

EVALUATION OF THE ANTIPLASMODIAL POTENTIAL OF THE AQUEOUS LEAF EXTRACT OF *Ricinus communis* L (EUPHORBIACEAE)

ABSTRACT

AIM: As the problem of resistance to antiplasmodial agents persists, the need to develop new and more effective drugs continue to arise. The antiplasmodial potential of the aqueous leaf extract of *Ricinus communis* using Albino mice injected with Plasmodium berghei NK65 was studied using the suppressive, prophylactic and curative models.

METHODS: Animals were divided into five groups consisting of six mice each. Artesunate (5mg/kg/day) and pyrimethamine (1.2mg/kg/day) served as positive controls while distilled water (10ml/kg/day) served as negative control. The extract was administered at doses of 73, 145 and 217mg/kg/day through the intraperitoneal route.

RESULTS: Results obtained showed that the extract achieved a Chemosuppression of 81.50% 89.90% and 92.90% which are greater than that achieved by the standard drug pyrimethamine in the prophylactic model experiment. **CONCLUSION:** The leaf of *R. communis* has good potentials for the development of a new antiplasmodial.

Key words: Antiplasmodial; *Plasmodium berghei*; Extract; Chemosuppression; *Ricinus communis*

INTRODUCTION

Malaria is a haematological disease caused by a protozoan of the genus Plasmodium and transmitted through the bite of an infected female anopheles mosquito. Malaria is one of the common killer diseases of tropical Africa with pregnant women and children under the age of five being the most vulnerable (Olashinde *et al*, 2010). The World Health Organisation (WHO) malaria report in 2019 confirmed that an estimated 228 million cases of malaria occurred worldwide in 2018 with 405,000 deaths within the same period with the African region accounting for 94% of deaths. It is one of the major public health problems in Nigeria contributing to a quarter of the malaria burden in Africa. The burden of malaria has remained high in most African countries [Griffin *et al*, 2010] and the emergence of malaria resistance to artemisin derivatives has become one of the greatest challenges to its control and elimination (Ariey *et al*, 2014). This resistance has been reported to be caused by a nucleotide polymorphism in parasites K13 gene which leads to deregulated protein response. This has manifested in slow parasite clearance in patients and is observed as increased survival of early ring stage parasites *in-vitro*. (Fairhurst and Dondorp, 2016)

Malaria is usually associated with poverty (Francesco Ricci, 2012) and has a major effect on economic development (Oriol *et al*, 2013). These facts along with the high cost of

orthodox medicine, have led most African countries to encourage the use of herbs in the treatment of malaria.

R. communis belongs to the Euphorbiaceae family and is widely distributed across the world (Eudinar *et al*, 2001). It invades grassland and farmlands (Weber, 2003). Although it is indigenous to Eastern Africa and the Mediterranean basin, today, it is widespread in tropical regions. The leaves of *R. communis* are alternate, curved and cylindrical ovate. They have found so much application in traditional medicine in so many countries where they are found. The leaves, for example, are used to relieve flatulence in children (Haider *et al* 2018), while they are recommended in the form of a decoction for women as a lactagogue (Bentley, 2018). Different parts of the plant and seed have also been used for so many other purposes due to a wide range of activities which they exhibit including antiasthmatic, anti-fertility, anti-inflammatory, wound healing, antiulcer and anti-diabetic activities.(Sarfraz *et al*, 2017). Remarkably, three unique peptides referred to as RCB 1-3 which have both antibacterial and antifungal properties have been reported [Delgarbat *et al*, 2015].

MATERIALS AND METHODS

Plant collection and Identification

Fresh leaves of *R. communis* were collected from a farm at Itak Ikot Atap village in Ikono local Government Area of Akwa Ibom State, Nigeria. The plant was identified with the aid of taxonomic keys provided by the Department of Pharmacognosy and Natural Medicine, the University of Uyo where a sample with herbarium number UUPH31P was deposited for future reference.

Plant extraction

Leaves of *R. communis* were washed, air dried for 18 days and then pulverized using a mortar and pestle. Three hundred gramme (300g) of the powdered leaves were soaked in 1L of water for 72 hours. The liquid extract was then obtained by filtration using a clean muslin cloth. This was further filtered using a grade1 Whatman filter paper and the filtrate was then evaporated to dryness in vacuo at 4°C. The extract obtained was stored in the refrigerator at 4°C until used.

Animals

Male and female albino mice weighing 20-25g were used. They were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. The animals were kept in standard cages with proper ventilation, fed with feed from Grand cereal limited, Jos, Nigeria and given clean drinking water *ad libitum*. Approval for the use of animals in the experiment was granted by the animal ethics committee of the Faculty of Pharmacy, University of Uyo, Nigeria

and all the animal experiments followed the ethical standards for the care and use of laboratory animals. (Guide for the Care and Use of Laboratory Animals, 2011)

Phytochemical studies

The leaf extract of *R. communis* was screened for the presence of certain phytochemical constituents using the methods described by (Harborne, 1984)

Acute toxicity studies

This was done using a modified Lorke method to determine the safety of the leaf extract using Swiss albino mice. The experimental animals were divided into three groups with three mice in each group. Each group was administered with different doses of the extract from 500, 1000, 1500 up to 5000mg/kg body weight. The animals were then observed within a 24 hour period for visible signs of toxicity and death. The median lethal dose (LD₅₀) was calculated using the method of Lorke (1983) thus:

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

Where A=Maximum dose producing 0% mortality

B= Minimum dose producing 100% mortality

Parasite

Plasmodium berghei (NK65) obtained from the National Institute for Medical Research (NIMR) Lagos was used for the study. They were preserved by the serial passage of 0.2mL parasitized blood from an infected mouse to an uninfected mouse

Parasite inoculation

The mice used for the experiments were first confirmed to be free from malaria parasites by the absence of these parasites in the smears made with blood obtained from the tails of the animals. Those confirmed to be free were then used for the experiments by the administration of 0.2mL (1.0×10^7) parasitized blood for the infection of each mouse (Etebong et al, 2015).

Antiplasmodial activity studies

Suppressive activity

The 4- day suppressive test described by Peters et al., (1975) with modifications was used to evaluate the suppressive activity of the leaf extract. Thirty (30) mice were randomly allocated to five groups of six mice each. On day1 (D₀), 0.2mL standard inoculum containing approximately 1×10^7 parasitized erythrocyte was administered through the intraperitoneal route to all the mice. Groups 1-3 were given 73, 145 and 217mg of the of the leaf extract per kg/day respectively. Group 4 received 5mg/kg/day of Artesunate (Standard drug) while group 5 which served as the control received 10ml/kg bodyweight of distilled water. This continued at the same time for the next 4 days. On the fifth day (D₄) thin blood smears using blood from the tail tip of each mouse in the model were made on microscopic slides. The slides were Giemsa stained and examined under the microscope after which the percentage suppression was determined at each dose.

Average percentage suppression was calculated below

Average % parasitemia in negative control - Average % parasitemia in positive control

Average % parasitemia in negative control

Prophylactic or Repository activity

The method described by Okokon and Nwafor (2009) with modifications was employed for this experiment. Thirty (30) albino mice were randomly divided into five groups of six mice each. Groups 1-3 were administered 73, 145 and 217mg/kg of the extract while group 4 and 5 received 1.2mg/kg/day of pyrimethamine (Positive control) and 10mL/kg of distilled water (Negative control). The drug and extract administrations continued at the same time of the day for three days. On the fourth day, the mice were injected with 0.2mL of infected blood containing approximately 1×10^7 parasitized erythrocytes through the intraperitoneal route. After 72 hours, thin blood films were prepared from blood obtained from the tail of each mouse on a slide. The slides were Giemsa stained to show parasitized erythrocytes in fields observed under the microscope and the percentage suppression due to its prophylactic activity was determined at each dose.

Curative Activity

This test was employed to determine the schizontocidal activity of the extract after a confirmed infection and the method described by Okokon and Nwafor (2009) with modifications was employed. 0.2mL blood containing approximately 1×10^7 parasitized erythrocytes was administered to the mice through the intra peritoneal route and left for 72 hours. Before the administration of the extract, blood was taken from the tail of each mouse and the level of parasitaemia determined. Mice in groups 1-3 were given 73, 145

and 217mg/kg/ of the leaf extract respectively. Those in group 4 were given 5mg/kg of Artesunate while those in group 5 which served as the negative control group were given 10ml/kg of body weight of distilled water. The administration of the extract, distilled water and standard drug was done once daily for five days after which blood samples were collected from the tail of each mouse on each day of the treatment. The blood was Giemsa stained and the effect of the extract treatment determined by monitoring parasitaemia level. The **mean survival time (MST)** which is the period of survival (in days) of each group of mice after the administration of extracts and standard drugs over a period of 30 days was calculated thus

$$\text{MST} = \frac{\text{No of days survived}}{\text{Total No of days (30)}} \times 100$$

Evaluation of parasitaemia

The parasitaemia levels were determined using the method described by (Knight and Peters, 1980). The number of parasitized erythrocytes out of 200 in random fields of the microscope was noted and percentage parasitaemia was calculated thus

$$\frac{\text{Average \% Parasitaemia in negative control} - \text{Average \% Parasitaemia in positive control}}{\text{Average \% parasitaemia in negative control}}$$

Statistical analysis

The one-way analysis of variance (ANOVA) was used for the analysis of data which was presented as the mean of six dimensions \pm SEM while the Turkey Kramer post hoc was employed to check for multiple comparisons. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS

Phytochemical Constituents

Results obtained showed the presence of saponins, alkaloids, flavonoids and glycosides in the aqueous leaf extract of *Ricinus communis*

Acute toxicity (LD₅₀) test

On the administration of varying doses of the extract, different signs of toxicity including convulsion and then death were observed. The animals which received 750-5000mg/kg body weight of the extract showed these visible signs of toxicity and died while those which received 700mg/kg all survived. The (LD₅₀) median lethal dose which is the geometric mean of the maximum dosage that produced zero percent lethality and the minimum dosage that produced 100% mortality was thus calculated to be 724.57mg/kg.

Pre-screening

Animals that were pre-screened for the prophylactic test had parasite count within the range of 59-69.5% while those pre-screened for the curative test had parasite count of 74-83.5%

Evaluation of Suppressive effect

Results obtained in this model showed a percentage chemo suppression of 47.80, 53.20 and 68.90% at doses of 73, 145 and 217mg/kg respectively, while Artesunate (standard drug) produced suppression of 88.0%. (Table 1)

Table1: Suppressive activity of aqueous leaf extract of *R. Communis* in mice infected with *Plasmodium berghei*

| Treatment | Dose(mg/kg) | Parasitaemia | Percentage suppression |
|-----------------|--------------|---------------------------|------------------------|
| Leaf Extract | 73 | 77.7± 2.43 ^{a,b} | 47.80 |
| Leaf Extract | 145 | 67.9±2.56 ^{a,b} | 53.20 |
| Leaf Extract | 217 | 46.3± 1.80 ^{a,b} | 68.90 |
| Artesunate | 5 | 17.3± 0.92 ^a | 88.0 |
| Distilled water | 10mL/kg | 149.0± 2.28 | - |

Values are expressed as mean± SEM, ^a=p<0.05 (Significant relative to control (distilled water)), ^b=p< 0.05 significant relative to control (artesunate), n=6 Turkey Kramer (Post hoc), ANOVA

Evaluation of Propylactic effect

The extracts on administration to the *P. berghei* produced chemosuppression of 81.50, 89.90 and 92.90% at doses of 73, 145 and 217mg/kg respectively, while Pyrimethamine (standard drug) caused a suppression of 63.9%. (Table 2)

Table 2: Prophylactic activity of aqueous leaf extract of *R. communis* in mice infected with *Plasmodium berghei*

| Treatment | Dose(mg/kg) | Parasitaemia | Percentage suppression |
|-----------------|-------------|---------------------------|------------------------|
| Leaf Extract | 73 | 27.0± 3.52 ^{a,c} | 81.50 |
| Leaf Extract | 145 | 14.7±0.92 ^{a,c} | 89.90 |
| Leaf Extract | 217 | 10.3± 0.76 ^{a,c} | 92.90 |
| Pyrimethamine | 5 | 52.7± 2.70 ^a | 63.90 |
| Distilled water | 10mL/kg | 146.0± 3.600 | - |

Values are expressed as mean± SEM, ^a=p<0.05 (Significant relative to control (distilled water)), ^b=p< 0.05 significant relative to control (pyrimethamine), n=6, Turkey Kramer (Post hoc), ANOVA

Evaluation of curative effect

Results obtained showed that after 6 days post administration of extract a significant decrease in parasitaemia ($p < 0.05$) was observed (Table 3.)

Table 3: Curative activity of aqueous leaf extract of *R. Communis* in mice infected with *Plasmodium berghei*

| Treatment | Dose(mg/kg) | Parasitaemia Levels | | |
|-----------------|-------------|--------------------------|----------------------------|----------------------------|
| | | D2 | D4 | D6 |
| Leaf Extract | 73 | 125.0± 0.56 ^c | 122.0± 1.10 ^{a,b} | 102.0± 1.38 ^{a,b} |
| Leaf Extract | 145 | 123.0±0.15 ^a | 117.0± 0.76 ^{a,b} | 79.7± 0.69 ^{a,b} |
| Leaf Extract | 217 | 121.0± 0.73 ^a | 113.0± 0.81 ^{a,b} | 53.0± 1.12 ^{a,b} |
| Artesunate | 5 | 122.0± 0.76 ^a | 103.0± 0.52 ^a | 26.7± 0.39 ^{a,b} |
| Distilled water | 10mL/kg | 135.0± 0.86 | 147.0± 1.18 | 158± 0.88 |

Values are expressed as mean± SEM, ^a= $p < 0.05$ (Significant relative to control (distilled water)), ^b= $p < 0.05$ significant relative to control (artesunate), n=6 Turkey Kramer (Post hoc), ANOVA

Mean survival time

A significant ($p < 0.05$) increase in the mean survival time was observed when the result of the extract administration was compared with the negative control (Table 4)

Table 4: Mean Survival time of mice infected with *Plasmodium berghei* after treatment with different doses of the aqueous leaf extract of *R. communis*

| Treatment | Dose(mg/kg) | Survival Time(Days) |
|-----------------|-------------|---------------------|
| Leaf Extract | 73 | 10.7±0.33 |
| Leaf Extract | 145 | 13.0±0.58 |
| Leaf Extract | 217 | 14.7± 0.67 |
| Artesunate | 5 | 21.7± 0.88 |
| Distilled water | 10mL/kg | 8.67± 0.33 |

Values are expressed as mean± SEM, ^a= $p < 0.05$ (Significant relative to control (distilled water)), ^b= $p < 0.05$ significant relative to control (artesunate), n=6 Turkey Kramer (Post hoc), ANOVA

DISCUSSION

While malaria has continued to be a significant health problem especially in the tropics, plant products which are known to be a rich source of bioactive chemicals have continued to give scientists hope of the development of novel antiplasmodial drugs for its control (Homburger, 1989; Das *et al*, 2007)

Phytochemical analysis of the extract showed the presence of alkaloids, saponins, flavonoids and glycosides. Several of these phytochemicals are known to be responsible for antimalarial activity. Flavonoids for instance have been reported to have antiplasmodial activity which they exhibit by inhibiting the fatty acid biosynthesis of the parasite (Freundlich *et al*, 2005). They have also been reported to chelate with the nucleic acid base pairing of malaria parasites (Etebong *et al*, 2015). Alkaloids have been reported to show antimalarial properties by blocking protein synthesis in plasmodium (Dawodu *et al*, 2014). Saponins and tannins act as primary antioxidants or free radical scavengers which can counteract oxidative damage induced by malaria parasite (David *et al*, 2004). This antioxidative property is believed to present yet another mechanism that

contributes to antiplasmodial activity (Abdulelah et al, 2010). These secondary metabolites may be responsible for the observed antiplasmodial activity.

The greatest problem with the use of plant parts extract in the management of most infections has been that of toxicity. Results obtained however showed that the plant extract was only slightly toxic. This is confirmed by the fact that the experimental animals which received doses of the extract above the LD₅₀ (724.57mg/kg) first showed visible signs of toxicity and then died.

A decrease in parasitaemia with an increasing dose levels of the extract was observed through the experiment with the highest dose (217.37mg/kg) showing highest chemosuppression confirming a dose dependent activity of the extract.

Although the standard drug artesunate produced a higher percentage of suppression (Table 1) the effect due to the extract is significant ($p < 0.05$) relative to the control. It is important to note that the extract used for the experiments are still in the crude form. When the active compounds responsible for this activity are obtained and purified, their effect will no doubt be better.

The result of the prophylactic test showed a better effect of the extract than the standard drug pyrimethamine (Table 2). This highlights the good potentials of the extract as a prophylactic agent.

Upon established infection there was also an observed significant ($p < 0.05$) reduction in parasitaemia level at various doses of the leaf extract when compared with the control

(Artesunate) (Table 3). Altogether, these significant reduction in parasitaemia levels by the extract in all the models of experiments, translated into a longer mean survival time when compared with water as a control (Table 4). Generally, a chemical compound is considered to be active when it is able to achieve a percentage parasitaemia suppression of at least 30% (Adugna *et al*, 2014). The chemosuppression effect of the aqueous leaf extract used in the study against *P. berghei* was found to be above 30% (tables1-3) and is therefore considered effective when used either for prophylactic or curative purposes.

CONCLUSION

Results obtained from this study showed that the leaf extract of *R. communis* has a significant suppressive, prophylactic and curative antiplasmodial activity when compared to the standards used. This explains why the people of Itak Ikot Akap village in Ikono Lcal Government Area of Akwa Ibom State, Nigeria use this leaf extract as an antimalarial. The dose dependent activity is believed to be due to the presence of certain secondary metabolites found in the leaves which could serve as a lead for the development of new and effective antiplasmodial.

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CONFLICT OF INTEREST

There are no conflict of interest regarding the publication of this paper

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