracts revealed that 400 mg/kg methanol extract treated group had the highest efficacy (81.89%) amongst the various dosages administered. The Artemisinin, which is a known drug for malaria treatment had 90.49% suppression on the parasites.

The superior performance noticed for artemisinin when compared with the extract in this study concurred with report of Matsuoka et al. (Matsuoka et al., 2000), that when a standard antimalarial drug is used in the management of *Plasmodium berghei* in mice, it suppresses parasitaemia. The highest percentage chemo-suppression activity of artemisinin recorded in the study showed that it still serves as effective antimalarial drug (Fidock et al., 2004). Although the suppression of parasitaemia was never complete (100% inhibition of parasite growth), the aqueous and methanol extracts both showed antiplasmodial effects with methanol extract showing better result. The antiplasmodial potency of this plant could be as result of its phytochemical composition which have been established to have antimalarial activities (Anjuwon et al., 2015).

Glyphaea brevis may elicit its antimalarial effect through one or a combination of the mechanisms of action that have been proposed for antimalarial compounds isolated from plants; these are: inhibition of hemozoin polymerization in the parasite; intercalation with the parasite DNA; inhibition of *Plasmodium falciparum* lactate dehydrogenase (pLDH); alkylation; inhibiting the formation of mobile microgametes; inhibition of proteolytic processing of circumsporozoite protein (Adebayo and Krettli, 2011).

The decrease in PCV observed in mice in negative control and extract administered groups are expected and this agreed with the research of Balogun *et al.* (Balogun et al., 2009), that the growing parasite consumes and degrades the intracellular proteins which are mainly haemoglobin, a major constituent of red blood cells.

The reduction in body weight in the group administered with extracts as well as the infected control group may be due to combined effect of plasmodial infection and possible catabolic effect of the extract on stored lipids and or short duration of the experiment (Zelege et al., 2017, Alli et al., 2011)

Liver injury can affect metabolic activities in the body due to its role in general metabolism. Within an hour of plasmodium infection, degenerative changes in the hepatocytes occurs due to invasion of the liver cells by *Plasmodium*, this may significantly alter the levels of serum liver marker enzymes. This study probed the possible effect of *G. brevis* in ameliorating this cellular damage. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are considered indicators of hepatocellular health (Yang and Chen, 2003). A significant increase in the activities of ALT, AST and ALP in the blood serum of control parasitized mice as compared with all the other groups was observed. The observed increase in enzyme

activities may be as a result of liver injury and altered hepatocyte integrity caused by the *Plasmodium* infection and the consequent release of these enzymes into the bloodstream. Serum elevations of ALT, AST and ALP activities are rarely observed except in parenchymal liver disease, muscular dystrophy, and organ damage; ALT is a more liver-specific enzyme than AST. The administration of *G. brevis* tends to normalize these enzymes (ALT, AST and ALP) activities which is in agreement with a reported investigation (George et al., 2011). This plant is extensively considered as vegetables. Treatment with extract from this study has shown to protect hepatocyte integrity of parasitized mice.

In conclusion, aqueous and methanol leaves extracts of *Glyphaea brevis* showed significant antiplasmodial activities making it a good candidate for the development of new antimalarial. Additional research should be carried out on the isolation and characterization of the components responsible for antiplasmodial activity in *G. brevis* leaves.

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