Histological Study of Ethanol Leaf Extract of Vernonia amygdaлина in Cerebellum of Young Mice Malaria Model

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MAA designed the study, wrote the protocol and the first draft of the manuscript. Author SAM performed the statistical analysis. Author SSA managed the analyses and composed the result. Authors MSA and LM managed the literature and the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria is still a major health problem around the world especially in Africa. Report has shown major cause of morbidity and mortality amongst children population to be malaria disease with P. falciparum as a causative organism.

Aim: This study is designed to investigate the effects of ethanol leaf extract of Vernonia amygdalina, (ELVA) leaf on the Brain tissue (cerebellum) of young mice inoculated with Plasmodium berghei NK 65.

Place and Duration of the Study: The study was conducted at Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, from May, 2017 to December, 2017.

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Study Design: The Chloroquine-sensitive parasites *P. berghei* (1 x 10⁵) intraperitoneally were used to induce malaria infection in the young mice. Twenty-five young mice were randomly divided into five groups of five mice each. Group 1 negative control (distilled water), group 2 positive control (*Pb*KNK65), groups (3, 4 and 5) were (*Pb*KNK65 + ELVA 250mg/kg), (*Pb*KNK65 + ELVA 125mg/kg), (*Pb*KNK65 + CQ 10mg/kg).

Methodology: The fresh leaves of *Vernonia amygdalina* plant were collected based on Ethnobotanical description and the leaves were cleaned from extraneous materials, air-dried under shade at room temperature then pounded into powder. The Powdered *Vernonia amygdalina* (500g) was macerated with 80 % ethanol for 72 hours with intermittent agitation by Orbital shaker at 120 revolutions per minute. The supernatant part of agitated material filtered with 15 cm whatman grade1 filter paper two times. The Parasitized erythrocytes were obtained from a donor-infected mouse by cardiac puncture in heparin and diluted with sterile blood from similar age group mice. Animals were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 10⁸ parasitized erythrocytes lethal inoculum on day 0. At the end of the administration, mice were sacrificed and brain tissue dissected out, fixed immediately in Bouin’s fluid. Brain tissue were then passed through routine histological procedure.

Results: ELVA were found to reduce *P. berghei* percentage parasitaemia at 250mg/kg (2.48 ± 0.22), 125mg/kg (3.76 ± 0.35) and CQ 10mg/kg (2.64 ± 0.10) compare to the positive control (4.34±0.57) with a significant difference of (p<0.05). Body weight and temperature was sustained with no significant difference across the groups.

Conclusion: Conclusively, our findings shows that ELVA and Chloroquine clear blood parasite level with a significant difference at a dose dependent level and exalt effect on the histology of the cerebellar cortices with an optimal side effect thus, as it continues to serve as remedy with traditional medicine practitioner.

Keywords: *P. falciparum*; *Vernonia amygdalina*; cerebellum; *P. berghei*; chloroquine.

## 1. INTRODUCTION

Malaria, an infectious disease caused by *Plasmodium spp.* parasites carried by female *Anopheles* mosquitoes and transmitted through bite [1,2,3], remains one of the challenges of the health sector majorly in the tropics. Malaria is a global epidemic, the incidence and severity are higher especially in the sub Saharan Africa, where pregnant women and children are the most susceptible [4,5,6,7]. Approximately 300,000 infant’s deaths and 2,500 deaths of pregnant women are attributable to malaria in the endemic areas [8]. Both mother and fetus are at risk of poor outcome with pregnancy. Malaria complications like premature delivery, anemia, abortion, intrauterine growth retardation, low birth weight, perinatal and maternal mortality are well [9&10] known. Many literature has highlight most cases of endemic *P. falciparum* malaria to be uncomplicated; however few numbers of severe complication including cerebral malaria, severe anemia and placental malaria have been reported. Severe malaria occurs when the parasite reaches the erythrocytic phase of the infection and starts to proliferate inside the erythrocytes. The brain is one of the major organs that express the precipitating effect of severe malaria through cytoadherence and resetting leading to occlusion of microvasculature [11] hence contributing directly to the pathogenesis of severe malaria disease. The cerebellum (or small brain) lies in the caudally cranial fossa. In the human adult, the weight of the cerebellum is about 150g. The cerebellum has a superficial layer of grey matter and the white matter. The cerebellum lies behind the pons and the medulla. It is separated from the cerebrum by a fold of dura matter called the tentorium cerebelli [12&13]. Currently, malaria control is a complicated task due to insecticide resistance in vector populations, as well as to the development of *Plasmodium* strains resistant to a growing number of antimalarial drugs. The increasing problem of resistance to the classical drugs (Chloroquine, Atovaquone, Sulphadoxine and Pyrimethamine) and the problem of recrudescence of Artemisinin stress the need to look for new antimalarial agents [14]. Recently, herbal plants have been used more widely than synthetic drugs due to its non-toxic, higher efficacy, less side effects and easy to process. Herbal plant extracts are noble source for a variety of drugs in many countries. Medicinal plants have formed the basis of healthcare worldwide. Hence, the use of plant derived natural products as part of herbal preparations for alternative sources of medicine continues to
play a major role in chemotherapy especially in third world countries [15]. Vernonia amygda|lina is commonly known as bitter leaf which is a shrub or small tree of 2-5m belonging to the family; “Asteraceae”. The leaves are green with characteristic odour and a bitter taste [16] the bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides [17]. Vernonia amygda|lina produces no seeds. Hence, they are distributed through cutting [18]. It is known as “Ewuro” in Yoruba, “Etidot” in Ibibio, “Onugbu” in Igbo and “Chusa” in Hausa tribes of Nigeria [19]. The leaves are mostly used as vegetables for preparation of soup to stimulate the digestive system and also used for treatment of fever in South Southern and South Eastern Nigeria. Generally drugs in use produces poor outcome which has created avenue for continuous study into new drugs or better understanding of resistance pattern. Efforts in creating more potent antimalarial drugs that is readily assessable to the larger population is yielding according several literature but more is still expected. In this study we examine histological effect of ELVA in cerebellar cortices of young mice inoculated with malaria parasite.

2. MATERIALS AND METHODOLOGY

2.1 Materials

Digital Microscope (Celestron and Olympus), Distilled water, Computers, Bouins fluid, Beakers, Specimen bottles, Digital weighing balance, 1ml and 5ml syringes and injecting needles, Dissecting tray, Chloroform and Dissecting kit.

2.2 Plant Collection

The fresh leaves of Vernonia amgdalina plant were collected based on Ethnobotanical description and with the help of local traditional healers around Mada Area Development Council in Gusau local government area of Zamfara state, Nigeria, in May 2017. The plants were identified and authenticated at Botany Department, Faculty of Life Science, Ahmadu Bello University Zaria, with voucher number (12063). The Fresh leaves of Vernonia amgdalina were cleaned from extraneous materials, air-dried under shade at room temperature then pounded into powder in Biochemistry Laboratory, of Umaru Musa Yar|dua University Katsina state, Nigeria. The powdered plant material was weighed (500 g) using SCIENTECH Mode No SL 3100D Rev-c accuracy class (II).

2.3 Ethanol Extract Preparation

Powdered Vernonia amgdalina (500 g) was macerated with 80% ethanol for 72 hours with intermittent agitation by Orbital shaker at 120 revolutions per minute. The supernatant part of agitated material filtered with 15 cm whatman grade1 filter paper two times. The filtrate of Vernonia amgdalina was then concentrated using Rotary evaporator (BUCHI R250, Switzerland) at 40°c to remove methanol and further dried using in a lyophilizer (CHRIST, 3660 Osterode/harzl, France) to remove water and the extract were kept at −20°C until used [20]. The total percentage yield is represented in Table 1.

2.4 Experimental Animal Preparation

The animals employed for this study were premature young male and female 2-5 weeks of Albino Swiss mice (5 - 7g). The mice were obtained from the Animal Facility, Faculty of Pharmaceutical Sciences of Ahmadu Bello University Zaria, Nigeria. The mice were put to full experimental condition because the experiment took place in the same venue of purchase by first screening the mice for any possible parasitic infection. They were then housed in cages and had unrestricted access to a standard pellet diet and water ad libitum. The animals were maintained under the natural light-dark cycle throughout the duration of the study. For in-vivo anti-malarial assays of plant extracts, the mouse-infective chloroquine sensitive strain of P. bergh|ei (NK65) obtained from National Institute of Medical agency Lagos State was kept alive by continuous intraperitoneal (i.p.) serial blood passage from mice to mice on research demand by the laboratory staff of the animal facility of Faculty of Pharmaceutical science, Ahmadu Bello University Zaria.

2.5 Inoculation

Parasitized erythrocytes were obtained from a donor-infected mouse by cardiac puncture in heparin and diluted with sterile blood from similar age group mice. Animals were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 10⁵ parasitized erythrocytes lethal inoculum on day 0. Infected mice with parasitemia were grouped and treatment commence [21].
Table 1. Percentage yield of ethanol leave extract of *Vernonia amygdalina*

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Solvent</th>
<th>Weight of powder</th>
<th>Vol. of solvent</th>
<th>Yield (g)</th>
<th>% of yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vernonia amygdalina</em></td>
<td>Ethanol</td>
<td>500g</td>
<td>5000ml</td>
<td>235</td>
<td>47</td>
</tr>
</tbody>
</table>

Table 2. Grouping and treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>0.2ml distilled water orally (7g)</td>
</tr>
<tr>
<td>GROUP 2</td>
<td><em>P. berghei</em> parasite (7g)</td>
</tr>
<tr>
<td>GROUP 3</td>
<td><em>P. berghei</em> + ELVA 250mg/kg body weight of mice (7g)</td>
</tr>
<tr>
<td>GROUP 4</td>
<td><em>P. berghei</em> + ELVA 125mg/kg body weight of mice (7g)</td>
</tr>
<tr>
<td>GROUP 5</td>
<td><em>P. berghei</em> + Chloroquine 10mg/kg body weight of mice (7g)</td>
</tr>
</tbody>
</table>

2.6 Grouping and Treatment

Evaluation of curative anti-malarial potential of the extract was done using a method described by [22] and modified by [23]. To assess the curative potential of the ethanol leaf extract on established *P. berghei* infection, 25 Swiss albino mice pups were group into 5 mice per group with a total of five groups as shown in Table 2.

After 72 hours, Post inoculation is ELVA. ELVA: Ethanol Leaf Extract of *Vernonia amygdalina* i.p route of inoculation, oral administration, All for the period of 7days and sacrifice on 8th day

2.7 Parasite and its Quantification

Blood sample were collected from tail snip of each mouse according to methods of [24]. The smear were prepared and visualized using microscope such that eye piece shows about 100 red blood cells per field [25].

The parasitemia level was determined by counting minimum of five fields per slide with about 100 RBC in random field of the microscope.

Percent parasitemia and percentage of suppression was calculated using formula described in all model [26].

\[
\% \text{ Parasitaemia} = \frac{\text{Number of Parasitized RBC}}{\text{Total RBC Counted}} \times 100 \%
\]

\[
\% \text{ Suppression} = \frac{(\text{Parasitaemia in normal control} – \text{Parasitaemia in treated group})}{\text{Parasitaemia in normal control}} \times 100 \%
\]

2.8 Animal Sacrifice and Tissue Samples

At the end of the administration, mice were sacrificed and brain tissue dissected out, fixed immediately in Bouin’s fluid [27]. Brain tissue were then passed through routine histological procedure. In the histology lab it was then dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Afterwards coronal sections were then taken for the various individual staining procedure which includes the H & E and Cresyl violet.

2.9 Statistical Analyses

Data collected were presented as Mean ± SEM and were statistically analysed using Statistical Package for Social Science (SPSS), Version 20 (IBM, Incorp, NY). A \( p \)-value < 0.05 was considered significant. ANOVA was used to compare the levels of parasitaemia of the *P. berghei* infected mice between the controls and extract treated groups at a fixed time. The results were presented as the Mean ± SEM (Standard Error of the Mean) and statistical significance was considered at a 95% confidence interval (\( P < 0.05 \)).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Parasitaemia quantification

On established infection, both dose (high, 250mg/kg and low, 125mg/kg) of ethanol extract caused a significant parasite reduction in comparison to normal control which showed a daily increment of parasitaemia (Table 3). The result indicates that the parasitaemia level of the mice in group 5 (2.64±0.10) was significantly lower compare to Group 2 (4.34±0.57) and group 4 (3.76±0.35) with a \( P \) value of 0.015 while there is no significant difference noted in the parasitaemia level at day 3 which is 72 hrs
after inoculation and onset of treatment with (P value) of 0.619.

The ethanol extract of Vernonia amygdalina at doses (125 and 250) mg/kg and CQ 10mg/kg had shown statistically significant (p<0.015) parasitaemia suppression of 45.6%, 59.5% and 50.6% as compared to normal control group, respectively. From the investigation, it was observed that the extract exhibited a dose dependent curative effect with a slight difference between doses.

3.1.2 Body weight determination

ELVA treated mice exhibited statistically significant (p<0.05) increments of body weight across all groups between day 7 (Table 4). The result indicates that the body weight of the mice on day 3 in group 3 (10.76±0.56) was significantly lower compare to Group 2 (12.98±0.35), group 4 (14.06±0.54), group 5 (13.04.033) and group 5 (11.08±0.49), while at day 7 which is the final day of the design just before sacrifice in which group 3 (10.94±0.59) was significantly lower compared to group 2 (12.62±0.49), group 4 (14.08±0.52) and group 5 (13.18±0.32) with (P value) of 0.001.

3.1.3 Temperature

The mean temperature of the mice was taken and represented in (table 5). Group 1 administered with distilled water shows no temperature difference throughout the duration of the research compare to Group 2 which was inoculated with P. berghei parasite and treated present high temperature and a corresponding feverish condition with reduction in feeding appetite. There is no significant amongst the treated and untreated group throughout the research.

### Table 3. Percentage parasitaemia and suppression

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>% Parasitaemia</th>
<th>% Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before (D3)</td>
<td>Post D7</td>
</tr>
<tr>
<td>1</td>
<td>Dist. H₂O</td>
<td>p/bw</td>
<td>--------</td>
<td>3.58±0.24</td>
</tr>
<tr>
<td>2</td>
<td>Pb</td>
<td>0.2ml</td>
<td>4.00±0.71</td>
<td>2.48±0.22</td>
</tr>
<tr>
<td>3</td>
<td>ELVA</td>
<td>250mg/kg</td>
<td>4.32±0.39</td>
<td>3.76±0.35a</td>
</tr>
<tr>
<td>4</td>
<td>ELVA</td>
<td>125mg/kg</td>
<td>3.60±0.24</td>
<td>2.64±0.10ab</td>
</tr>
<tr>
<td>5</td>
<td>CQ</td>
<td>10mg/kg</td>
<td>3.60±0.24</td>
<td>2.64±0.10ab</td>
</tr>
</tbody>
</table>

Pb-Plasmodium berghei, ELVA- Ethanol leave extract of Vernonia amygdalina, CQ- Chloroquine Where N = Numbers of animal, P/bw- Per body weight, Pre D3- before (Day 3), Pos D7- After (Day 7), D3- Day 3, D7- Day 7. D3 and D7 shows no significant difference at p-value 0.619 and 0.015 respectively.

### Table 4. Body weight at day 3 and day 7

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>Body Weight(g)</th>
<th>Pre D3</th>
<th>Post D7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled H₂O</td>
<td>P/bw</td>
<td>11.62±0.31</td>
<td>11.96±0.39</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>P.b</td>
<td>0.3ml</td>
<td>12.98±0.35</td>
<td>12.62±0.49</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>V.A</td>
<td>2500mg/kg</td>
<td>10.76±0.56</td>
<td>10.94±0.59</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>V.A</td>
<td>1250mg/kg</td>
<td>14.06±0.54</td>
<td>14.08±0.52</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CQ</td>
<td>10mg/kg</td>
<td>13.04±0.33</td>
<td>13.18±0.32</td>
<td></td>
</tr>
</tbody>
</table>

P.b- induced with Plasmodium berghei, V.A- Vernonia amygdalina treated, CQ- Chloroquine Where N = Numbers of animal, P/bw- Per body weight, Pre D0- before (Day 0), Post D7- After (Day 7), D3- Day 3, D7- Day 7. D0 and D4 shows significant difference at p-value 0.001 and 0.002 respectively.

### Table 5. Mean temperature

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before inoculation</th>
<th>After inoculation</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.0 ± 0.62</td>
<td>32.0 ± 0.36</td>
<td>32.2 ± 0.28</td>
</tr>
<tr>
<td>2</td>
<td>32.5 ± 0.64</td>
<td>33.0 ± 0.67</td>
<td>33.8 ± 0.93</td>
</tr>
<tr>
<td>3</td>
<td>33.0 ± 0.10</td>
<td>33.5 ± 0.21</td>
<td>33.2 ± 0.57</td>
</tr>
<tr>
<td>4</td>
<td>31.0 ± 0.43</td>
<td>32.5 ± 0.39</td>
<td>32.5 ± 0.34</td>
</tr>
<tr>
<td>5</td>
<td>31.0 ± 0.31</td>
<td>32.0 ± 0.42</td>
<td>32.5 ± 0.56</td>
</tr>
</tbody>
</table>

No significant difference at P < 0.617
**Fig. 1A.** Photomicrograph of the transverse section of cerebellar cortex (Group 1) showing: PC Purkinje cells, ML Molecular layer, GC Granular cells. H&E stain. Mag. X 250.

**Fig. 1B.** Photomicrograph of the transverse section of cerebellar cortex (Group 1) showing: PC Purkinje cells, ML Molecular layer, GC Granular cells. Cresyl echt violet stain. Mag. X 250

**Fig. 2A.** Photomicrograph of the transverse section of cerebellar cortex (Group 2) showing: DPC Distorted Purkinje cells, ML Molecular layer, GC Granular cells. H&E stain. Mag. X 250.

**Fig. 2B.** Photomicrograph of the transverse section of cerebellar cortex (Group 2) showing: PC Purkinje cells, ML Molecular layer, GC Granular cells. Cresyl echt violet stain. Mag. X 250

**Fig. 3A.** Photomicrograph of the transverse section of cerebellar cortex (Group 4) showing: PC Purkinje cells, ML Molecular layer, GC Granular cells. H&E stain. Mag. X 250.

**Fig. 3B.** Photomicrograph of the transverse section of cerebellar cortex (Group 4) showing: PC Purkinje cells, ML Molecular layer, GC Granular cells. Cresyl echt violet stain. Mag. X 250
3.1.4 Histopathology

The histological findings in the control group as obtained from (Fig 1) showed normal evenly distributed supporting cells in molecular layer and pyramidal cells in pyramidal layer as compared a fair photomicrograph obtained from the treatment group i.e group 3, 4 and 5 respectively (Fig 3, 4 & 5) There is great difference in neuronal arrangement and integrity when comparing the treated group from the untreated group (group 2) which is characterized with distorted supporting layers, degenerative features of pyramidal cell and non-differentiated granular layer. This characteristics were mildly noted in the treated group especially the group with high dose of ELVA. In this study it implies that ELVA shows some ameliorative effect by mitigating cellular necrosis.

3.2 Discussion

Several researches have been conducted on therapeutic value of herb drug but unifying the inference is a great challenge. Around the world now interest in antimalarial herb-drug is how to achieve to safety and overcome resistance. Approaches to understanding disease pathogenesis are not new, and have involved experimental infections in animal model and subsequent monitoring of the pathogenic processes [28]. Plasmodium berghei infection in mice causes a change in histopathology features...
in various organs. This is due to *Plasmodium* parasite infection can induce inflammatory cells that can cause changes in pulmonary microcirculation leading to increased hypoxia microenvironment thus necrosis or degeneration [29]. In this study, we used ELVA to treat *P.berghei* NK 65 infection in cerebellar cortices of young mice. From our study treated group displayed parasitaemia level that gradually decrease during treatment, a remarkable effect against CQ-sensitive *P. berghei*, at the therapeutic doses of 250 mg/kg drug is achieved. There is a significant reduction in parasitaemia, but total parasite clearance was not achieved compared to the report of [30], were a 100 percent clearance is achieved with (CQ + extract). Also there was a reversal in temperature in the treated group which is significant but no significant difference in temperature for our study. Potentiation of both antimalarial substance is suggested to be the explanation for the difference in parasite suppression and temperature.

Iwalokun [31] shows that VA against CQ resistant *P. berghei*, present antimalarial activity of (62.7%) merely comparable to CQ alone (57.2%) which is in accordance with our study where high doses of 250mg/kg gave (59.5%) against 10mg/kg CQ (50.6%). The mode of extraction and dosage might be responsible for the difference in percentage suppression. The dose dependent activity of ELVA in our study can be corroborated with the work of [32]. Also the significant difference in weight of mice across the groups is in relation with work [25], who report increase in weight across the group but fluctuate along the period of the research, this could be explained as the variety in the constituent of the extract and drug. This could be as result of natural selection since the mice were randomized before starting the experiment.

In a report by [33], where decrease bioavailability of ethanol extract of *N. latifolia* decreases parasite load in *P. berghei* and shows moderate neuroprotection to hippocampus of infected mice this in line with our report where high dose of extract group shows reduced cellular distortion compare to the non-treated group.

Our study also shows that 10mg/kg Standard drug (CQ) group moderate the cellular structure compared to the untreated group and this is not in agreement with study of [34], who reported that severe distorted neurons are seen in the section of hippocampus of infected mice treated with 5mg/kg standard drug Artemisinin/Lumefantrine. The age and mechanism of action could be the rationale behind the difference in safety of both standard drugs.

### 4. CONCLUSION

The study observed a significant difference in percentage parasitemia and resultant suppression but slight difference in the histological effects. Thus considering the hypothesis of the studies two theories can be extrapolated which include ELVA shows some ameliorative effect or the autoimmune cells of the nervous intervene in the entire process thereby reducing the level of degeneration of cells.

### 5. RECOMMENDATION

Further study is advised where stereological methods are used in assess the effects of the extract and parasitic agent on the neurons.

### CONSENT

It is not applicable.

### ETHICAL CONSIDERATION

Ethical approval was collected from Ahmadu Bello University, research and ethics committee on the use of animals with an approval number of ABUCAUC/2017/029.

### ACKNOWLEDGEMENT

Our immense gratitude goes to the entire staffs of the animal facility of Faculty of Pharmaceutical science, Staffs of Histology unit of Department of Human Anatomy, and Friends from hematological laboratory of Veterinary Medicine for providing the bench space.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

### REFERENCES

2. Gandhi PR, Jayaseelan C, Kamaraj C, Rajasree SR, Mary RR. In vitro


23. Carvalho LH, BRANDAO MG, Santos-Filho D, Lopes JL, Krettli AU. Antimalarial activity of crude extracts from Brazilian plants studied in vivo in Plasmodium


