

**IN VIVO ANTIPLASMODIAL AND EFFECTS OF SUBCHRONIC ADMINISTRATION OF *TRICHILIA EMETICA* LEAVES EXTRACTS****Sulaiman S. Rukayyah<sup>1\*</sup>, Ali A. Jigam<sup>2</sup> and Mohammed T. Aisha<sup>1</sup>**<sup>1</sup>Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria.<sup>2</sup>Department of Biochemistry, Federal University of Technology, Minna, Nigeria

**ABSTRACT:** *Objective: The leave extracts of Trichilia emetica were investigated for antiplasmodial activity against Plasmodium berghei infections in mice and chronic dose effects of the methanolic extract were also studied. Methods: The plant leaves were successively extracted into three (3) extract forms (Hexane, ethylacetate and methanolic extracts). Plasmodium berghei (NK 65 Chloroquine sensitive strain) was inoculated in to twenty mice assigned for 5 groups of 4 mice each. Group I, II and III were treated with 300mg/Kg bw hexane, ethylacetate and methanol extracts respectively. Group IV with 5mg/kg bw chloroquine phosphate (standard) and group V with 20ml/kg bw normal saline (control). Another set of 40 mice were divided into two groups of twenty each (test and control) and some serum parameters were studied. The test animals were gavaged with 300mg/kg bw extract while controls were given normal saline over a period of 5weeks on alternate days. Histology of the liver, and kidney were carried out. Results: The presence of alkaloids, saponins, phlobatannins, flavonoids, cardiac glycosides and phenolic compounds have been detected and quantified. T. emetica extracts of hexane and methanol suppressed parasitemia in mice by 79.19 % and 95.83% respectively while ethylacetate extract has no activity. The weight of the test group was on a continuous decrease compare to the control while the reverse was the case in terms of the PCV. Glucose, total proteins, triacylglycerides, ALT and ALP levels all decrease significantly compared to the control group. AST level of the test group was significantly higher compare to the control. Histology revealed no damage to the kidney and liver. Conclusion: Hexane and methanolic extracts of Trichilia emetica have strong efficacy against malaria and a possible mechanism for this efficacy is its ability to lyses erythrocytes.*

**KEYWORDS:** *Plasmodium berghei, curative, phytochemicals, spectrophotometer, serum.*

**INTRODUCTION**

Malaria is the most important eukaryotic parasitic disease, threatening the livelihood of over 3.3billion people [1]. Efforts to disrupt the life cycle of the parasite by controlling the vector have had only limited success, while the usefulness of antimalaria drug is hampered by their lack of availability to those most in need and rapid evolution of drug resistant parasites. An effective, safe vaccine remains the most promising approach to controlling the disease [2]. Immense benefits have been derived by man from using medicinal herbs in disease management because they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs [3]. Therapeutic properties ascribed to most of these herbs are linked to the phytochemical compounds contained in them. Phytochemicals such as alkaloids, glycosides, phenols, saponins,

titerpenoids, flavonoids, etc have been suggested to possess antimalaria properties [4,5]. The increase in discovery of more medicinal plants has demanded for increased scientific scrutiny of their bioactivity so as to provide data that will help physician and patients make wise decision before using them [3].

*Trichilia emetica* is a tree native to Savannah belt and open woodland of Africa [6]. It has many different traditional uses [7] including treatment of convulsion, fever, jaundice, cold, epilepsies, scabies, pneumonia and also as purgative, diuretic agents [6]. Some of these claims have been buttress by scientific proves [8, 9, 10, 11, 12]. One major and overriding criterion in the selection of herbal medicines for use in health services is safety. Plant extracts should not only be efficacious but safe for consumption. Therefore, closely associated with screening of plant extracts for their activities against microorganism or disease conditions is the need to know their toxic potentials [13]. Although there has been earlier reports of its in vitro antiplasmodial activity but in vivo antiplasmodial has not been found in literature. A plant with high in vitro activity many have no in vivo activity and vice versa [14]. Therefore considering its wide ethnomedicine uses and broad spectrum activity against microbes there is the need also to determine the effect of *Trichilia emetica* on some vital body organs and key enzyme markers.

## **MATERIALS AND METHOD**

### **Plant Materials**

Fresh leaves of *Trichilia emetica* were collected in Bida Local Government Area of Niger State of Nigeria between July and September, after identification by herbal practitioner and authentication at the Biological Sciences Department Federal University of Technology, Minna. These leaves were air dried at room temperature and pounded into powdered form using pestle and mortar. The powder was packaged in an air tight container, labelled and stored until analysed.

### **Preparation of Crude Extracts**

Eighty grams of air dried sample *Trichilia emetica* was extracted exhaustively (48hours) in the cold sequentially with two liters each of n-hexane, ethylacetate and methanol (Sigma-Aldrich Europe) in that order separately. The marc was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator. Each of the paste was poured into beaker and placed on a water bath for complete evaporation of the organic solvent. The green pastes were weighed and labelled prior to further analysis

### **Animals**

Healthy Swiss albino mice of either sex of about 7weeks old weighing between 20-30g were obtained from Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used for the experiments. The mice were conveniently housed under standard environmental conditions, temperature  $27 \pm 2^{\circ}\text{C}$ , and 70% relative humidity, free access to commercial food pellets, water and natural 12hours day light/night cycles. The experiments were conducted in strict compliance with internationally accepted principles for laboratory animals' use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review [15].

## Parasites

*Plasmodium berghei* Nk65 chloroquine sensitive strain was obtained from Nigerian Institute of Medical Research (NIMR) Lagos, Nigeria and maintained in the laboratory by serial passage in mice.

## Phytochemical Screening

Phytochemical screening was carried out with aqueous extract and pulverized sample using standard procedures as described by Edeoga *et al.* (2005), Akinyemi *et al.* (2005) and Kwada and Tella (2009)[16,17,18]. Tannins, phlobatannins, saponins, and flavonoids were screened according to the methods described by Edeoga *et al.* (2005) [16]. Cardiac glycosides (Keller-killani Test) and alkaloids were screened according to the methods described by Akinyemi *et al.* (2005)[17]. Harbone's (1973) Method of alkaloid determination was also used. Five grams of the samples were weighed into 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath at 90°C to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [16]

Quantitative determination of flavonoids. Ten grams of the pulverized stem bark was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125mm). The filtrate was transferred into evaporating dish and placed on a water bath at 40°C until a constant weight was maintained [18]. The Obadani and Ochuka (2001) method was used in saponin determination. Twenty grams of each plant sample was weighed into a conical flask and 100cm<sup>3</sup> of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hour with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer recovered while the ether layer was discarded. The purification process was repeated. 50ml of n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath at 40°C. After evaporation the samples were dried in the oven to a constant weight, the saponin content was calculated as a percentage of the sample used [16] Total phenol was determined using Edeoga *et al.* (2005) method. Two grams of the plant sample was defatted with 100ml of diethyl ether using a soxhlet apparatus for 2 hours. The fat free sample was boiled with 50ml of ether for 15 minutes for the extraction of the phenolic component. Five millilitre of the extract was pipetted into a 50ml flask, then 10ml of distilled water was added. Two millilitre of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was measured at 505nm [16].

Quantitative determination of Cardiac glycosides: The quantity of glycosides in the pulverized sample was evaluated using Baljet's reagent (95ml 1% picric acid and 5ml 10% NaOH) as Oluwaniyi *et al.* (2007). Digitalis cardiac glycosides develop an orange-red colour with Baljet's reagent. The intensity (absorbance) of the colour produced is proportional to the concentration of

cardiac glycosides. This colour formation is made use of for the quantitative estimation of cardiac glycosides present in *B. aegyptiaca*. One gram of sample was extracted by soaking overnight with 10ml of 70% alcohol and filtered. The extracts were then purified using lead acetate and  $\text{Na}_2\text{HPO}_4$  solutions before the addition of freshly prepared Baljet's reagent. The intensity (absorbance) of the colour produced was measured using a spectrophotometer at 495nm. A blank was carried out at the same time using distilled water and Baljet's reagent. The absorbance of the colour produced is proportional to the concentration of the glycoside. A 0.02% solution of digitoxin solution was prepared in chloroform-methanol (1:1 v/v) mixture (1ml = 0.2mg). Different volumes, viz. 1, 2, 3, 4 and 5ml (equivalent to 0.2, 0.4, 0.6, 0.8 and 1mg of digitoxin respectively) of the solution were transferred, each into a dry Erlmeyer flask. The solvent in each flask was evaporated on a water bath and dissolved in 0.35ml 90% alcohol. Ten ml of distilled water and 10ml of freshly prepared Baljet's were added. Absorbances were read and standard curve was plotted. The concentration of cardiac glycoside in *B. aegyptiaca* was then extrapolated from the standard curve [19].

Determination of condensed tannins (Proanthocyanidins): The method described by Iqbal *et al.* (2001) was followed for the determination of condensed tannins in the extract. The samples were extracted to quantitatively diffuse the phenolics present in the materials to liquid phase. For the extraction process, aqueous acetone (70%) was used. Each of the dried (finely ground) sample (200 mg) was taken in a glass beaker of approximately 25mL capacity. Ten mL of aqueous acetone (70%) was added and the beaker was suspended in an ultrasonic water bath and subjected to ultrasonic treatment for 20min at room temperature. The contents of the beaker was then transferred to centrifuge tubes and subjected to centrifugation for 10 min at approximately 3000 g at 4°C using a refrigerated centrifuge. The supernatant was collected and kept on ice. The pellet left in the tube was transferred to the beaker using two portions of 5 mL each of 70% aqueous acetone and again subjected the contents to ultrasonic treatment for 20 min. The supernatant was again collected as described above. Butanol-HCl reagent (butanol-HCl 95:5 v/v) was prepared by mixing 95 mL of n-butanol with 5 mL concentrated HCl (37%). Ferric reagent (2% ferric ammonium sulfate in 2N HCl) was prepared by dissolving 2.0 g of ferric ammonium sulfate in 2N HCl (16.6 mL of concentrated HCl was made up to 100 mL with distilled water to make 2N HCl). The reagents were stored in dark bottles. In a 100 mm x 12 mm glass test tube, 0.5 mL of the tannin extract diluted with 70% acetone was pipetted. The quantity of acetone was large enough to prevent the absorbance (550 nm) in the assay from exceeding 0.6. Three mL of the butanol-HCl reagent and 0.1 mL of the ferric reagent was added to the tubes. The tubes capped with a glass marble were shaken using a Vortex and then placed on a heating block adjusted at 97 to 100°C for 60 min. After cooling the tubes, absorbance was recorded at 550 nm. Absorbance of the unheated mixture (considered as a suitable blank) was subtracted from the absorbance of heated mixture, which was actual reading at 550 nm to be used for calculation of condensed tannins. Condensed tannins (% in dry matter) as leucocyanidin equivalent were calculated by the formula:

(A 550 nm x 78.26 x Dilution factor)/(% dry matter)

This formula assumes that the effective  $E^{1\%, 1\text{ cm}, 550\text{ nm}}$  of leucocyanidin is 460 [20].

#### Safe dose and acute toxicity (LD<sub>50</sub>)

Five groups of four mice were used and the animals were given extracts intraperitoneally (i.p) at doses of 200, 400, 800, 1600, and 3000mg/kg body weight (b/w) respectively. Extracts were dissolved with 2ml of dimethylsulphoxide (DMSO) (Sigma Chemical St Louis, MO, USA) and

the volume was made up to 10ml with distilled water. A control group was given normal saline (0.9% w/v NaCl) at 20ml/Kg bw. Mice were observed over 72 hours for clinical signs and mortality was recorded. LD<sub>50</sub> was obtained as the intercept of % mortality (y-axis) and dosages (x-axis) [21].

### **Antiplasmodial Screening**

Mice were pre-screened by microscopy of thin and thick tail tip blood smears. This was necessary to exclude the possibility of test animals harbouring rodent *Plasmodium species*.

### **Curative Test**

This is a procedure whereby mice are infected and left for 72 hours before treatment with test and standard drug as in the Rane test or established infection [22]. Twenty albino mice were selected and divided into five groups. One group served as control, another group as standard and the others as the test groups for hexane, ethylacetate and methanolic extracts respectively. The mice were inoculated with 0.2ml of diluted donor blood (*Plasmodium berghei* approximately  $1 \times 10^7$  infected red cells) by the intraperitoneal route. The animals were left for 72 hours for the infection to be established. The control group was given 0.2ml of 0.9w/v of normal saline. 300 mgkg<sup>-1</sup> bw day<sup>-1</sup> dose was selected for the plant extract, because it was the safe dose. On D3 i.e, after 72 hours of infection, the plant extracts were administered subcutaneously once daily for 4 days from D3 to D7. Thick and thin blood smears from the tail blood were examined for parasite every morning. Chloroquine (5mg/kg bw) was used as standard drug because the parasites are sensitive to this compound (Chloroquine sensitive strain of *Plasmodium berghei*) and was hence ran for comparison.

### **Evaluation of the medium term effect dosage of crude extract in mice**

Forty mice were kept in two groups (A and B) of the twenty each. Group A was used as test and gavaged with 300mg/Kg bw of the extracts and group B given 20ml/Kg bw normal saline on alternating days. All animals were monitored for different biochemical parameters at weekly intervals for five weeks.

Weight of mice were taken with Avery Balance (W and T) Avery Ltd, Birmingham, UK. Packed Cell Volume (PCV) was determined using the microhaematocrit method [23]. Serum glucose was determined using Randox Glucose Kit (Cat GL364) based on the Glucose oxidase reaction. The estimation serum total protein was carryout with Total Protein Randox kit (Cat No. TP 245) on cupric ions in an alkaline medium, interact with protein peptides bonds resulting in the formation of a coloured complex compound which is proportional to the amount of protein present in the sample. Triglyceride was evaluated using Randox Triglycerides kit (Cat No. TR 210). The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophonazone and 4-chlorophenol under the catalytic influence of peroxidase [24]. Estimation of AST and ALT was done using AST Randox kit (Cat No AS 101 and Cat No. AL 100 respectively) based on method of Marghoob et al (2013) [25]. The estimation ALP activity was done using Randox ALP kit (Cat. No 542) ALP hydrolyses colourfulness p-nitrophenyl phosphate (pNPP) producing phosphate and coloured p-nitrophenol at alkaline pH [26].

### Histopathological screening of tissues

At the end of the treatment period, mice were sacrificed and liver and kidney collected in sterile saline. Freshly dissected organs from each animal were cut rapidly and fixed in buffered neutral formalin (10%). The tissues were dehydrate in ascending grades ethanol (70%, 80%, 90%, 95% and 100%), cleared in 2 changes of Xylene, impregnated with 2 changes of molten paraffin and finally embedded in wax. Tissue sections of 4-5 $\mu$ m in thickness were cut with a microtome and stained with hematoxylin and eosin [27].

### Statistical Analysis

Results are expressed as means  $\pm$  standard error of the mean. While Analysis of variance (ANOVA) coupled with Duncan Multiple Range Test (DMRT) was used to test for the significant differences between groups for Antiplasmodial bioassay, paired sample t-test was used to test for significant differences for the biochemical parameters, PCV, mean weight change and enzyme assay data using Statistical Package for Social Sciences (SPSS) version 16.0. A value of  $P < 0.05$  was accepted as significant.

## RESULTS

The extract yields of *Trichilia emetica* obtained with different solvents are in the order: methanol > hexane > ethyl acetate 10.48, 6.00 and 2.42% respectively.

### Phytochemical Screening

Cardiac glycosides, pholobatannins, flavonoids, saponins and alkaloids were detected during phytochemical screening with total phenols been most abundant (64.71%).

**Table 1.0 Phytochemical contents of *T. emetica* and *B. aegyptiaca* extracts**

Phytochemicals	Test	T.emetica	
			%
Tannins	0.1% FeCl		
	Bromine H <sub>2</sub> O	-	
Pholobatannins	Lead acetate	-	
	1% HCl	+	
Saponins	Frothing	+	13.40
	Emulsion	+	
Flavonoids	Dilute NH <sub>3</sub> /Conc H <sub>2</sub> SO <sub>4</sub>	-	32.20
	Lead Acetate	+	
	Dilute NaOH	-	
Cardiac glycosides	Keller-killeni	+	
Alkaloids	Mayer's test	+	19.40
<b>Total phenol</b>	<b>Spectrophotometric test</b>		<b>64.71</b>

+ = present, - = absent

**Table 2.0 Results of safe dose determination (Pre LD<sub>50</sub>) for *Trichilia emetica***

Dose bw. ip.)	Observations	Mortality	(mg/kg
200	Animal appears normal	0/4	
400	No observable Changes	0/4	
800	Initial restlessness but normal	0/4	
1600	Slow activity, laboured breathing	0/4	
3000	Somnolence with a single mortality	1/4	

Selected dose = 300mg/kg bw

The suppression of parasitaemia in mice treated with different fractions *T. emetica* of is in figure 1.0. Ethylacetate fraction of *T. emetica* is not effective in the treatment of malaria, because the mice in this particular group died before the control group. Hexane and methanolic fraction of *T. emetica*, show promising effect on malaria parasite, with the methanolic fraction having higher activity than the hexane extract with parasite suppression of 95.83% and 79.17% respectively. The methanolic extract of *Trichilia emetica* completely clear *B. berghei* from circulation in the infected mice after 23 days, the cured mice remained aparasitemic for over 60 days.

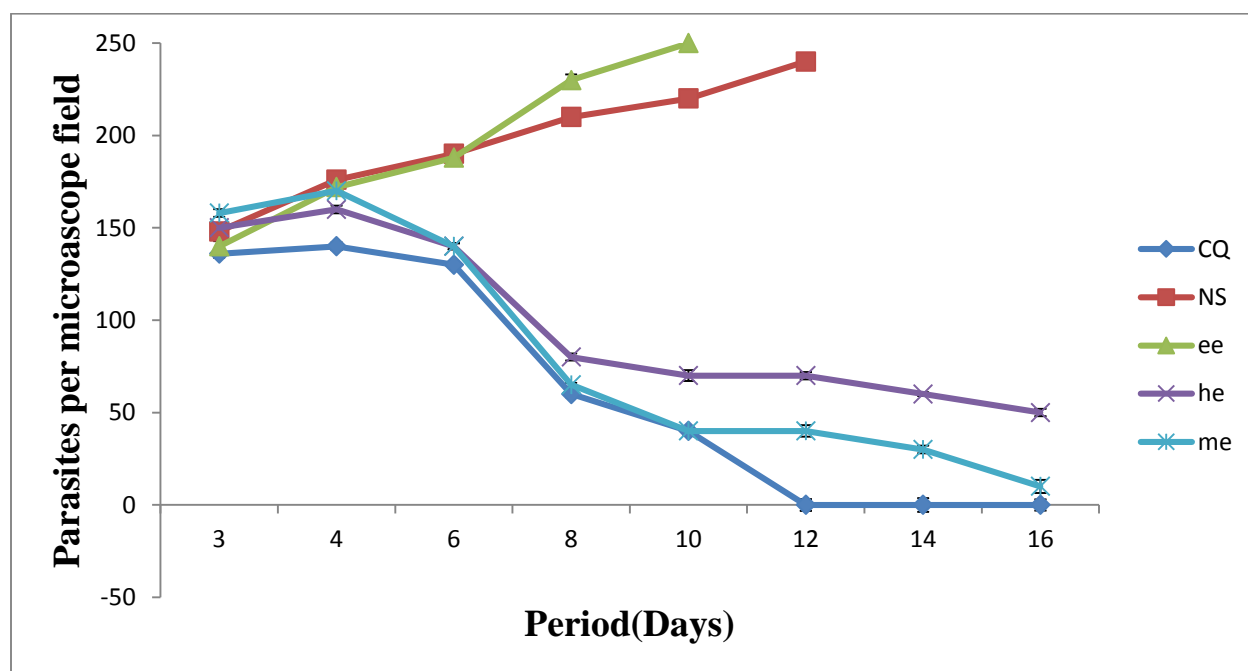


Figure 1.0: Suppression of parasitaemia in mice treated with different fractions of *T. emetica*

### Weight Variations

The whole body weights of mice (Fig.2.0) administered *T. emetica* exhibited a drastic decrease in week one (1) and two (2), with a minimal increase to week. However the weight decreased steadily

after week three (3). The weight of control mice continue to increase from week one (1) to week five (5). The results of fresh organ weights of mice expressed as percentage of whole body weights are in Table 2.0. Only the stomach of the test showed some variations. The other organs of the test mice were comparable in weight with those of the control.

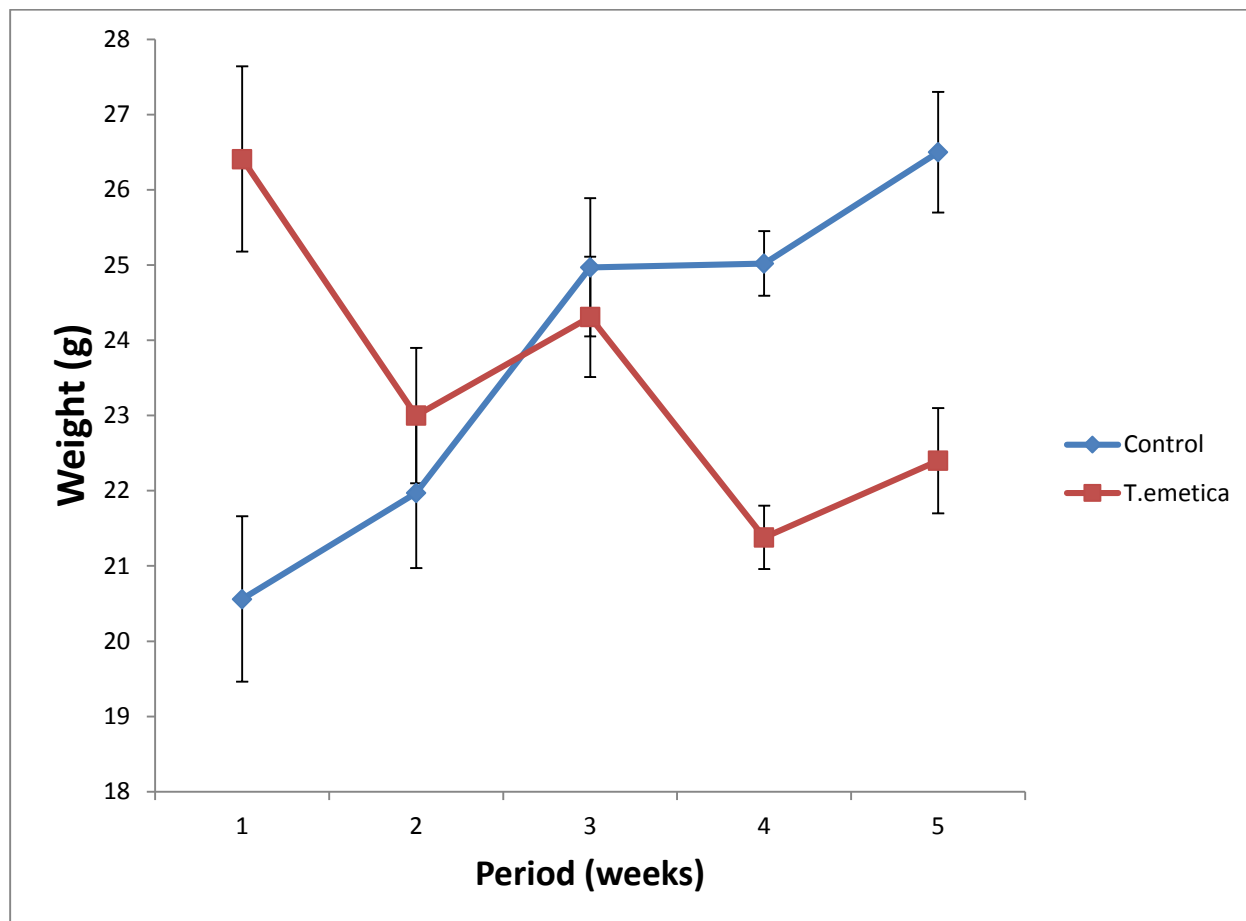


Figure 2.0: Variations in weight (g) of mice dosed with *T. emetica*.

**Table 3.0 Results of fresh organ weights of mice dosed with *T. emetica* extract**

Organs	Control	Test
Liver	4.55	4.69
Kidney	1.27	1.25
Intestine	11.32	11.30
Spleen	0.39	0.45
Stomach	2.34	2.68*

\* means the sample result is significantly different at 5% ( $p < 0.05$ ) across the row.

### Packed Cell Volume

Variations in Packed Cell Volume in mice chronically dosed with *T. emetica* and the control is represented in figure 3.0. The PCV of *T. emetica* were progressively decreasing from week one to



three, with a peak at week four and a decline afterward while the PCV for the control was increasing in all the weeks

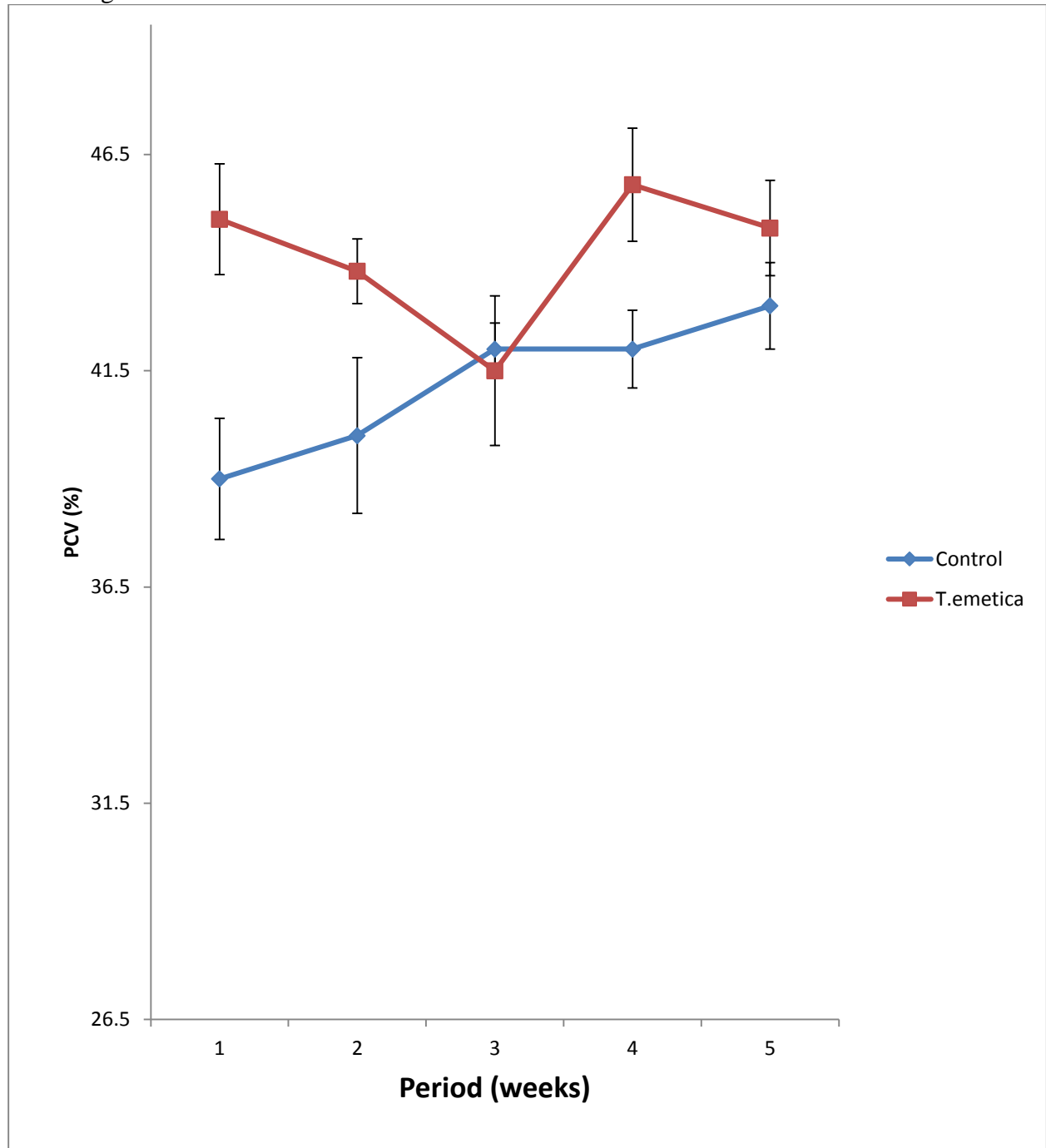


Figure 3.0: Variations in packed cell volume in mice dosed with *T. emetica*.

**Serum Glucose, Total Proteins and Triglycerides**

Table 4.0 contains values for glucose, total proteins and triacylglycerides. Glucose levels in the test mice were significantly ( $p < 0.05$ ) elevated in week 1 ( $110.13 \pm 2.6$ ) and in week 5 ( $95.65 \pm 2.05$ ) decrease significantly ( $p < 0.05$ ). There was significant change in the level of total proteins between the test and the control over the five week period. However, the triacylglycerides levels declined significantly ( $p < 0.05$ ) from week 1 ( $144.87 \pm 3.13$ ) to week 5 ( $100.05 \pm 2.88$ ).

**Table 4.0: Effects of *T.emetica* extracts on some serum biochemical parameters in mice.**

Period	Parameters (mg/dl)		
	Glucose	Total proteins	Triacylglycerides
WK1 Ctrl	$96.00 \pm 1.84$	$7.00 \pm 1.11$	$187.40 \pm 5.13$
Test	$110.13 \pm 2.6^*$	$7.80 \pm 0.34$	$144.87 \pm 3.13^*$
WK 5 Ctrl	$109.08 \pm 1.28$	$8.00 \pm 1.20$	$223.00 \pm 2.94$
Test	$95.65 \pm 2.05^*$	$6.10 \pm 0.22$	$100.05 \pm 2.88^*$

The results are expressed as mean  $\pm$  standard error of mean ( $\bar{X} \pm SEM$ ) with ten sample size ( $n=10$ )  
\* means the result is significantly different at 5% ( $p < 0.05$ ) on the same column.

**Serum Transaminases (AST and ALT) and Alkaline phosphatase (ALP)**

The values of Transaminases (AST and ALT) and Alkaline phosphatase (ALP) are in Table 5.0. AST level was significantly ( $p < 0.05$ ) higher in week 1 ( $60.00 \pm 2.85$ ) and week 5 ( $23.00 \pm 1.15$ ) compared to the level in the control. No significant change in the ALT level in the week 1 but there was a significant ( $p < 0.05$ ) decrease in week 5 ( $3.50 \pm 0.76$ ) compared to the control. Steady decrease in ALP level was also observed to week 5 ( $185.20 \pm 4.25$ ) were there was a significant decrease of ALP levels when compared to the control mice.

**Table 5.0: Effects of *T. emetica* extracts on some serum enzymes in mice.**

Period	Serum enzymes ( $\mu/L$ )		
	AST	ALT	ALP
WR1 Ctrl	$9.80 \pm 1.40$	$12.00 \pm 1.14$	$202.32 \pm 8.18$
Test	$60.00 \pm 2.85^*$	$12.00 \pm 1.20$	$199.00 \pm 4.02$
WK 5 Ctrl	$11.60 \pm 1.11$	$12.60 \pm 2.79$	$200.00 \pm 3.89$
Test	$23.00 \pm 1.15^*$	$3.50 \pm 0.76^*$	$185.20 \pm 4.25^*$

The results are expressed as mean  $\pm$  standard error of mean ( $\bar{X} \pm SEM$ ) with ten sample size ( $n=10$ )  
\* means the result is significantly different at 5% ( $p < 0.05$ ) on the same column.

**Histopathology**

The histology of the test mice showed no difference in the liver and kidney compared to the control.

## DISCUSSIONS

Medicinal plants are considered to be the main sources of biologically active compounds that can be used for the treatment of malaria [28]. The presence of phytochemicals such as alkaloids, saponins, flavonoids, phlobatannins and cardiac glycosides observed during qualitative screening for secondary metabolites suggests that the hexane and methanolic extracts may exert some mechanisms that counter the pathological processes of *P. berghei* infection. These metabolites with profound antioxidant properties, among other mechanisms [29], may exert their antiplasmodial effect by decreasing nitric oxide production in kupffer cells, resulting in killing the parasites [30]. In addition, secondary metabolites such as alkaloids and glycosides have been shown to possess direct antiplasmodial effects [31, 32]. It is imperative that hexane and methanolic extracts contain more of these active phytochemicals, accounting for their antiplasmodial effects in vivo. The result has clearly indicated that different solvent extracts of the same plant can exhibit different antiplasmodial activities, just as extracts of different parts of the same plant [14]. Total phenolic content showed positive correlation with the reducing power and lipid peroxidation inhibition as earlier reported [29].

The continuous decrease in PCV value, for the first three weeks, could be attributed to either chronic kidney or bone marrow disease [33] or ability of the extract to bind to essential minerals, like iron required in the synthesis of red blood cells, causing a decrease in the concentration of circulating red blood cells. After the third week, increase in PCV value indicates hemcentration due to increase in red blood cells mass. The increase in red blood cells is suggestive of polycythemia and a positive erythropoietic effect, thus enhancing the carrying capacity in the mice [34]. This could be another possible mechanism (destruction of the infected red blood cells and stimulation of its synthesis afterwards) with which this extract elucidate its antiplasmodial activity. The fresh organ weight of kidney and spleen which are hematopoietic organs were not significantly different ( $p > 0.05$ ) when compared to the control; this is the same with the liver and heart muscle. The fresh organ weight of the stomach was significantly higher when compared to the control. The decrease in glucose and triacylglycerides levels suggests that methanolic extract of *T. emetica* contain active principle(s) that have antidiabetic property. This could be due to its ability to stimulate the pancreas for insulin production [35]. Decrease in serum proteins could generally be early indication of renal or liver or nutritional deficiency. Similar effects of some plant extract in experimental animals have been documented [36].

In the assessment of organ damage by a xenobiotic, the determination of enzyme levels such as ALT, AST and ALP are largely used [37]. In the present study, the activities of ALT and ALP were decreased indicating that the extract has some hepatoprotective function and biliary advantage [38]. The observed increase in AST level signifies damage to the liver, kidney, heart muscle, erythrocyte or skeletal muscle, but there is no corresponding increase in the activity of ALT (more specific and better parameter to detect liver injury) or ALP to suggest liver or kidney damage. Histology of the liver, kidney and heart muscle revealed that no damage was done to these organs.

The adverse effect of the extract reflected by highest AST activity (60u/l) in week one shows how important these enzyme markers easily detect organ damage. Apart from the liver, kidney, heart and skeletal muscle, AST is also found in erythrocytes. The decline in PCV value from the first week with corresponding increase in AST activity in the first week suggest that destruction of erythrocytes have contributed in the rise in AST activity. Also, when the extract stimulated erythropoiesis, the activity of AST was on a decrease with increase in PCV value. However, the possibility skeletal muscle contributing to rise in AST level cannot be over rule

This report of in vivo antiplasmodial activity of *T. emetica* extract is also a confirmation of the earlier reports of its antiplasmodial activity in vitro by Kamanzi *et al.*, 2004 and Bah *et al.*, 2007 [39,40]. Thus *T. emetica* has both in vitro and in vivo antiplasmodial activity [41,42].

### IMPLICATION TO RESEARCH AND PRACTICE

The outcome of this research is of great importance to pharmaceutical industries for the development of therapeutical active drug against malaria parasites. Isolation of the bioactive phytochemical from these plant extracts will be of immense significance to drug manufacturers.

### CONCLUSION

The present studies has confirmed that *T. emetica* does not only have an in vitro antiplasmodial activity but also has in vivo antiplasmodial activity. It was also observed that long term administration of the methanolic extract of *T. emetica* causes damage to erythrocytes and skeletal muscle.

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#### FUTURE RESEARCH

Fractionation of plant extracts to determine the active compound(s)