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ANTIPLASMODIAL EFFICACY AND TOXICITY PROFILE OF METHANOLIC LEAF EXTRACT OF TITHONIA DIVERSIFOLIA IN PLASMODIUM BERGHEI-INFECTED MICE

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Abstract

Malaria is a mosquito borne infection of human and animals caused by parasitic protozoan's belonging to the plasmodium species. Malarial is a public health problem most especially in tropical countries where majority bear the burden of the disease. Due to emergence of resistance of malaria to some of the drug used, medicinal plant has also proved a potent means of treatment. Medicinal plant or herbs derived from plant and used for the treatment and prevention of disease have been important in all culture throughout history. The significant of their ameliorative and preventive effectiveness in various diseases is seen today in their continued use. Herbal remedies for different ailment including malaria. broadens the horizon of knowledge in the area of natural product chemistry as well as benefiting people immensely by lowering the cost of treatment of ailment. The antiplasmodal effect of methanolic extract of Tithonia diversifolia were evaluated in swiss albino mice infected with chloroquine sensitive Plasmodium berghei. Activities investigated were curative effect against established infection, in which the parasitaemia level were assessed by counting the parasitized and total red blood cells in Giemsa- stained smears. Toxicity effect on haematological parameters, using haematology analyser and serum electrolytes using spectrophotometetry analysis and histological analysis of the liver and kidney in mice. The extract shows a dose dependent blood schizonticidal activity. The invivo antiplasmodal effect of the extract (100,200, and 400mg/kg.body weight) show 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 show a significant increase in the white blood cell and red blood cell, also a significant increase ($p<0.05$) was observed in potassium ion and alkaline phosphatase concentration. The histological analysis shows that the extract does not have a significant effect on the selected organ based on the doses used. The results of this study suggest that the extract have a considerable antiplasmodal property but care must be taken by the local producer and consumer in avoiding indiscriminate use of the plant as increase dose may be lethal.

Keywords: Methanolic Leaf Extract, Malarial, Dried leaf, Antiplasmodial.

Introduction

Malaria is a mosquito borne infection disease of human and animals caused by parasitic protozoan's (a group of single-celled microorganism) belonging to the Plasmodium species. Malaria causes symptoms that typically include fever. fatigue, vomiting and headaches. In severe cases it can cause yellow skin, seizures, coma, or death (Caraballo 2014). Symptoms usually begin ten to fifteen days after being bitten. If not properly treated, people may have recurrences of the disease months later. (WHO 2014). In those who have recently survived an infection, re-infection usually causes milder symptoms. This partial resistance disappears over months to years if the person has no continuing exposure to malaria.

Malaria is a public health problem most especially in the tropical countries where majority bear the burden of the disease. It is one of the six killer disease in the world today and it has been estimated that 40% of the world's population is at risk and 500 million people suffer from the disease annually. The disease is widespread in the tropical and subtropical regions that exist in a broad band around the equator (Caraballo 2014). This includes much of sub-Saharan Africa. Asia, and Latin America. In 2015, there were 214 million cases of malaria worldwide. This resulted in an estimated 438,000 deaths. 90% of which occurred in Africa (WHO, 2016). Rates of disease have decreased from 2000 to 2015 by 37% (WHO 2016) but increase from 2014 during which there were 198 million cases (WHO. 2014). Malaria is commonly associated with poverty and has a major negative effect on economic development, (Gollin and Zimmermann 2007. Worrall et al., 2005). About two million children, nostly less than five years and pregnant women die from malaria related illness cach year and nine out of ten cases are found in sub-saharan Africa. (WHO, 2010). Most vulnerable group in the endemic areas constitutes people in the rural environments who often had little or no access to modern medicine. Despite various declarations by African governments and complementary efforts promised in the main content of the Roll back malaria declaration, malaria remains a major health challenge in Africa and Nigeria. The vulnerable groups are mostly pregnant women and children under five (5) years (WHO 2010).

The first effective treatment for malaria came from the bark of Cinchona tree, which contains quinine. This tree grows on the slopes of the Andes, mainly in Peru. The indigenous people of Peru made a tincture of cinchona to control fever. Its effectiveness against malaria was found and the Jesuits introduced the treatment to Europe around 1640; by 1677, it was included in the London Pharmacopoenia as an antimalaria treatment. (Kaufman and Ruveda 2005). It was not until 1820 that the active ingredient, quinine, was extracted from the bark, isolated and name by French chemists (Pelletier and Caventou 1820), Kyle and Shampe (1974). Quinine became the Dredominant malarial medication until the 1920s, when other medications began to be developed. In the 1940s. Chloroquine replaced quinine as the treatment of both uncomplicated and severe malaria until resistance supervened, first in

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Southeast Asia and South America in the 1950s and then globally in the 1980s. (Achan et al., 2011).

A medicinal plant is any plant, in which one or more of its organs contain active ingredients which can be used for therapeutic purpose or contain foundation compounds that can be used for the synthesis of useful drug (Sofowora, 2008). It has also been reported that, the African continent have a long history with the use of plants and in some African countries, up to 90% of the population rely on medicinal plants as a source of drugs (Hostettmann et al.,2000).

Thus plants such as Tithonia diversifolia in the family Asteraceae are used in herbal medicines to cure diseases and heal injuries due to its tradomedicinal potentials.

The stem and leaves of Tithonia diversifolia are used as a fodder, poultry feed (Roothaert and Paterson, 1997), In Nigeria, the decoctions of various parts are used for the treatment of malaria, diabetes mellitus, sore throat, liver and menstrual pains (Elufioye and Agbedahunsi, 2004: Owoyele et al.,2004; Moronkola et al., 2007). Oral decoction of the leaves and stem is used for the treatment of hepatitis in Taiwan and gastrointestinal disorderin Kenya and Thailand (Johns et al., 1995). Mahecha and Rosales (2005) stated that, the protein in Tithonia diversifolia foliage is highly soluble and hence is quickly fermented in the rumen of goats.

Based on the background of this study, the study therefore examined the antiplasmodal activities of methanolic extract of dried leaf of Tithonia diversifolia and its toxicity effect using swiss albino mice. Specifically, the study:

i. evaluate antiplasmodal activities of methanolic extract of dried leaf of Tithonia diversifolia on Plasmnodium berghei.
ii. observe the effect of methanolic leaf extract of Tithonia diversifolia on heamatological indices and serum biochemistry of Swiss albino mice.
iii. determine histopathological effect of methanolic leaf extract of Tithonia diversifolia on some internal organs of Swiss albino mice.

Methodology

Plant material, extraction, and acute toxicity testing: Fresh leaves of Tithonia diversifolia were collected from Ikere-Ekiti, Nigeria, authenticated, air-dried, and pulverized. Methanolic extraction was performed by macerating 100 g of powder in 500 mL of 100% methanol for 72 hours, followed by filtration (Whatman paper) and vacuum concentration (rotary evaporator). Acute toxicity was assessed using Lorke's method (1983). Phase I involved oral administration of 10, 100, and 1000 mg/kg doses to three mouse groups (n=3/group), while Phase II tested 1000–5000 mg/kg in individual mice (n=4). Mortality and behavioral changes (e.g., stretching, nose-rubbing) were monitored for 14 days. The LD50 was calculated as $\sqrt{(A \times B)}$, where A = highest non-lethal dose and B = lowest lethal dose.

Antimalarial evaluation and analyses: Chloroquine-sensitive Plasmodium berghei (Institute of Advanced Medical Research, Ibadan) was maintained in mice. Infected donor blood (>30% parasitemia) was diluted to 1×107 parasitized erythrocytes/mL and injected intraperitoneally (200 µL/mouse) into 25 albino mice. After 72 hours, mice were divided into six groups (n=5): Group I (uninfected/untreated), Groups II–IV (100, 200, 400 mg/kg T. diversifolia extract), Group V (chloroquine, 10 mg/kg), and Group VI (distilled water, negative control). Treatments were administered orally for 5 days. Daily parasitemia was assessed via Giemsa-stained blood smears using the formula: % Parasitemia = (Parasitized RBCs / Total RBCs) × 100. Post-treatment, blood was collected via cardiac puncture for hematological (Abacus 380 analyzer) and biochemical (spectrophotometry/Reflotron) profiling. Liver and kidney tissues were fixed in 10% formal saline, processed through graded alcohol dehydration, xylene clearing, paraffin embedding, and microtomy (5 µm sections), then stained with hematoxylin and eosin for histopathology.

Results

Table l show the activity of the methanolic extract of Tithonia diversifolia on the parasite density following tive davs treatment of established Plasmodium berghel infected mice. Though Chloroquine reduced the level of parasites than the extract. The percentage clearance of parasitaemia by the extract and Chloroquine as represented this figure is in the order of: Chloroquine 90.16%. Extract (400mg/kg.bw), 75.94%, Extract (200mg kg.bw), 70.40%. Extract (100nmg/kg.bw), 66.39%.

The result of the haematological parameters showed some significant changes in the uninfected untreated and treated groups. The infected and treated with distilled water (Negative control) mice showed a significant difference in the progressive development of severe anaemia while those infected and treated with extract and Chloroquine developed a mild and insignificant anaemia. There is significant boost in the red blood cell of those group treated with the extract as compared to the negative control. Table 2 show that the mice in the test group showed a positive increase in the leucocytes. The extract significantly increase the serum concentration of potassium ion. However, the extract did not significantly increase the concentration of urea in all the doses administered compare to control, table 3. There was no significant increase in the chloride ion concentration as compare to the uninfected group and negative control. Table 3. The extract had no significant effect on alkaline phosphatase at 100mg/kg.bw while there was significant increase at both 200 and 400mg/kg.bw.

Table 1: Effects of different doses of Methanolic extract of *T. diversifolia* on chloroquine sensitive *P. berghei*-infected mice 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}

Table 2: Effect of Methanolic Extract of Tithonia diversofolia on Haematological parameters of swiss albino mice

Parameters	**Uninfected Group**	**Negative Control (Distilled Water)**	**100mg Extract**	**200mg Extract**	**400mg Extract**	**Positive Control (10mg Chloroquinne)**
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$^{12/1}$)	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^{C}	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^{0} / 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 ± 0.44i
PLATELET(10^{9}/l)	680.00±29.40^{k,l}	910.40±6.52^{k,m}	963.00±38.76^{m}	769.00±12.82^{l,m}	611.5± 3.88^{l}	894.50 ± 52.56^{m}
NUETROPHIL(%)	12.40 ± 0.29	50.00 ± 0.13^{0}	52.40 ± 0.08^{0}	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}

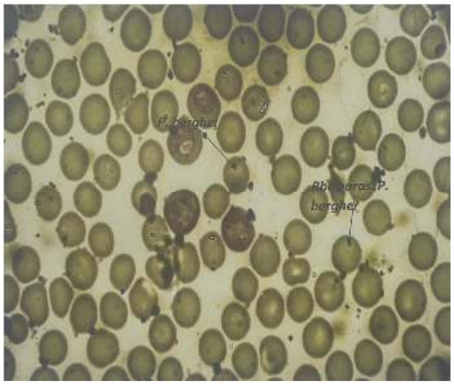
Data are expressed as mean ± SEM, n=3, values with same letters are not significantly different (*P>0.05*), values with different superscripted letters are significantly different (*P>0.05*).

Table 3: Effect of Methanolic Extract of T. diversofolia on selected electrolysis of swiss albino mice

Parameters	Uninfected Group	Negative Control (Distilled Water)	100mg Extract	200mg Extract	400mg Extract	Positive Control (Chloroquinne)
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 ± 0.21^{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}

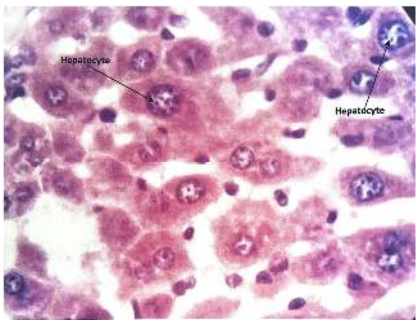
Data are expressed as mean ± SEM, n=3, values with same letters are not significantly different (*P>0.05*), values with different superscripted letters are significantly different (*P>0.05*).

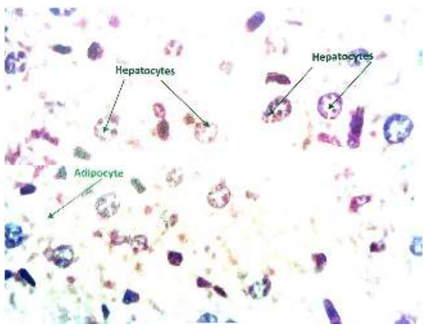
Slide 1: Slide of Plasmodium berghei of both infected and non-infected Red Blood Cells.



The slide shows some of the parasitized cells with the Schizont stage and the unparasitised cells.

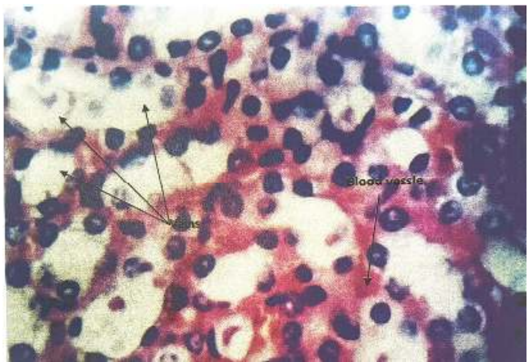
Slide 1: Negative Control, Liver (Dist. Water) **Slide 2:** Extract 400mg Liver

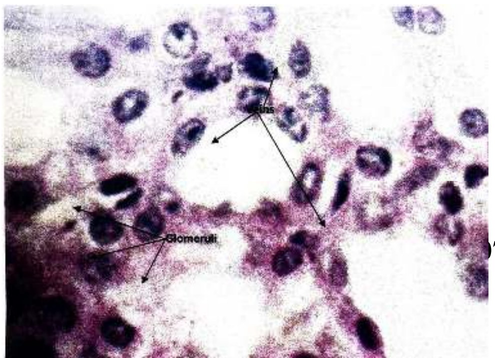




Slide 1 vs. 2: Liver histology (negative control vs. 400 mg/kg).

Slide 3: Negative Control, Kidney (Dist. Water) **Slide 4:** Extract 400mg Kidney





Slide 3 vs. 4: Kidney histology (negative control vs. 400 mg/kg).

Discussion

Methanolic extract of the leaves of Tithonia diversifolia showed signiticant antiplasmodal activities as depicted by the percentage parasitaemia and chemosuppressoive properties of the extract. The highest curative cffect was observed for the plant extract when the highest dose 400mg/kg.bw was administered, and this make it dose dependent. 78.94% curative effect was observed in the 400mg/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 agrec with report by Kanmel et al. (2000), that when a standard antimalarial drug is used in the management of plasnmodium 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. (Fidock et al., 2004). The antiplasmodal properties of this plant could be as result of its phytochemical composition which have been established to have antiplasmodal and antiprotozoal activities. (Arise et al., 2012). Indeed, tagitinin c and some other sesquiterpene lactone isolated from Tithonia diversi folia might be responsible for the anti-malaria property of the plant. (Goffin er al., 2002).

White blood cell play an important role in the immune system. The significant increase observed in wlhite blood cell count indicate a boost in the immune system because increase in white blood cell increases the immunological action of the body. The significant increase in the red blood cell indicate an increase in oxygen carrying capacity of the blood and amount of oxygen delivered to the tissue. The boost in the red blood cell production also indicate that the extract has the ability to stimulate erythropoietein release in the kidney which is the humoral regulator of red blood cell production. (Sanches et al., 2004). The significant reduction in the packed cell volume (pcv) observed may be as a result of the malaria parasite which parasitized the cells. The significant increase in serum potassium level may be as a result of the increase potassium load in the body luid which could be atributed to kidney dy stunction possibly by a defective mechanism of tubular potassium exeretion. (Kes 2001). The increase in the serum urca obserned in the study could be altributed to reduced functionality of the kidney at clearing metabolic by product.

The Alkaline phosphatase. (ALP), Alanine amino transterase (ALT), and Aspartate amino transferase in tissues and blood are important marker enzymes which are used to asses the integrity of the cell membrane, cytosolic activity and cell death (Akanji et al. 1993). 1ncreased ALP activities in liver suggest the possibility of the extract causing membrane damage in these organs at higher concentration or longer period of exposure of the animal to the extract, though there was no significant different 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 (Adebayo et al., 2009). 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.

The histological analysis of the mouse liver shows that the liver hepatocytes are dilated in group one (negative control), but gradually coming to normal in the other groups treatcd with the extract. The enlargement of this hepatocyte may be as a result of the malaria parasite invasion. The adipocyte also is enlarged in negative control as compared to other groups. The morphology of the kidney is normal based on the doses used for this study as compared to the control.

Conclusion

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

Recommendations

Isolate and Characterize Active Compounds: Prioritize the purification of sesquiterpene lactones such as tagitinin C, identified as the primary antiplasmodial constituent to enhance therapeutic efficacy and reduce required dosages. Phytochemical profiling will clarify structure-activity relationships.

Conduct Chronic Toxicity Studies: Implement OECD Guideline 408-compliant 90-day subchronic toxicity assays in rodent models. This addresses electrolyte imbalances (K^+ /urea elevation at 400 mg/kg; Table 4) and potential renal stress observed in acute settings.

Advance Standardized Clinical Trials: Develop quality-controlled extracts using HPLC fingerprinting for batch consistency. Initiate Phase I trials evaluating safety in healthy volunteers, focusing on hematological and hepatic parameters normal histology at ≤400 mg/kg; Slides 6/12.

Launch Public Health Campaigns: Partner with Nigeria's NAFDAC to disseminate dosage guidelines (max 400 mg/kg/day) via community health networks. Emphasize risks of self-medication (electrolyte dysregulation) while validating traditional use.

Explore Synergistic Formulations: Test extract combinations with sub-therapeutic artemisinin derivatives to combat resistance, leveraging T. diversifolia's immunostimulatory effects.

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