Toxicity Studies and in vivo Antiplasmodial Evaluation of Leaf and Stem Bark Extracts of Terminalia avicennioides

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Abstract

Malaria is one of the most serious infectious diseases found in the tropics and subtropics. P. falcifarum resistance to almost all anti-malarial drugs has necessitated the search for anti-malarial compounds. The aim of this study is to investigate the antiplasmodial effects of crude leaf and stem bark extracts of T. avicennioides, a plant utilized by traditional healers in Northern Nigeria to treat malaria and other ailments. Brine shrimp lethality (cytotoxicity) assay and acute toxicity were also evaluated, which are considered a useful tool for preliminary assessment of toxicity in plant extracts. The median lethal dosage ($LD_{50}$) was calculated by administering different doses of the extract (100–4000 mg/kg) intraperitoneally to three groups of three mice each for 24 hours and observing the animals for physical sign of toxicity. Three models were employed to assess the extracts antiplasmodial effectiveness in vivo against the rodent malaria parasite Plasmodium berghei: suppressive, curative, and repository. The leaf and stem bark extracts were found to show significant toxicity to brine shrimp larvae of Artemia salina in the brine shrimp lethality test with $LC_{50}$ values of >1000 and 29.6 respectively. Excitation, paw licking, and death were symptoms of acute toxicity of the extracts based on physical and behavioral observations. Death was recorded at 1000 mg/Kg of the stem bark extract and the $LD_{50}$ is ≤100 mg/Kg body weight while for the leaf extract it was estimated to be ≤600 mg/Kg with no death record. In vivo evaluation revealed that the extracts significantly (P <0.05) reduced parasite count in all models (suppressive, curative and prophylactic), with high average percentage inhibition of parasitaemia (54.68, 58.67 and 65.61%) seen in both T. avicennioides leaf and stem bark extracts, which was comparable to that produced by chloroquine and artesunate, respectively (64.31&93.1; 70.08&80.67; 63.40&75.20%). Both extracts had moderate antiplasmodial activity and were non-toxic to mice and brine shrimps. This finding validates the plant’s traditional use in malaria treatment.

Keywords: Malaria, Cytotoxicity, Acute Toxicity, T. Avicennioides, Brine Shrimps.

INTRODUCTION

Malaria is a parasitic disease caused by a single-celled protozoan from the genus Plasmodium, and it is one of the main causes of morbidity and mortality worldwide, outnumbering all other eukaryotic diseases (Conway, 2015; WHO, 2022). P. falciparum is responsible for about 80% of all malaria cases and about 90% of the deaths from malaria (WHO, 2022). This high morbidity and mortality has a significant economic and social consequence, especially in developing countries, where public health subsidies account for 40% of total expenditures (Taye and Alexander, 2022). The urgent need to define novel antimalarial agents that are cheaper and more effective is justified by parasite resistance to the current arsenal of preventative and treatment medications, as well as the fact that the current antimalarial drugs are prohibitively expensive in poor settings.

Terminalia avicennioides Guill and Perr., is a tree widely distributed in the Savannah region of West Africa (Burkhill, 1985). The plant is locally referred to as “Baushe” among the Hausa, “Kpace” in Nupe, “Kpayi” in Gwari, “Idi” among the Yoruba (Mann, 2007; Atawodi et al., 2011; Azeez et al., 2015). The aqueous decoction of the dried leaves and stem bark has
wide traditional medicinal application in teeth and skin infections; malaria and severe jaundice; gastrointestinal disorders among others (Mann, 2007; Abdullahi et al., 2001). Several bioactive hydrolysable tannin compounds including ellagic acid, punicalagin, flavogallonic acid and terchebulin (Shuaibu et al., 2007) have been isolated from this plant. In this study, cytotoxicity and anti plasmodial activity of the stem bark and leaf extract of T. avicennioides was evaluated to confirm its efficacy and safety of its use.

2. METHODS

Plant Collection

Fresh plant material of Terminalia avicennioides was collected from Gwarzo Local Government area of Kano State. They are identified and authenticated by Dr Yusuf Nuhu at the herbarium of Plant Biology Department, Bayero University, Kano, with Herbarium Accession Number BUKHAN 609

Extraction of Plant Materials

The plant materials were air dried and ground using mortar and pestle. 1Kg was macerated with 96% ethanol (2.5L) for two weeks (Adoum, 2009). The macerate was filtered and evaporated to dryness using a rotary evaporator (R200) at 40°C. The ethanol crude residues obtained from the leaves and stem bark of T. avicennioides were labeled as TA-01 and TA-02 respectively.

Brine Shrimp Lethality Test (BSLT)

Screening of extracts against Artemia salina (brine shrimps) was carried out according to the method described by Meyer et al., (1982) and Adoum, (2009). Brine shrimp eggs were added to sea water (250 ml) in a hatching chamber. The eggs were allowed to hatch for 48 hours. Samples were prepared by dissolving each of the extracts (20 mg) in methanol (2 ml). This was labeled as the stock solution. From the stock solution, aliquots of 500 μl, 50 μl and 5 μl were pipetted and poured into separate vials which contained 1000 μg/ml, 100 μg/ml and 10 μg/ml of the samples respectively. Usually three vials for each concentration were prepared making a total of 9 vials per extract or fraction plus a control vial containing solvent without the extract. The solvent in each vial was evaporated at room temperature overnight. 2 drops of DMSO (dimethyl sulphoxide) was added to each vial plus sea water (4 ml). Ten shrimps were transferred to each vial using a Pasteur pipette and the volume of the liquid in each vial was adjusted to 5 ml with sea water. After 24 hours, the numbers of surviving shrimps were counted and the LC<sub>50</sub> was determined at 95% confidence interval using regression analysis.

Acute Toxicity Study

Test Animals

Mice (18-22 Kg) of age between 6-12 weeks used as test animals were obtained from the Animal House, Department of Pharmacology and Therapeutics, Bayero University Kano. The mice were maintained at 22°C (50-70% humidity), feed with diet and water ad libitum according to NIH, (2011). Acute toxicity test was carried out according to Lorke’s method (Lorke, 1983; Chinedu et al., 2013) which consists of two phases:

Phase 1

Twelve (12) animals were divided into four (4) groups of three mice (3) each. Single dose of the extracts (10, 100, 1000 mg/kg) were administered intraperitoneally to groups 1, 2 and 3 while the control Group (group 4) received normal saline.

Phase 2

The extracts (1600, 2900 and 5000 mg/kg) were administered intraperitoneally to the first three mice in the group of four (4) mice. The fourth (control) received normal saline. Toxicity signs such as paw licking, salivation, stretching of the entire body, weakness, sleep, weight, food and water consumption, respiratory distress, coma and/or death were observed for the first 4 hours and subsequent 14 days. Recovery and weight gain were seen as an indication that they have survived the acute toxicity. LD<sub>50</sub> was calculated as geometric mean of the highest non-lethal dose and the lowest lethal dose.

In Vivo Antimalaria Assay

Test Animals

Swiss albino mice (18-22 g) of either sex aged 6-12 weeks were used for the in vivo study. They were obtained and maintained in the Animal House, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University Kano. The animals were kept based on the guidelines of the National Institutes of Health (NIH 2011) and fed with standard animal feeds and water ad libitum. Ethical approval was obtained from the Research Ethics Committee, Aminu Kano Teaching Hospital, Kano, with the no: NHREC/21/08/2008/EC/1998

Rodent Plasmodium Parasite

Chloroquine-sensitive Plasmodium berghei NK65 strain was used to induce malaria in the experimental mice. The parasite was obtained from a donor infected mouse maintained at the Animal Facility Centre, National Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria.

Parasite Inoculation

Parasitized blood was collected from donor mouse with rising parasitemia (34%) retro-orbitally. The blood was diluted with 0.9% saline in EDTA sample bottle, such that every 0.2 ml of blood contained 1× 10⁷ infected erythrocytes. Each mouse used in the study was inoculated with 0.2 ml of infected blood intraperitoneally (Abdulkadir et al., 2022).
Peter’s 4- Days Plasmodium Berghei Suppression Test

As described by Peters (1965), thirty-six (36) mice were given a P. berghei inoculum (1 x 10⁷) intraperitoneally, and they were divided into six groups: Group I (negative control) received 10 ml/Kg of normal saline. Groups II-IV received doses of the extracts (150, 300, 600 mg/Kg (TA-01) and 25, 50, 100 mg/Kg of TA-02 respectively), and Groups V and VI (positive controls) were treated with chloroquine 5 mg/Kg and artemunate 5 mg/Kg. Two-Four hours post infection, the extracts were administered, and treatment continued every day for four days (i.e., from day 0 to day 3). Blood was collected from the tail of each mouse and smeared onto a microscope slide to make a thin film. The blood films were fixed with methanol, stained with 10% Giemsa, and examined microscopically to determine the mean parasitemia level by counting the number of parasitized RBCs in three random microscopic fields (Chinwendum et al., 2012; Abdulkadir et al., 2022).

Curative Test (Rane’s Test)

The method of Ryley and Peters (1970) was employed in this study. On the first day (day 0), 36 mice were injected with 1 x 10⁷ P. berghei infected erythrocytes intraperitoneally. The mice were kept for 72 hours (days 0-3) in order for the infection to be established. The mice were divided into six groups at random: Group I served as the negative control and received 10 ml/Kg of normal saline; Groups II-IV received doses of the extracts (150, 300, 600 mg/Kg (TA-01) and 25, 50, 100 mg/Kg body weight of TA-02 respectively). Group V and VI served as the positive controls and received doses of 5 mg/Kg artemunate, respectively. The treatment continued for 5 days (day 3–7). Twenty-four hours after the last treatment, blood was collected from the tail of each mouse and smeared onto a microscope slide to make a thin film. The blood films were fixed with methanol, stained with 10% Giemsa, and examined microscopically to determine the mean parasitemia level by counting the number of parasitized RBCs in three random microscopic fields. The mean survival time of the animals was determined by finding the average survival time (days) of the mice in each group over a period of 28 days (day 0-27) after infection with P. berghei as given by the formula below (Fentahun et al., 2017; Abdulkadir et al., 2022).

\[
\text{Percent infected erythrocytes} = \frac{A}{1,000} \times 100
\]

Prophylactic (Repository) test

This test was done as described by Peters (1965). Thirty-six mice (36) were randomly distributed into 5 groups. Group I were treated with 10 ml/ Kg of normal saline (negative control), groups II-IV were treated with doses of the extracts (150, 300, 600 mg/Kg (TA-01) and 25, 50, 100 mg/Kg body weight of TA-02 respectively); and group V and VI were treated with Pyrimethamine 1.2 mg/kg and Chloroquine 5 mg/Kg (positive controls). Treatment continued orally for five days (from day 0 to day 4). On the 6th day (i.e., day 5), the mice were inoculated intraperitoneally with 1 x 10⁷ P. berghei infected erythrocytes. After 72 h, blood was collected from the tail of each mouse and the parasitemia level was determined (Chinwendum et al., 2012; Abdulkadir et al., 2022).

3. RESULTS AND DISCUSSION

Cytotoxicity of the Crude Leaf and Stem Bark Extract of T. Avicennioides

Brine shrimp lethality test of the leaf and stem bark extracts showed significant toxicity against brine shrimp larvae of Artemia salina at LC₅₀ values of 29.6 and >1000 µg/ml respectively (Table 1). Brine shrimp lethality test is a preliminary and convenient assay for monitoring biological activities in plant extracts, and the results in many instances correlate with their cytotoxic and antitumor properties (Ramachandran, 2011). The lower the LC₅₀ value, the more cytotoxic the extract (Nguta & Mbaria, 2013). Cytotoxicity of plant extracts was determined using the Clarkson’s toxicity index: non-toxic (LC₅₀ >1000 µg/ml), low toxic (LC₅₀ 500 - 1000 µg/ml), moderate toxic (LC₅₀ of 100 - 500 µg/ml) and highly toxic (LC₅₀ of 0 – 100 µg/ml) (Clarkson et al., 2004; Meyer et al., 1982). The stem bark extract of T. avicennioides (TA-02) exhibited the highest lethality on brine shrimps at LC₅₀ value 29.6 µg/ml (14.2-52.9) which is in accordance with Ukwade et al., (2020) and Mann et al., (2011) at LC₅₀ of 25.87µg/ml and 63. 2µg/ml respectively while the leaf extract (TA-01) showed no toxicity with LC₅₀ of >1000 µg/ml as reported by Mann et al., (2011)

| Table 1: Brine Shrimp lethality test of the crude extracts |
|-----------------|-----------------|
| Plant Extract   | BST LC₅₀(µg/ml) |
| TA-01           | >1000           |
| TA-02           | 29.6 (14.2-52.9)|

Acute Toxicity of Crude Leaf and Stem Bark Extracts

One major and overriding criterion in the selection of herbal medicines for use in health services is safety. Plants extracts should not only be efficacious but safe for consumption. Therefore, closely associated with screening of plants extracts for their biological activity is the need to know their toxic potentials. The acute lethal effect of the extracts on mice (Table 2)
recorded death at 1000 mg/Kg of the stem bark extract and this implies that the $LD_{50}$ of the extracts to be $\leq$100 mg/Kg body weight, while the leaf extract was estimated to be $\leq$600 mg/Kg. Other studies involving oral administration of the aqueous infused extract showed no mortality up to a dose of 3000 mg/kg and no signs of toxicity during 14 days of observation in Wistar rats (Bulus et al., (2011; Owusu & Antwi-Adjei, 2017)) investigated acute toxicity effect of aqueous extract of stem bark of $T. avicennioides$ on white albino rats and reported $LD_{50} >5000$ mg/Kg body weight. There was no significant weight decrease among the treated groups up to 1000 mg/Kg body weight.

### Table 2: Acute toxicity of $T. avicennioides$ crude extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>$T. avicennioides$ leaf extract (TA-01)</th>
<th>$T. avicennioides$ stem bark extract (TA-02)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment</strong></td>
<td><strong>Dose (mg/Kg)</strong></td>
<td><strong>Mortality after 24hrs</strong></td>
</tr>
<tr>
<td>Phase I</td>
<td>10</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0/3</td>
</tr>
<tr>
<td>Control</td>
<td>N/S</td>
<td>0/3</td>
</tr>
<tr>
<td>Phase II</td>
<td>1600</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>2900</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>N/S</td>
<td>0/1</td>
</tr>
<tr>
<td>$LD_{50}$</td>
<td>600, 300 &amp; 150 mg/Kg</td>
<td>100, 50, 25 mg/Kg</td>
</tr>
</tbody>
</table>

*Experiment was conducted in two phases; each dose group of phase I is made up of 3 mice each while those in phase II have 1 mice per group.
N/S- Normal saline 10 mls/Kg

### Antiplasmodial Effect of Leaf and Stem Bark Extract of $T. avicennioides$

The highest suppression of parasitaemia produced by $T. avicennioides$ extracts was observed in the stem bark extract at 54.68%. This effect was produced by 100 mg/Kg of both the extract and was comparable with that produced by 5 mg/Kg chloroquine CQ (64.31%) and artesunate ART (93.10%) as shown in Fig 1 & 2. In curative potential evaluation of $T. avicennioides$ crude extracts, all the three doses significantly reduced ($P < 0.05$) parasitaemia level on day 7 as compared to day 3. On day 7, the highest parasite clearance of 65.61% was seen by 100 mg/Kg dose of the stem bark extract. Chloroquine and artesunate treated group showed almost total clearance of the established $P. berghei$ infection on day 7 at 70.08% and 80.67% respectively. However, moderate curative effect of 52.73% was shown by the leaf extract of $T. avicennioides$ (Fig 1 &2) at 600mg/Kg. The mice in the extract-treated groups survived longer than those in the negative control group; those treated with high doses of 100 and 600 mg/kg survived for 9-16 days, while those in the negative control group survived for 4-6 days only. The mice in the chloroquine and artesunate-treated groups survived for 22-24 days, respectively. In the residual test, the activity of $T. avicennioides$ extracts were significant on day 8 ($P < 0.05$), at the tested doses. High prophylactic activity of 58.67% at 600 mg/Kg body weight was recorded for the leaf extracts. The activity was comparable to 5 mg/Kg pyrimethamine PYR and chloroquine at 63.40% and 75.20%.
The extracts *T. avicennioides* contain different classes of secondary metabolites, such as terpenoids, flavonoids, polyphenols, saponins and alkaloids which were known to have antimalarial activity (Ginsburh and Atamna 1994). All doses *T. avicennioides* extracts displayed significant and dose dependent inhibition in comparison with untreated control. Rodent models continue to be relevant in discovery of effective antimalarial agents. Rodent models have been validated through the identification of several conventional antimalarials such as chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives (Iyiola et al., 2011). *Plasmodium berghei berghei* parasite (NK 65 strain) is used in predicting treatment outcomes of any suspected anti-malaria agent because of its susceptibility to chloroquine. The significant suppressive antimalarial effect produced by the extracts of *T. avicennioides* support the traditional use of the plants as herbal medication against malaria in Northern part of Nigeria. In the curative study, a daily increase was observed in the parasite count of the negative group, which is consistent with normal proliferation of the parasite. The mean survival time is important to evaluate the antimalarial activity of plant extracts (Peters, 1975). The extract prolonged survival time of mice at all dose levels which is associated with suppression of parasitaemia in the curative model. The antimalarial effect produced by *T. avicennioides* leaf and stem bark extracts were similar to that produced by the positive controls. This implies that the extracts may act through mechanisms which are similar to those through which chloroquine and artesunate act. The parasite suppression exhibited by these extracts is comparable to results of former studies conducted on
methanol extract of the stem bark of *T. avicennioides* (Omonkhuwa et al., 2013; Akanbi, 2013; Akanbi et al., 2020). Although the antiplasmodial activity of both methanol leaf extracts of *A. leiocarpos* and *T. avicennioides* was reported to be higher at 400 mg/kg body weight (Akanbi et al., 2017). In vivo antiplasmodial activity can be classified as moderate, good and very good if an extract displayed respective percent parasite suppression equal to or greater than 50% at doses of 500, 250 and 100 mg/kg body weight per day (Deharo et al., 2001). Based on this classification, the extracts of *T. avicennioides* are considered to have exhibited good antiplasmodial activity, with dose dependent inhibition against *P. berghei* infection in mice.

4. CONCLUSION

The results of this study indicate that the leaf and stem bark extracts of *T. avicennioides* plant possesses significant in vivo anti plasmodial activity against *P. berghei* infection in mice and it is considered to be safe since it appears to be non-toxic. These findings justify and confirm the ethno botanical usage to be safe since it appears to be non-toxic. Further research on ethanol leaf and stem bark extracts and fractions of *T. avicennioides* could be carried out in order to isolate, identify and characterize the active principle from this plant.

5. ACKNOWLEDGEMENT

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6. ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

7. COMPETING INTEREST

Authors have declared that no competing interests exist.

REFERENCE


