Journal of Advances in Microbiology

8(2): 1-7, 2018; Article no.JAMB.38996
ISSN: 2456-7116

Antiplasmodial Activities of Methanol Leaf Extract of *Tithonia diversifolia*, and It's Toxicity Effect on Swiss Albino Mice

A. Agbadaola[1*] and W. O. Tanimowo[2]

[1]Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ogun State, Nigeria.
[2]Department of Biological Sciences, Anchor University, Lagos, Nigeria.

Authors' contributions

This work was carried out in collaboration between the two authors. Author AA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author WOT managed the analyses of the study, managed the literature searches and the last draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/38996
Editor(s):
(1) Grzegorz Cieslar, Professor, Department and Clinic of Internal Diseases, Angiology and Physical Medicine, Medical University of Silesia, Poland.
Reviewers:
(1) Anjuwon Tayo Micheal, Ahmadu Bello University, Nigeria.
(2) Voravuth Somsak, Walailak University, Thailand.
Complete Peer review History: http://www.sciencedomain.org/review-history/23139

Original Research Article

Received 25th November 2017
Accepted 3rd February 2018
Published 12th February 2018

ABSTRACT

Due the ceaseless use of plants in the treatment of diseases by indigenous people of various countries and continents, it is necessary to evaluate possible health risks associated with the consumption of crude preparation of various parts of plants. This study has been aimed at evaluating the antiplasmodial activities of methanol extract of dried leaf of *Tithonia diversifolia* and its toxicity effect using swiss albino mice. The antiplasmodal effect of methanol extract of *Tithonial diversifolia* were evaluated in swiss albino mice infected with chloroquine sensitive *plasmodium berghei*. Result showed a dose dependent blood schizonticidal activity. The *in vivo* antiplasmodial effect of the extract (100,200, and 400 mg/kg.body weight) against *P. berghei* showed significant increase ($p<0.05$) in dose dependent activity for curative test, significant increase in percentage parasitaemia clearance as 66.39%, 70.40%, and 78.94% respectively. Extract showed a significant increase in the white blood cell and red blood cell ($P<0.05$), also a significant increase ($p<0.05$) was

**Corresponding author: E-mail: akinbaba4@gmail.com;*
E-mail: wtanimowo@aul.edu.ng;

observed in potassium ion and alkaline phosphatase concentration. The result of this study suggests that the extract have a considerable antimalaria property but the adverse effect on the function of liver and kidney, if used indiscriminately may be lethal.

Keywords: Antiplasmodial; parasitaemia; methanol; chloroquine; antimalaria.

1. INTRODUCTION

Malaria is caused by parasitic protozoan's belonging to the *Plasmodium* species [1]. Basic symptoms are fever, fatigue, vomiting, and headaches, which usually begin ten to fifteen days after being bitten. In addition, it can cause yellow skin, seizures, coma, or death in severe cases [2]. People may develop recurrences of the disease months later, if not well treated [1]. Re-infection usually causes milder symptoms in those who have recently survived an infection, this partial resistance however, disappears over months to years if the person has no continuing exposure to malaria.

Five species of the genus plasmodium can infects and spread by humans [2]. Most death are caused by *Plasmodium falciparum* because *Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae* generally cause a milder form of malaria [2]. Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic test [2]. Methods that use the polymerase chain reaction to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity [3].

Nature has provided plants in abundance for all living creatures, which possess medicinal virtues. The essential medicinal values of some plant have long been published but a large number of them remain unexploited as yet. In spite of the advent of modern medicine, nearly about 80% of the total human population still depends on traditional remedies together with folklore system based mainly on phytotherapy [4]. Although, there is an historic role of medicinal herbs in the therapeutics and prophylactics of disease, these do not, however, assure their safety for uncontrolled use by the uncontrolled public [5]. These notwithstanding, the available evidences show that plants are playing a leading role in the therapy [6].

Tithonia diversifolia is a species of flowering plant that belongs to family Asteraceae commonly known as the wild sunflower, tree marigold, mexician sunflower, or Japanese sunflower. *Tithonia diversifolia* have various indigenous medicinal uses in many countries. In Nigeria for instance, the decoction of its various parts are used for the treatment of ailments such as malaria, diabetes mellitus, sore throat, liver and menstrual pain [7]. Also, an oral decoction of the leaves and stem is used for the treatment of hepatitis in *Taiwan* and gastro-intestinal disorder in Kenya and Thailand [8]. Some of these indigenous medicinal uses have been scientifically proved. As a result of it's continues use in the treatment of diseases by indigenous people of various nativity, it is necessary to evaluate possible risks that the consumption of crude preparation of various parts of this plant may cause to the health of the people. This study has been aimed at evaluating the antiplasmodial activities of methanol extract of dried leaf of *Tithonia diversifolia* and its toxicity effect using swiss albino mice.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extract Preparation

The fresh leaf of *Tithonia diversifolia* was obtained along state specialist hospital road Ikere-Ekiti, identified and authenticated. The leaf was subsequently cleaned and air dried at room temperature before it was grounded into a coarse powder using electric blender (SONIC JAPAN, SFP-2210, CHINA). 100 g powder plant was macerated in 500 ml of 100% methanol for 72 hours. It was filtered using Whatman filter paper and concentrated *in vacuo*, using rotary evaporator. The extract was stored in well-closed container and kept for further usage.

2.2 Animal Collection

Swiss Albino mice were obtained from Animal House of the Botany Department, Olabisi Onabanjo University, Ago- Iwoye, Ogun State.

2.3 Acute Toxicity

The method of Lorke was used to determine the median lethal dose (LD) of the extract. In the first

phase, nine mice were randomly divided into three groups of three mice each and each group received the extract at 10, 100 and 1000 mg/kg body weight orally (*via* a feeding cannula). The mice were then observed for signs of adverse effects and mortality for the first 48 h and then for 12 more days. In the second phase, four mice were divided into 4 groups of 1 mouse each and were each similarly treated but at doses of 1000, 1600, 2900 and 5000 mg/kg orally. The animals were then monitored for any sign of toxicity like stretching, rubbing of nose on the floor and wall of cage, change in body weight and mortality over a period of 14 days [9].

The LD_{50} was calculated using the formular:

LD_{50}= [A× B] $^{1/2}$
LD- lethal dose
A= Highest dose that gave no mortality,
B = Lowest dose that produced mortality

2.4 Parasite Strain

Chloroquine (CQ)-sensitive *Plasmodium berghei* NK65 strain was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University of Ibadan, Nigeria. The parasites were maintained by continuous blood passage in mice. A standard inoculum of 1×10^7 parasitized erythrocytes was prepared by dilution of blood collected through cardiac puncture from a donor mouse (> 30% parasitaemia) with normal saline and administered intraperitoneally (200 µl) to each test mouse.

2.5 Evaluation of Schizonticidal Activity in Established Infection (Curative or Rane's) Test Model

Thirty (30) mice were inoculated with *P. berghei*, 72 hrs prior to drug administration using a modified method of Ryley and Peters [10]. The mice were randomly divided into 6 groups of 5 mice each and mice in Group 1 was neither injected with parasite nor treated with extract, Groups II – IV were orally treated with methanol extract of *T. diversifolia* at 100, 200 and 400 mg/kg/day while Chloroquine at 10 mg/kg/day and distilled water (0.2 ml) were administered to Groups V and VI as positive and negative controls, respectively. The drug administration, preparation of blood smears collected from the tail and microscopic examination of parasitized cells to assess the parasitaemia levels were carried out daily for 5 days (D_1 – D_5). The parasitaemia levels were assessed by examining and counting the parasitized and total red blood cells in the Giemsa-stained blood smears.

The percentage chemosuppression was determined by recording the number of parasitized red blood cells out of 500 red blood cells counted in random fields under light microscope and calculating the average percentage parasitaemia suppression using the formula for percentage parasitaemia = $100\{Np/Nt\}$,

where Np is the number of parasitised red blood cells and Nt is the total number (parasitised + unparasitised) of red blood cells per view of count.

Percentage chemosuppression = $100\{(A-B)/A\}$,

where A is the average percentage parasitaemia in the negative control group and B is the average parasitaemia in the test group.

2.6 Collection of Blood Sample for Haematological and Biochemical Parameters

Each of the thoracic cavities of the mice was carefully dissected and the heart was exposed. The required blood sample was withdrawn from the heart (left ventricles) by cardiac puncture with a needle and syringe. The blood was quickly transferred into an EDTA bottle for estimation of haematological parameters and lithium heparin bottle for estimation of serum electrolytes. The haematological parameter; packed cell volume (PCV), haemoglobin level, white blood corpuscle (WBC), mean corpuscular haemoglobin concentration (MCHC) were estimated using automated haematological analyzer (Abacus 380).

Serum Potassium and Chloride concentration was determined using spectrophotometer. Alkaline phosphate, Aspartate aminotransferase, alanine aminotransferase were determined using Reflotron machine.

2.7 Data Analysis

The percentage parasitaemia reduction was expressed as mean ± standard error of mean (SEM). The variation in a set of data was analyzed through the one-way analysis of

variance while the difference among the means was considered at 95 % confidence level using the post-hoc method of Newman-Keuls (GraphPad Prism® 4 San Diego, USA [2011]).

3. RESULTS

The effect of the methanol extract of *Tithonia diversifolia* on the parasite density following five days treatment of established *Plasmodium berghei* infected mice. The percentage clearance of parasitaemia by the extract and Chloroquine is represented in (Table 1). The percentage clearance is in the order of; Chloroquine, 90.16%, Extract (400 mg/kg.bw), 78.94%, Extract (200 mg/kg.bw), 70.40%, Extract (100 mg/kg.bw), 66.39%.

The result of the haematological parameter showed significant increase in red blood cell, White blood cell ($p<0.05$) with increase in the concentration of the extract. There is significant boost in the red blood cell of those group treated with the extract as compare to the negative control.

The extract significantly increased the serum concentration of Potassium ion. However the extract did not significantly increase the concentration of urea concentration in all the doses administered compare to control (Table 4). There was no significant increase in the chloride ion concentration as compare to the uninfected group and negative control (Table 4). The extract had no significant effect on alkaline phosphatase at 100 mg/kg body weight while there was significant increase at 200 and at 400 mg/kg body weight. The three doses used for this study show no significant effect on ALT and AST (Table 3).

4. DISCUSSION AND CONCLUSION

Methanol extract of the leaves of *Tithonia diversifolia* showed significant antiplasmodial activities as depicted by the percentage parasitaemia and chemo-suppressive properties of the extract. The highest curative effect was observed for the plant extract when the highest dose 400 mg/kg.bw was administered, and this made it dose dependent. 78.94% curative effect was observed in the 400 mg/kg b.w, extract as compared with 90.16% obtained for chloroquine phosphate.

The better performance observe for chloroquine compare with the extract in this study agree with report by Kamel et al. [11], that when a standard antimalaria drug is used in the management of *plasmodium berghei* in mice, it suppress parasitaemia. The highest percentage chemosuppression activity of chloroquine recorded in the study showed that it could still serve as antimalaria drug [12]. The antiplasmodial properties of this plant could be as result of its phytochemical composition which has been established to have antiplasmodial and antiprotozoal activities [13]. Indeed, tagitinin c and some other sesquiterpene lactone isolated from *Tithonia diversifolia* might be responsible for the anti-malaria property of the plant [14].

The significant increase in serum potassium level observe in the extract may be as a result of the increase potassium load in the body fluid which could be attributed to kidney dysfunction possibly by a defective mechanism of tubular potassium excretion [15]. The increase in the serum urea observed in the study could be attributed to reduce in functionality of the kidney at cleaning metabolic by product.

The Alkaline phosphatase,(ALP) ,Alanine amino transferase (ALT), and Aspartate amino transferase in tissues and blood are important marker enzymes which are used to assess the integrity of the cell membrane, cytosolic activity and cell death [16]. Increased ALP activities in liver suggest the possibility of the extract causing membrane damage in these organs at higher

Table 1. Effects of methanol extract of *T. diversifolia* on chloroquine sensitive *P. berghei*– infected mice at different doses under the curative test after day 5 administration

Dose (mg/kg)	% Parasitaemia	% Clearance
Negative Control	29.97 ± 1.21	0.0
100.00	10.07 ± 0.59[a]	66.39 ± 1.95
200.00	8.87 ± 1.47[a]	70.41 ± 4.92
400.00	6.31 ± 1.40	78.94 ± 4.66[b]
Chloroquine (10)	2.95 ± 0.50	90.16 ± 1.66[b]

Data are expressed as mean ± SEM, n=5, values with same letters are not significantly different (P>0.05), values with different superscripted letters are significantly different (P<0.05)

Table 2. Effect of methanol extract of *Tithonia diversifolia* on haematological parameters of Swiss albino mice

Parameters	Uninfected group	Negative control (Distilled water)	100 mg extract	200 mg extract	400 mg extract	Positive Control (10 mg Chloroquine)
Hb (g/dl)	15.41 ± 0.09	11.97± 0.13	14.06 ± 0.12	14.93 ± 0.37^{a}	15.40± 0.08^{a}	16.37 ± 0.34
PCV (%)	50.73±0.06	41.70±0.43	42.58±0.37	43.320.50	45.900.35	49.68±0.25
RBC (X10$^{12/}$l)	9.71±0.16^{b}	8.63 ±0.09	8.80 ± 0.26^{c}	8.84 ± 0.12^{c}	9.04 ± 0.39^{b}	10.56 ± 0.14
MCV (fl)	52.00 ± 1.42^{e}	48.00 ± 0.41^{d}	47.00 ± 0.17^{d}	49.00 ± 0.10^{d}	51.00 ± 0.45^{e}	47.00 ± 0.25^{d}
MCH (pg)	15.90 ± 0.42^{f}	15.60 ± 0.22^{f}	15.30 ± 0.08^{f}	15.75 ± 0.02^{f}	15.30±0.12^{f}	14.30 ±0.04
MCHC (g/dl)	30.45 ± 0.37^{g}	32.40 ± 0.28^{h}	32.75 ± 0.11^{h}	31.75 ±0.10	30.15 ± 0.17^{g}	30.40 ± 0.14^{g}
WBC (x10^{9} /l)	8.70 ±0.08	8.63 ±0.06	13.25 ± 0.32^{i}	16.15 ± 0.31^{j}	16.60 ± 1.09^{j}	14.86 ± 0.44^{i}
Platelet (10^{9} /l)	680.00 ± 29.40^{k,l}	910.40 ± 66.52^{k,m}	963.00 ± 38.76^{m}	769.00 ± 12.82^{l,m}	611.5 ± 43.88^{l}	894.50 ± 52.56^{m}
Neutrophil (%)	12.40 ±0.29	50.00 ± 0.13^{o}	52.40 ± 0.08^{o}	35.60 ± 0.18^{n}	35.35 ± 0.27^{n}	35.00 ± 0.30^{n}
Lymphocyte (%)	82.20 ± 0.28^{p}	31.10 ±1.17	36.40 ± 0.31	46.40 ±0.28	48.90 ± 0.05	54.40 ± 0.23^{p}

Data are expressed as mean ± SEM, n=3, values with same letters are not significantly different (P>0.05), values with different superscripted letters are significantly different (P<0.05)

Table 3. Effect of methanol extract of *T. diversifolia* on liver of Swiss albino mice

Parameters	Uninfected group	Negative control (Distilled water)	100 mg extract	200 mg extract	400 mg extract	Positive control (Chloroquine)
Alkaline phosphatase.	20.20 ± 0.26^{a}	19.10 ± 0.60^{a}	35.70 ± 0.99^{a}	52.30 ± 1.19^{a}	88.40 ±10.14	22.10 ± 0.48^{a}
Aspartate amino transferase (AST)	70.70 ±0.12	69.90 ± 0.65^{b}	66.10 ± 0.29^{b}	68.20 ± 0.33^{b}	72.10 ± 0.37^{b}	73.20 ±0.46
Alanine amino transferase (ALT)	42.70 ± 0.37^{d}	39.50 ± 0.16^{c,d}	36.00 ± 0.41^{c}	37.60 ± 0.53	41..40 ± 0.26^{c,d}	38.80 ± 0.37^{c,d}

Data are expressed as mean ± SEM, n=3, values with same letters are not significantly different (P>0.05), values with different superscripted letters are significantly different (P<0.05)

Table 4. Effect of methanol extract of *T. diversifolia* on selected electrolyte of Swiss albino mice

Parameter	Uninfected group	Negative control (Distilled water)	100 mg extract	200 mg extract	400 mg extract	Positive control (Chloroquine)
Potassium	1.50 ± 0.17	1.60 ± 0.21^{a}	1.90 ± 0.24^{b}	2.60 ± 0.43^{b}	3.10 ±0.17	1.90 ± 0.08^{a}
Chloride	112.10 ± 0.38^{d,e}	105.90 ± 0.39^{c}	102.00 ±0.51	106.0 ± 00.31^{c,d,e}	103.00 ± 0.45^{c,d}	109.60 ± 0.12^{e}
Urea	5.10 ± 0.16^{f}	5.80 ± 0.06^{f}	6.80 ± 0.31^{f}	7.60 ±0.36	10.30 ±0.14	6.36 ± 0.15^{f}

Data are expressed as mean ± SEM, n=3, values with same letters are not significantly different (P>0.05), values with different superscripted letters are significantly different (P<0.05)

concentration or longer period of exposure of the animal to the extract, though there was no alteration in serum AST and ALT activities at the concentrations used in this study. This observation is reflective of the response of the cellular system to offset the stress imposed on the enzyme by exposure to the extract which may result from the inhibition of the enzyme by exposure to the extract which may result from the inhibition of the enzyme activity in situ [17]. Increase alkaline phosphatase is needed during strees to produce adequate amount of phosphate groups for oxidative phosphorylation to generate ATP which, in turn is required for the phosphorylation of some biomolecules like ethanolamine and choline, to form phosphatidyl ethanolamine and phosphatidyl choline, which are vital phospholipid component of the plasma membrane thereby trying to stabilize the integrity of the membrane.

This study has shown that the extract has antiplasmodial properties, administration of *Tithonia diversifolia* enhance Red blood cell production and it also cause slight electrolyte imbalance. Though, presumptive treatment is common practice. Indiscriminate use and consumption of the crude extract of *Tithonia diversifolia* leaves may be lethal. The results shows that the administration of methanol extract of *Tithonia diversifolia* may adversely affect liver and kidney function at high concentration. Thus, indiscriminately use of the plant should be discouraged.

ETHICAL APPROVAL

The study was approved by the Ethics Committee of the Olabisi Onabanjo University, Ago- Iwoye. Furthermore, the mice were well fed with standard commercially available feed and given water as desired by the researcher. They were subjected to optimal environmental condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. World Malaria report. Geneva, Switzerland World Health Organization. 2014;32-42.
2. Caraballo H. Emergency Department Management of mosquito-borne illness: malaria, Dengue, and West Nile virus. Emergency Medicine Practice. 2014;16(5).
3. Nadjm B, Behrens RH. Malaria: An update for physicians. Infection Disease Clinics of North America. 2012;26(2):243-59.
4. Azaizeh H, Fulder S, khalil K, Said O. Ethnomedicinal knowledge of local Arab Practioners in the middle East region, Fitoterapia. 2003;7(8):98-108.
5. Mattehews HB, Lucier GW, Fisher KD. Medicinal herbs in the United State: Research needs. Environ. Health Perspect. 1999;158:2200-211.
6. Srivastava R. Studying the information needs of medicinal plant stakeholder in Europe. Traffic Dispatches. 2000;15:5.
7. Elufioye TO, Agbedahunsi JM. Anti-malaria activities of *Tithonia diversifolia* (Asteraceae) and Crossopteryx febrifuga (rubiaceae) on mice *in-vivo*. Journal of Ethnopharmacology. 2004;93(2-3):167-71.
8. Johns T, Faulbert GM, Kokwaro JO, Mahunnah RLA, Kimanani EK. Anti-giardial activity of gastrointestinal remedies of the Luo of East Africa. Journal of Ethnopharmacology. 1995;46:17-23.
9. Lorke DA. New approach to practical acute toxicity testing. Archives of Toxicology. 1983;275–287.
10. Ryley JF, Peter W. The antimalarial activity of some quinolone esters. Ann. Trop. Med. Parasitol. 1970;84:209-222.
11. Kamel K, Matsuaka H, Furuhata S, Fujisaki R, Kawakami T, Mogi S, Yoshihara H, Aoki N, Ishil A, Shibuya T. Antimalaria activity of leave-extract of *Hydragea macrophylla*, a common Japanese plant. Acta. Medica Okayama. 2000;54:227-232.
12. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalaria drug discovery. Efficacy models for compound screening. Nature Reviews Drug Discovery. 2004;3: 509-520.
13. Arise RO, Malomo SO, Lawal MM. Comparative antimalarial and toxicological effect of artemisinin with methanol extract of *Carica papaya* leaves and bark of *Aistonia broonai* in animal models. Advances in Natural & Applied sciences. 2012;6:116-123.
14. Goffin EE, Ziemons P, De Mol C, de Madureira M, Martin AP. *In-vitro* antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent in Tagitinin C. Planta Medica. 2002;68:543-545.

15. Akanji MA, Olagoke OA, Oloyede A. Effect of chronic consumption of metabisulphite on the integrity of rat kidney cellular system. Toxicology. 1993;81:173-179.
16. Adebayo JO, Balogun EA, Oyeleke SA. Toxicity study of the aqueous extract of *Tithonia diversifolia* leaves using selected biochemical parameters in rat. 2009;1:143-147.
17. Kes P. Hyperkalemia, a potentially lethal. Acta Clinica Croatica. 2001;40:215-225.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23139