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ANTIPLASMODIAL ACTIVITY OF METHANOL LEAF EXTRACT OF *Cryptolepis oblongifolia* (APOCYNACEAE) (MEISN) SCHLTR IN MICE INFECTED WITH *Plasmodium berghei-berghei*

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ABSTRACT
Malaria remains a major public health problem in Sub-saharan Africa, where 85-90% of all global burden of the disease exist. In traditional medicine, Cryptolepis oblongifolia is used to cure cough, malaria, stomachache and diarrhoea. The aim of this study is to evaluate the antiplasmodial activity of the methanol leaf extract of Cryptolepis oblongifolia in mice infected with Plasmodium berghei. Oral median lethal dose (LD_{50}) and phytochemical screening of the extract were carried out using OECD 425 guidelines and method of Trease and Evans respectively. The antiplasmodial activity of the plant was studied using chloroquine-sensitive Plasmodium berghei-berghei in three rodent malaria models: Curative, suppressive and prophylactic. Data were analyzed using one way ANOVA followed by Dunnett's post hoc test. The oral median lethal dose (LD_{50}) of the extract was found to be > 5000 mg/kg. The extract contained alkaloid, saponin, flavonoid, phenol, cardiac glycosides, steroids, terpenoids and tannins. The extract at the doses tested (375, 750 and 1500 mg/kg) produced significant ($p < 0.001$) curative effect with percentage parasite clearance of 15.8, 40.3 and 57.9, respectively. The extract also produced significant ($p < 0.001$) suppressive antiplasmodial effect with percentage chemosuppression of 24.9, 41.2 and 55.5 respectively. A significant prophylactic ($p < 0.001$) effect was recorded with percentage chemoprophylaxis of 5.7, 20.1 and 44.8, respectively. The extract at the highest dose (1500 mg/kg) prolonged the survival time of the treated mice, in the curative group, to 28 days compared to those in the negative control group that survived for 7.8 days. The results of this study indicate that the methanol leaf extract of Cryptolepis oblongifolia has significant antiplasmodial activity in mice.
Keywords: Antiplasmodial, Cryptolepis oblongifolia, Plasmodium, Berghei-berghei, Chloroquine

INTRODUCTION

Malaria is a parasitic infection causing significant morbidity and mortality especially in sub-Saharan Africa. Recent data from the WHO indicate that 241 million cases were recorded in 2020 with 627000 deaths. Nigeria has the highest number of malaria cases (26.8%) and deaths (31.9%) globally (WHO, 2021). The global burden of mortality is dominated by countries in Sub-Saharan Africa, with the Democratic Republic of Congo and Nigeria together accounting for more than 35% of the global total estimated malaria deaths (WHO, 2015). Resistance to antimalarial drugs is a major drawback in effective treatment and control of malaria globally, *P. falciparum* has developed high levels of resistance to the available cheap and safe drugs such as chloroquine and sulfadoxine-pyremethamine (WHO, 2015). *Cryptolepis oblongifolia* is a multi-stemmed shrub in the family Apocynaceae. It is called red-stemmed milk rope and is native to moist and mesic regions of southern Africa, where it occurs in rocky grassland, grassy woodland or riverine vegetation (Bingham and Mike, 2013). In Mozambique the leaves are used as a cure for malaria (Bullock, 1963). In traditional medicine in Tanzania and Zimbabwe root preparations are used to cure cough, stomachache and diarrhoea in children (Bullock, 1963).

The aim of this study is to evaluate the antiplasmodial activity of methanol leaf extract of *Cryptolepis oblongifolia* in mice infected with P*lasmodium berghei* parasite. To the best of our knowledge, antiplasmodial activity of the leaf extract of *Cryptolepis oblongifolia* in mice has not been reported previously.

MATERIALS AND METHODS

Plant collection and identification

The leaves of *Cryptolepis oblongifolia* were collected from Karau-Karau village at Giwa local government area of Kaduna State-Nigeria in July, 2021. The plant was identified and authenticated by a botanist at the Department of Plant Biology, Faculty of Life Sciences, Ahmadu Bello University, Zaria. The voucher specimen number (ABU03359) was collected for feature reference.

Preparation of the plant extract

Fresh leaves of *Cryptolepis oblongifolia* were sorted and shade dried. The leaves were size-reduced and 1 kg of the powdered plant was extracted with 7.5 L of 70% (v/v) methanol using cold maceration method for five days with occasional shaking. The extract was filtered, concentrated and evaporated to dryness using water bath maintained at 45^{o}C as described by Ingle, *et al.,* 2017.

Experimental animals

Adult Swiss albino mice of either sex (18-32 g) were obtained from the Animal House Facility of Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were maintained in a well-ventilated cages, feed with vita feeds and allow free access to water *ad libitum*. Ethical clearance was obtained from Ahmadu Bello University, Zaria Committee on Animal Use and Care (ABUCAUC) with approval number: ABUCAUC/2022/010

Preliminary phytochemical screening

The qualitative preliminary phytochemical screening of methanol leaf extract of *Cryptolepis oblongifolia* was done using the methods of Trease and Evans, 2009.

Acute toxicity study

The median lethal dose (LD_{50}) of *Cryptolepis oblongifolia* was determined using OECD guidelines number 425 (OECD, 2008). A Limit test was done and five rats were dosed with 5000 mg/kg of the extract sequentially. Each rat was monitored frequently within the first 30 minutes, then once per hour for the first four hours and later daily, for signs of toxicity and/or death. All the rats were observed for two weeks for the signs of late onset toxicity and/or mortality.

Plasmodium parasite inoculation

Blood sample was collected, retro-orbitally into EDTA bottle, from a donor mouse with parasitaemia of 28%. The blood was then diluted with normal saline in such a way that each 0.2 ml contains approximately 1 x 10^7 infected RBCs. Each mouse was inoculated with 0.2 ml of the infected blood intraperitoneally (Peters, 1965)

Antiplasmodial activity of *Cryptolepis oblongifolia* in mice with established infection (Curative test)

Antiplasmodial potential of methanol leaf extract of *Cryptolepis oblongifolia* against established infection was carried out using the method of Ryley and Peters (1970). On the first day (D_0), adult mice were inoculated with the parasite and left untreated for 72 hours (D_0-D_3) for infection to be established. On day three post parasite inoculation, each mouse was tail-bled, thin blood smear was prepared, fixed with absolute methanol and stained with Giemsa. Pre-treatment parasitaemia levels were determined by counting the number of parasitized erythrocytes in ten random fields. After the baseline blood sampling, all inoculated mice were randomly divided into 5 groups of 5 mice each and treated orally with distilled water (10 ml/kg, group I [negative control]), graded doses of the extract (375, 750 and 1500 mg/kg body weight, groups II-IV) and standard drug, chloroquine (5 mg/kg body weight, group 5 [positive control]) respectively for 5 days (D_3-D_7). Post-treatment parasitaemia levels were determined on day seven of the experiment, as described above, using light microscope (Celestron CB1000CF 40-1000) at x100 magnification. All the mice were monitored and the mean survival time for each group was determined arithmetically by finding the average survival time (post-inoculation) in each group over a period of 28 days (Ryley and Peters, 1970).

Antiplasmodial activity of *Cryptolepis oblongifolia* against early infection (4-Days Peter's suppressive test)

The method described by Peters (1980) was used in this test. On the first day (D_0), adult mice were inoculated with *Plasmodium berghei-berghei* and thereafter randomly divided into 5 groups of 5 mice each. On the same day (D_0), treatment, with the graded doses of the extract (375, 750 and 1500 mg/kg) was started four hours after inoculation and continued daily for three days. Twenty-four hours (24 hours) after administration of the last dose (D_4), blood sample was taken from the tail of each mouse and thin film was prepared, fixed in absolute methanol, stained with Giemsa solution and examined under microscope at X 100 magnification for determination of parasitaemia.

Prophylactic Antiplasmodial activity of Cryptolepis oblongifolia (Repository) test in mice

The prophylactic activity of the extract was evaluated using the method described by Peters (1965) Thirty adult mice were randomly grouped into 5 groups of 5 mice each and treated with the graded doses of the extract (375, 750 and 1500 mg/kg) and standard drug (Pyrimethamine 1.2 mg/kg) orally for five 5 days (D_0-D_4). On the sixth day (D_5), the mice were inoculated with 0.2 ml of blood infected with *Plasmodium berghei-berghei* intraperitoneally. Smears were made from tail blood of each mouse 72 hours after inoculation and parasitaemia level was determined.

Data analysis

Results were expressed as mean plus or minus standard error of mean (Mean ± SEM) and analysed using One-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test for multiple comparisons among groups. Results were considered statistically significant at $p \leq 0.05$. SPSS software version 20 was used for data analysis.

RESULTS

Preliminary phytochemical screening

The preliminary qualitative phytochemical screening of the methanol leaf extract of *Cryptolepis oblongifolia* (MLECO) revealed the presence of alkaloid, saponin, flavonoid, phenol, cardiac glycosides, steroids, terpenoids and tannins (Table 1).

Acute Toxicity Study

The MLECO at 5000 mg/kg was found to be non-toxic to the rats using the OECD guidelines (425) for acute toxicity testing. The rats were found to have some restlessness and hyperventilation within the first four hours of MLECO administration. All the five rats survived up to the 14 days observation period with no signs of late onset toxicity or mortality. The oral median lethal dose (LD_{50}) of MLECO in rats was found to be > 5000 mg/kg.

Antiplasmodial activity of *Cryptolepis oblongifolia* on established infection (Curative test)

The methanol leaf extract of *Cryptolepis oblongifolia* (MLECO) at tested doses of 375, 750 and 1500 mg/kg produced significant ($p < 0.004$ and 0.001), dose dependent parasitaemia suppression (15.8, 40.3 and 57.9%) compared to the negative control group. The standard drug (Chloroquine) at tested dose of 5 m g/kg produced significant ($p < 0.001$) parasitaemia suppression of 88.7%. All the mice in the MLECO (1500 mg/kg) group and those in the standard drug (Chloroquine, 5mg/kg) group survived throughout the 28 day observation period. The mice in the other groups (MLECO 375 and 750 mg/kg) survived for 23.1 and 27.6 days respectively; while those in the negative control group survived for only 7.8 days (Table 2).

Antiplasmodial activity of *Cryptolepis oblongifolia* on 4-Days Peter's Suppressive Test

The MLECO at tested doses of 375, 750 and 1500 mg/kg produced significant ($p < 0.001$) dose dependent parasitaemia suppression (24.9, 41.2 and 55.5%) compared to the negative control group. The standard drug (Chloroquine) at the tested doses of 5 mg/kg produced significant ($p < 0.001$) parasitaemia suppression of 89% (Table 3).

Antiplasmodial activity of *Cryptolepis oblongifolia* on Prophylactic Test

The MLECO at the tested doses of 750 and 1500 mg/kg produced significant ($p < 0.001$) chemoprophylactic parasitaemia suppression (20.1, and 44.8%) compared to the negative control group. The standard drug (Pyrimethamine) at the tested doses of 1.2 mg/kg produced significant ($p < 0.001$) chemoprophylactic parasitaemia suppression of 79.9% (Table 4).

Table 1: Phytochemical Constituents of Methanol Leaf Extract of *Cryptolepis oblongifolia*

PHYTOCHEMICAL	INFERENCE
Anthraquinones	-
Tannins	+
Saponin	+
Flavonoids	+
Cardiac glycosides	+
Terpenoids	+
Steroids	+
Phenols	+
Alkaloids	+

+ = Present, - = Absent

TABLE 2: Curative Effect of Methanol Leaf Extract of *Cryptolepis oblongifolia* in *Plasmodium bhergei- bhergei* Infected Mice

Treatment	Parasitaemia suppression (Mean ± SEM)			
	D_3	D_7	Parasite Clearance (%)	Mean Survival Time (Days)
DW 10 ml/Kg	19.1 ± 0.37	22.1 ± 0.71	-	7.8 ± 1.19
MLECO (375)	18.9 ± 0.63	18.6 ± 0.50*	15.8	23.1 ± 1.38
MLECO (750)	17.8 ± 0.80	13.2 ± 1.13**	40.3	27.6 ± 0.33
MLECO (1500)	18.3 ± 0.43	9.3 ± 0.43**	57.9	28.0 ± 0.00
CQ (5)	16.7 ± 1.01	2.5 ± 0.24**	88.7	28.0 ± 0.00

Values presented as Mean ± SEM, n = 6, * and ** significantly different from DW group at $p < 0.004$ and 0.001 using one way ANOVA and Dunnett's *post hoc* test. DW = Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis oblongifolia*, CQ= Chloroquine, D_3 and D_7 = Days Three and Seven

TABLE 3: Suppressive Effect of Methanol Leaf Extract of *Cryptolepis oblongifolia* in *Plasmodium berghei- berghei* Infected Mice

Treatment (mg/Kg)	Parasitaemia suppression (Mean ± SEM)	Chemosuppression (%)
DW 10 ml/Kg	24.5 ± 0.80	-
MLECO (375)	18.4 ± 0.26*	24.9
MLECO (750)	14.4 ± 0.90*	41.2
MLECO (1500)	10.9 ± 0.72*	55.5
CQ (5)	2.7 ± 0.30*	89

Values presented as Mean ± SEM, n = 6, * significantly different from DW group at $p < 0.001$ using one way ANOVA and Dunnett's *post hoc* test. DW= Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis oblongifolia,* CQ = Chloroquine

TABLE 4: Prophylactic Effect of Methanol Leaf Extract of *Cryptolepis oblongifolia* in *Plasmodium berghei- berghei* Infected Mice

Treatment (mg/Kg)	Parasitaemia suppression (Mean ± SEM)	Chemoprophylaxis (%)
DW 10 ml/Kg	19.4 ± 0.28	-
MLECO (375)	18.3 ± 0.20	5.7
MLECO (750)	15.5 ± 0.57*	20.1
MLECO (1500)	10.7 ± 0.44*	44.8
PYR (1.2)	3.9 ± 0.49*	79.9

Values presented as Mean ± SEM, n = 6, * significantly different from DW group at $p < 0.001$ using one way ANOVA and Dunnett's *post hoc* test. Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis oblongifolia,* PYR = Pyrimethamine

DISCUSSION

The presence of alkaloids, saponins, flavonoids, phenol, cardiac glycosides, steroids, terpenoids and tannins in the extract has revealed the secondary metabolites contained in the plant; and these are responsible for numerous pharmacological activities. The oldest antimalarial drug, quinine, is an alkaloid, isolated from the bark of *cinchona* tree (Biamonte *et al.*, 2013); and the currently acceptable artemisinin, is a terpenoid (Sesquitrepine lactone) isolated from *Artemisia annua* (Bray *et al.*, 2005). This shows that the secondary metabolites in MLECO are responsible for the observed antiplasmodial effect. The MLECO contains flavonoid; and Rasoanaivo, *et al.* (2011) and Kaur, *et al.*, (2009) reported that flavonoids produce antiplasmodial effect by inhibiting the parasite fatty acid biosynthesis there by disrupting the parasite metabolism with subsequent parasite killing. It was also reported that flavonoids exert their antiplasmodial action by targeting certain functional biomolecules of the parasite such as enzymes, proteins and DNA (Nijveldt, 2001). Dua and his colleagues isolated *conessine*, an alkaloid, from the bark of *Holarrhena antidysenterica* and was found to have significant antiplasmodial effect in mice (Dua *et al.*, 2013). The MLECO was found to contained flavonoid and alkaloid which may be responsible for the observed antiplasmodial effect. These secondary metabolites may act singly or synergistically to account for the observed antimalarial effect.

The extract of *Cryptolepis oblongifolia* was found to have significant effect on blood and tissue stages of the *plasmodium berghei*. The extract also prolonged the survival time of the treated mice to more than twice that of the negative control (28 versus 7.8 days) which is an indication of significant antiplasmodial activity by the plant extract. (Ryle and Peters, 1970).

CONCLUSION

The results of this study suggested that the methanol leaf extract of *Cryptolepis oblongifolia* has significant antiplasmodial activity on curative, suppressive and prophylactic rodent malaria models at the tested doses.

Conflict of interest

The authors declared no conflict of interest.

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