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# ANTIPLASMODIAL ACTIVITY AND SAFETY ASSESSMENT OF METHANOL LEAF EXTRACT OF *Detarium microcarpum* GUILL. & PERR. (FABACEAE)

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## ABSTRACT

Malaria is an endemic infectious disease that is widespread in the tropical and sub-tropical areas of the world, leading to morbidity and mortality. *Detarium microcarpum* Guill. & Perr. (Fabaceae) is used traditionally in the treatment of malaria, diabetes, hypertension, and pneumonia. The aim of this study is to evaluate the antiplasmodial activity and safety profile of methanol leaf extract of *Detarium microcarpum*. Phytochemical screening and oral median lethal dose ($LD_{50}$) estimation of the extract were carried out. The antiplasmodial activity was evaluated in mice infected with chloroquine sensitive *Plasmodium berghei-berghei* using curative, suppressive and prophylactic experimental models. For toxicity studies, rats were orally administered with the extract of *Detarium microcarpum* daily for 28 days, biochemical assay and hematological analysis were conducted. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, triterpenes and glycosides. Oral $LD_{50}$ of the extract was estimated to be > 5000 mg/kg. The extract at all doses tested produced a significant ($p < 0.001$) curative, suppressive and prophylactic effects. The extract also significantly prolonged the survival time of the treated mice up to 19 days compared to the negative control group. The extract revealed some significant increase in AST ($p < 0.01$) and ALP ($p < 0.001$) at the highest dose. However, kidney function tests and hematological analysis were not significantly affected in all the treatment groups as compared to the control. The results of this study suggest that the methanol leaf extract of *Detarium microcarpum* possesses curative, suppressive and prophylactic anti-plasmodial activity and is relatively safe.

**KEYWORDS**: **Antiplasmodial; *Detarium microcarpum*; *Plasmodium berghei-berghei*; Chloroquine; Artesunate.**

## INTRODUCTION

Malaria is a life-threatening parasitic disease transmitted by the bites of female *anopheles* mosquitoes infected with *Plasmodium* specie [1]. In humans, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi* have been identified to cause malaria. Clinically, *Plasmodium falciparum* is the most fatal and the primary cause of malaria incidences. Malaria is an endemic infectious disease that is widespread in tropical and sub-tropical areas of the world [2] leading to morbidity and mortality [3, 4]. Some population groups are considerably at higher risk of contracting malaria and developing severe complications, which include children (below 5 years), pregnant women, sicklers, patients with HIV/AIDS as well as non-immune immigrants [1].

Malaria treatment using medicinal plant extracts and isolated compounds has a long and successful tradition [5]. For example, quinine was isolated from *Cinchona* (Rubiaceae) and artemisinin from

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*Qinghaosu* (Asteraceae) [6]. Resistance to various antimalarial drugs (quinolones and antifolate family) including artemisinin derivatives has been reported and documented [7, 8], causing a major impediment in the fight against malaria and its attendant complications.

Remedies from natural plant origin are believed to be harmless and have no risk; however, some plants are inherently toxic [9] leading to adverse effects. Traditional herbal medicines have been used for the treatment of various ailments for centuries globally; however, the traditional use of any plant for medicinal purposes does not warrant its safety. Therefore, toxicity studies on medicinal plants must be conducted so as to evaluate and establish their short- and long-term safety [10], for proper validation of their therapeutic uses.

*Detarium microcarpum* is a tree legume that belongs to the family of Fabacaea, which grows naturally in the drier regions of West and Central Africa. It is known locally as *taura* (Hausa), *ofo* (Igbo), *ogbogbo* (Yoruba), *gatapo* (Kanuri), *gkungorochi* (Nupe), *aikperlarim*i (Etsako), and *gwogwori* (Gwari). Previous scientific work on *Detarium microcarpum* revealed that the plant possesses antimicrobial activity [11]; inhibitory activity against hepatitis C virus [12]; anti-inflammatory and analgesic effects [13]; anti-bacterial activity [14]. In Nigeria, *Detarium microcarpum* is used traditionally by the Lala tribe of Adamawa to cure malaria and jaundice [15] and the Gwaris of the Federal Capital Territory (FCT) to cure malaria and dysentery [16] but the efficacy has not been scientifically investigated. The rodent malaria parasite model known as *plasmodium berghei berghei* which is chloroquine sensitive is employed to evaluate the *in-vivo* antimalarial activity [17, 18]. The aim of this study is to determine the antiplasmodial activity and safety profile of methanol leaf extract of *Detarium microcarpum*.

## MATERIALS AND METHODS

### Collection and authentication of plant materials

Fresh leaves of *Detarium microcarpum* were collected from Gwarzo village, Gwarzo LGA, Kano State, Nigeria. The plant was identified and authenticated by Baha'uddeen Said Adam of the herbarium unit of Bayero University Kano, Nigeria. A voucher specimen number of (0071) was collected for future reference and compared with the already deposited plant specimen.

### Preparation of methanol leaf extract

Fresh leaves were cleaned and air-dried under shade at room temperature until the attainment of a constant weight and then crushed into fine powder. One thousand grams (1000 g) of the powdered material was extracted with four liters (4 L) of 70 % $^{v}/_{v}$ methanol using cold maceration process for 72 hours with regular shaking. Afterwards the mixture was filtered using gauze and Whatman No.1 filter paper, and then evaporated to dryness in an oven at a temperature of about 45$^{o}$C to obtain 14.06% $^{w}/_{w}$.

### Experimental animals

Adult Swiss albino mice (16-24 g) and Wister rats (170-200 g) of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Bayero University Kano. They were allowed to acclimatize for one week with food and water *ad libitum* and maintained under standard laboratory conditions in accordance with the National Academy of Sciences, guides for the care and use of Laboratory animals (1996). Ethical approval was obtained from the animal rights committee of the College of Health Sciences, Bayero University, Kano (BUK/CHS/REC/VII/44).

### Drugs and chemicals

Chloroquine (Fluka, Germany), artesunate (Mekophar, Vietnam), pyrimethamine (SKG, Nigeria), methanol (JHD Sci-Tech. Co. Ltd, China).

### Plasmodium parasites

Chloroquine-sensitive *Plasmodium berghei berghei* was obtained from the National Institute of Medical Research (NIMR) Yaba, Lagos, Nigeria and was maintained by continuous intraperitoneal inoculation every four days in fresh mice [19, 20].

### Parasite inoculation

A *Plasmodium berghei berghei* infected mouse (parasitemia of 34 %) was used as a parasite donor and blood sample was collected retro-orbitally into an EDTA containing bottle. The inoculum was prepared by determining the percentage parasitemia and erythrocyte count of the donor mouse and further diluting the blood with isotonic saline [21] in such a way that 0.2 ml of the blood solution administered intraperitoneally contained approximately $1\times10^{7}$ parasitized erythrocytes [22].

### Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on the extract to detect the presence of secondary metabolites using established methods [23].

### Acute toxicity ($LD_{50}$) study

Acute oral toxicity of the extract was evaluated in mice using Lorke's method [24]. The study was conducted in two phases; in phase one, nine mice were divided into three groups of three mice each and were administered with 10, 100 and 1000 mg/kg of the extract orally; they were observed for the first 4 hours, then 24 hours for signs and symptoms of toxicity including death. In phase two, three groups of one mouse each were treated with 1600, 2900 and 5000 mg/kg orally and were observed for the first 4 hours, then 24 hours for signs and symptoms of toxicity including death. The $LD_{50}$ was calculated as the geometric mean of the lowest dose that caused death of the animal and the highest dose for which the animal survived.

### Antiplasmodial activity against established infection (Curative test)

Evaluation of the schizontocidal activity of the extract against established infection was carried out as described by Ryley and Peters [25]. Adult mice were inoculated with *Plasmodium berghei berghei* on the first day ($D_0$). 72 hours later ($D_3$), the mice were divided randomly into six groups of six mice each (n=6). Group I received 10 ml/kg of distilled water (negative control), Group II, III & IV received 250, 500 and 1000 mg/kg of the leaf extract respectively, Group V received 10 mg/kg of chloroquine and Group VI received 5 mg/kg of artesunate (positive controls) for five consecutive days ($D_3$–$D_7$) orally. Blood was collected from each mouse by tail-bleeding on day three (post parasite inoculation) and day seven (post treatment) and parasitemia was determined by microscopic examination of Giemsa stained thin blood smear. The mean survival time (MST) of the mice in each treatment group was determined over a period of 28 days ($D_0$–$D_{27}$) by finding the average survival time (days) of the mice (post inoculation).

$$\text{MST} = \frac{\text{Sum of survival time of all the mice in a grp}}{\text{Total number of mice in the group}}$$

### Antiplasmodial activity against early infection (Suppressive test)

Evaluation of the schizontocidal activity of the extract against early *Plasmodium berghei berghei* infection in mice as described by Peters [26]. Adult mice were randomly divided into six groups of six mice each (n=6). On the first day ($D_0$), the mice were infected with *plasmodium berghei berghei* and all treatment started 4 hrs after inoculation. Group I received 10 ml/kg of distilled water (negative control), Group II, III & IV received 250, 500 and 1000 mg/kg of the leaf extract respectively, Group V received 10 mg/kg of chloroquine and Group VI received 5 mg/kg of artesunate (positive controls) for four consecutive days ($D_0$ – $D_3$) orally. On the fifth day ($D_4$), blood was collected from each mouse by tail-bleeding and smeared on to a microscope slide in order to make a thin film and parasitemia was determined by microscopic examination of Giemsa stained thin blood smear.

### Prophylactic (repository) test

The prophylactic activity of the extract was assessed using the method described by Peters [26]. Adult mice were randomly divided into five groups (n=6). Group I received 10 ml/kg of distilled water (negative control), Group II, III & IV received 250, 500 and 1000 mg/kg of the leaf extract respectively and Group V received 1.2 mg/kg of pyrimethamine (positive control) orally. Treatment was done for five consecutive days ($D_0$–$D_4$). On the sixth day ($D_5$), the mice were inoculated with *Plasmodium berghei berghei*. 72 hours later, blood was collected by tail-bleeding and parasitemia was determined.

### Parasitemia determination

Thin blood smears were applied on microscope slides, fixed with absolute methanol for 10 minutes and stained with 10% Geimsa stain for 30 minutes. The slides were moderately washed over running water and dried at room temperature. The number of parasitized red blood cells was counted using light microscope with an oil immersion eye piece of 100x magnification power. An average of three fields counted was taken in each slide [27] and percentage parasitemia and suppression were determined as;

$$\% \text{ parasitemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100$$

$$\% \text{ Suppression} = \frac{Po - Pt}{Po} \times 100$$

Where Po is the average parasitemia of the control group and Pt is the average parasitemia of the test group.

### Sub-chronic toxicity study

This study was carried out in accordance with the OECD 407 guidelines [28]. Rats were randomly divided into four groups (n=6). Group I received distilled water; Groups II, III & IV received 250, 500 and 1000 mg/kg of the leaf extract orally for 28 days respectively. Animals were visually observed for changes in behavioral patterns, changes in physical appearance, symptoms of illness and mortality. At

the end of the treatment period, the animals were euthanized and blood sample was collected for the measurement of biochemical and hematological parameters.

### Biochemical analysis

The blood sample was collected in plain dry bottles, allowed to clot then centrifuged at 3500 rpm for 10 minutes to obtain the serum. Alanine amino transferase (ALT) and Aspartate amino transferase (AST) were determined by the method of Reitman and Frankel [29]. Alkaline phosphatase (ALP) was determined by the method of Rec [30]. Total protein (TP) was assayed by biuret method of Gornal et al. [31]. Albumin (ALB) was determined by the method of Doumas et al. [32]. Bilirubin was assayed by the method of Jendrassik and Grof [33]. Urea was determined by the method of Kaplan [34] and Creatinine was determined by the method of Butler [35]. Sodium ($Na^+$) was determined by the method of Trinder [36]. Potassium ($K^+$) was determined by the method of Henry et al. [37]. Chloride ($Cl^-$) was determined by the method of Schonfeld and Lewellen [38] and bicarbonate ($HCO_3^-$) was determined by the method of Forrester et al. [39]. All parameters of liver function and renal function were analysed using standard kits (Agappe Diagnostic kit, Switzerland and Randox Diagnostic kit, UK).

### Hematological analysis

The blood sample was collected in EDTA bottles to prevent clotting. The parameters were white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT). The hematological analysis was performed using Biobase automatic hematological analyzer (BK6300).

### Data analysis

Results were expressed as Mean ± Standard Error of Mean (S.E.M) and were analyzed using One -way analysis of variance (ANOVA) followed by Dunnett's post hoc test (SPSS Version 16.0). Values of $p \leq 0.05$ were considered statistically significant.

## RESULTS

Phytochemical analysis of the extract revealed the presence of alkaloids, glycosides, flavonoids, saponins, triterpenes, and tannins (Table 1). The oral median lethal dose ($LD_{50}$) of the extract was above 5000 mg/kg.

For the curative test, the extract showed a significant ($p < 0.001$) reduction in the average % parasitemia level in the treatment group when compared to the negative control in a dose dependent manner. The extract produced 83.52, 86.65 and 87.21 % parasite clearance at the doses of 250, 500 and 1000 mg/kg respectively. The standard drugs chloroquine (10 mg/kg) and artesunate (5 mg/kg) also showed a significant ($p < 0.001$) reduction in the average % parasitemia level with the % clearance of 88.85 and 91.07 respectively. The extract significantly ($p < 0.001$) prolonged the survival time of the mice in each group when compared to the negative control. Chloroquine and artesunate extended the mean survival time up to about 28 days (Table 2).

For the suppressive test, the extract showed a significant ($p < 0.001$) reduction in the average % parasitemia level at the doses tested when compared to the negative control with the % chemo suppression of 77.54, 85.46 and 80.92 at the doses of 250, 500 and 1000 mg/kg respectively. The standard drugs chloroquine and artesunate showed 86.33% and 87.05% chemo-suppressive effect respectively (Table 3).

For the prophylactic test, the extract (250, 500 and 1000 mg/kg) showed a significant ($p > 0.001$) reduction in the average % parasitemia level compared to the negative control, with a chemoprophylaxis effect of 75.64%, 91.26% and 82.03%. The standard drug pyrimethamine produced a significant ($p > 0.001$) chemoprophylaxis effect of 83.16% (Table 4).

For the liver function test, the extract did not cause significant ($p > 0.05$) change in ALT, total protein, albumin and total bilirubin level in the treatment groups when compared to the control. However, significant increase in AST ($p < 0.01$) and ALP ($p < 0.001$) was observed in the group treated with 1000 mg/kg of the leaf extract (Figure 1).

For the kidney function test, there was no significant ($p > 0.05$) change caused by the extract in the level of urea, creatinine and serum electrolytes (Sodium, potassium, chloride and bicarbonate) in all the treatment groups when compared to the control (Figure 2).

For the hematological analysis, the extract did not significantly ($p > 0.05$) affect the analyzed hematological indices in all the treatment groups when compared to the control (Figures 3).

## DISCUSSION

In this study the phytochemical screening of the methanol leaf extract of *Detarium microcarpum*

**Table 1:** Phytochemical constituents of *Detarium microcarpum*

| Constituents | Inference |
|---|---|
| Alkaloids | + |
| Glycosides | + |
| Flavonoids | + |
| Saponins | + |
| Triterpenes | + |
| Tannins | + |

+ =Present

**Table 2:** Curative effect of the extract on *Plasmodium berghei berghei* infected mice

| Treatment (mg/kg) | Average % Parasitemia | | % Clearance | Mean Survival Time (Day) |
|---|---|---|---|---|
| | Pre- (D3) | Post- (D7) | | |
| D/W 10 ml/kg | 42.74 ± 2.45 | 84.96 ± 3.35 | - | 5.50 ± 0.30 |
| LEXT (250) | 43.41 ± 1.29 | 14.00 ± 1.15* | 83.52 | 16.16 ± 0.60* |
| LEXT (500) | 42.95 ± 1.02 | 11.34 ± 1.18* | 86.65 | 18.00 ± 0.40* |
| LEXT (1000) | 43.28 ± 1.00 | 10.87 ± 1.28* | 87.21 | 19.33 ± 0.70* |
| CQ (10) | 44.45 ± 0.92 | 9.47 ± 0.85* | 88.85 | 27.00 ± 0.30* |
| ART (5) | 43.62 ± 0.68 | 7.59 ± 0.52* | 91.07 | 27.33 ± 0.30* |

D/W= Distilled Water, LEXT= Leaf Extract of *Detarium microcarpum*, CQ= Chloroquine, ART= Artesunate, D3= Day 3, D7= Day 7, *p < 0.001, n= 6

**Table 3:** Suppressive effect of the extract on *Plasmodium berghei berghei* infected mice

| Treatment (mg/kg) | Average % Parasitemia | % Chemosuppression |
|---|---|---|
| D/W 10 ml/kg | 70.50 ± 1.88 | - |
| LEXT (250) | 15.83 ± 0.77* | 77.54 |
| LEXT (500) | 10.25 ± 1.43* | 85.46 |
| LEXT (1000) | 13.45 ± 2.24* | 80.92 |
| CQ (10) | 9.64 ± 0.71* | 86.33 |
| ART (5) | 9.13 ± 0.65* | 87.05 |

D/W= Distilled Water, LEXT= Leaf Extract of *Detarium microcarpum*, CQ= Chloroquine, ART= Artesunate, *p < 0.001, n= 6

**Table 4:** Prophylactic effect of the extract on *Plasmodium berghei berghei* infected mice

| Treatment (mg/kg) | Average % Parasitemia | % Chemoprophylaxis |
|---|---|---|
| D/W 10 ml/kg | 55.75 ± 3.26 | - |
| LEXT (250) | 13.58 ± 1.72* | 75.64 |
| LEXT (500) | 4.87 ± 0.50* | 91.26 |
| LEXT (1000) | 10.02 ± 0.97* | 82.03 |
| PYR (1.2) | 9.39 ± 1.07* | 83.16 |

D/W= Distilled Water, LEXT= Leaf Extract of *Detarium microcarpum*, PYR= Pyrimethamine, *p < 0.001, n= 6

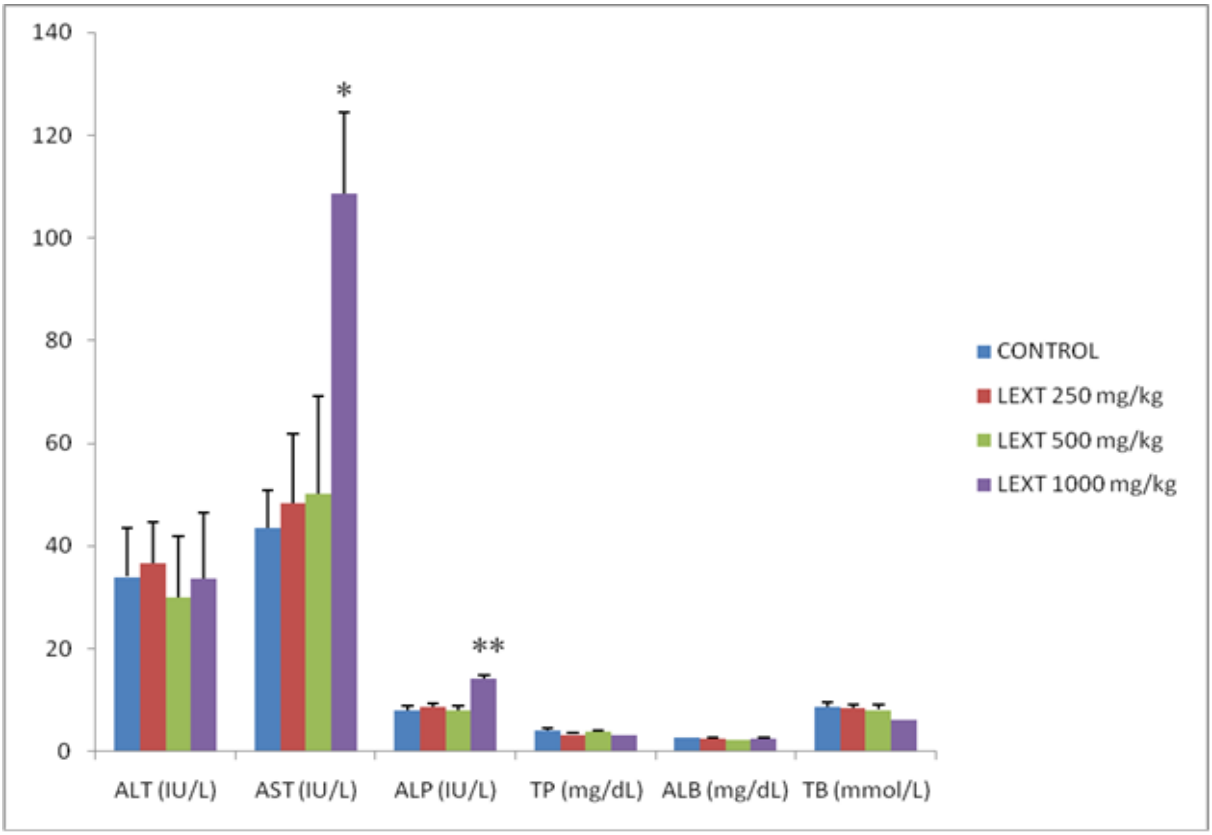


**Figure 1**: Effect of 28 days oral administration of the extract on liver function test in rats.
Key: Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Total Protein (TP), Albumin (ALB), Total Bilirubin (TB), Leaf Extract of *Detarium microcarpum* (LEXT), *p < 0.01, **p < 0.001, n= 6.

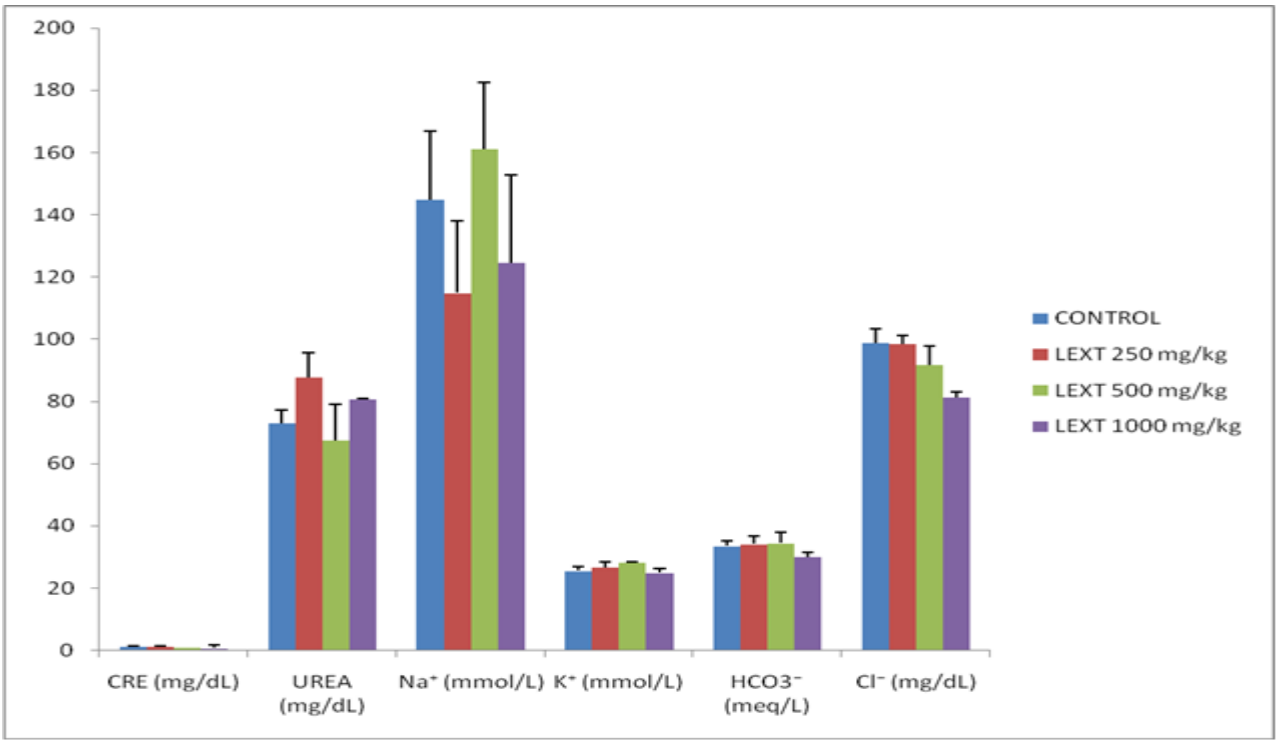


**Figure 2**: Effect of 28 days oral administration of the extract on kidney function test in rats.
Key: Sodium ($Na^+$), Potassium ($k^+$), Chloride ($Cl^-$), Bicarbonate ($HCO_3^-$), Creatinine (CRE)Leaf Extract of *Detarium microcarpum* (LEXT), n= 6.

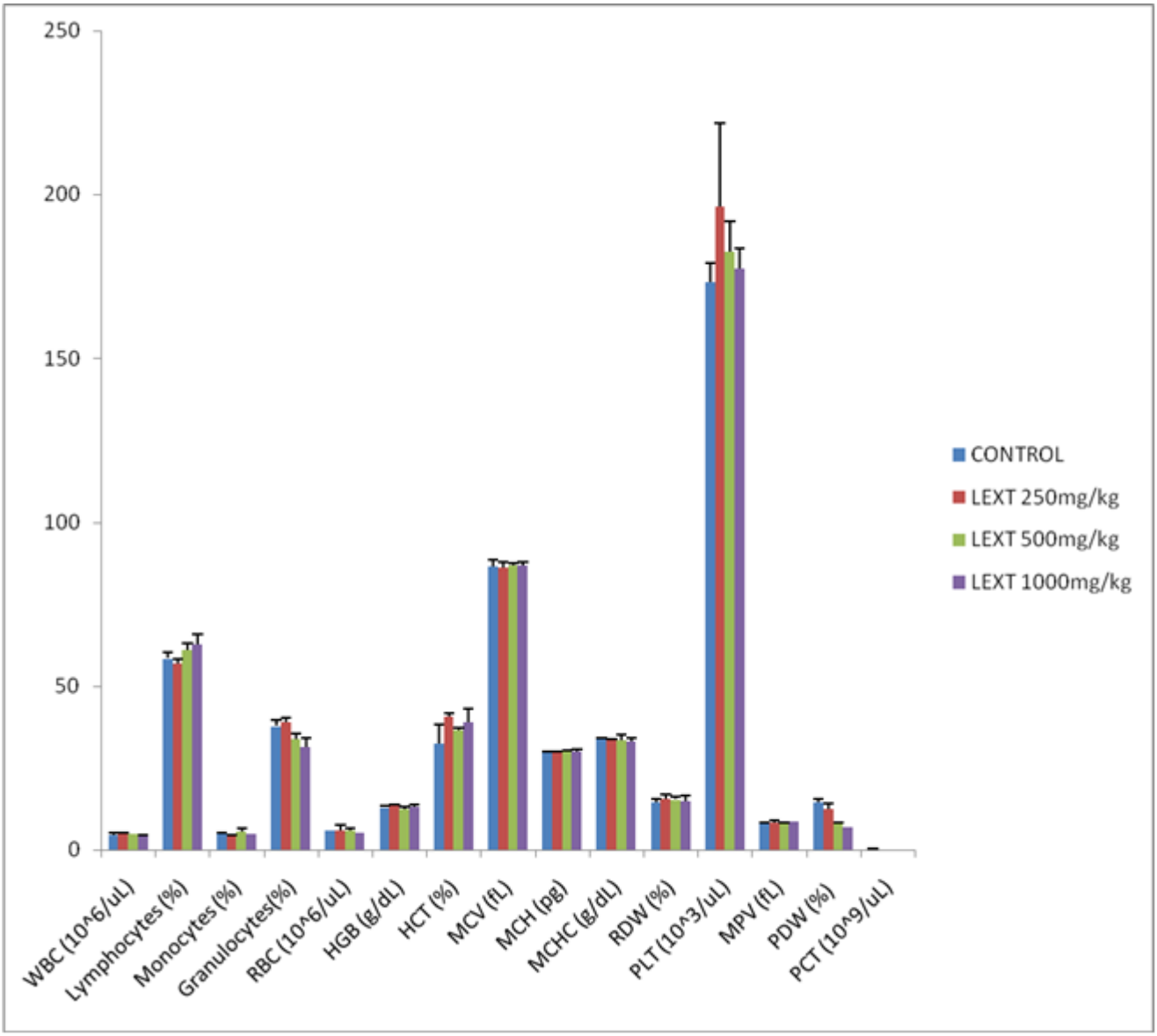


**Figure 3**: Effect of 28 days oral administration of the extract on hematological indices in rats
Key: White blood cell (WBC), Lymphocytes, Monocytes, Granulocytes, Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red distribution width (RDW), Platelet (PLT), Mean platelet volume (MPV), Platelet distribution width (PDW), Plateletcrit (PCT), Leaf Extract of *Detarium microcarpum* (LEXT), n= 6

revealed the presence of alkaloids, saponins, tannins, flavonoids, glycosides and triterpenes which have numerous pharmacological activities, as earlier reported [14, 16, 40 - 41]. Acute toxicity studies are conducted to determine the dose that causes death or serious toxicity in animals within a specific time [13]. According to Lorke's [24] toxicity scale; the oral $LD_{50}$ of methanol leaf extract of *Detarium microcarpum* (> 5000 mg/kg) is practically safe, which is in conformity with the study conducted by David et al [41].

Curative test and Peter's 4-day suppressive test are used to evaluate the activity of the extract against established and early form of malaria infection respectively [27]. The extract showed significant ($p < 0.001$) curative (blood schizonticidal) and suppressive (tissue schizonticidal) effects on the parasite density at the doses tested, which also prolonged the mean survival time (MST) of the infected mice in the treated groups when compared to the negative control, indicating that the extract was able to suppress the effect of the parasite. In anti-malarial study, any test compound that is capable of extending the MST beyond 12 days is considered as having a good suppressive effect [42, 43].

The antiplasmodial effect of *Detarium microcarpum* may be due to the presence of alkaloids, saponins, flavonoids, triterpenes and tannins which have been reported to be responsible for the anti-malarial activity in some plants [44 – 46]. Alkaloids have been reported to generate its anti-malarial activity by inhibition of protein synthesis in the *plasmodium* parasite [47]. Saponins, tannins and flavonoids are natural antioxidants that can prevent oxidative damage induced by the parasite [48]. The antiplasmodial activity of methanol leaf extract of *Detarium microcarpum* may be due to the presence of these constituents exerting their various functions through one or more of these mechanisms or by different mechanism.

The liver is a vital organ that plays a central role in drug biotransformation and its normal function can be assessed by determining the various activities of serum biomarker enzymes (ALT, AST and ALP) [49]. ALT is a cytoplasmic enzyme found primarily in the liver [50]. AST is found in the cytoplasm and mitochondria of different tissues such as the liver, kidney, heart, skeletal muscles and brain [51]. An increase in the level of serum ALT and AST signifies hepatocellular damage; However, ALT is a more sensitive marker of hepatocellular damage than AST [52]. ALP is a hydrolase enzyme found in cells which line the biliary ducts of the liver and also found in other organs such as the kidney, bone, placenta and intestine [53]. An increase in the level of serum ALP indicates hepatobiliary effects and cholestatic liver disease [54]. Bilirubin is a waste product of hemoglobin catabolism; an increased serum bilirubin level is associated with biliary cirrhosis and hepatic cholestasis [55]. Serum protein level roughly reflects the major functional variation in the liver and kidney; an abnormal level may be associated with liver infections or chronic inflammation [56]. Albumin is the main protein synthesized in the liver; an altered level may indicate liver damage. In this study there was no significant change in the level of serum ALT, total protein, albumin and total bilirubin in the treated groups when compared to the control group. However, a significant increase in AST ($p < 0.01$) and ALP ($p < 0.001$) levels was observed at the dose of 1000 mg/kg. The increase may signify that the extract at a higher dose is hepatotoxic or extra hepatotoxic or may be due to enzyme induction rather than pathology.

The kidney is the major organ of excretion of waste products. Assessment of kidney damage can be determined by measuring the level of urea, creatinine and electrolytes. Urea is a waste product of protein breakdown; elevated serum urea signifies a toxic effect on renal tubules, renal parenchyma, cardiac injury, dehydration, shock, blockage of the urinary outflow [57]. Creatinine is a waste product of muscle catabolism; an increase in the concentration of serum creatinine is associated with impaired glomerular filtration [58]. The results showed an insignificant difference in the level of urea and creatinine in the treated groups when compared to the control group. The finding may indicate that the extract at the doses tested is not nephrotoxic, which was further supported by serum electrolytes analysis.

Hematopoietic system is a very sensitive target for toxic compounds and gives an insight on the pathological and physiological status of the body [59]. It was observed that there was no significant alteration in the hematological indices analyzed (i.e. WBC, RBC, Hb, HCT, Monocytes, Lymphocytes, Granulocytes, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, and PCT) in the treated groups when compared to the control group. This signifies that the extract is not hematotoxic and may not cause alteration in the production and circulation of RBC, WBC and platelets.

## CONCLUSION

The results obtained revealed that the methanol leaf extract of *Detarium microcarpum* has significant anti-plasmodial activity which could be due to the

presence of some bioactive substances in the extract, thus provide scientific credence in the use of the plant for the treatment of malaria traditionally. Assessment of serum liver and kidney function parameters and hematological indices suggest that the extract is relatively safe at the doses tested after short term treatment in rats. However, an evidence of toxicity was observed in the liver at the highest dose.


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