IN VIVO ANTI-PLASMODIAL SCREENING OF Nicotiana tabacum AND ITS EFFECTS ON HEPATIC AND RENAL FUNCTION IN SWISS ALBINO MICE

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ABSTRACT
Malaria remains one of the leading public health diseases in developing countries and efforts to keep the infection at bay have suffered a lot of setbacks compounded by the increasing cases of resistance and cross resistance to first line antimalaria drugs. In this study, we investigated the anti-plasmodial efficacy of ethanol and aqueous extracts of Nicotiana tabacum on Plasmodium berghei infected Swiss albino mice and its effects on liver and kidney function. The comparison of changes in parasite load of the infected mice before and after treatment showed that the parasitemia level reduced significantly (p<0.05) in the mice treated with both ethanolic and aqueous extracts of N. tabacum, while there was a significant (p<0.05) increase in parasitemia level in the untreated mice. The activities of Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT) and creatinine varied marginally in the treated groups but the values were statistically comparable with control group (untreated) (P>0.05). However, the concentration of urea was statistically higher in treated groups than the control (p<0.05). Our results therefore demonstrate the anti-plasmodial potential of N. tabacum and its relative safety for human consumption at the tested doses.

Keywords: Nicotiana tabacum, anti-plasmodial effects, plasmodium heighlei, biochemical marker

INTRODUCTION
Malaria and its attendant mortality and morbidity has been a bane confronting many countries in different part of the world with the impacts mostly felt in Sub-Saharan Africa due to poor socio-economic development, inaccessibility to health facilities and incessant resistance associated with vector and parasite control strategies. The latest report from World Health Organization indicated that over 219 million people were infected in 2011 with 660,000 deaths. Nigeria and Democratic republic of Congo however accounted for over 40% of the death (WHO, 2012).

Herbal treatment has been part of healthcare system in tropical region of the world. The herbs are consumed to accentuate the challenges posed by the conventional drugs, which among many others include exorbitant prices and declining level of efficacies (Ismail et al., 2011). Zirhi et al (2005) estimated that about 80% of the World’s population uses herbal remedies. This estimate plausibly conforms with the situation in Africa where wide use of herbal products had been documented in both rural and urban areas (Aijayeoba et al., 2003; Aijayeoba et al., 2006, Mathaura et al., 2007; Ahmed et al., 2010; Odgehe et al., 2012). The high patronage of traditional birth attendants by the pregnant women (Idowu et al., 2008) is also an indication of acceptability of herbal treatment among Nigerian populations. Nicotiana tabacum (tobacco) is common in many parts of the world. In a recent publication by Israeli scientists, evidence was adduced that tobacco leaves contain a natural compound artemisinin, an ingredient that was formally isolated from sweet wormwood plant which has been well documented for its potency against malarial symptoms (Xinhua, 2012). This recent finding contradicts known cases of resistance and cross resistance to first line antimalaria drugs. In this study, we investigated the anti-plasmodial efficacy of ethanol and aqueous extracts of Nicotiana tabacum on Plasmodium berghei infected Swiss albino mice and its effects on liver and kidney function using mice model.

MATERIAL AND METHODS
Plant materials
Tobacco leaves were obtained from herbal vendors at Oja-Oba market, Osogbo and later authenticated at the Department of Biological Science, Osun State University, Osogbo, Nigeria.

Ethanolic extract
The tobacco leaves were air dried and ground into fine powder. 20g of the powdered leaves was weighed into a timble of a 250ml soxhlet apparatus and plugged with wad of cotton wool. The extraction was done with 200ml absolute ethanol as a solvent for 6hrs. The resulting ethanolic extract was concentrated in a rotary evaporator to give a paste and was used for this experiment.

Aqueous extract
The 2% aqueous extract of tobacco leaves was prepared by weighing 20g of the tobacco powder into one litter (1000ml) of distilled water. The solution was left for 72hours and filtered with muslin cloth. The infusion was kept in the refrigerator until use.

Experimental animal
30 mice weighing between 18 and 22g were obtained from the Animal House of the College of Health Sciences, Osun State University, Osogbo, Nigeria. The mice were kept in locally constructed cages 0.5m2 per mouse (at room temperature, 24±2°C; 12h dark cycle and 60-70% RH). The mice were maintained on food and water ad libitum at the Central Animal house of the College of Sciences, Engineering and Technology, Osun State University Osogbo in accordance with current ethical guidelines for the care of the laboratory animals.
Malaria Parasite

The chloroquine susceptible *Plasmodium berghei* used for the experiment was obtained from the donor mice at Institute of Malaria Research and Training (IMRAT) University College Hospital, Ibadan, Nigeria. Parasitized erythrocytes were obtained from these donor mice by bleeding of the eyes and then diluted with phosphate buffer saline (PBS). The mice were inoculated intraperitoneally with 0.2ml of blood suspension containing the parasitized erythrocyte on day 0, after seven days of acclimatization.

### Treatment regime

The thirty mice were randomly allocated into five groups with each group containing six mice. Post infection treatment was done on establishment of parasitemia in the mice after three days of inoculation. Treatment was based on body weight and was done ad libitum once daily for five days. All the experimental animals were grouped as follows:

- **Group 1**: treated with 0.2ml of olive oil (uninfected group).
- **Group 2**: treated with 0.2ml ethanolic extract of *Nicotiana tabacum* dissolved in olive oil.
- **Group 3**: given 2% aqueous infusion of *Nicotiana tabacum* which replaced their drinking water throughout the experimental period.
- **Group 4**: treated with 0.2ml of chloroquine (65mg/kg)
- **Group 5**: infected but not treated with any of the extract, infusion or chloroquine throughout the experimental period.

### Parasitemia determination

Thick smear of blood films were prepared three days after inoculation of the mice and five days after the commencement of treatment using the peripheral blood collected from the tail of the mice. The films were stained with Giemsa and the number of parasitized erythrocytes was enumerated in 50 high power fields.

### Extraction and preparation of tissue samples

The mice were sacrificed by cervical dislocation. The liver and the kidney tissues of the experimental animals were harvested rinsed in 1.15% KCl, homogenized in 4 parts of 0.25M sucrose solution, and then centrifuged at 4°C, 10,000rpm for 10mins. The supernatants were put in well labelled bottles and kept in the freezer for analysis.

### Kidney function tests

Creatinine and Urea concentrations were determined in the kidney homogenate supernatant using Randox kits (England) following manufacturer’s instructions.

### Biochemical analysis of the kidney toxicity

Kidney function test

The concentrations of creatinine and urea determined in the supernatant obtained from the homogenate of kidney of the mice are presented in table 2. The results revealed that the concentration of urea was statistically lower in uninfected mice and group treated with chloroquine as compared with groups treated with extracts and the group infected but not treated (P<0.05). There was no significant difference in the concentration of urea in the group treated with the extracts and untreated group. There concentration of creatinine was statistically similar across the groups (P>0.05).

Liver function test

The concentrations of AST, ALT and ALP as determined across the group are presented in table 3. The concentrations of the biochemical enzymes varied marginally among the groups but the differences were not statistically significant.

### Results

#### Statistical analysis

The data were expressed as mean ± Standard Error mean of six animals. Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, Standard version 10.0.1. P-values < 0.05 were considered statistically significant for differences in mean.

#### Antiplasmodial activities of the plant extracts

The results on parasitemia level before and after treatment showed that the parasite load reduced significantly (P<0.05) in the mice treated with ethanolic extract, aqueous extraction and chloroquine by 46.15%, 31.46% and 56.52% respectively. There was however significant increase in parasitemia in the mice infected but not treated (Table 1). No mortality was recorded during the experiment.

### Table 1

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>BEFORE TREATMENT</th>
<th>AFTER TREATMENT</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (uninfected)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>26.3 ± 2.9</td>
<td>14.3 ± 1.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>26.7 ± 0.4</td>
<td>18.3 ± 0.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>23.3 ± 3.6</td>
<td>10.3 ± 1.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Infected but not treated</td>
<td>20.0 ± 4.0</td>
<td>38.8 ± 2.8</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

**Legend:** The results are mean ± standard error mean of six mice in 50 high power fields; P value <0.05 is significant.

### Table 2

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>UREA  mg/dl</th>
<th>CREATININE mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (uninfected)</td>
<td>3.79±0.20 a</td>
<td>0.776±0.81 b</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>4.74±0.33 a</td>
<td>0.496±0.70 a</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>4.35±0.27 a</td>
<td>0.557±0.50 a</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>3.65±0.28 b</td>
<td>0.513±0.11 a</td>
</tr>
<tr>
<td>Infected but not treated</td>
<td>4.71±0.27 a</td>
<td>0.442±0.11 a</td>
</tr>
</tbody>
</table>

**Legend:** The results are mean ± standard error mean of six mice; figures with different letters along the column are significant at P <0.05.
**DISCUSSION**

The present study assessed the efficacy of the ethanolic extract and aqueous infusion of tobacco leaves on parasite load and biochemical markers of liver and kidney toxicities. The doses used in the present study had been previously known to exhibit less neurological misbehaviour and higher survival in mice treated with tobacco leaves (Adeniyi et al., 2010). The aqueous and ethanolic extracts demonstrated significant reduction in parasite load five days post treatment which compared favourably but less potent as chloroquine. This observation confirmed the earlier publication on anti-plasmodial potency of the leaf (Xianhu, 2012).

The ethanolic extract demonstrated higher potency over aqueous extract which was in conformity with previous observation that the extraction method plays major role in the potency of herbal plants (Mathaure et al., 2007). Various researchers had reported that most of the herbal plants only achieved chemosuppressive but not total curative effect on malaria parasites (Salawu et al., 2010; Ismail et al., 2011; Ejebe et al., 2011). However, it is not clear whether the extracts could achieve a curative potential if the treatment continues for longer days since the standard malaria drug (chloroquine) did not also cure the parasite five days post treatment. On the other hand, whether increase in consumption of the extracts from once to twice daily could double and fast track the curing potential of the extracts is also an issue waiting for elucidation. These unattended issues are part of the limitations of the present study and would be addressed in our subsequent investigations on the plant.

The biochemical enzymes analyzed in the present study have been previously described as potential markers in determining the liver and renal integrity of the animals being exposed to pharmacology agent(s) described as potential markers in determining the liver and renal integrity. The biochemical enzymes analyzed in the present study have been previously described as potential markers in determining the liver and renal integrity of the animals being exposed to pharmacology agent(s) (Nwogu et al., 2008; Uboh et al., 2010). With exception of urea, the results of the renal and liver function tests showed that most of the values obtained for the biomarkers in treated groups are statistically comparable with the control (untreated). These findings showed that tobacco leaf did not portend any serious threat to liver and renal functioning when consumed. Similar observations have also been reported elsewhere on other herbal products (Uboh et al., 2010). According to Odeyemi et al. (2010), the high concentrations of these biochemical enzymes are normally released into the blood stream from the cytosol and sub-cellular organelles when hepatic and renal injuries occur. However, the significantly higher concentration of urea in the groups treated with extracts calls for worry and may signal the gradual cellular damage by the ethanolic extract. The area is the principal end product of protein catalysis and may signal the gradual cellular organellar when consummed.

**Table 3 Biochemical analysis of the liver toxicity biomarkers**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>ALT U/L</th>
<th>AST U/L</th>
<th>ALP U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.54 ±3.46 a</td>
<td>106.6 ±22.12 a</td>
<td>77.65 ±25.6 a</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>71.59 ±11.43 a</td>
<td>94.86 ±11.13 a</td>
<td>57.40 ±27.87 a</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>73.59 ±6.05 a</td>
<td>106 ±8.11 a</td>
<td>81.70 ±27.69 a</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>60.81 ±11.04 a</td>
<td>88.93 ±6.45 a</td>
<td>37.54 ±14.10 a</td>
</tr>
<tr>
<td>Infected but not treated</td>
<td>79.42 ±3.43 a</td>
<td>99.83 ±11.52 a</td>
<td>62.01 ±16.08 a</td>
</tr>
</tbody>
</table>

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