Pharmacological Potential of Asthma Weed (Euphorbia hirta) Extract toward Eradication of Plasmodium berghei in Infected Albino Mice

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ABSTRACT
Malaria, a mosquito borne infectious disease of human and other animals affects a large number of the world’s population, and it’s caused by parasitic protozoan of the genus plasmodium. The search for new antimalarial chemotherapies has however become urgently increasing due to the parasite resistance to available drugs. Asthma weed (Euphorbia hirta) which is a natural therapeutic herb used for the management of gastrointestinal disorders, respiratory diseases and other ailments was therefore investigated in order to establish the whole plant aqueous-methanolic extract in vivo antiplasmodial effects on chloroquine-sensitive Plasmodium berghei in infected mice, using the suppressive and prophylactic test models.

The in vivo antimalarial activity of the Euphorbia hirta extract doses (200, 400 and 800 mg/kg body weight) against P. berghei showed that it has significant (p<0.05) suppressive activity of 51 – 59 % and prophylactic activity of 25 – 50 % when compared with Chloroquine that gave 95 and 81 % suppressive and prophylactic antiplasmodial activities respectively. Result further showed that antiplasmodial action may not be through the oxidation of red blood cell membrane lipids as increasing extract concentration results in the reduction of the enzymatic activities of SOD and GPx, and concentrations of GSH and TBARS. We assumed that the antiplasmodial effect of the extract may have been contributed by its phytochemical components. This thus suggests that E. hirta plant extract has a very good potential pharmacological activity, and this could be beneficial in the development of new drugs for treatment or prophylaxis against malaria.

Keywords: Antimalaria, Chemosuppression, Erythrocytes, New Drug Discovery, Phytochemicals, Prophylactic Effect

INTRODUCTION
Malaria constitutes one major public health problems in tropical Africa, and it is estimated that over 250 million Africans are infected by malaria parasites with nearly 90 million clinical cases annually¹. Deaths occurring from this African endemic diseases have increased to an estimated of 2 million every year¹. A number of preventive medications are available to travelers into malaria-endemic countries (prophylaxis). However, the occurrences of multi-drugs resistance result to reduction in the effectiveness of these drugs in curing malaria or improving patients' symptoms, consequently leading to serious impediments to improved health care issues. These diversities of resistance types will require that public health measures to control malaria are regionally attended to². This urgency generated by drug-resistant strains of malaria parasites has thus accelerated anti-malarial drug research over the last two decades³. While synthetic pharmaceutical agents continue to dominate research, attention has increasingly been directed to natural products⁴. The success of quinine (QN) and artemisinin, isolated from Artemisia annua and its derivatives, for the treatment of resistant malaria has focused attention on plants as a source of anti-malarial drugs⁵. Moreover, plants have been the basic source of sophisticated traditional medicine systems for thousands of years and were instrumental in early pharmaceutical drug discovery and industry⁶. The world’s poorest are the worst affected, and many treat themselves with traditional herbal medicines. These are often more available and affordable, and sometimes are perceived as more effective

Figure 1: A typical Euphorbia hirta plant¹³.
than conventional anti-malarial drugs. Plasmodium falciparum, the most widespread etiological agents for human malaria has been reported to be increasingly resistant to standard antimalarial drugs and this situation necessitates a continued effort to search for new drug entities, particularly with novel modes of action. The family Euphorbiaceae consists of 2000 species. The genus Euphorbia is the largest genus of medicinal plants widely distributed in most parts of the China and Pakistan. The Euphorbias are characterized by the presence of milky latex which is toxic. It can grow to a height of 40 cm. The plant; Euphorbia hirta, is commonly called Pill-bearing springe and asthma herb. In indigenous Nigerian folks it is called Ege-ile or Emi-ile (in Yoruba), Udani (in Igbo) and Nonan kurchiya (in Hausa). It is also known as Rooi euphorbia in South Africa. E. hirta has been reported to be beneficial in the treatment of gastrointestinal tract disorders, respiratory and bronchial diseases and in conjunctivitis. The Terpenoids present in E. hirta was reported for various biological activities such as antitumor and anticanancer, anti-inflammatory and antiviral/antibacterial. The Flavonoids were found to confer beneficial biological activities such as antimutagenic, anticarcinogenic, immune-stimulating and anti-inflammatory and arterosclerosis inhibiting effects on human. The polyphenols were reported to act as antioxidant, inhibiting lipid peroxidation and scavenging superoxide. From the aforementioned biological effects of E. hirta on human health and coupled with its traditional medical uses among native Nigerian folks as an antimalarial plant, we therefore see the imperative need to scientifically evaluate the potentials of extracts from E. hirta in order to validate its antimalarial potentials and possibly for wider acceptability as an antimalarial remedy.

**Table 1**: Relative phytochemical constituents of various solvent extract of Euphorbia hirta

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Aq-Met</th>
<th>Met</th>
<th>Aq</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Where: + = presence; - = absence; Aq-Met = Aqueous-Methanolic; Met = Methanolic; Aq = Aqueous

**MATERIALS AND METHODS**

**Animals Used and Parasites Maintenance**

Albino Swiss mice (12-25g) of either sex also obtained from Nigeria Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, were used for this study. The animals were kept in cages at standard room temperature and relative humidity (25°C and 50 %, respectively) and under naturally illuminated environment of 12:12 hour dark/light cycle. The animals were used in accordance with National Institutes of Health (NIH) Guide for the care and use of laboratory animals. The Plasmodium berghei strain used was also obtained from NIMR Lagos, Nigeria where the parasites are maintained by weekly hosting in Albino Swiss mice.

**Phytochemicals Screening**

Standard screening tests were carried on the plant extracts out for the presence of various phytochemicals such as alkaloids, anthraquinones, flavonoids, saponins, steroids, tannins and terpenoids, using standard procedures as described in. The healthy E. hirta plants were uprooted from field and thoroughly washed in distilled water blotted dry in-between clean sterile paper towel and oven dry in Genlab Oven (Genlab Ltd, Widnis, Cheshire, WA8 OSR) at 50°C until a constant weight was achieved. The dried plants were ground in electric blender (Waring Products Division, Torrington, USA), resulting fine powder were stored in air tight properly labeled container until further use. The dried ground whole plants of E. hirta (500g) were extracted with mixture of water and methanol at a ratio of 3:1.
After 72 hours, the method—treated cells on a stainless metallic feeding cannula were orally administered to the albino mice with the aid of a positive control (chloroquine 10 mg/kg/day and the infected group). The animals were divided into groups of six mice each and were orally administered with 200, 400 and 800 mg/kg/day doses of the E. hirta whole plant aqueous-methanolic extract, chloroquine 10 mg/kg/day and the infected group has access to water for four consecutive days (day 0 to day 3). On the fifth day (day 4), thick films were made from blood from the tail of each mouse and fixed with methanol, stained with Giemsa and parasitaemia was determined by manually counting the parasitized red blood cells on at least 500 red blood cells. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected group with those of treated mice.

**Evaluation of Repository Activity of Extract**

The repository activity was determined using the method described by Peters21. In this method, the mice were divided into five groups of six mice each in a cage and the animals were administered doses of 200, 400 and 800 mg/kg of extract/body weight/day, 10 mg/kg/day of chloroquine and water (for the infected group) for 4 days (day 0 to day 3). On the fifth day (day 4), the animals were inoculated with P. berthelotii. After 72 hours, the parasitaemia level was determined by thick blood smears and Giemsa-staining as described above.

**Microscopy and Percentage Parasitaemia Determination**

Parasitaemia, which is the percentage of infected erythrocytes, is used to monitor the progress of infection and recovery in infected mice. The most widely used technique for parasitaemia determination in mouse blood is manual microscopic enumeration of Giemsa-stained blood films22. In this study, parasitaemia was determined by microscopic examination of Giemsa stained blood smears taken from the tail of an infected mouse and spread on a microscope slide to make a thin film using Olympus CX21 microscope, (Olympus Corporation, Tokyo, Japan) following Cheebrough23. The parasite count was recorded and the suppression of parasitaemia was expressed as

<table>
<thead>
<tr>
<th>Drug/extract</th>
<th>Dose (mg/kg)</th>
<th>Parasitaemia count</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (Infected Group)</td>
<td>-</td>
<td>24.97±2.17*</td>
<td>-</td>
</tr>
<tr>
<td>Euphorbia hirta</td>
<td>200</td>
<td>12.24±2.44*</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>11.24±2.49*</td>
<td>55.0</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>10.20±2.05*</td>
<td>59.1</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>10</td>
<td>1.10±0.15*</td>
<td>95.6</td>
</tr>
</tbody>
</table>

Data are represented in Mean ± SE when n=5 and the values with * are significantly different from chloroquine groups and with * are significantly different from the infected group at p<0.05.
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Table 4: Levels of Thiobarbituric acid reacting species (TBARS) and Reduced Glutathione (GSH) in mice administered extracts of E. hirta during suppressive test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control groups (P. Berghii Infected)</th>
<th>Test groups (Chloroquine mg/kg)</th>
<th>(10) 200 mg/kg</th>
<th>(10) 400 mg/kg</th>
<th>(10) 800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/ml)</td>
<td>25.78±0.75</td>
<td>22.03±0.60</td>
<td>23.65±3.29</td>
<td>22.85±0.99</td>
<td>22.39±1.21</td>
</tr>
<tr>
<td>GSH (µmol/ml)</td>
<td>0.25±0.03</td>
<td>0.23±0.04</td>
<td>0.25±0.05</td>
<td>0.24±0.01</td>
<td>0.21±0.01</td>
</tr>
</tbody>
</table>

Values are statistically significant at p<0.05 along the control and test. Values are given as mean ± SEM. The data was analyzed using One-way ANOVA.

Table 5: Levels of Thiobarbituric acid reacting species (TBARS) and Reduced Glutathione (GSH) in mice administered extracts of E. hirta during repository test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control groups (P. Berghii Infected)</th>
<th>Test groups (Chloroquine mg/kg)</th>
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<th>(10) 400 mg/kg</th>
<th>(10) 800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/ml)</td>
<td>24.23±0.48</td>
<td>24.14±0.89</td>
<td>23.11±1.28</td>
<td>23.51±0.88</td>
<td>23.02±2.86</td>
</tr>
<tr>
<td>GSH (µmol/ml)</td>
<td>0.28±0.04</td>
<td>0.24±0.03</td>
<td>0.20±0.05</td>
<td>0.22±0.03</td>
<td>0.22±0.04</td>
</tr>
</tbody>
</table>

Values are statistically significant at p<0.05 along the control and test. Values are given as mean ± SEM. The data was analyzed using One-way ANOVA.

percent for each dose, by comparing the parasitaemia in the infected group with the treated one.

Average Suppression = \( \frac{APC - APT}{APC} \times 100 \)

Where APC = Average parasitaemia in the infected group.

A PT = Average parasitaemia in the test group.

Lipid Peroxidation Activity of Extract

Lipid peroxidation as evidenced by the formation of Thiobarbituric acid reactive substances (TBARS) and Hydroperoxides (HP) were measured according the method of Niehaus and Samuelsson⁹ and Jiang et al.²⁵ respectively. In brief, 0.1ml of the sample was treated with 2ml of TBA-TCA-HCl reagent in ratio 1:1:1 and placed in water bath for 15mins, cooled and centrifuged at room temperature for 10mins at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535nm and the amount of MDA was calculated using the formula;

\[
MD = \frac{OD \times V_i/V_2 \times 1.56 \times 10^5}{X/V_2} \text{ (Nmol/ml)}
\]

1.56 x 10⁵ = Molar Extinction Coefficient

OD= Absorbance Reading

V₁=Total Volume of reaction mixture

V₂=Volume of Sample

Estimation of Reduced Glutathione

Reduced glutathione (GSH) was determined by the method of Ellman²⁶. 0.4 ml 10% TCA was added to 0.2 ml of the sample, centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellman reagent (19.8 mg of 5, 5-dithiobisnitrobenzoic acid in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm and the amount of GSH is expressed per milliliter of whole blood using the formula:

\[
GSH = \frac{OD \times V_i/V_2 \times 10^5}{1.3600} \text{ (µmol/ml)}
\]

13600 = Molar Extinction Coefficient

OD= Absorbance Reading

V₁= Total volume of reaction mixture

V₂= Volume of Sample

Determination of Superoxide Dismutase and Glutathione Peroxidase

Glutathione Peroxidase (GPX) and superoxide dismutase (SOD) are the most important enzymes of the cell antioxidant defense system. However, these molecules are themselves susceptible to oxidation. Superoxide dismutase (SOD) was assayed utilizing the techniques of Kakkar et al.²⁷. A single unit of enzyme was expressed as 50 % inhibition of NBT (Nitro blue tetrazolium) reduction/min/mg protein. Glutathione Peroxidase (GPX) activity was measured by the method described by Ellman²⁶. Briefly, reaction mixture contained 0.2 ml of 0.4 M phosphate buffer pH 7.0, 0.1ml of 10 mM sodium Azide, 0.2 ml of extract, 0.2 ml glutathione and 0.1 ml of 0.2 mM hydrogen peroxide. The contents were incubated at 37°C for 10 mins. The reaction was arrested by 0.4 ml of 10% TCA, and centrifuged. Supernatant was assayed for glutathione content by Ellman’s reagent. and the activity of GPX is expressed per millilitre of whole blood using the formula:

\[
GPX = \frac{OD \times V_i/V_2 \times 10^5}{1.3600} \text{ (µmol/ml)}
\]

13600 = Molar Extinction Coefficient

OD= Absorbance Reading

V₁= Total volume of reaction mixture

V₂= Volume of Sample

SOD = \( \frac{OD \times V_i/V_2 \times 10^6}{4020} \text{ (SOD/min/mg protein)} \)

4020 = Molar Extinction Coefficient

OD= Absorbance Reading

V₁= Total volume of reaction mixture

V₂= Volume of Sample

Statistical Analysis
ids, etc, have been produce disease biological effect in symptoms observed in the human plasmodial infection of parasite in activity.

10 mg/kg/day chloroquine caused a chemo suppression of 95.6 %, which was higher than those of the 200, 400 and 800 mg/kg body weight of mice when infected with P. berghei. The highest extract suppression of the 200 mg/kg, 400 mg/kg and 800 mg/kg and Chloroquine at 10 mg/kg body weight of mice yielded 51.0%, 55.0%, 59.1% and 95.6% chemo suppression respectively when compared to the infected group. The observed highest

The aqueous methanolic extract of E. hirta showed dose dependent chemo suppressive effect at various doses in mice infected with Plasmodium berghei malaria parasite. The highest extract suppression of parasitaemia was observed at the dose of 800mg/kg body weight of mice with a mild suppression of parasite in 400mg/kg and 200mg/kg body weight of mice when compared to the infected group (water) with the highest % parasitaemia as illustrated in Table 2. Percentage suppression was observed to increase as extract concentration increased. From Table 2, the extract caused a statistically significant (P<0.05) chemo suppression of 51.0 %, 55.0 % and 59.1 % for the 200, 400 and 800 mg/kg/day respectively when compared to the infected group. The standard drug, chloroquine caused chemo suppression of 95.6 %, which was higher than those of the extract treated groups and significantly different from the values of the extract.

DISCUSSION

The therapeutic properties ascribed to most medicinal plants have been linked to the presence of phytochemical compounds contained in them. Phytochemicals such as alkaloids, terpenes, sapomins, flavonoids, steroids, tannins and terpenoids as shown in Table 1.

The results of the 4-day suppressive study of the extract E. hirta showed dose dependent chemo suppressive effect at various doses in mice infected with Plasmodium berghei malaria parasite. The highest extract suppression of parasitaemia was observed at the dose of 800mg/kg body weight of mice with a mild suppression of parasite in 400mg/kg and 200mg/kg body weight of mice when compared to the infected group (water) with the highest % parasitaemia as illustrated in Table 2. Percentage suppression was observed to increase as extract concentration increased. From Table 2, the extract caused a statistically significant (P<0.05) chemo suppression of 51.0 %, 55.0 % and 59.1 % for the 200, 400 and 800 mg/kg/day respectively when compared to the infected group. The standard drug, chloroquine caused chemo suppression of 95.6 %, which was higher than those of the extract treated groups and significantly different from the values of the extract.

Table 6: Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activities in mice administered extracts of E. hirta during suppressive test

Table 7: Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activities in mice administered extracts of E. hirta during repository test.

Statistical analysis was done by analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). Values were considered statistically significant when p<0.05.

RESULTS

Phytochemical Screening

The phytochemical screening of the aqueous-methanolic whole plant extract of E.hirta revealed the presence of saponins, alkaloids, flavonoids, steroids, tannins and terpenoids as shown in Table 1.

Four-Day Antiplasmodial Activity of Extract

The results of the 4-day suppressive study of the extract E. hirta showed dose dependent chemo suppressive effect at various doses in mice infected with Plasmodium berghei malaria parasite. The highest extract suppression of parasitaemia was observed at the dose of 800mg/kg body weight of mice with a mild suppression of parasite in 400mg/kg and 200mg/kg body weight of mice when compared to the infected group (water) with the highest % parasitaemia as illustrated in Table 2. Percentage suppression was observed to increase as extract concentration increased. From Table 2, the extract caused a statistically significant (P<0.05) chemo suppression of 51.0 %, 55.0 % and 59.1 % for the 200, 400 and 800 mg/kg/day respectively when compared to the infected group. The standard drug, chloroquine caused chemo suppression of 95.6 %, which was higher than those of the extract treated groups and significantly different from the values of the extract.

Repository Effect of Extract on Plasmodium berghei

The aqueous-methanolic extract of Euphorbia hirta produce a significant (P < 0.05) dose-dependent prophyllactic activity at the different doses with a reduction in the level of parasitaemia in 25.1 %, 43.1 % and 50.4 % for 200, 400 and 800 mg/kg/day extract treated groups while 10 mg/kg/day chloroquine caused a chemo suppression of 80.9 % when compared to control as shown in Table 3. The comparison between the prophyllactic and suppressive effect E. hirta aqueous-methanol extract on P. berghei infected mice is illustrated in Figure 2. The figure showed that E. hirta shows more biological effect in restraining the proliferation of the P. berghei parasite than in its prevention. Table 4 (suppressive) and 5 (prophylactic) showed the mean levels of Thiobarbituric acid and non-enzymic antioxidant reduced glutathione status in the P. Berghei Infected, Chloroquine and E. hirta extract treatment after P. Berghei infection. The tables revealed significant reduction in both groups. Similar trend were observed for Superoxide Dismutase and Glutathione Peroxidase activities as revealed in Table 6 (suppressive) and Table 7 (prophylactic).

DISCUSSION

The therapeutic properties ascribed to most medicinal plants have been linked to the presence of phytochemical compounds contained in them. Phytochemicals such as alkaloids, terpenes, sapomins, flavonoids, etc, have been reported to exhibit anti-plasmodial activity. The anti-plasmodial activity exhibited by this extract was perhaps due to the possible presence of active compounds. Although rodent models do not produce exactly the same signs and symptoms observed in the human plasmoidal infection but they have been reported to produce disease features similar to those of human plasmoidal infection, when infected with P. berghei. Moreover, several studies have employed P. berghei in predicting treatment outcome of suspected antimalarial agents, because of its high sensitivity to chloroquine, making it appropriate for this study. Substances that reduce parasite multiplication (anti plasmodial effect) in the host were considered to possess antimalarial activity.

The data was analyzed using One-way ANOVA

Stable values of the extract.

The aqueous methanolic extract of Euphorbia hirta produce a significant (P < 0.05) dose-dependent prophyllactic activity at the different doses with a reduction in the level of parasitaemia in 25.1 %, 43.1 % and 50.4 % for 200, 400 and 800 mg/kg/day extract treated groups while 10 mg/kg/day chloroquine caused a chemo suppression of 80.9 % when compared to control as shown in Table 3. The comparison between the prophyllactic and suppressive effect E. hirta aqueous-methanol extract on P. berghei infected mice is illustrated in Figure 2. The figure showed that E. hirta shows more biological effect in restraining the proliferation of the P. berghei parasite than in its prevention. Table 4 (suppressive) and 5 (prophylactic) showed the mean levels of Thiobarbituric acid and non-enzymic antioxidant reduced glutathione status in the P. Berghei Infected, Chloroquine and E. hirta extract treatment after P. Berghei infection. The tables revealed significant reduction in both groups. Similar trend were observed for Superoxide Dismutase and Glutathione Peroxidase activities as revealed in Table 6 (suppressive) and Table 7 (prophylactic).
chemo suppression effect in the standard drug, Chloroquine may be due to the inability of the parasite to develop resistance against the drug. Result of the 4 days treatment showed a significant (p<0.05) difference between the various extract concentrations and Chloroquine. Furthermore, percentage chemo suppression was also observed to increase as extract concentration increased. The prophylactic study of the aqueous-methanolic extract of E. hirta produced a significant (p<0.05) dose dependent reduction in the level of parasitaemia and possesses blood schizontocidal activity which is in agreement with the suppressive test of this study. Although, the mechanism of action of the extract has not been elucidated, some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation or by inhibiting protein synthesis depending on their phytochemical constituents. The extract could have exerted its action through either of the two mechanisms mentioned above or by some other mechanisms. Antiplasmodial effects of natural plant products have been attributed to some of their active phytochemical components. Some of these phytochemicals such as terpenes, saponins and flavonoids (detected in E. hirta) were reported to have antiplasmodial activity. Earlier studies by Etkin, reported the oxidant generation potential of some plant extracts, based on the ability of the extract to increase conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG). Increased oxidation has also been shown to create an intracellular environment that is unfavourable to plasmodial growth. The mechanism of action of artemisinin, which depends on oxidant action for its potent antimalarial activity, validates this. These groups of compounds collectively referred to as oxidant drugs hold the promise for effective treatment of multidrug resistant Plasmodium parasites. These drugs cause enhanced production of oxygen radicals inside parasitized erythrocytes or act to render parasites (or their host cells) more susceptible to attack by oxygen radicals. Accordingly, antimalarial oxidant drugs are structurally diverse and include seemingly unrelated compounds such as primaquine (i.e., its redox-active metabolites), methylene blue, and the endoperoxide artemisinin. While these agents are among the most potent antimalarial agents ever developed, there remains considerable interest in identification of compounds which act in combination and synergistically with the oxidant drugs to decrease host toxicity and to counter the development of drug resistance. However, lack of oxidizing action in some plants does not rule out antiplasmodial activity since they may be active through other biochemical mechanisms. The antiplasmodial effect of aqueous-methanolic whole plant extract of E. hirta may therefore be due to the phytochemical components (alkaloids, flavonoids and terpenes) but not likely the oxidant generation potential as evident from the various oxidative status parameters such as the Thiobarbituric acid reacting species (TBARS), Reduced Glutathione (GSH), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activities and that were measured. Subsequently, it was evident that the extract and drug did cause significant decrease in the lipid peroxidation following extract and drug administration as determined by TBARS level in mice plasma. The GSH content and enzymatic activities of SOD and GPx were also statistically different from that measured from the infected group. This implies the activity of the extract in scavenging the free radicals that could have been generated as a result of oxidative stress induced by P. berghei, consequently, the activities of these enzymes and that concentration of GSH were found to reduce as the concentration of the extract increases. This is in agreement with Williams and co who reported that the polyphenols, which are found in extracts of medicinal plant, can act as a good antioxidant inhibiting Lipid peroxidation and scavenging superoxide. As evident from this study, in which the highest administered dose, which was 800 mg/kg body weight, was able to show comparable activity with a well-known antiplasmodial drug (Chloroquine); and being far lower than the LD50 as previously determined by Rajeh et al., (over 5000 mg/kg body weight), indicates that the aqueous-methanolic whole plant extract of E. hirta possesses antimalaria activity by its ability to suppress Plasmodium berghei infection in mice. This further establishes the safe use of the plant extracts by the natives of Southern Nigeria, who have been using it for traditional treatments of various ailments, and thus could possess pharmacological potentials that is beneficial in the development of new drug for treatment or prophylaxis against malaria. Additional studies such as identification, purification and characterization of the exact phytochemicals that are involved in the antiplasmodial actions, the elucidation of their molecular/structural properties and establishing their mechanism of actions should therefore be considered using E. hirta whole plant extracts. These properties may be used as targets in the design of synthetic drugs that may confer same/similar biological activities.

REFERENCES


47. Williams RJ, Spencer JPE and Rice-Evans C. Flavonoids antioxidants or signaling molecule. *Free radical biology and Medicine*, 2004; 7, 36:838-849