

Evaluation of Biochemical and Haematological Changes in *Plasmodium-berghei*-Infected Mice Administered With Aqueous Extract of *Tithonia diversifolia*

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Abstract- This work was done to investigate the biochemical and haematological parameters of aqueous extract of *Tithonia diversifolia* plant, in mice induced with *Plasmodium berghei* a plant that is used locally to treat malaria. A total of 25 mice were used in this study and were divided into 5 groups. Group A (control), group B group C, group D and group E were infected with *Plasmodium berghei* and treated with normal saline, artesunate (50mg/kg), 300mg/kg and 600mg/kg body weight of the extract respectively. MCH – mean cell hemoglobin; MCHC – mean cell hemoglobin concentration; MCV– mean cell volume; PCV – packed cell volume; PLT – platelet count; RBC – red blood cells; WBC – white blood cell count/leukocyte count, N – Neutrophil Count, L-Lymphocyte Count, Hb- Hemoglobin count, M- monocyte count and AST –Aspartate Aminotransferase, ALT- Alanine Aminotransferase and ALP Alkaline phosphatase were evaluated and analysed statistically. The results revealed that groups treated with 300mg/kg, 600mg/kg *Tithonia diversifolia* leave extract and artesunate had reduced parasitaemia level of 76.69%, 83.02% and 84.72% respectively. The administration of (300mg/kg) dose of the extract and artesunate (50mg/kg) caused a decrease in the activities of AST, ALT and ALP that may indicate safety while administration of 600mg/kg dose of the extract caused an increase, this may suggest that 600mg/kg dose exposure of the extract could pose a risk resulting to organ damage. The administration 300mg/kg cause an increase in Hb, PCV, WBC, N with decrease in PLT while a decrease was observed for the Hb, PCV with significantly increase in WBC of group administered with the 600mg/kg dose of the extract when compared with the negative control. It was concluded that although *T. diversifolia* is reported to have health benefit as it offer treatment and protection against malaria, the observed changes in biochemical and haematological parameters suggest that it may have some clearly definable safety and toxicity properties especially at 300mg/kg and 600mg/kg dose in *Plasmodium berghei* infected mice.

Index Terms- *Plasmodium berghei*, *T. diversifolia*, malaria, biochemical and hematological parameters

I. INTRODUCTION

Arising from their biodiversity and perhaps the rich complement of phytochemicals and secondary metabolites, plants have from antiquity been used as sources of medicament

against various ailments (Farombi, 2003). In rural areas where access to modern health facilities is limited by the level of development, plants/herbs remain the mainstay of the health care system (TMP, 2007). An increase reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998).

Malaria has been a pervasive scourge and a global public health problem for more than a century, claiming the lives of millions each year and reducing the quality of life of many others, especially in sub-Saharan Africa (SSA) where it still remains a serious concern (WHO, 2011). It remains the most significant parasitic disease in the tropics where it causes ~200 million clinical cases and is reported to claim up to 1.2 million lives each year (Murray *et al.*, 2012). It annually affects hundreds of millions of people, principally in sub-Saharan Africa, Asia and South America, with young children and pregnant women being particularly at risk.

The disease remains a major cause of morbidity and mortality, exacting its greatest toll in sub-Saharan Africa where over 80% of cases and 90% of deaths occur (WHO, 2012). These huge burden could be ascribed to efficient afro-tropical malaria vectors with strong vectorial capacities that maintain high levels of transmission, environmental factors and climatic changes, population movement, deteriorated socioeconomic situation, lack of access to effective anti-malaria treatment and use of fake anti-malarial drugs (Sinka *et al.*, 2012).

Anemia is a hallmark of malaria infection that occurs as a result of intense hemolysis (destruction) of infected RBCs due to higher parasitemia caused mainly by *P. falciparum* (Ghaliwal *et al.*, 2004). Anaemia is a fairly common problem encountered in malaria and it poses special problems in pregnancy and in children. It can be due to multiple causes, repeated hemolysis of infected red cells is the most important cause for a reduction in hemoglobin levels. Anemia depends on the degree of parasitemia, duration of the acute illness and the number of febrile paroxysms. The pathogenesis of malarial anaemia is complex and undoubtedly involves multiple processes relating to both the destruction of erythrocytes and their reduced production.(Claire, 2004). Massive destruction of red cells accounts for rapid development of anemia in *P. falciparum* malaria. Nonparasitized RBCs are also removed from the circulation by complement-mediated lysis and phagocytosis

resulting from immune complex deposition and complement activation (Claire, 2004). During *P. falciparum* infections, reticulocyte levels are inappropriately low, reflecting suppression of the normal response of erythropoietin (EPO) (Claire, 2004). Some of these mechanisms may perpetuate anemia even after completion of the treatment.

Tithonia diversifolia (Hemsl.) A. Gray commonly referred to as Mexican sunflower or tree marigold belonging to the Compositae family has been put into many uses which include treatment of stomach pains, indigestion, sore throat and liver pains (Kokwaro, 1976).

The bioactive compounds in the leaves of the plant has been isolated and these include sesquiterpenes, alkaloids, tagitinin, saponin and flavonoids etc. (Kuo and Chen, 1998; Gu *et al.*, 2002). Also in 2002 Gu *et al.* isolated three new sesquiterpenoids; 2-alpha-hydroxytirodunin, tithofolinolide and 3-alpha-acetoxydiversifolol, along with eight known sesquiterpene lactones some of which are, 3 beta-acetoxy-8 beta-Isobutyroxyreynosin, tagitinin A and tirodunin in an ethyl acetate extract of the aerial parts of *T. diversifolia*. A reliable reversed phase high performance liquid chromatographic method revealed the presence of Tagitinin C and A, an antiplasmodial sesquiterpene lactones (Goffin *et al.*, 2002). Ether extract from aerial parts of the plant has been shown to have good antiplasmodial activity against three strains of *Plasmodium falciparum* (Bidla *et al.*, 2004)

It is known to be used in folk medicine to treat various illnesses including malaria, diarrhea, inflammation, haematomas, as well as bacterial and parasitic infections (Wanjau *et al.*, 1997; Tona *et al.*, 1998; Rungeler, *et al.*, 1998, Kuo And Chen, 1998; Goffin *et al.*, 2002; Gu *et al.*, 2002). In addition infusion from its leaves has been used for subduing swelling, dissolving lumps and treating enteritis and gastritis in local folk medicine (Tona *et al.*, 1998).

In Nigeria, the crude extract of *T. diversifolia* has been extensively used locally as anti-malaria without possible recourse to its possible deleterious effect on the individual that uses it. However, because some of the anti-malaria drugs; for example, chloroquine has been associated with increased anaemia (Hamer *et al.*, 2003). There is need to scientifically evaluate or investigate antiplasmodial activity, biochemical and haematological parameters to assess the potency and relative safety of *T. diversifolia* in the plasmodium-berghei-infected mice.

II. MATERIALS AND METHODS

A. Host Animals

Twenty five pure strain Laboratory adult Swiss albino mice, mean weight of 22 g were obtained from the animal house, Department of Science and Computer Technology, Federal

Polytechnic Ado Ekiti. They were kept in clean cages in the laboratory and fed on chow diets and water ad-libitum for one week for acclimatization at room temperature of $29 \pm 2^\circ\text{C}$ before being exposed to the reference drug and plant extracts. The animals were weighed and placed into five groups per assay.

B. Plant Material

Fresh leaves of the plant were collected within the premises of Federal Polytechnic Ado Ekiti, Ekiti State, Nigeria in March 2014 and identified at department of Botany Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. It was air dried inside a well-ventilated room and milled into powder using electric blender/mill grater (model MS-223, Taiwan). 100 g powdered plant was soaked in 400ml distilled water for 48 hrs. The resultant mixture was filtered with cheesecloth and the filtrate concentrated under reduced pressure at 40°C for 48hrs using an oven to give 15 g of a dark solid extract which was stored at a very low temperature room until required for use.

C. Parasites and Inocula

Plasmodium berghei (NK₆₅ strain) obtained from Institute of Medical Research and Training (IMRAT) Ibadan, Oyo State, Nigeria was maintained by blood passaging in white mice and a dose of 10^5 parasitized red blood cells (RBC) was inoculated intraperitoneally (i.p). The Parasite density was assessed using giemsa stained blood smear preparation from infected animals 6-10 days after inoculation. Infected red blood cells were counted 5 times using the haemocytometer and the mean calculated. The number of parasitized cells per ml was then determined for the corresponding dilutions for the required inocula.

D. Mice Grouping and Treatment

Owoyele *et al.*, (2005) was adopted with modification (Rane's test). After one week period of acclimatization in the laboratory, the mice (n = 25) were grouped into five categories (A-E), each containing 5 mice. Group A (control), Group B. Group C, Group D and Group E were infected with *Plasmodium berghei* and treated with normal saline, artesunate (50mg/kg), 300mg/kg and 600mg/kg b.w of the extract *Tithonia diversifolia* respectively for 7 days.

E. Anti-plasmodial Activity of the Extract

Blood sample was taken from tail vein of each mouse on the fourth day. Methanol fixed and 10% Giemsa stained thin film was examined microscopically. Percentage parasitemia of the extract were determined following the formula described below (Hilou *et al.*, 2006).

Mean survival time of each mouse in all groups was determined by calculating the average survival days of mice in each group over 7 days.

F. Collection of Blood Samples for Estimation of Biochemical and Haematological Parameters

Each of the animals was first anaesthetized with chloroform, then the thoracic cavities were carefully dissected (opened) and the heart was exposed. The required blood sample (3 ml) was withdrawn from the heart (left ventricle) by cardiac puncture

with a needle and syringe. The blood was quickly transferred into an EDTA bottle for estimation of haematological parameters and plane bottle for biochemical parameter. The haematological parameters were estimated as in a previous study (Owoyele *et al.*, 2004) The Red Blood Cell (RBC) counts was done using a haemocytometric method. The haemoglobin (Hb) and haematocrit (PCV) values were determined by the cyanomethaemoglobin and microhaematocrit methods respectively. All other erythrocyte indices – mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC) were calculated from the haematological data obtained. The platelet count and differential leucocyte counts were determined as described by Jain (1986) using Automated Haematology Analyzer-k-x-21 by systmex, Japan.

Enzymatic assays: Determination of plasma albumin (ALB) and total protein (TP) for liver functionality, aspartate amino transferase (AST) and alanine amino transferase (ALT) for cellular integrity of the liver and alkaline phosphatase (ALP) for condition linked to the biliary tract were analysed using standard methods using Randox reagent kit.

G. Statistical analysis

Results obtained (Mean \pm SD) were subjected to statistical analysis by unpaired comparison using the student t-test, p-values < 0.05 were accepted as significant. The results were also subjected to analysis of variance (ANOVA).

III. RESULTS

The changes in the parasitemia level in mice administered *Tithonia diversifolia* leave extract is shown in the table 1. The group treated with 300mg/kg and 600mg/kg leaf extract and artesunate reduced parasitemia level by 76.69%, 83.02% and 84.72% respectively. The percentage parasitemia in group administered the doses of the leave extract and artesunate decreased throughout the period of observation while a increase was observed in the infected treated with normal saline (positive control) group. The extract produced a dose-dependent effect at the doses employed in this study.

IV. DISCUSSION

The result from this investigation shows that aqueous extract of *T diversifolia* leaves has antiplasmodial activity with the property that showed curative effects on malaria parasite. This study also indicates that the parasite clearance ability of the extract is dose-dependent. This was evident in our observation whereby the mice treated with 600mg/kg of the extract has a lower parasite count compared with 300mg/kg dose. The present study support the earlier reports on the antiplasmodial activity of *T. diversifolia* of Goffin *et al.*, 2002; Bidla *et al.*, 2004; Elufioye and Agbedahunsi 2004; and Oyewole *et al.*, 2008. However, the dose of dependent property of *T. diversifolia* initiates risk in biochemical and haematological parameters of the mice.

The antiplasmodial activities of several medicinal plants have been attributed to the presence of some phytochemicals like

saponins, alkanoids, flavonoids tagitinin, and sesquiterpenes (Gu *et al.*, 2002) which is also presence in the crude extract of *T. diversifolia* (Reference)

The determination of levels of some liver enzymes in the blood could be one of the ways of detecting liver damage, increase beyond limit of the serum hepatic enzyme and levels of increase is said to be proportional to the extent of level damage (Crook 2006).

In this study administration of (300mg/kg) dose of the extract and artesunate (50mg/kg) caused a decrease in the activities of AST, ALT and ALP while administration of 600mg/kg dose of the extract caused an increase. This may suggest 600mg/kg dose exposure of the extract could pose a risk resulting to organ damage. Some plant extracts have been known to possess different levels of hepatotoxicity which depends mainly on the levels of anti-nutrients inherent in the plants (Sofowora 1993). Phytochemical analysis of *T. diversifolia* revealed its content of alkaloids and saponins amongst others. The mechanisms of action of alkaloids and saponins are similar and its involves complexing with cholesterol to form pores in the cell membrane bilayers (wink, 1993; francis *et al.*, 2002). This may be possible mechanism by which *T. diversifolia* acted on the liver cells to bring about the observed increase in the level of AST and ALT at 600mg/kg dose in this study.

This investigation also revealed that there was a significant decrease in serum creatinine level in infected, 300mg/kg, 600mg/kg doses *T diversifolia* extract and artesunate treated group compared with infected and untreated group. Significant increase in serum creatinine could implies renal functional impairment. The same trend was observed in total protein evaluation in all the groups under investigation.

In this study the 300mg/kg, 600mg/kg of the extract and (50mg/kg) of artesunate produced a significant difference on the hematological PCV, Hb, WBC, Platelets, MVC, RBC, MCHC, N, L and M. when compared with the negative control as shown in Table 3. The slight increase in hematological values demonstrated an improvement in disease progression (Chang and Stevenson, 2004; Weartheral *et al.*, 2002). Literature has shown that ingestion of medicinal compounds or drugs can alter the normal range of haematology parameters (Ajagbonna *et al.*, 1999).

There was a decrease in Hb and PCV at 600mg/kg dose of the extract, infected and control groups. The observed anaemia in *P. berghei* infected mice may be due to RBC destruction caused either by parasite multiplication or by spleen reticuloendothelial cell action (chinchilla *et al.*, 1998). This decrease may also be due to multiple causes of which repeated heamolysis of infected red blood cells is the most important. The haemolysis may be due to non-immune destruction of parasitized red cells in case of high parasitemia or immune mediated destruction of parasitized as well as non-parasitized red cells because the changes in the red cell antigen structure brought about by the parasitic invasion stimulation the production of antibodies against the red cell. This triggers immunemediated red cell lysis. In addition the growing parasite and degrades the intracellular proteins which are mainly

Table 1: Parasitemia levels

Groups	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Group A	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Group B	24.6±1.14	27.8±0.45	33.4±1.14	41.4±1.52	46.6±1.14	53.4±2.07	56.2±0.84
Group C	21.2±0.84	15.6±0.55	4.2±0.45	2.0±0.71	0.00±0.00	0.00±0.00	0.00±0.00
Group D	20.8±0.83	20.8±0.84	9.8±0.84	7.0±0.71	5.6±0.89	3.0±0.71	1.2±0.45
Group E	21.6±1.14	16.4±0.55	4.8±0.84	2.0±0.71	1.6±0.055	1.0±0.00	0.4±0.55

Results are presented as Mean ± Standard deviation of mean.

- GROUP A- (Control)
- GROUP B- (*Plasmodium-berghei* + Normal Saline) – negative control
- GROUP C- (*Plasmodium-berghei* + 50mg/kg artesunate)- positive control
- GROUP D- (*Plasmodium-berghei* + 300 mg/kg *Tithonia diversifolia*)
- GROUP E- (*Plasmodium-berghei* + 600 mg/kg *Tithonia diversifolia*)

The biochemical investigation result showed a statistical significant ($P>0.05$) difference in the *Tithonia diversifolia* extract and artesunate treated mice groups when compared to the negative control (normal saline) from three to tenth day. (Table2). In this study administration of 300mg/kg dose of the *Tithonia diversifolia* extract and artesunate caused a decrease in the activities of AST, ALT and ALP thus improving renal and hepatic functions.

Table 2: Results of *Tithonia diversifolia* on biochemical parameters of Albino mice

GROUPS	TP(g/dL)	AST (U/L)	ALT(U/L)	ALP(U/L)	Creatinine (mg/dl)	Uric Acid (mg/dl)
A	2.398±0.0993 ^{be}	7.430±2.365 ^{be}	13.94±3.792 ^{be}	18.08±4.189 ^b	7.475±1.152 ^b	7.220±1.612 ^{bcd}
B	4.335±0.4246 ^{acde}	18.04±3.408 ^{acd}	34.07±2.270 ^{acde}	35.44±4.048 ^{acde}	13.89±3.246 ^{acde}	27.33±2.742 ^{acde}
C	2.438±0.0563 ^b	5.224±1.692 ^{be}	13.86±4.126 ^{be}	17.98±2.667 ^b	7.244±1.192 ^b	13.40±2.144 ^{ab}
D	2.808±0.2706 ^b	7.716±2.568 ^{be}	13.75±2.997 ^{be}	20.75±1.759 ^b	7.757±1.322 ^b	13.42±2.238 ^{ab}
E	2.933±0.3474 ^{ab}	14.47±3.384 ^{acd}	22.91±7.936 ^{abcd}	24.20±9.759 ^b	8.444±1.656 ^b	9.867±2.242 ^b

Results are presented as Mean ± Standard deviation of mean. One way ANOVA was used to test mean difference significance using Tukey's multiple comparison. P-value <0.05 was considered statistically significant.

- GROUP A- (Control)
- GROUP B- (*Plasmodium-berghei* + Distilled water)
- GROUP C- (*Plasmodium-berghei* + 50mg/kg artesunate)
- GROUP D- (*Plasmodium-berghei* + 300 mg/kg *Tithonia diversifolia*)
- GROUP E- (*Plasmodium-berghei* + 600 mg/kg *Tithonia diversifolia*)

The result of haematological parameter showed a significant change in the infected and untreated group as well as those infected but treated with the extract and artesunate as presented in table 3. Conversely the infected and treated with normal saline (negative control) mice showed a significant difference in the progressive development of severe anemia while those infected and treated with the extract and artesunate developed a mild and insignificant anemia.

Table 3: Results of *Tithonia diversifolia* on Haematological parameters of Albino mice

GRO UPs	PCV %	WBC 10 ⁹ /L	RBC 10 ¹² /L	Hb g/L	Platelet1 0 ⁹ /L	MCH pg	MCHC g/L	MCV fL	N 10 ⁹ /L	L 10 ⁹ /L	M 10 ⁹ /L
A	29.00±1.00 ^{bde}	24533.33±305.51 ^{bcd}	4.83±0.17 ^b	9.66±0.35 ^{be}	234.00±4.00 ^{bc}	19.98±0.04	33.29±0.07	60.00±0.00	13.67±0.58 ^b	75.66±0.58	8.33±0.57 ^d
B	16.33±0.57 ^{ad}	6266.67±57.74 ^a	2.72±0.09 ^{ad}	5.42±0.21 ^{ad}	61.67±2.89 ^{ad}	19.92±0.08	33.20±1.13 ^d	59.99±0.00	9.00±1.00 ^{ad}	58.66±7.64 ^e	4.33±0.57 ^e
C	19.67±8.08	9666.67±305.51 ^a	4.05±1.35	8.07±2.66	105.00±5.00 ^a	20.05±0.04	33.41±0.07	60.00±0.00	12.66±1.15	66.66±1.53	9.00±1.00 ^d
D	30.67±4.04 ^{abe}	13133.33±2193.93 ^a	4.722±0.67 ^{be}	9.47±1.33 ^{be}	213.33±8.94 ^b	20.05±0.04	33.54±0.18 ^b	60.00±0.00	13.33±1.15 ^b	69.67±1.201	14.67±4.16 ^{abc}
E	17.00±1.73 ^{ad}	11700±7188.18 ^a	2.67±0.289 ^{ad}	5.33±0.58 ^{ad}	148.33±1.55	20.00±0.00	33.33±0.15	60.00±0.00	11.33±2.31	78.33±1.15 ^b	11.33±1.15 ^b

Data are expressed as mean ± SD.

- GROUP A- (Control)
- GROUP B- (*Plasmodium-berghei* + Distilled water)
- GROUP C- (*Plasmodium-berghei* + 50mg/kg artesunate)
- GROUP D- (*Plasmodium-berghei* + 600 mg/kg *Tithonia diversifolia*)
- GROUP E- (*Plasmodium-berghei* + 600 mg/kg *Tithonia diversifolia*)

hemoglobin (Gavigan *et al.*, 2001). This may account further for decrease in Hb and also at 600mg/kg dosage the extract may contain chemicals that may trigger those actions. These decreases however were considerably reversed in 300mg/kg dose extract and artesunate treated groups. This suggests that at 300mg/kg dosage the extract may have some stimulatory effect on the production of red cells (erythropoiesis). This might have contributed to the increase in Hb and PCV observed in 300mg/kg dose extract-treated group.

There was a significant increase in WBC of 300mg/kg, 600mg/kg extract and artesunate-treated group as compared with control group. WBCs function mainly to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce, transport and distribute antibodies in the immune response. A decrease in WBC of control group may imply a reduction in the ability of the mice to resist the infection (Yakubu *et al.*, 2007). However WBC in the 300mg/kg dose of extract and (50mg/kg) artesunate treated groups were higher than the 300mg/kg dose of extract treated and control groups. This suggests a boost in the immune system by the extract at 300mg/kg dosage and reference drug which indicate an improved ability of the mice to combat the infection as a result of treatment. Although 600mg/kg dose extract treated group shown a reduced parasitemia level but a decrease in WBC was observed, this may suggest that at 600mg/kg dose of the extract may have deleterious or harmful effect. The significant decrease in WBC in the control group which correlates with high parasitaemia and other derangement is as a result of infection.

Like RBC, platelets are nuclear and discoid; they measured 1.5-3.0 μm in diameter. The body has very limited reserve of platelet, so they can be rapidly depleted (Wagner and Brugger, 2003). Decrease platelet counts also common in malaria may result from sequestration of platelet in the spleen (Horstmann *et al.*, 1981). There was a significant decrease in the level of platelet in the control group as a result of continued infection and a decrease in platelet count is also observed in 300mg/kg dose in extract-treated groups compared with 600mg/kg dose extract-treated group, suggesting a stimulatory effect of the extract at 300mg/kg dosage. The increase in WBC count is an indication of the ability of the extract at 300mg/kg dose indicates increase in the production of the cells of the immune system. Lymphocytes are the main effector of cells of immune system (McKnight *et al.*, 1999). The increase in lymphocyte in this study may affect the effector cells of the immune system. The significant increase in the neutrophil by 300mg/kg dose of the extract could possibly suggest the ability of the extract to enhance blood component to undergo phagocytosis.

V. CONCLUSION

From this study, it can be noted although *T. diversifolia* is reported to have health benefit as it offer treatment and protection against malaria, the observed changes in biochemical and hematological parameters suggest that it may have some clearly definable safety and toxicity properties especially at 300mg/kg and 600mg/kg dose of aqueous extract of *T. diversifolia* in *Plasmodium berghei* infected mice.

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