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In vivo antiplasmodial activity of crude extracts of *Vernonia cinerea* Less (Asteraceae) against *Plasmodium berghei* infection in mice in Bobo-Dioulasso, Burkina Faso.

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Abstract: Resistance of *Plasmodium falciparum* against antimalarial drugs constitutes a veritable obstacle to malarial control in the world and particularly in Sub-Saharan Africa. Nowadays the artemisinin resistance was observed in the South-east Asian region. Several investigations were made on the medicinal plants species coming from *Asteraceae* family above all *Vernonia cinerea* Less and were showed that the plant own several pharmacology activities tested in pre-clinical trials. The plant material was collected in Banfora (Burkina Faso). The antiplasmodial activity of the 80% methanolic extract, 50/50 % hydro-methanolic and aqueous extract have been tested against *Plasmodium berghei*in mice by using the Peter's 4-day suppressive test was conducted. The NMRI female mice have been used and treated with the distilled water, 100, 250, and 500 mg/kg body weight of the extract, respectively. Percentage of parasitaemia and parasitaemia suppression were determined. The standard deviation and the IC95 were calculated. The analyzes were made by the Excel software and ANOVA. The results revealed 21.4 %; 13.8 % and 43.1 % ($p>0.05$) inhibition at 100, 250 and 500 mg/kg bwt dose levels, respectively by the 80 % methanolic extracts. The 50/50% hydro-methanolic extracts showed a percent reduction of parasitemia of 42.7 %; 25.6 % and 40.6 % ($p>0.05$) respectively for the same dose levels. About the aqueous extracts, the suppression produced 9.5 %, 14.6 % and 3.2 % ($p>0.05$) at 100, 250 and 500 mg/kg bwt dose levels, respectively.These results suggest that the extract of *Vernonia cinerea* Less possesses antiplasmodial activity and these results may justify its use in ethnomedicine to treat malaria disease.

Keywords: Antimalarial activity, Malaria, NMRI mice, *Plasmodium berghei*, *Vernonia cinerea* Less

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I. INTRODUCTION

The resistance of *Plasmodium falciparum* to antimalarial drugs is a real obstacle to the control of malaria in the world, especially in Sub-Saharan Africa[1]. Today the artemisinin resistance was observed in the region of Southeast Asia. This molecule is extracted from *Artemisia annua* (Asteracea) and is the basis of Artemisinin-based Therapeutic Combinations[2].This resistance is due to K13 propeller *Plasmodium falciparum* gene mutation[3]. Face in this situation, many investigations were realized on the medicinal plants species of the family of *Asteraceae* especially *Vernonia cinerea* Less. This study revealed that the plant have owned several pharmacological activities tested in pre-clinical phase[4–6].The extracts of the plant were studied for different pharmacological activities above all larvicidal activity[7], the repellent propriety[8] , antibacterial activity[9], antipyretic,analgesic and anti-inflammatory activity [10]and *in vitro*antiplasmodial potent[11-12]. In Burkina Faso, our previous studies have allowed to corroborate the *in vitro* efficacy of crude extracts of *Vernonia cinerea* Less against *Plasmodium falciparum* strains [11].The aim of the study consisted to evaluate the *in*

*vivo*antiplasmodial activity of 80% methanolic, 50/50% hydro-methanolic and aqueous crude extracts of *Vernonia cinerea* Less against *Plasmodium berghei* infection on mice in Burkina Faso.

II. MATERIAL AND METHODS

Collection of plant material

The whole plant of *Vernonia cinerea* Less was collected in March 2017, in the village of Lemouroudougou village located at 15 km from Banfora (Burkina Faso).This area is permanently watered where the plant grows in abundance. After collect, the drug sample was washed with water and then dried out of the sun to prevent degradation of any thermosensitive molecule and in a dry and ventilated place to avoid any deterioration due to the action of mold.The plant material was sent to the IRSS/DRO(Institut de Recherche en Science de la Santé/Direction Régionale de l'Ouest) inBobo-Dioulasso after sprayingto evaluate the *in vivo* antiplasmodial activity.

Preparation of plant extract

Extracts were prepared from the plant powder. We obtained three organic extracts and one aqueous with water. Crude organic extracts were prepared by maceration for 16 h successively with dichloromethane, 80 % methanol and 50/50 % hydro-methanol solvents. The plant powder (100 g) was used for these organic extraction methods with 1000 mL of each solvent. The dichloromethanolic extract was air dried at room temperature. The 80% methanolic and hydro-methanolic extracts were freeze-dried with lyophilisator (Brand) after total evaporation of solvents. Aqueous extracts were prepared by boiling 200 g of plant powder in 2000 mL of purified water for 30 min. After cooling, solutions were filtered on cotton wool and freeze-dried. Plant powder was obtained by applying the classical natural product extraction method by Sanon et al. [13].The extracts were lyophilized in CNRFP (Centre National de Recherche et de Formation sur le Paludisme).The lyophilizates were used to prepare the stock solution that were used for the *in vivo* antiplasmodial tests and stored at 4°C.

Experimental animals

Seventy-two (72) female NMRI mice aged 6 to 8 weeks and weighing 23 to 31 g were used for tests. They were obtained from CIRDES (Centre International de Recherche Développement sur l'Elevage en zone Sub humide)of Bobo-Dioulasso. The mice were housed in plastic cages containing soft wood chips. They were maintained under standard laboratory conditions. They were exposed to a 12:12 dark-to-light cycle and fed adequately with the feed for fattening 29 % from the livestock department of the Regional Directorate of Livestock of Bobo-Dioulasso and drinking water. All mice were acclimatized at 25°C ± 2°C to the working environment four days before the beginning of the experiment. The experiment was conducted according to international principles for the use of laboratory animals (EEC Directive of 1986; 86/609 / EEC). The consent of the Institutional Ethics Committee of the IRSS/DRO was obtained for the research.

Parasites

Chloroquine sensitive *Plasmodium berghei* ANKA strain (MRA-311, MR4, ATCC Manassas Virginia) stored at -80°C was obtained from the IRSS/DRO in Bobo-Dioulasso. The parasites were maintained by serial passage from infected mice to non-infected mice on weekly basis to maintain viability of the strain[14].

Doses preparation for treatment

The lyophilized methanolic crude extract, hydro-methanolic and aqueous extracts were used to prepare doses 100, 250 and 500 mg/kg body weight for treatment. For each dose preparation, the masses of extract were calculated from themean weights of the lots of mice. The extracts were weighed using an analytical balance and solubilized in 1.4 mL of distilled water before being administered to mice.

Inoculum preparation

Parasitic red blood cells (RBCs) from donor mice maintained at the laboratory is used to infect test mice. The parasitaemia of each donor mouse was evaluated to determine the amount of parasitized red blood cells needed to obtain 1 x 10^7 parasitized RBCs to be injected into test mice. The blood smears were made from blood drawn from the tail of the donor mice,fixed with methanol; colored with Giemsa 10% and read with an optical microscope at 100X magnification. The determination of parasitaemia was made by reading 10 fields each containing 100 RBCs per field[15].

Red Blood Cells numeration

Five (5µL) of blood were taken from the sectioned end of the mouse tail and diluted 1/5000 in Phosphate Buffered Saline (PBS).Then 10 µL of dilutedblood were introduced betweenthe cell of the Mallassez

blade by capillarity. Blood solution was allowed to sediment for 10 min on a horizontal plane, then the elements were counted under a microscope at 400 X magnification. The RBCs were counted in a grid of 16 cells.The RBCs obtained represent the number of RBCs in 0.01 µL of dilutedblood. The number of RBCs per microliter of blood in the mouse was obtained by multiplication with the dilution factor.

Infection of test mice with Plasmodium berghei ANKA strain

Donor mice of PAC 6-7 parasitaemia between 12-25% previously infected with *Plasmodium berghei* were used.Mice were anesthetized with petroleum ether in a bottle containing cotton. The blood from donor mice was collected successively with a glass Pasteur pipette through the eye vein and introduced into a heparin tube. The parasitized blood collected was diluted taking into account parasitaemia and counting of RBCs in physiological saline (1% PBS) so that 200 µL of blood contained 1x 10^7 infected RBCs. Before the infection test mice were weighed and pooled in groups of 6 mice. All mice of the different test groups received an injection of 200 µL of parasitized RBCs intra-peritoneal using a hypodermic 1 mL syringe with 1 x 10^7 infected RBCs by mouse.

Grouping and dosing of animals

The mice were divided into 4 groups of 6 mice each per extract. Group I mice were received the distilled water (negative control), and the test group II, III and IV mice received orally different doses of extracts of *Vernonia cinerea* Less (100, 250, and 500 mg/kgbody weight).

Four-day suppressive antimalarial test

This test was used to evaluate the schizontocidal activity of extracts in *Plasmodium berghei* infested mice according to the method described by Peter et al. [16].Infected mice were previously grouped.Treatment started 2 hours after inoculation of the parasite in mice in day 0.As people traditionally use the plant material for treatment and prevention of malaria orally [17], each treatment was administered through oral route using oral gavage to ensure safe ingestion of the preparations. Mice received orally the doses of extracts (100, 250 and 500 mg /kg).The doses were administered once daily for 4 days consecutively(from day 0 to day 3).At day4 of the treatment, thin blood smears were prepared from the tail of each mouse on microscope slides to determine the parasitaemia and the percentage inhibition of parasites.The animals were sacrificed at the end of the test to avoid their suffering. The parasitaemia of each blade was obtained by the median of reading results of 3 field'scontaining 100 RBCs. The mean of 3 reading was realized by different technicians.

$$(1) \qquad \%\, Parasitaemia = \frac{(\text{Nomber of infected RBCs})}{\text{Tota l Nomber of RBCs}} x\ 100$$

The activity of extracts on parasites was expressed depending parasitaemia reduction in treated mice compared to mice in the control group.The Percent Reduction (PR) of parasitaemia per mouse was calculated according to the formula:

$$(2) \qquad \%\, Suppression = \frac{(\%\, parasitaemia \quad of\ Negative\ control\ -\%\, parasitaemia \quad of\ Treated\ group)}{\%\, parasitaemia \quad of\ Negative\ control} x\ 100$$

III. RESULTS

The 4-day suppressivetest

The results indicated that the crude extract of *Vernoniacinerea* Less showed interesting antiplasmodial activity against chloroquine sensitive *P. berghei* infected NMRI mice. Level of suppression of 80 % methanolic extract at concentrations of 100 mg/kg, 250 mg/kg and 500 mg/kg following the 4-day test was 21.4 %; 13.8 % and 43.1 % respectively($p>0.05$) (Table 1). The hydro-methanolic extracts showed 42.7 %; 25.6 % and 40.6 % respectively for the same dose levels ($p>0.05$) (Table 2). For the aqueous extracts, the % suppression was gave 9.5 %, 14.6 % and 3.2 % at 100, 250 and 500 mg/kg body weight dose levels, respectively that were mentioned in the(Table 3). There was no statistically significant difference among the percentage reduction of all the doses of each plant extract ($p>0.05$).

Table 1.*In vivo* suppressive activity of crude 80 % methanolic extracts of *Vernonia cinerea* less (4-day test) against *Plasmodium berghei* infection in mice.

Drug/extracts	Dose (mg/kg)	% Parasitaemia	% Suppression	Confidence Interval (IC95)
Negative Control (distilled water)	00	32±10.8	0.00	(20.66-43.33)
80% methanolic extracts	100	25.2±11.4	21.4	(13.23-37.16)
	250	27.6±8	13.8	(19.20– 35.99)
	500	18.2±18.8	43.1	(-1.52 -37.92)

Notes: Results are expressed as Mean ± Standard Deviation. (*n* = 6).

Table 2.*In vivo* suppressive activity of crude 50/50 v/v hydro-methanolic extracts of *Vernonia cinerea* less (4-day test) against *Plasmodium berghei* infection in mice

Drug/extracts	Doses (mg/kg)	% Parasitaemia	% Suppression	Confidence Interval (IC95)
Negative Control (distilled water)	00	32±10.8	0.00	(20.66-43.33)
Hydro-methanolic extracts (50/50%)	100	18.3±14.3	42.7	(3.29-33.30)
	250	23.8±13.7	25.6	(9.42-38.17)
	500	19±9.3	40.6	(9.24-28.75)

Notes: Results are expressed as Mean ± Standard Deviation. (*n* = 6).

Table 3.*In vivo* suppressive activity of crude aqueous extracts of *Vernonia cinerea* less (4-day test) against *Plasmodium berghei* infection in mice

Drug/extracts	Doses (mg/kg)	% Parasitaemia	% Suppression	Confidence Internal (IC95)
Negative control (distilled water)	00	31.6±2.7	0.00	(28.76-34.43)
Aqueous extracts	100	28.6±6,2	9.5	(22.09-35.10)
	250	27±1	14.6	(25.95-28.04)
	500	30.6±5.4	3.2	(24.93-36.26)

Notes: Results are expressed as Mean ± Standard Deviation. (*n* = 6).

Statistical analysis

Data were entered and cleaned using Excel 2013 software then transferred into OpenEpi version 3.01 software. One-way analysis of variance (ANOVA) was used to determine statistical significance for comparison of parasitaemia suppression among groups. In this analysis, the data valueswere expressed as mean of parasitaemia ±standard deviation(M± SD) for six mice per group. The analysiswas performed with 95% confidence interval and $p<0.05$ was considered to be statistically significant.

IV. DISCUSSION

Antimalarial activity of the crude extract of *Vernonia cinerea* Less, used in traditional medicine in Burkina Faso and elsewhere, against *Plasmodium berghei* infection in mice in four-day suppressive test model are reported. *In vivo* models are usually employed in antimalarial studies because they take into account the possible prodrug effect and probable involvement of the immune system in eradication of the pathogen [18]. The percentage suppression of parasitaemia was evaluated and data corroborate that *Vernonia cinerea* Less has therapeutic activity against malaria and further confirm the *in vitro* antiplasmodial activities reported earlier [11]. The percentage suppression of parasitaemia of the extract treated groups are different from those in the negative control group. The values are showed 43.1 % inhibition at 500 mg/kg body weight dose levels, by the 80% methanolic extracts. Even, the hydro-methanolic extracts showed 40.6 % for the same dose level. According to Deharo et al., 2001[19], *in vivo* antimalarial activity of plant extracts can be categorized as: moderate, good and very good if the extract showed 50 % or more chemosuppression at 500, 250 and 100 mg/kg/day extract dose, respectively.

Hence, the current finding revealed that the extracts can be considerate have moderate 4-day suppressive antimalarial activity. This might be explained by the presence of various secondary plant metabolites in the crude extract that may have a complex biochemical effect and lack of very strong antimalarial activity that could ameliorate all pathologic changes [20].In our previously studies, phytochemical screening of the constituent plant extracts of *Vernonia cinerea* Less showed the presence of alkaloids, triterpenes and sterols, anthracenosids, tannins , saponins [11].

This suggests that the antiplasmodial activity of the plant could be attributed to these constituents of plant extracts. This study provides evidence that *Vernonia cinerea* Less is sure and possesses moderate *in vivo* suppressive activities against *Plasmodium berghei*.

V. CONCLUSION

That present study revealed that the crude extracts of *Vernonia cinerea* Less possesses antiplasmodial activity and necessitates further scientific validation. The plant may contain biologically active lead molecules which are relevant in the treatment of malaria and may be a possible source of antimalarial drug development.

Abbreviations

RBC: Red Blood Cells
IC95: Confidence Interval 95%
WHO: World Health Organization.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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