

# Anti-Plasmodial Effects of Ethanolic Extract of Florida Beggar Weed (*Desmodiumtortuosum*) on Albino Mice-Infected with *Plasmodium Bergheibergeri*

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**Abstract:** The aim of this study is to investigate the anti-plasmodia effects of ethanolic extract of Florida beggar weed on albino mice experimentally infected with *Plasmodium bergheibergeri* Malaria infection in mice was initiated by intraperitoneal (IP) inoculation of 0.5 ml blood, diluted to contain  $2 \times 10^7$  parasitized red blood cells (PRBC) from a donor mouse infected with *P. b. berghei*. Controls to malaria-infected mice were given an equivalent volume and dilution of normal uninfected red blood cells inoculation period was for four days. Nine groups in a plastic wire cages were created. Three control groups were created viz: Normal (non-inoculated) negative (inoculated but untreated) and positive control (inoculated and treated with 10mg/kg Chloroquine). Two categories with three sub-groups were created with each category representing the ethanolic extracts of *Desmodiumtortuosum* leaf and stem respectively. The three sub-groups in each category represent the three serial dilutions of 100, 200 and 500mg/kg ethanolic extracts each of the leaf and stem administered for four days for the treatment of the parasite. To Measure the parasitaemia levels in the animals, thick and thin film smears were made on slides for microscopic viewing. Slides were viewed under light microscopy with oil immersion (1000x magnification). The average Parasitaemia was calculated as well as the average percentage parasite inhibition (suppressive effect) was obtained. Result showed that the parasitemia level for the treated groups decreased progressively for the five days period. This is indicative in the mean number of the percentage parasitized red cells of 500mg/kg doses as  $3.50 \pm 1.25$  and  $4.00 \pm 1.22$  on the first day post inoculation and  $1.50 \pm 0.28$  and  $1.50 \pm 0.28$  for *Desmodiumtortuosum* leaf and stem respectively by the fifth day. The decrease is also observed in the 100 and 200mg/kg groups of each sample. Except for the untreated group which showed a progressive increase in parasitemia level showing the mean number of the percentage parasitized red cells as  $3.50 \pm 0.64$  on the first day post inoculation and  $13.25 \pm 1.31$  by the fifth day. The hematological result showed a significant ( $p < 0.05$ ) decrease in values of RBC, PCV, Hb, and Neutrophils in the inoculated groups especially the untreated group. As compared to the treated groups, these parameters are seen increasing progressively as concentrations increases. The potency of the leaf and stem on the fifth day was observed to be the same. The plant generally showed dose independent behaviour. The biochemical assays of the leaf and stem extracts showed no toxicity effects. In conclusion, the ethanolic extracts of *Desmodiumtortuosum* leaf and stem showed moderate anti-

plasmodial properties and are less toxic to the body therefore can be pursued for the development of an antimalarial drug.

**Keywords:** *Desmodiumtortuosum*, *Plasmodiumbergheibergeri*, parasite, ethanolic, albino mice.

## I. INTRODUCTION

Malaria remains the most severe and complex health challenges in the sub-Saharan Africa (FMH, 2007). It is a major cause of global infectious diseases leading to infant morbidity and mortality mostly in Africa (WHO, 2009). Malaria has contributed to between 200-500 million episodes and between 1-3 million death annually (WHO, 2009) and irrespective of the various declarations by African governments in the 1990s and complementary effort promised in the main content of the roll back malaria declaration in 2000 at Abuja, malaria stand to be a major health drawback or challenges (Olufunke and Olunuyiwa, 2003). In areas of high endemicity most of the malaria associated infant morbidity and mortality rate are recorded in pregnant women and children (WHO, 2009). The disease equally had negative impacts on the economy of prevalent countries (WHO, 2005).

Malaria causes between 200-2005 million episodes and between 1-3 million death per year (WHO, 2013). It is estimated that about 3.3 billion people are at risk of malaria globally (WHO, 2015). Malaria infection has makes a large but unquantifiable contribution to low birth weight in newborn in developing nations, severe maternal anemia and perinatal mortality, it is a major causes of morbidity and mortality in infants and children (Omoga, 2017). Malaria leads to about 5-12% of all low birth weights in children worldwide (WHO, 2010).

Malaria was responsible for one out of ten cases of deaths in pregnant women which caused the Federal Government of Nigeria to spend over one billion naira yearly in treating malaria (Government in Action, 2005). The high mortality rate is broadly associated to resistance of the parasite to commonly used anti-malaria drugs (Aliiet al., 2011). The emergence of the inactiveness of chloroquine in fighting malaria led to additional research which produced a new and

active anti-malaria drug, Artemisin (WHO, 2005; Tolu *et al.*, 2007).

Despite the success attributed to Artemisin combination therapy (ACT), many malaria endemic communities rely actively on traditional herbal medicines which are often readily available and affordable (Etkin, 2003). Therefore, parasite resistance to the drug has been detected in southeast Asia which also reduces the audibility of the drug and place us in an emergency situation (Alker *et al.*, 2007). It becomes too imperative to seek for an effective alternatives to overcome the drawback of contemporary anti-malaria therapies. The search for an alternative anti-malaria option has resulted to different studies on several plants in order to find out active anti-malaria activity in them. Anti-malaria activities of a member of these plants have also been reported (Kaura *et al.*, 2009). According to UNESCO (1998), the usefulness of these medicinal plants may hold the key to another new and effective anti-malaria drug in the future (Hill, 1952). The use of plants parts for medicine has been in existence and is well documented in records kept in ancient China, Egypt and India ( Faisini and Taiwo, 2005). The use of plants for medical activities has produced results of proven efficacies compared to conventional modern medicines. Hippocrates the father of medicine depended totally on the use of herbs for his successes (Morah, 2010). Globally, in recent times, herbal medicine has become increasingly useful and a potent part of primary health care system (Chang and Stevenson, 2000).

In consonance with the enormous dependency on herbal medicine/traditional health practice and in recognition of the crucial role of herbal medicine in health care delivery, the use of these natural products in the Alma mata declaration of Health for all by the year 2000 and was approved by the world Health organization (WHO) in 1978. The program defined traditional medicine as “the sum total of knowledge and practice, whether explicable or not used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation whether verbally or in writing” (Dhawan *et al.*, 1980; Morah, 2007) according to WHO, about 80% of the world inhabitants rely absolutely on traditional medicine for primary health care. Plant natural products equally play a vital role in the health care system of the remaining 20% of world population who dwell mainly in the development communities (Morah, 2000). WHO has also described traditional medicine as one of the surest means to achieved total health coverage of the world population (Rukangira, 2004). In African plant extracts are employed or used in the various ailments due to their anti-bacteria, Audifugal, and Audi-parasitic properties (Raly and Peter, 1970; Pamplona Rogers, 2004). It is equally noted that over 400,000 species of tropical flowering plants have medicinal properties and thus, made traditional medicine more cheaper than modern medicine (Ladipo and Doherty, 2011). These plants are generally grouped or referred to as medicinal plants. Medicinal plants are refers to any plant which in one

part or more of its organs, contains substances that can be used for therapeutic purposes or which are precursor for the synthesis of useful drugs. The medicine value or content of the plant lies in or depends on some chemical substances that generate or produce a physiological action on the human or animal body (Eduegy *et al.*, 2005). Some plant decoctions are of essential value in treatment of malaria, diarrhea, typhoid fever, gastrointestinal disorder among others (Meyer *et al.*, 1996).

In Nigeria, the Fulani herdsmen and others who keep animals as a means of livelihood have been involved in the treatment of animal diseases prior to the onset of modern medicine (Nwude, 1997) which remedies against parasitism accounted for the highest means of intervention (Ibrahim *et al.*, 1984). However, majority of the population in malaria endemic areas still depends on traditional medical remedies from plants due to the limitations of pharmaceutical industries (Quiroz *et al.*, 2014). Plant has proven to be the most successful in the treatment of almost all ailments and they equally provide a vital source of raw materials in the pharmaceutical industries. Virtually all cultures from time immemorial to the recent time used plants as sources of one form of medical care or the other. Plant has always played a crucial role in the treatment of human traumas and diseases (Sofowora, 2006). One of the plants which is also very prominent in the traditional medical systems is *Desmodium tortuosum*, a Florida beggar weed is an erect, self-pollinating, annual Legume introduced in the late 1800's into the southeastern United States as a forage crop. The distribution of Florida beggar weed ranges from the eastern communities to North Carolina to mid. Florida and west to Texas it is considered the most troublesome weed on the sandy coastal plain soil of the southeast where peanuts are grown. Among other members of the *Desmodium* genus, Florida beggar weed exhibits heteroblastic development characterized by an abrupt change from Juvenile to adult morphology.

Malaria continues to represent an undesirable global issue and scourges in the world. The incidence continue to increase and survive in the different environmental condition which affects the control of malaria in late pregnancy and increase cases of morbidity and mortality as a result of the efficacy of convectional drugs against both endo-and ecto parasitic diseases has been reported with variable success. (Maleney, 1982, Basan and Halder, 1994). The toxic effects of these chemical on human and the development of resistance to its by parasites as well as the high cost of drugs have paved way for herbal remedies as the most alternatives. Therefore, it is imperative to investigate the medicinal plants that aid in the combat of eliminating the deadly illness of malaria.

This study provided the essential information about the medicinal properties and the potency of Florida beggar weed with respect to malaria treatment. The phytochemical properties of the plant was estimated which formed basis for studies on this plant. The cytotoxicity studies was determined to draw attention to the toxicological perspective of this plant.

### Aim and Objectives

The aim of this study is to investigate the anti-plasmodia effects of Florida beggar weed on albino mice experimentally infected with *Plasmodium bergheberghei* with the following objectives:-

1. To investigate the anti-plasmodial effects of ethanolic leaf and stem extract of *Desmodiumtortuosum* on mice experimentally infected with plasmodium bergheberghei.
2. To determine the efficacy of the extract at different concentrations (doses) on the parasite.
3. To investigate the effects of ethanolic extracts of the leaf and stem of *Desmodiumtortuosum* on some hematological and biochemical parameters of the mice infected with *P. bergheberghei*.
4. To investigate the phytochemical constituents of the leaf and stem extracts of *Desmodiumtortuosum*.
5. To compared the potency of Florida beggar weed and chloroquine on the parasites.

## II. MATERIALS AND METHODS

### Study Area

Ignatius Ajuru University of Education is located at Rumuolumeni Port Harcourt, Rivers State with coordinates 4.8055<sup>0</sup> N 6.9324<sup>0</sup> E. the topography is characterized by flat plains with a network of rivers, tributaries and Creeks which have a high potential for breeds of mosquitoes. Malaria transmission is intense throughout the year with a peak during the raining season from March to November and dry season from December to February. The vegetation of part of Port Harcourt is characterized by mangrove and rain forest ecosystems which form part of the rich Funa and flora of the state.

### Collection of Plant Materials

The plant materials (Leaves and stem of *Desmodiumtortuosum*) were obtained from a garden inKaa community, Khana Local Government Area of Rivers State, Nigeria where it is grown as a garden plant. The plants were properly identified using appropriate identification keys by a plant taxonomist. Voucher specimens of the plants were deposited in the herbarium of the department of Plant Science and Biotechnology, Rivers State University, Port Harcourt.

### Acute toxicity studies (LD<sub>50</sub>)

To determine the lethal dose (LD<sub>50</sub>) of the ethanolic leaf extract, thirty six (36) mice weighting between 16g-26g were used in this study. These mice were divided into nine (9) groups of four (4) mice each. Group 1, received food and water only; group 2, 3, 4, 5, and 6 received 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of ethanolic leaf extract of *Desmodiumtortuosum* respectively. Similarly Group 7, 8 and 9 received 1000, 2000, and 3000 mg/kg body weight of ethanolic stem extract of the same plant (*D. tortuosum*). All mice were subjected to 24 hours fasting with only water

before administration of extracts. The extract was dissolved in 20% tween-80 and administered orally. The first group of four mice served as the control and received only 20% tween-80. The mice in all groups were then observed for toxicity and fatality over 72 hours (3 days). Physical observations such as their degree of restiveness, aggressions and calmness were observed. The LD<sub>50</sub> was calculated using the formula of Lock (1983).  $LD_{50} = \sqrt{ab}$

Where a = least tolerable dose

b = maximum tolerable dose.

### Preparation of Ethanolic extracts of leaf and stem of *Desmodiumtortuosum*

The leaves and stems of *D. tortuosum* were separately washed to remove impurities and air dried to a stable weight before weighing on a weighing balance. About 50g of the leaf was put or weighed into an electric blender and was blended using 100% absolute ethanol (1000mls). The blended solution was allowed for about 9hours to settled before filtration. The blended extracts were filtered and the filtrate was then concentrated using a rotary evaporator to get crude extract of the leaf. The same procedure was also carried out on the stem of plant but 100g of the stem was used.

### Reconstitution of the Extracts for Administration

Preparation of 20% Tween-80; 20ml of tween-80 was measured into a clean glass cylinder containing 80ml of distilled water. The mixture was slightly heated to ensure the formation of a homogenous solution. This constituted stock solution of 20% tween-80 was used to dissolve the dried plant extracts into solution for oral administration. The volume (dosage) to be administered was prepared daily according to the average weight of each group of mice.

### Acquisition of *Plasmodium bergheberghei*

Mice and chloroquine for the experiment mice already parasitized with *plasmodium bergheberghei* (NK65) were bought from the Nigeria institute for medical research (NIMR), Lagos and maintained alive. The mice for the study were obtained from the animal house of the faculty of basic medical sciences, University of Port Harcourt, Nigeria. The mice were housed in standard cases in the laboratory and stabilized for 7 days during which they were fed on standard livestock feed (vital feed growers). The body was conducted in the animal house and the laboratory of the department of biochemistry, University of Port Harcourt. Standard chloroquine (diphosphate salt) obtained from pharmacy was used in this study. The mice were handled in accordance with the guidelines for the care and use of laboratory animal by the institute for animal research, United States of America National Research council, (2003).

### Experimental Design

At the commencement of the experiment, 36 albino mice weight between 16-26g and were divided into 9 groups of 4

mice each. These group were labeled as Group 4H, 4B, 4T, 4P; Group 5H, 5B, 5P, 5T; Group 6H, 6B, 6P, 6T; Group 7H, 7B, 7P, 7T, Group 8H, 8B, 8T, 8P; Group 9H, 9B, 9P, 9T and control groups 1,2 and 3.

#### *Inoculation of the mice with the parasites*

The parasitized mice with *Plasmodiumbergheberghei* (NK65) were sacrificed after 6 days having observed to have shown clinical symptom of malaria. The mice were anaesthetized in a glass jar containing cotton wool soaked in chloroform. Blood was collected from the sacrificed mice by cardiac puncture using stentsyringes and needles. The blood was diluted in normal saline in the ratio of 1:10, that is 1ml of blood in 10ml of normal saline. The parasitized erythrocyte in volume of 0.3ml was used to infect each of the experimental mice intraperitoneally 6 days before treatment.

#### *Determination of baseline parasitaemia*

Six days after inoculation of parasite, blood was collected from the tail of each mouse in the various groups before administration of extracts. This was used to make thin and thick blood smear to determine the baseline parasitaemia.

#### *Preparation of thin and thick films*

Thin film: Both thin and thick blood films were made on the same slide about 2ml of fresh free slide and with the edge of another clean slide at an angle of about 45°. A smear was made these slides were stained with Giemsa for microscope examination and determination of percentage parasitaemia at each dose level was made by comparing the parasitaemia in infected but untreated control group 2 with those infected and treated groups.

Thick film: About 12µl of fresh blood was collected using a precious variable micropipette and with the cover of a clean slide, the blood was spread circularly to make an even spread. The blood was allowed to dry and prevented from insects and dust by drying under mosquito net.

#### *Staining Procedure*

The staining process was done using the method recorded by chearsbrough (2005). Only thin films was fixed with methanol and allowed to stand for about 10-25 seconds before staring, this was done to fix the blood film. 10% Giemsa solution was prepared by mixing 90ml of buffer solution at PH7.2 with 10ml Giemsa stock solution in 100ml cylinder. The mixture was powered into a staring dish both thin and thick films were placed in the dish and allowed for about 10minutes. The slides were flushed with tap water and air dried before observation was made under the microscope. A drop of immersion oil was drop or applied on the slides and examined under the microscope using x40 and x100 objectives.

#### *Calculation of Parasitaemia*

Percentage of parasitaemia was determined by counting the member of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Percentage parasitaemia and

average percentage parasitaemia were calculated according to the following formula as adopted by Ablishek et al (2010)

$$PP = \frac{\text{Total member of PRBC}}{\text{Total member RBC}} \times 100$$

Total member RBC

Where: PP= percentage parasitaemia

PRBC = parasitized Red Blood Cells

RBC = Red Blood Cells

#### *Determination of Percentage Average Suppression*

The percentage chemosuppression was determined using the method of Ebiloma et al (2012). It was calculated by subtracting the averaging percentage parasitaemia in the lest group from average percentage parasitaemia in control group 2 (infected untreated group). The value obtained was expressed as a percentage of the average percentage parasitaemia in the control group 2

$$As = \frac{APC - APT \times 100}{APC}$$

Where: As = Average suppression

APC = Average parasitaemia in control

APT = Average parasitaemia in test group

#### *Administration of the plant extracts to the mice in the different groups*

The reconstituted extracts of the leaves and stem of the plants were administered to the mice 6 days after inoculation with plasmodium bergheberghei. The extracts were administered orally with the help of feeding cannula. Group 4-6 were treated for 6 days with 100, 200 and 500mg doses of leaf extracts of *Desmodiumtortuosum*/kg body weight orally daily respectively; Three control groups were used. Control group 3 was inoculated with the parasite and treated with 10mg chloroquine/kg weight orally daily. Group 2 was infected with the parasite but was not treated with any extract. Group 1 was not infected with the parasite and was not treated with any extract but only fed on food and water.

#### *Sacrifice and Collection of Samples for Analysis*

The extracts were administered for 6 days and on the first day post administration, the mice were sacrificed and blood collected from each mouse in all the groups by cardiac puncture using sterile syringes and needles. The blood was collected into well labeled specimen bottles containing anticoagulant (EDTA) for analysis of hematological parameters. The blood for biochemical parameters was also collected into well labeled plain bottles without anticoagulant. Fresh blood was used to make thin and thick blood films for parasite count and determination of parasitaemia.



*Hematological Parameters*

The mice were sacrificed at the end of the 6<sup>th</sup> day experimental period and blood collected by cardiac puncture into well labeled specimen bottles containing anti-coagulant (EDTA). Changes in hematological and biochemical parameters of the blood samples owing to the application of the plant extracts were investigated. The following hematological parameters were estimated using an automated hematological analyzer: hemoglobin concentration (HB), packed cell volume (PCV), Red blood cell count (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin (MCHC), mean corpuscular volume (MCV), white blood cell count (WBC) and platelet count (PLT). The method adopted by the committee on enzymes of the Scandinavian society for clinical chemistry and clinical physiology (1974) was used.

*Biochemical Parameters*

The blood samples collected in well labeled heparin bottles for biochemical analysis were centrifuged at 3,00rpm for 10 minutes to recover the serum from the whole blood. The Sera were collected with sterile syringes equipped with needles and frozen in a refrigerator until used for biochemical analysis. The biochemical parameters to be tested included: Aspartate aminotransferase (AST), alanine aminotransferase (ALT). Alkaline phosphatase (ALP), total protein, urea and creatinine. For biochemical analysis, 0.5ml of blood was collected from the plain bottle with the help of a variable volume micropipette into centrifuge tube. The specimens were centrifuge at 2000rpm for 10 minutes. The serum settle on top of the blood and the serum was collected by means of sterile syringes and needles into plain tubes.

*Data Analysis*

All tests were performed at statistical significance of P<0.05 using statistical package for social sciences, SPSS version 23 and values were expressed as mean± SEM (standard error of mean) and comparisons made using ANOVA.

III. RESULT AND DISCUSSION

*Phytochemical Composition*

The qualitative phytochemical screening of the ethanolic extracts of leaf and stem of *Desmodiumtortuosum* were conducted indicating the following:

Table 1:Result of the qualitative phytochemical composition of leaf and stem of *desmodiumtortuosum*

S/N	Phytochemical	Leaf extract	REMARK	Stem extract	REMARK
1	Alkaloids		+++		++
2	Flavonoids		++		++
3	Tannin		++++		+++
4	Cyanogenic glycoside		++		+++
5	Saponin		+++		+++

6	Terpenoid		+++		++
7	Phenol		++++		++++
8	Steroid		+		+
9	Oxalate		+++		+++
10	Resin		+++		++

Key: ++ = Moderately present; + = slightly present; +++ = Highly positive; - = Absent

Table 2: Quantitative Phytochemical Composition of Ethanolic Leaf and stem Extracts of *desmodiumtortuosum*

S/ N	SAMPLE IDENTITY	ALKA LOID	TANNI N	CYNO GENIC GLYC OSIDE	FLAV ONOI D	SAPO NIN
1	D. tortuosum leaf ethanolic extract	2.57± 0.21	3.38± 0.15	1.71± 0.04	3.38± 0.13	8.43± 0.54
2	D. tortuosum stem ethanolic extract	2.09± 0.28	2.65± 0.10	2.16± 0.12	1.61± 0.12	8.73± 0.02

*Acute Toxicity Studies (LD<sub>50</sub>)*

Five doses of 1000, 2000, 3000, 4000 and 5000mg/kg body weight were administered and observed for activity after the first 30 minutes of administration. No mortality were observed after seven days. The same was applicable for the stem extracts and no mortality were observed also after seven days. The median lethal dose of the plant extracts in mice was estimated at about 3000mg/kg body weight (Table 3).

Table 3: The average percentage mortality of ethanolic extracts of the leaf and stem of *Desmodiumtortuosum* administered orally in mice during acute toxicity study

Group	Dose (mg/kg BW)	No. of mice in each group	No of Deaths in leaf extract	Mortality %	No. of mice in each group	No of Deaths in stem extract	Mortality %
1	NORMAL CONTROL	4	0	0	4	0	0
2	1000	4	0	0	4	0	0
3	2000	4	0	0	4	0	0
4	3000	4	0	0	4	0	0
5	4000	4	0	0	4	0	0
6	5000	4	0	0	4	0	0

### Evaluation Of Anti Malarial Potential

#### Suppressive activities of ethanolic extracts of *Desmodiumtortuosum*

The results showed a significant difference ( $p < 0.05$ ) in percentage parasitemia suppression for the treated groups relative to the non-treated group. Whereas the control group treated with standard drug (Chloroquine<sup>®</sup>) 10mg/kg dose showed a percentage suppression of 90.57%, the 100mg/kg doses of *Desmodiumtortuosum* leaf and stem had percentage suppression of 79.25% and 86.79% respectively. The plant showed a dose independent behaviour between the 200mg/kg and the 500mg/kg ethanolic leaf extracts (88.68%) and the 100mg/kg and 200mg/kg ethanolic stem extracts (86.79%) respectively (Table 4).

Table 4: Percentage suppression of *Desmodiumtortuosum*

S/N	GROUP/ TREATMENT	Av % parasitemia	Av % Inhibition
1	NORMAL CONTROL (NOT INFECTED)	0.00±0.00	100%
2	NEGATIVE CONTROL(INFECTED BUT NOT TREATED)	13.25±1.31	0.00%
3	POSITIVE CONTROL(CHLOROQUINE)	1.25±0.25	90.57%
4	100mg/kg leaf extract	2.75±0.85	79.25%
5	200mg/kg leaf extract	1.50±0.28	88.68%
6	500mg/kg leaf extract	1.50±0.28	88.68%
7	100mg/kg stem extract	1.75±0.47	86.79%
8	200mg/kg stem extract	1.75±0.47	86.79%
9	500mg/kg stem extract	1.50±0.28	88.68%

#### Curative activity of *Desmodiumtortuosum*

The result of the effect of the treatment with different concentrations of plant extracts on parasitemia density in mice is presented in Table 4.3b. The parasitemia density for the non-treated group progressively increased for the five days period, showing the mean number of the percentage parasitized red cells as 3.50±0.64 on the first day post inoculation and 13.25±1.31 by the fifth day. Treatment with the doses of the plant extracts (500mg/kg) showed a significant parasitemia reduction ( $p < 0.05$ ) when compared to the non-treated group. The reduction in parasitemia levels in the treated groups were not similar to that observed for the reference drug. The highest parasitemia reduction was seen in Chloroquine<sup>®</sup> followed by 500mg/kg ethanolic extracts of *Desmodiumtortuosum* of leaf and stem on the fifth day. The 10mg/kg dose Chloroquine<sup>®</sup> showed a significant reduction of parasitemia from 3.25±0.47 on the first day to 1.25±0.25 on the fifth day. The parasitemia density for the treated groups progressively decreased for the five days period, showing the mean number of the percentage parasitized red cells as 3.50±1.25 and 4.00±1.22 on the first day post inoculation and 1.50±0.288b and 1.50±0.28 for the 500mg/kg of

*Desmodiumtortuosum* leaf and stem respectively by the fifth day. The potency of the leaf and stem on the fifth day was observed to be almost the same. The plant generally showed dose independent behaviour (Table 5).

Table 5: Curative activity of ethanolic extracts of *Desmodiumtortuosum* of leaf and stem on *Plasmodium berghei*

S/N	GROUP	DAY 0	DAY 3	DAY 5
1	NORMAL CONTROL	0.00±0.00	0.00±0.00 <sup>beh</sup>	0.00±0.00 <sup>b</sup>
2	NEGATIVE CONTROL	3.50±0.64	7.50±1.89 <sup>acdfg</sup>	13.25±1.31 <sup>acdefghi</sup>
3	POSITIVE CONTROL	3.25±0.47	1.50±0.28 <sup>beh</sup>	1.25±0.25 <sup>b</sup>
4	100mg/kg leaf extract	4.00±1.35 <sup>a</sup>	3.25±0.47 <sup>b</sup>	2.75±0.85 <sup>b</sup>
5	200mg/kg leaf extract	3.75±0.75 <sup>a</sup>	6.75±0.47 <sup>acef</sup>	1.50±0.28 <sup>b</sup>
6	500mg/kg leaf extract	3.50±1.25	2.00±0.57 <sup>beh</sup>	1.50±0.288 <sup>b</sup>
7	100mg/kg stem extract	3.00±0.70	2.25±0.25 <sup>beh</sup>	1.75±0.47 <sup>b</sup>
8	200mg/kg stem extract	4.25±0.75 <sup>a</sup>	6.25±1.10 <sup>acfg</sup>	1.75±0.47 <sup>b</sup>
9	500mg/kg stem extract	4.00±1.22 <sup>a</sup>	3.75±0.62	1.50±0.28 <sup>b</sup>

Data are Mean± SEM of four determinations. Values found in a column and bearing common superscript letters a,b,c,d,e,f,g,h,i, are significantly different ( $p < 0.05$ ) when compared to the normal control, negative control, positive control, 100mg/kg, 200mg/kg, 500mg/kg leaf extract and 100mg/kg, 200mg/kg and 300mg/kg stem extract respectively. Each alphabet denoting each group from group 1-9

#### Effect of Ethanolic Extracts of *Desmodiumtortuosum* of leaf and stem on some hematological Parameters

The result represented indicated a general reduction in RBC in the treated group (ranging values of 3.00- 4.73) as compared to the normal control (uninfected group) which showed a value of 5.15±0.15. The untreated group (negative control) also had a reduction in RBC 2.76±0.17 having the least value of RBC. On a more general note, the total white blood count and absolute lymphocytes were significantly higher in cases of the infected groups compared to the uninfected normal control ( $p < 0.05$ ) group. The results show a higher number of leukocytes, predominantly of the lymphocyte cells, being a major feature in the malaria infected mice these are probably typical findings in malaria infection. However, the PCV, Hb, absolute neutrophils and RBC were significantly lower in the malaria infected groups than in the uninfected control ( $p < 0.05$ ) group. A lower PCV in the malaria infected patients may reflect anaemia which is often mainly due to mechanical destruction of parasitized red cells as well as splenic clearance of parasitized and defected erythrocytes. Also it is most probably that relative neutropenic leukocytopenia develops subsequently in malaria infected mice with a relative increase in lymphocyte cells as reflected by the significantly lower neutrophils and higher

lymphocytes in the infected mice. The ethanolic stem extracts of *Desmodiumtortuosum* showed more potency as compared

to the ethanolic leaf extracts (Table 6).

Table 6: Effect of Ethanolic Extracts of *Desmodiumtortuosum* of leaf and stem on some hematological Parameters

S/N	GROUP	RBC	WBC	PCV	Hb	NEU	LYMP
1	NORMAL CONTROL	5.15±0.15 <sup>bcddefgh</sup>	6.32±0.18 <sup>bdeg</sup>	47.25±1.31 <sup>bcddefg</sup>	14.77±0.20 <sup>bcddefgh</sup>	75.50±1.19 <sup>bde</sup>	16.00±1.47 <sup>bdefg</sup>
2	NEGATIVE CONTROL	2.76±0.17 <sup>acefghi</sup>	13.23±0.31 <sup>acefghi</sup>	24.00±1.52 <sup>acefg</sup>	8.23±0.54 <sup>acefghi</sup>	60.66±1.76 <sup>acefghi</sup>	33.66±1.45 <sup>achi</sup>
3	POSITIVE CONTROL	4.53±0.14 <sup>abdeg</sup>	6.66±0.26 <sup>bdeg</sup>	40.33±2.02 <sup>abdeg</sup>	12.83±0.50 <sup>abdeg</sup>	73.33±0.88 <sup>bd</sup>	19.33±1.20 <sup>bc</sup>
4	100mg/kg leaf extract	3.10±0.05 <sup>achfi</sup>	12.46±0.43 <sup>acefghi</sup>	27.00±0.57 <sup>achfi</sup>	8.93±0.20 <sup>acefghi</sup>	63.00±1.73 <sup>achi</sup>	32.33±1.76 <sup>achi</sup>
5	200mg/kg leaf extract	3.70±0.11 <sup>abci</sup>	9.90±0.51 <sup>abcdfhi</sup>	32.33±0.88 <sup>abci</sup>	10.76±0.08 <sup>abcdfhi</sup>	67.66±1.76 <sup>ab</sup>	27.66±2.84 <sup>a</sup>
6	500mg/kg leaf extract	4.23±0.08 <sup>abd</sup>	7.33±0.26 <sup>bdeg</sup>	37.66±1.20 <sup>abd</sup>	13.33±0.14 <sup>abdeg</sup>	69.66±1.20 <sup>b</sup>	27.33±2.96 <sup>a</sup>
7	100mg/kg stem extract	3.70±0.10 <sup>abci</sup>	9.10±0.30 <sup>abcdfghi</sup>	32.00±1.00 <sup>abci</sup>	10.90±0.30 <sup>abcdfi</sup>	70.00±1.00 <sup>b</sup>	27.00±3.00 <sup>a</sup>
8	200mg/kg stem extract	4.20±0.10 <sup>abd</sup>	6.95±0.25 <sup>bdeg</sup>	37.00±1.00 <sup>abd</sup>	12.60±0.30 <sup>abde</sup>	72.50±0.50 <sup>bd</sup>	20.50±0.50 <sup>bd</sup>
9	500mg/kg stem extract	4.73±0.08 <sup>bdeg</sup>	6.06±0.14 <sup>bdeg</sup>	42.00±1.10 <sup>bdeg</sup>	14.03±0.14 <sup>bdeg</sup>	72.33±1.20 <sup>bd</sup>	19.00±1.73 <sup>bd</sup>

Data are Mean± SEM of four determinations. Values found in a column and bearing common superscript letters a,b,c,d,e,f,g,h,i, are significantly different (p<0.05) when compared to the normal control, negative control, positive control, 100mg/kg, 200mg/kg, 500mg/kg leaf extract and 100mg/kg, 200mg/kg and 300mg/kg stem extract respectively. Each alphabet denoting each group from group 1-9

*Effect of Ethanolic Extracts of Desmodiumtortuosum of leaf and stem on some Biochemical Parameters*

The ethanolic leaf and stem extracts of *Desmodiumtortuosum* in. Showed a significant decrease in the enzymes ALT, AST and ALP in plasma of groups treated with varied concentrations of the ethanolic extracts of both the leaf and the stem compared with the negative control group. In Table 4.5, the Total protein concentrationiceions were shown to be 71.75±1.93 and 54.33±2.72 for the normal and non-treated group respectively. Comparing the T.P concentrations for the normal control to the non-treated group showed a statistically significant (p<0.05) decrease. The T.P concentrations for the treated groups show no linearity with the normal control as well as the non-treated group especially for the ethanolic stem extracts. Though, a trend of progressive increase in the value of total protein can be deduced as concentrations of extracts increased. Total Bilirubin concentrations were shown to be 8.25±0.85 and 29.66±1.20 for the normal and negative control respectively. Comparing the T.Bil concentrations for the

normal control to the negative control showed a statistically significant (p<0.05) increase. The T.Bil concentrations for the treated groups were significantly (p<0.05) lowered when compared to the negative control. The concentrations of T.Bil for the treated groups showed a progressive decrease with increase in the doses of each category of plant extracts administered. The Urea concentrations were shown to be 2.50±0.18 and 5.03±0.31 for the normal and non-treated group respectively. Comparing the Urea concentrations for the normal control to the non-treated group showed a statistically significant (p<0.05) increase. The Urea concentrations for the treated groups were significantly (p<0.05) lowered when compared to the non-treated group. The treated groups also responded to dose administration evident by the progressive trend of decrease observed in each plant extracts. The Creatinin concentrations were shown to be 55.00±1.58 and 77.00±2.64 for the normal and non-treated group respectively. Comparing the Creatinin concentrations for the normal control to the non-treated group showed a statistically significant (p<0.05) increase. The Creatinin concentrations for the treated groups were significantly (p<0.05) lowered when ethanolic leaf extract were administered giving values of 71.74±1.45, 68.00±1.15 and 63.33±1.85 compared to the ethanolic stem extract were an increased trend was observed 59.50±1.50, 62.00±6.00 and 63.00±0.57 for 100mg/kg, 200mg/kg and 500mg/kg ethanolic leaf and stem respectively (Table 7).

Table 7: Effect of Ethanolic Extracts of *Desmodiumtortuosum* of leaf and stem on some Biochemical Parameters

S/N	GROUP	AST	ALT	ALP	T.P	T.B	UREA	CREA
1	NORMAL CONTROL	9.50±0.86 <sup>bdefg</sup>	11.00±0.70 <sup>bdefgh</sup>	117.50±2.21 <sup>bdefg</sup>	71.75±1.93 <sup>bdh</sup>	8.25±0.85 <sup>adg</sup>	2.50±0.18 <sup>bde</sup>	55.00±1.58 <sup>bde</sup>
2	NEGATIVE CONTROL	37.66±3.17 <sup>acdefghi</sup>	28.66±0.89 <sup>acdefghi</sup>	177.00±3.46 <sup>acdefghi</sup>	54.33±2.72 <sup>bfi</sup>	29.66±1.20 <sup>acdefghi</sup>	5.03±0.31 <sup>acefghi</sup>	77.00±2.64 <sup>acefghi</sup>
3	POSITIVE CONTROL	12.33±0.88 <sup>bdeg</sup>	13.67±0.67 <sup>bdg</sup>	128.00±2.51 <sup>bde</sup>	62.00±2.08	13.00±1.52 <sup>b</sup>	3.13±0.23 <sup>bd</sup>	64.67±2.60 <sup>b</sup>

4	100mg/kg leaf extract	25.66±1.45 <sup>abcghi</sup>	20.33±0.88 <sup>abc</sup>	156.00±4.61 <sup>abcghi</sup>	60.00±1.15 <sup>a</sup>	18.67±2.60 <sup>abfi</sup>	4.37±0.23 <sup>aci</sup>	71.74±1.45 <sup>eg</sup>
5	200mg/kg leaf extract	21.00±1.52 <sup>abc</sup>	17.67±0.67 <sup>abi</sup>	150.00±1.73 <sup>abcghi</sup>	64.66±1.20	12.67±1.20 <sup>b</sup>	3.86±0.14 <sup>ab</sup>	68.00±1.15 <sup>a</sup>
6	500mg/kg leaf extract	17.00±1.15 <sup>abd</sup>	16.67±0.88 <sup>abi</sup>	132.33±2.02 <sup>abdei</sup>	66.67±2.33 <sup>b</sup>	11.00±0.57 <sup>bd</sup>	3.40±0.20 <sup>b</sup>	63.33±1.85 <sup>b</sup>
7	100mg/kg stem extract	21.50±0.50 <sup>abc</sup>	21.00±1.00 <sup>abc</sup>	142.00±1.00 <sup>abhi</sup>	61.00±3.00	18.00±0.00 <sup>abi</sup>	3.35±0.25 <sup>b</sup>	59.50±1.50 <sup>bd</sup>
8	200mg/kg stem extract	16.50±1.50 <sup>bd</sup>	16.00±1.00 <sup>abg</sup>	121.50±1.50 <sup>bddeg</sup>	55.00±2.00 <sup>ai</sup>	13.00±1.00 <sup>b</sup>	3.20±0.20 <sup>b</sup>	62.00±6.00 <sup>b</sup>
9	500mg/kg stem extract	12.66±1.20 <sup>bddeg</sup>	11.67±0.88 <sup>bddeg</sup>	115.00±1.15 <sup>bddeg</sup>	67.33±2.72 <sup>bi</sup>	10.33±0.64 <sup>bdg</sup>	3.00±0.10 <sup>bd</sup>	63.00±0.57 <sup>b</sup>

Data are Mean± SEM of four determinations. Values found in a column and bearing common superscript letters a,b,c,d,e,f,g,h,i, are significantly different ( $p<0.05$ ) when compared to the normal control, negative control, positive control, 100mg/kg, 200mg/kg, 500mg/kg leaf extract and 100mg/kg, 200mg/kg and 300mg/kg stem extract respectively. Each alphabet denoting each group from group 1-9

#### Discussion

Ethnomedicinal plants hold a great promise in antimalarial therapy as a result of their phytochemical composition. Pharmaceutical drugs designed for the treatment of malaria elicit specific actions; this grossly limits their ability to combat the emergence of resistance especially in monotherapy. Herbal products on the other hand elicit a complementary or synergistic action which is due to the various secondary metabolites found in the plant materials. (Treasure, 2000). The results presented in showed that the secondary metabolites present in the plant consists of alkaloids, flavonoids, tannin, cynogenic glycosides and saponins. According to Ojewole (2015), the pharmacological relevance of ethnomedicinal plants is due to their phytochemical composition. As such the active ingredients present in the plant under study is highly implicated in its pharmacological property. For instance there has been reports of the anti-plasmodial property of alkaloids (Tylore *et al.*, 2011) (Tables 4.1a and 4.1b).

The result of the acute toxicity studies conducted showed that the plant leaf and stem ethanolic extracts did not cause mortality when administered orally at concentrations of 5000mg/kg, 3000mg/kg, 2000mg/kg and 1000mg/kg body weight. The LD<sub>50</sub> (median lethal dose) was estimated at 3000mg. This means that the plant extract could be used within these ranges of concentrations without causing hepatic damage or being cytotoxic. Going by the statement made by Hodge and Sterner (2005) and Ghosh (1984), at this concentration, the plant extract could be considered relatively safe.

The treatment with the plant extracts showed a lowering of the parasitemia density in all extract treated group. It also showed that there was a significant decrease ( $p<0.05$ ) when the plant

extracts was given to the inoculated mice, for all three doses of 500mg/kg, 200mg/kg and 100mg/kg body weight for both leaf and stem respectively. Its use in the traditional treatment of malaria has been reported although the scientific basis for this has not been established. The results of the present study demonstrated that *Desmodium tortuosum* ethanolic leaf and stem extracts showed significant antimalarial activity against *Plasmodium berghei in vivo*; this lends credence to the claims of its antimalarial property. The highest anti-plasmodial activity was observed with the concentrations of 500mg/kg. This showed a parasite growth inhibition of 88.68% for both the leaf and the stem. This suggests that the plant's antimalarial property can be mimicked as being modern medicine. This finding is in line with the research finding of Deharo *et al.*, (2001) which states that *in vivo* anti-malarial activity of plant extracts can be categorized as modern medicine, good and very good if the extract showed 50% or more chemosuppression at 500, 250 and 100mg/kg /day extract dose respectively. Flavonoids have been detected in the *Artemisia* species and have been reported to show significant anti-malarial activity against *P.falciparum* (Chanphenet *et al.*, 1998). These set of compounds ( alkaloids and flavonoids ) were identified in the plant, hence the allusion that the presence of these secondary metabolites could be the reason for the plant's therapeutic action.

Haematological parameters as an investigating tool for cases of early malaria infections may help to detect early complications associated with serious malaria infection so as to help in the care for the patients and prevent death that may result from such complications. The haematological parameter changes in malaria infected blood sample have been reported (Kakomaet *et al.*, 1992). They reported that the infected patients tended to have significantly lower PCV, haemoglobin, and red blood cell counts, which is in agreement with the present study where the RBC counts in malaria infected blood were significantly lower than that of non-infected mice. The PCV level were also noted to be significantly lower in the untreated controls ( $P<0.05$ ). The result in this study, which indicates significant increase in the white blood cell count of the malaria infected mice ( $p<0.05$ ) when compared to the non-inoculated control, probably is as a result of an increase in the release of leukocytes at the early stage of the infection, to



content and fight against the infection. Increase in WBC count in malaria mice in this study corroborates to that reported (Fisher *et al.*, 1970). The mean values of haematological parameters were examined between the malarial infected mice. Significantly lower PCV conforms to the report of (Ekvall H., 2003) which showed parasitaemia and haematological alterations in malaria. The level of neutrophils in infected mice were found to be significantly lower than that in the normal control ( $P < 0.05$ ), which is in line with the report of (Ekvall H., 2003). The alteration in counts including relative lymphocytosis and decrease in packed cell volume were observed in this study just as reported by (Kakoma *et al.*, 1992). Haematological abnormalities are considered a hallmark of malaria and are reported to be most pronounced in *P. falciparum* infections. This study showed that *P. berghei* malaria infections can lead to significant changes of various blood cell parameters similar to infections with *P. falciparum*.

In general, the values of AST, ALT, ALP, Urea and Creatinin were increased due to inoculation with the *P. berghei* parasite, but are seen restoring back to normal as it was seen in the non-inoculated group (normal control). Leaves and stem of *Desmodium tortuosum* showed no physiological effects when used as anti-malaria drug. Hence *Desmodium tortuosum* stem and leaf presents some degree of hepato-protective, cardio-protective therapeutic effects.

#### IV. CONCLUSIONS

Based on these findings, it is clear to us that the oral administration of *Desmodium tortuosum* leaf and stem ethanolic extracts (100–500 mg/kg) to mice for 4 days significantly reduced parasitemia of *P. berghei* in experimental mice with non-toxicity. The implication of this finding is that *Desmodium tortuosum* leaf and stem ethanolic extracts possess potent antimalarial effect and may therefore serve as potential sources of safe, effective, and affordable antimalarial drugs. The displayed high *in vivo* antimalarial property and lack of toxic effect render *Desmodium tortuosum* leaf and stem a candidate for future isolation of compounds which could develop into new lead structures and candidates for drug development programs against human malaria. *Desmodium tortuosum* leaf and stem are generally non-toxic and have cardiac, hepato and renal protective abilities when consumed within tolerable physiological range.

#### V. CONTRIBUTION TO KNOWLEDGE

The outcome of the study is expected to contribute to the knowledge of pharmacology. The availability of natural products like medicinal plants will greatly help to solve the healthcare problems of rural communities.

- The plant extracts showed a moderate antimalarial property.
- Looking at the dose administered and its effect on the enzyme markers, it can be deduced that *Desmodium tortuosum* leaf and stem has healing effect based on the dose range administered

#### VI. RECOMMENDATIONS

On the basis of the findings of this research, the following are recommended:

1. Further work should be done to ascertain the use of the plant in preventive malarial therapy.
2. The plants should be used in combination with other plants with known antimalarial activity to understand how this synergy can boost the antimalarial property of these plants.
3. Other models for investigating antioxidant properties should be adopted to further establish the plant's antioxidant property and possibly elucidate its mechanism of action.
4. Attempts should be made to isolate the active substance responsible for specific therapeutic action.
5. Further studies should also be conducted on the root of the said plant in order to ascertain its anti-plasmodial effect.

#### ACKNOWLEDGEMENT

I wish to acknowledge my Dr. R. B. Bob-Manuel for her continual motherly encouragement and my Post graduate students in the Department and unit of Biology and Parasitology respectively. Not forgetting my family was stood tall with me and copy in my absents.

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