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In vivo antiplasmodial potential of aqueous seed extract of *Ricinus communis*

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ABSTRACT

Introduction: *Ricinus communis* is used by the people of Niger-Delta region of Nigeria, for the treatment of various ailments, especially malaria. This study evaluated the antiplasmodial potentials of the aqueous seed extract of *R. communis,* using *Plasmodium berghei berghei.*
Methods: Acute toxicity study was carried out to determine the median lethal dose (LD_{50}) of the extract. Antiplasmodial effect of the extract was assessed in suppressive, repository/prophylactic and curative models, using Swiss albino mice (15-29 g). Mice were infected intraperitoneally with 0.2 mL of parasitized blood. Extract doses administered were 54.77, 109.54 and 164.32 mg/kg/d of the seed extract and each dose had 6 replicates. Artesunate (5 mg/kg/d) and pyrimethamine (1.2 mg/kg/d) were used as standard drugs, while distilled water (10 mL/kg/d) served as control.
Results: Acute toxicity study produced LD_{50} of 547.72 mg/kg. The extract demonstrated a dose-dependent reduction in parasitaemia in all tests. At the end of 4-day test, suppressive effect of 20.80, 49.00, 75.00 and 88.40% were obtained for doses 54.77, 109.54 and 164.32 mg/kg/d of the seed extract and artesunate, respectively. In the repository test pyrimethamine was more potent (72.26%) than the seed extract (9.47%–51.42%). The extract also exhibited appreciable curative effect. The activity of the seed extract was significant when compared with the control ($P<0.05$). Mice treated with the seed extract and drugs survived for longer duration than the control group.
Conclusion: The aqueous seed extract of *R. communis* has antiplasmodial potential and its active principle should be elucidated and further investigated to help in the ongoing fight against malaria.

Implication for health policy/practice/research/medical education:
The aqueous seed extract of *Ricinus communis* has antiplasmodial potential which may be useful in the ongoing fight against malaria.
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Introduction

Malaria is a public health challenge with several people at risk of infection. The populations at risk reside mainly in tropical and sub-tropical regions of the world with African countries accounting for the largest burden of the disease (1). The vast majority of cases occur in children under the age of five years and pregnant women. The disease is caused by *Plasmodium* parasites, transmitted through the bite of infected female *Anopheles* mosquitoes. Different species of *Plasmodium* affect humans. Of these, *Plasmodium falciparum* is the most deadly, leading to many fatal complications (2,3). Tremendous gains have been made in the fight against malaria, as attributed to the adoption of artemisinin-based combination therapy (ACT) and the scale up intervention efforts such as the use of long-lasting insecticide-treated net (LLIN), intermittent preventive therapy (IPT) for pregnant women, vector control measures and increased funding (4). However, owing to the development of resistance by malaria parasites to currently available drugs current tools and treatments are insufficient to achieve elimination in many countries (5). Besides, there is no commercially available malaria vaccine despite intense research and development effort (6). The aforementioned reasons

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make the development of novel antimalarial agents urgent. There have been many validations of traditional remedies through scientific research (7-11). *Ricinus communis* Linn., also known as castor oil plant is a species of flowering plant, in the family Euphorbiaceae. Although indigenous to Eastern Africa and the Mediterranean basin is distributed widely across the world and widespread in tropical regions, tropical Africa inclusive (12). It is commonly called Ogiri-Okpei and Eto adan-ukebe (the plant of enema) in the Igbo and Ibibio speaking parts of Nigeria, respectively. Its pharmaceutical and industrial uses have been reported and these are attributed to its rich phytochemical components (13).

This study was carried out to explore the *in vivo* antiplasmodial efficacy of crude aqueous seed extract of *R. communis* in *P. berghei* infected albino mice.

Materials and Methods

Plant preparation

The plant *R. communis* was obtained from a local farm in Itak Ikot Akap Village, Ikono Local Government Area, Akwa Ibom State, Nigeria, in November, 2016. Taxonomic keys for the identification of this plant were obtained from the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. Herbarium specimen with voucher number UUPH31P was prepared and deposited in the herbarium of the same department, for future referencing. The seeds of *R. communis* were thoroughly washed and air–dried for 21 days. This was followed by pulverization and cold extraction, for 48 hours (with periodic stirring), using distilled water, as the extraction solvent. The crude liquid extract was obtained by filtration thrice using a sterile muslin cloth and twice using Whatman No. 1. filter paper and non-absorbent cotton wool in a filter funnel. The liquid extract was subsequently concentrated to dryness, *in vacuo*, at a temperature of 40°C, using a rotary evaporation. The concentrated extract obtained was stored in a refrigerator prior to use.

Experimental animals

Swiss albino mice of both sexes weighing 15–29 g were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. They were maintained according to standard guidelines and fed with animal feed pellets and water *ad libitum*.

Acute toxicity study

This was carried out to determine the safety profile of the seed extract. Doses of the aqueous seed extract of *R. communis* ranging from 50-5000 mg/kg were administered through the oral route to Swiss albino mice. Mice were first of all divided into 7 groups of 3 mice each. The animals were observed for visible signs of toxicity and death. The median lethal dose (LD_{50}) was determined using the method of Lorke (14) as:

$$LD_{50} = \sqrt{AB}$$

Where A = Maximum dose producing 0% mortality
B = Minimum dose producing 100% mortality

Parasite species used for the study and preparation of inoculum

Parasites used for the experiments reported in this study were *Plasmodium berghei berghei* (Strain no. NK 65 merozoites). They were obtained from Nigeria Institute for Medical Research (NIMR), Lagos. These parasites were maintained by sub passage in mice.

Blood samples were obtained from the parasitized donor mouse via cardiac puncture. This was stored in sterile heparinized bottle. The inoculum was prepared by diluting 2 mL of the parasitized blood with 10 mL of sterile normal saline, to obtain the final inoculum of 0.2 mL (1.0×10^7), which is the standard inoculum for the infection of a single mouse (15, 16).

Preparation of drugs and stock solution of the seed extract

The purity of the standard drugs (artesunate and pyrimethamine) were ascertained by identification test and melting point determination described by WHO in the International Pharmacopoeia (17).

Artesunate (50 mg) was dissolved in 100 mL distilled water while pyrimethamine (25 mg) was dissolved in 10ml distilled water to obtain the stock solutions, from which doses of 5 mg/kg/d and 1.2 mg/kg/d of artesunate and pyrimethamine respectively, were prepared for subsequent administration. Values for 10%, 20% and 30% of the median lethal dose (LD_{50}) were determined and used as the doses for the study.

Determination of antiplasmodial activity

Suppressive test

The suppressive activities of the seed extract and the standard drug, artesunate were evaluated using the method of Okokon and Nwafor (18). Thirty albino mice were used for the study and were randomly divided into five groups of 6 mice each. On the first day (D_0) each mouse received (intraperitoneally) 0.2 mL of infected blood containing *P. berghei*. Thereafter, mice in groups 1, 2 and 3 received 54.77, 109.54 and 164.32 mg/kg/d of the seed extract respectively, via the oral route. Mice in group 4 received 5 mg/kg/d of artesunate while group 5 mice received 10 mL/kg/d of distilled water (control). All administrations (extract, drug and distilled water) were done daily for 4 days. On the fifth day (D_4), thin blood smears were made on microscope slides, using blood obtained from the tip of the tail of each mouse. The smears were stained with Giemsa stain and examined under the oil immersion objective of a microscope to determine the extent of

parasite clearance. This was done by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Formulae used were:

$$\%\,parasitaemia = \frac{No.\ of\ parasitized\ erythrocytes}{Total\ No.\ of\ erythrocytes\ counted} \times \frac{100}{1} \quad (1)$$

$$Average\ percentage\ chemosuppression = \left[\frac{A-B}{A}\right] \quad (2)$$

Where *A* is the average percentage parasitaemia in the control group and *B* is the average percentage parasitaemia in the test group.

Prophylactic test

Mice were treated before being infected with the parasite *P. berghei berghei.* Thirty mice were also used for this test and they were divided into 5 groups of 6 mice per group. Albino mice in groups 1, 2 and 3 received the same treatment as in the suppressive model except that mice in group 4 each received 1.2 mg/kg/d of pyrimethamine as the standard drug. They received treatment for 3 days. On the fourth day (D_3), each mouse was infected Intraperitoneally with 0.2 mL of blood containing 1.0×10^7 *P. berghei berghei* parasitized erythrocytes. After 72 hours of parasite inoculation, blood samples were obtained from the tip of the tail of each mouse to assess the prophylactic effect of each treatment. This was done by counting the parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. The average percentage of chemosuppression was determined as described in the suppressive test (18).

Curative test

The method of Tekalign et al (19) was adopted in the evaluation of the schizonticidal potential of the aqueous seed extract of *R. communis*, during established infection. Thirty mice were used for this study. They were divided into 5 groups of 6 mice per group. Each mouse was infected intraperitoneally with the standard inoculum of 1.0×10^7 *P. berghei berghei* parasitized erythrocytes on the first day (D_0). Mice were left for 72 hours for parasitaemia to be established. Mice in the different groups received same dosages of extract, artesunate and distilled water as stated in the suppressive model. Treatment lasted for 5 days. Blood samples were collected from the tip of the tail of each mouse on alternate days within the treatment period (i.e., D_2, D_4, D_6). Blood samples collected were used to prepare thin films and observed for schizonticidal efficacy of the extract and drug, as described for the suppressive test.

The mean survival time (MST) was determined for each group, over a period of 30 days, using the formula:

$$MST = \frac{Number\ of\ days\ survived}{Total\ number\ of\ days\ (30)} \times \frac{100}{1} \quad (3)$$

Data analysis

Results were expressed as multiple comparisons of mean ± standard error of the mean (SEM). Significance was determined using one-way analysis of variance (ANOVA). A probability level of 5% or less was taken as significant.

Results

LD_{50} value of the seed extract of *Ricinus communis*

The medium lethal dose (LD_{50}) of the aqueous seed extract of *R. communis* was 547.72 mg/kg.

Antiplasmodial activity

After oral treatment with the various doses of the seed extract of *R. communis*, there was significant decrease in parasitaemia in all models. This decrease was observed to be dose-dependent (Tables 1, 2 and 3).

In the suppressive test, the doses of the aqueous extract of *R. communis* used in this test which were 54.77, 109.54 and 164.32 mg/kg resulted in suppressive effect of 20.80, 49.00 and 75.00%, respectively (Table 1). This decrease in parasitaemia was significant when compared with the control ($P<0.05$). Artesunate produced the highest chemosuppressive effect of 88.40%.

In the prophylactic test, average percentage chemosuppression of 9.47, 21.58 and 51.42% was obtained and the doses used were 54.77, 109.54 and 164.32 mg/kg of the extract, respectively. The decrease in parasitaemia observed was also significant when compared with the control group ($P<0.05$). The use of the standard drug (pyrimethamine) resulted in a decrease in parasitaemia (72.26%) and this decrease was also significant ($P<0.05$), when compared with the results obtained in the control group (Table 2).

In the curative test, there was a progressive dose- and time-dependent reduction in parasitaemia on

Table 1. Suppressive effect of the aqueous seed extract of *Ricinus communis* on *Plasmodium berghei* in infected mice

Treatment	Dosage (mg/kg)	Mean parasite density x 10^7	Mean % chemosuppression
Distilled water (control)	10	149.0 ± 0.28	-
Artesunate	5	17.30 ± 0.92*	84.40
R. communis	54.77	118.00 ± 0.35*	20.80
R. communis	109.54	76.00 ± 0.59*	49.00
R .communis	164.32	37.30 ± 0.73*	75.00

Values are expressed as mean ± SEM, n=6.
*Significant ($P < 0.001$) compared with control.

Table 2. Prophylactic effect of the aqueous seed extract of *Ricinus communis* on *Plasmodium berghei* in infected mice

Treatment	Dosage (mg/kg)	Mean parasite density x 10^7	Mean % chemosuppression
Distilled water (control)	10	190.0 ± 0.12	-
Pyrimethamine	1.2	52.7 ± 0.74*	72.26
R. communis	54.77	172.0 ± 1.32*	9.47
R. communis	109.54	149.0 ± 0.19*	21.58
R .communis	164.32	92.3 ± 1.37*	51.42

Values are expressed as mean ± SEM, n=6.
*Significant ($P < 0.001$) compared with control.

Table 3. Antiplasmodial effect of the aqueous seed extract of *Ricinus communis* during established infection (curative effect)

Treatment	Dosage (mg/kg)	% Reduction in parasitaemia/day Day 2	% Reduction in parasitaemia/day Day 4	% Reduction in parasitaemia/day Day 6
Distilled water	10	135.0 ± 1.17	147.0 ± 0.56	158.0 ± 1.12
Artesunate	5	102.0 ± 0.76*	83.3 ± 1.52*	16.7 ± 0.56*
R. communis	54.77	119.0 ± 0.57*	70.7 ± 0.76*	32.7 ± 0.74*
R. communis	109.54	82.7 ± 0.92*	63.3 ± 1.38*	21.0 ± 0.53*
R. communis	164.32	74.7 ± 0.56*	60.43 ± 0.43*	16.0 ± 0.36*

Values are expressed as mean ± SEM, n=6.
*Significant ($P < 0.001$) compared with control.

administration of the extract as revealed by the results of the curative test (Table 3) compared to control group. No curative effect was observed in the control, rather an increase in parasitaemia was observed.

Mice in extract treated groups survived longer (11.4 ± 0.21 and 14.3 ± 0.92 days) than mice in the control group (10.3 ± 0.21 days). Mice treated with artesunate survived longest for 20.7 ± 0.47 days (Table 4).

Discussion

Plasmodium berghei berghei is used in predicting treatment outcomes of any suspected antimalarial plant, due to its high sensitivity, making it the appropriate parasite for antiplasmodial study (20). The median lethal dose (LD_{50}) of the aqueous seed extract of *R. communis* which was established at 547.72 mg/kg indicates that the extract is only slightly toxic, according to Loomis and Hayes (21). Thus, it is relatively safe. Ezenobi et al (13) in their research on the phytochemical composition of *R. communis* seeds reported that phytochemicals in the seeds included alkaloids, saponins, flavonoids, tannins, phenols and steroids. The pharmacological activity of the seeds of this plant has been attributed to the presence of these phytochemicals. For instance, it is reported that the seeds of *R. communis* possess antimicrobial activity against important pathogenic organisms (22).

Table 4. Mean survival time of mice treated with aqueous seed extract of *Ricinus communis*, artesunate and distilled water (control)

Treatment	Dosage (mg/kg)	Mean survival time (days)
Distilled water	10	10.3 ± 0.21
Artesunate®	5	20.7 ±0.92**
R. communis	54.77	11.4 ± 0.21
R. communis	109.54	12.8 ± 0.42*
R. communis	164.32	14.3 ± 0.73**

Values are expressed as mean ± SEM, n=6.
Significance relative to control: *$P < 0.05$; **$P < 0.001$.

The antimalarial properties of a large number of medicinal plants have been attributed to the presence of alkaloids (23,24). Saponins are known to inhibit the growth of *P. falciparum* (25) and to elicit antimalarial efficacy (26). Saponins have also been found to be detrimental to many infections protozoans (27). The antimalarial potential of plant products containing flavonoids had earlier been reported. It has also been reported that flavonoids exhibited significant antiplasmodial activity against different strains of malaria parasite. Although the mechanism of action of the seed extract of *R. communis* has not yet been elucidated, the secondary metabolites present in the extract may have elicited the observed antiplasmodial activity either singly or in synergy (28,29). The decrease in parasitaemia observed in all models in this study is an indication that the seeds of *R. communis* have antiplasmodial potentials. A compound is considered active when percentage suppression in parasitaemia is 30% or more (30). Thus, the 49 and 75% chemosuppression obtained when extract doses 109.54 and 164.32 mg/kg respectively, were used in the 4-day test, for instance is appreciable. That the various doses of the seed extract could not produce suppression comparable to that of the standard drugs (artesunate and pyrimethamine), could be

attributed to the fact that the extract is in its crude form. It is hoped that purified fractions and subsequent elucidation of the active principle(s) in the seed of *R. communis* would give better results (improved performance).

The observed mean survival time (MST) of mice treated with various doses of the extract which was longer when compared with the control group, treated with distilled water is attributed to the antiplasmodial efficacy of the extract. However, the group treated with the standard drug (artesunate) survived for longer duration than the extract treated groups.

Conclusion

The aqueous seed extract of *R. communis* demonstrated appreciable suppressive, prophylactic and curative antiplasmodial activities *in vivo* and these effects were dose-dependent and significant when compared to the control group. The extract-treated groups survived longer than those of negative control group. These depict the antiplasmodial effectiveness of the extract. The plant is relatively safe and may be exploited in the ongoing fight against malaria.

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Author's contributions

UP designed the study, interpreted the results, and wrote the manuscript. EE did statistical analysis of the results and reviewed manuscript. UE did the experiments. IE and RS assisted in the writing of the manuscript. All read and confirmed the final edition for publication.

Conflict of interests

Authors declare that no competing interest exists.

Ethical considerations

All experiments involving the use of these mice were approved by the Animal Ethics Committee of the Faculty of Pharmacy, University of Uyo, Nigeria (UUP 17).

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