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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 firstline antimalaria drugs. In this study, we investigated the anti-plasmodial efficacy of ethanolic 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. tobacum* and its relative safety for human consumption at the tested doses.

Keywords: *Nicotiana tabacum*, anti-plasmodial effects, *plasmodium beighei*, 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)**. **Zirihi *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; Odeghe *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 tobacum (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 artemisin, 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 reports on tobacco. Literature abound on the adverse effects of tobacco smoke which has been proven to induce various ailments such as bladder and lung cancer **(Hartge *et al.*, 2006)**, pancreatic cancer **(Joan and Debra,2004),** neurological disorders **(Adeniyi *et al.*,2010),** nasal cavity and liver tumors **(Abraham *et al.*,1988).** Multiple dose of tobacco specific nitrosamine-4-(methylamino)-1-(3-pyridyl)-1-butanone(NNK) was reported to be involved in the oxidative DNA damage linked lung tumorigenesis **(Fung-Lung and Yong, 1992).** Despite the well documented research and evidences on adverse effects of tobacco smoking, little is known on toxicities of smokeless tobacco consumption on vital organs in the body. Our recent interactive sessions with herbal vendors in Osogbo, a city in Southwestern Nigeria showed that few people do request for tobacco leaves decoction for malaria treatment.

The objective of this study was to evaluate the in-vivo anti-plasmodial efficacy of ethanolic and aqueous extracts of *N. tabacum* and its effects on kidney and liver 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 grounded 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 muslein cloth. The infusion was kept in the refridgerator 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 0.5m^2 per mice (at room temperature ,24-25^oC; 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 *adlibitum* 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 *N. 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^{-1})
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 dislogement. The liver and the kidnery tissues of the experimental animals were harvested rinsed in 1.15% KCl ,they were homogenised in 4 parts of 0.25M sucrose solution,the resulting homogenates were centrifuged at 4^{o}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 and absorbances (nm) were read using UNISPEC SM7504UV Spectrophotometer, (UNISCOPE, England).

Liver function tests

The activities of aspartate aminotranferase (AST) and alanine aminotranferase (ALT) were determined using the method described by **Reitman and Frankel (1957)** and alkaline phosphatase (ALP) was determined using the method described by Deutsche Gesellschaft fur Klinische Chemie **(DGKC, 1972)**.

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.

RESULTS

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, aqeous 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.

Kidney function test

The concentrations of creatinine and urea determined in the supernatant obtained from the homegenate 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 comapred 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 accross 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.

Table 1 The mean parasitemia of the *P.berghei* infected mice before and after treatment with plant

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

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

Table 2 Biochemical analysis of the kidney toxicity

GROUPS	UREA mg/dl	CREATININE mg/dl
Control (uninfected)	3.79±0.20 ^{b}	0.776±0.81 ^{a}
Ethanolic extract	4.74±0.33 ^{a}	0.496±0.70 ^{a}
Aqueous extract	4.35±0.27 ^{a}	0.557±0.50 ^{a}
Chloroquine	3.65±0.28 ^{b}	0.513±0.11 ^{a}
Infected but not treated	4.71±0.27 ^{a}	0.442±0.11 ^{a}

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

Table 3 Biochemical analysis of the liver toxicity biomarkers

GROUPS	ALT U/L	AST U/L	ALP U/L
Control	82.54 ±3.46 [a]	108.66 ± 22.12 [a]	77.65 ± 25.6 [a]
Ethanolic extract	71.59 ±11.43 [a]	94.86 ± 11.13 [a]	57.40 ± 27.87 [a]
Aqueous extract	73.59 ±6.05 [a]	106 ± 8.11 [a]	81.70 ± 27.69 [a]
Chloroquine	60.81 ± 11.04 [a]	88.93 ±6.45 [a]	37.54 ±14.10 [a]
Infected but not treated	79.42 ± 3.43 [a]	99.83 ±11.52 [a]	62.01 ± 16.08 [a]

Legend: The results are mean ± standard error mean of six mice. Figures with different letters along the column are significant at P <0.05.
AST= Aspartate Aminotransferase; ALP= Alkaline phosphate; ALT= Alanine aminotranferase

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 **(Mathaura *et al.*, 2007)**. Various researchers had reported that most of the herbal plants only achieved chemo-surpressive 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 elucidiation. 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) **(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 plant. Urea is the principal end product of protein catabolism, a waste that is excreted by the kidney **(Khleifat *et al.*, 2002)**. Its accumulation in the kidney may signify the compromise in the integrity of the kidney. High level of urea in blood is associated with Nephritis, renal ischemia and urinary tract obstruction **(Oyewole *et al.*, 2012)**.

CONCLUSION

In conclusion, the present study has documented the anti-plasmodial potential of *N. tobacum* and the results showed that the aqeous and ethanolic extracts of the leaf are potent in causing significant reduction in malaria parasite load as the standard anti-malaria drug . The analysis of biochemical markers of the kidney and liver function showed that the extracts do not pose any serious threat on the organs when consumed at the tested doses. The findings from these study are remarkable and demonstrate that the leaf extract of *N. tobacum* is a promising antimalaria herb and could be explored in the manufacture of potent anti-malaria drugs.

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