Original Research Article

EFFECTS OF POWDERED STEM BARK OF *Terminalia avicennioides* MADE AS DIETARY FEED FED TO MICE INFECTED WITH *Plasmodium berghei*, ON LIVER FUNCTION

----------ABSTRACT----------

Aim: The aim of this study was to investigate the antiplasmodial activity and effect of stem bark of T*erminalia avicennioides* made as dietary feed fed to mice infected with *plasmodium berghei*, on some serum biochemistry.

Methodology Twenty (20) mice were divided into four groups. Group 1 was not infected with *Plasmodium berghei* (normal control), Group 2 was infected with *P. berghei* but not treated (negative control). Group 3 was infected and treated with 5.0 mg/kg of Arthemeter-Lumefantrine (positive control). Groups 4 was infected and fed with treated feed (*T. avicennioides*). Treatments were carried out for five days. Blood was taken daily from the tail of the mice before treatment for the assessment of parasitaemia. The animals were sacrificed on the fifth day and the whole blood was collected into EDTA bottle. Serum obtained was used to assay for biochemical parameters.

Results: Parasitaemia count was significantly lower ($p<0.05$) in all the treated groups when compared with the negative control group. The high density lipoprotein was significantly higher ($P<0.05$) in the normal control (123.14±3.19) when compared with the positive control (99.18±2.76), negative control (85.29±0.85) and the group treated with *T avicennioides* (86.14±3.21). The serum Alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase level in group treated with *T. avicennioides* (167.90±4.13, 15.87±1.32 and 17.50±1.95) respectively were significantly reduced ($p<0.05$) when compared with the negative control (197.25±5.44, 20.01±1.32 and 26.71±0.45) respectively. The mean bilirubin and albumin level in the negative control showed no significant difference when compared with the group fed with *T. avicennioides*.

Conclusion: The study concluded that *T. avicennioides* has antiplasmodial activity with mild adverse effect on liver function.

KEYWORDS*: Antiplasmodial, Terminalia avicennioides, arthemeter-lumefantrine, bilirubin, albumin.*

I. INTRODUCTION

Malaria is one of the most prevalent and deadliest protozoan tropical diseases. Although over a century of effort has been made to combat this disease, malaria eradication remains a global burden, with millions of clinical cases

reported worldwide each year and over three billion people living under its threat [1]. Five human *Plasmodium* species (*Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. knowlesi*, and *P. malariae*) are known to cause malaria infection [2]. The severe form of this disease is usually caused by *P. falciparum* [3]. This particular species *(P. falciparum*) is the most pathogenic of the five species of malaria parasites that infect man, *P. falciparum* is the major cause of almost every malaria mortality and morbidity in tropical and subtropical regions [4].

The most severe form of malaria infection is caused by *P. falciparum*; with variable clinical features which include; fever, headache, chills, vomiting, muscular aching and weakness, cough, diarrhoea and abdominal pain [5]. Other symptoms related to organ dysfunction may supervene, such as acute renal failure (ARF), generalized convulsions, pulmonary oedema, circulatory collapse, followed by coma and death [5].

The incessant development of malaria resistance to most of the widely available, affordable and first-line treatments are the greatest challenge [6,7]. The overall control of the mosquitoes which serve as malaria vector is also made difficult by their ceaseless resistance to a wide range of insecticides (Snow, 1996). Moreover, lack of access to indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) [8] and widespread production of fake antimalarial drugs contributes to the major problems that plague the management and control of malaria in Africa [8].

WHO made artemisinin combination therapy (ACT) drugs as the first line of treatment in all malaria endemic areas, there is still report of the resistance of malaria parasite to all malarial drugs including artemisinin in malaria-endemic areas, this has contributed to morbidity and mortality caused by malarial parasite [9]. The insurgence of drug resistance, scarcity and high cost of original convectional drug has led most people in malaria endemic areas to the use of medicinal herbs for the treatment of malaria [10].

Herbal medicine has shown to have genuine efficacy and about 75% of rural populations depend on it as first-line treatment [11]. The World Health Organization advocated for countries, especially those in malaria-endemic areas to study efficacy of traditional medicine in their area with the view of finding and exploiting plants that provide safe and effective remedies for ailments caused of both pathogens and microbial agents and non-microbial original [12].

Natural products are the direct or indirect sources of most of the drugs introduced in the past 30 years. Natural products from plants are a rich source of principal compounds for the development of new drugs against protozoan diseases such as malaria [13]. Some plants have been known to have relatively good antimalarial activity. *Vernonia amygdalina* [14], *Momordica balsamina* [15], *Azadirachta indica* [16], *Astonia boonei* [17], *Terminalia avicennioides* [12] and *Anogeissus leiocarpus* [18]. The antimalarial effect of *T. avicennioides* is comparable to that of Artesunate. *T. avicennioides* family combretaceae, have been reported to possess anti-plasmodial activity [12, 19]. *Terminalia avicennioides* of the family Combretaceae is a yellowish-brown, durable and hardwood, commonly found in the savannah region of West Africa [20]. Various extract of *Terminalia avicennioides* are used to treat ailments such as helminthiasis [21] and gastric and peptic ulcers [22]. It also has significant antimicrobial activities against *Staphylococcus aureus* [20], malaria parasite [23], *Salmonella typhi*, *Salmonella paratyphi*, and *Vibrio cholera* [24]. Akanbi, [12] in his research, discovered that the methanolic bark extract of *T. avicennioides* has antimalarial effect which is a clear indication of high antiplasmodial activity. Therefore, this study investigates the effects of powdered stem bark of *Terminalia avicennioides* made as dietary feed fed to mice infected with *plasmodium berghei* as a possible edge to overcome the damages done by the malaria parasite to humanity

II. MATERIALS AND METHODS

Experimental Animal

Adult Swiss albino mice used for this study were obtained from the Animal unit at Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria. The animals were kept in well aerated wired cages, fed with standard mouse feed (top feed) and were allowed to drink water freely. The animals were kept for two weeks to be acclimatized with the new immediate environment before they were infected with the malaria parasite.

Parasite Acquisition

Plasmodium berghei parasite used for this study was donated by the laboratory by Professor Ademowo, O. G. in the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University Teaching Hospital, University of Ibadan, Oyo state, Nigeria. The parasites were maintained in the experimental animals by serial passage of blood collected from a patent donor mouse to a naive recipient.

Plant Materials

The stem bark of *T. avicennioides* Tree (locally called Udi) was collected in Akungba-Akoko, Ondo State, Nigeria, and were identified by Dr. A. O. Obembe, from Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo state, Nigeria. The Herbarium specimen with voucher number UIH22319. *T. avicennioides* was deposited at the Herbarium unit of the University of Ibadan, Ibadan, Nigeria.

Feed Formulation

The stem bark was air dried extensively and milled into powder form. The standard commercial mouse feed was obtained from a retail outlet in Akungba-Akoko, Ondo state, Nigeria. The milled stem bark of *T avicennioides* was then sieved to further extract fine powder. Thirty percent (30%) of the powder was added to seventy percent (70%) standard commercial mice feed. 100cl of Water and 45cl of honey was added to the combination and mixed thoroughly for 15-25 minutes with the hands to enable homogenization of the ingredients. The prepared paste was then pelleted manually into size 2mm. The pelleted feed was dried at room temperature. The Swiss albino mice were fed with the treatment alongside with portable water, twice daily; after blood collection in the morning and later in the evening.

Parasitolocal Study

Thick blood film was prepared from blood collected from the tail vein of each mouse daily for five days, and slides were screened for malaria parasite using Giemsa stain. The number of parasite counted per 200 white blood cells was recorded and used to calculate parasite density on the basis of 8000 assumed leucocytes/µl of blood.

In-vivo Antimalarial Assay

Mice weighing between 15g-22 g were distributed into four groups (G1, G2, G3, and G4). Each group comprised of five mice. Mice in the groups 1, 2, and 3 were infected intraperitoneally with an aliquot of 0.2 ml of standard

inoculums ($1x10^7$ *Plasmodium berghei* strain NK 65 parasitized erythrocytes). Group 1 was infected and treated with formulated feed treatment. Group 2 was infected and not treated (negative control). Group 3 was infected and were treated with standard drug (Arthemeter-Lumefantrine). This served as positive control. Group 4 was not infected and fed with standard mice feed. This served as normal control. All the treatments were administered twice daily through feed for five consecutive days apart from group 3 which was once daily. Blood was taken daily from the tail vein of the mice, before treatment for the assessment of parasitaemia later in the day. Serum samples were kept in the deep freezer at -20°C until analysis was done.

Biochemical Assays

Serum obtained from mice were used to assay for the liver function using the spectrophotometric method with Randox test kits. Serum bilirubin concentration was determined by dimethyl sulphoxide principle [25]. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels were measured by the pyruvate and oxaloacetate methods, respectively [26] and serum albumin concentration was measured as given earlier [27]. Serum HDL-cholesterol concentration was measured by the NIHCDS [28] method, as described in the manual of the Randox HDL-cholesterol kit

Statistical Analysis

The differences among groups were analyzed by the one-way Analysis of Variance (ANOVA). The SPSS 20.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. The results were expressed as mean ± Standard Deviation (SD). The level of significance was estimated at $P<0.05$.

Results

The effect of the treatment of *Plasmodium berghei* infected mice fed with *T. avicennioides* mixed feed on the parasite density is shown in Table 1. The level of inhibition on the parasite count was determined by comparing the changes in parasite count in day 0 to those the subsequent post treatment days. There was an increase in the parasite density in the negative control group on day 4 and 5 (704±45.61) and (792±52.15) respectively when compared with day 0 (528±110.10). Among the treated groups, there was a significant reduction ($P<0.05$) in the parasite density when compared to the negative group. A significant decrease ($p<0.05$) was recorded in the group fed with *T. avicennioides* in day 5 (124±86.72) when compared with the initial parasite load (560±46.62) in day 0.

Table 1: Effect of treatment of mice infected with *P. berghei* with the stem bark of *T avicennioides* on parasite density.

Source of variance	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Artemether-Lumefantrine	$528±99.59^{d}$	$464±60.63^{d}$	$376±53.66^{d}$	$272±33.46^{c}$	$168±33.46^{b}$	$80±40.00^{a}$
T. avicennioides	$560±46.62^{d}$	$492±67.3^{c}$	$402±79.60^{bc}$	$344±93.81^{bc}$	$282±74.83^{ab}$	$124±86.72^{a}$
Negative Control	$528±110.10^{a}$	$568±91.21^{ab}$	$592±76.94^{ab}$	$640±56.57^{b}$	$704±45.61^{bc}$	$792±52.15^{d}$

*Mean values with the same superscript along the same column are not significantly different while means with different superscript are significantly different at $P<0.05$.

The effects of the treatment on high density lipoprotein (HDL) in mice infected with *Plasmodium berghei* are illustrated Figure 1. The HDL level was significantly higher (P<0.05) in the normal control (123.14±3.19) when compared with the positive control group (99.18±2.76), negative control group (85.29±0.85) and the group treated with *T. avicennioides* (86.14±3.21). The mean level of HDL was not significantly different (P>0.05) in negative control group (85.29±0.85) when compared with the group treated with *T. avicennioides* (86.14±3.21).

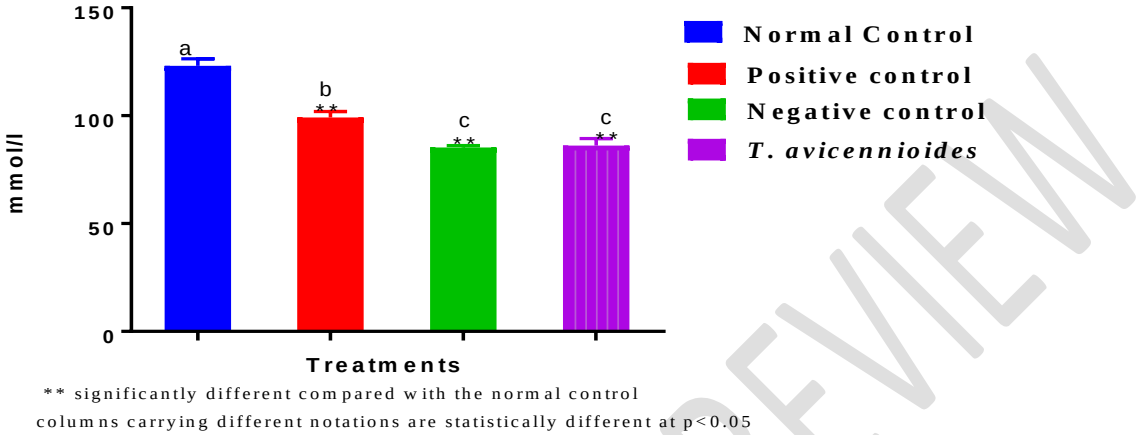

Figure 1: Effects of powdered stem bark of *T. avicennioides* fed to mice infected with *P. berghei* on serum high density lipoprotein level.

The effects of the treatment of the mice infected with *P. berghei* with *T. avicennioides* on serum alanine aminotransferase (ALT) are indicated in Figure 2. The mean serum ALT level in normal control (114.61±7.09) was significantly lower (P<0.05) when compared with mean value of positive control group (157.25±10.19). The negative control group (199.33±5.44) was significantly higher (P<0.05) when compared with the group fed with *T. avicennioides* (167.90±4.13).

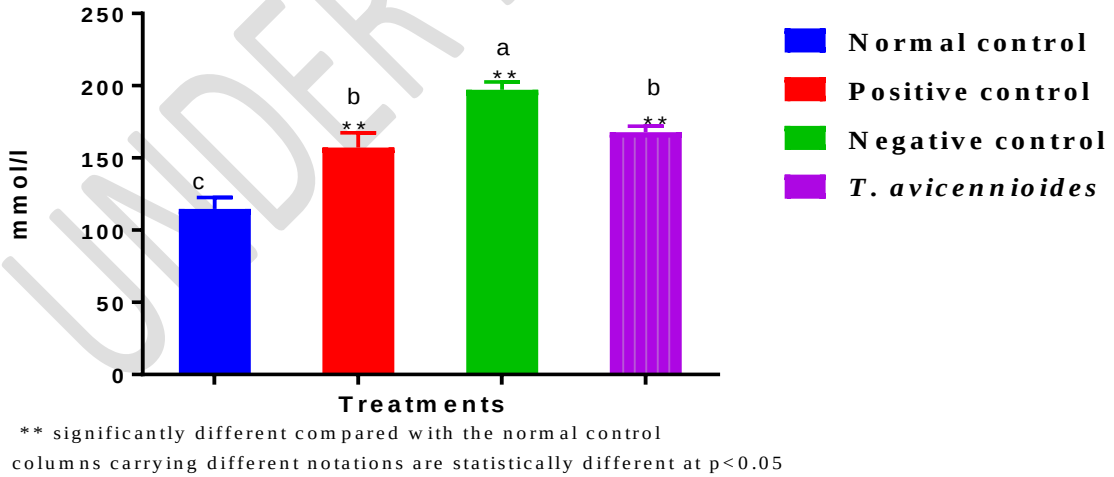

Figure 2: Effect of powdered stem bark of *T. avicennioides* fed to mice infected with *P. berghei* on serum Alanine aminotransferase

The effects of the treatment of the mice infected with *P. berghei* fed with *T. avicennioides* on serum alkaline phosphatase (ALP) are indicated in Figure 3. There was significant reduction (P<0.05) in ALP level in normal

control (6.44±0.92) when compared with mean serum level in mice fed with *T. avicennioides* (15.86±1.32), positive control (14.49±2.07) and negative control (20.01±1.32). The mean serum ALP in the group fed with *T. avicennioides* (15.86±1.32) was significantly reduced (P<0.05) when compared with the negative control group (20.01±1.32).

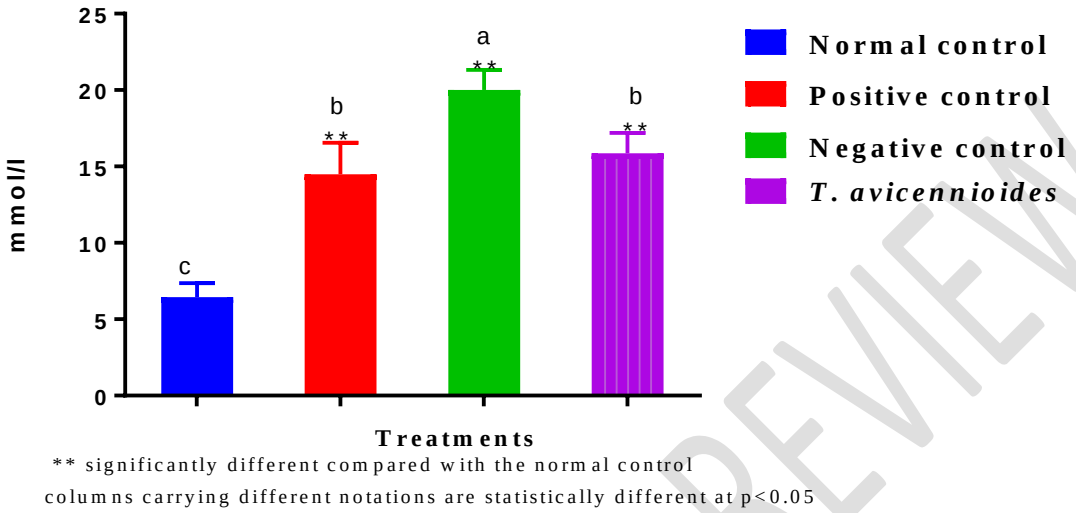

Figure 3: Effect of powdered stem bark of *T. avicennioides* fed to mice infected with *P. berghei* on serum alkaline phosphatase.

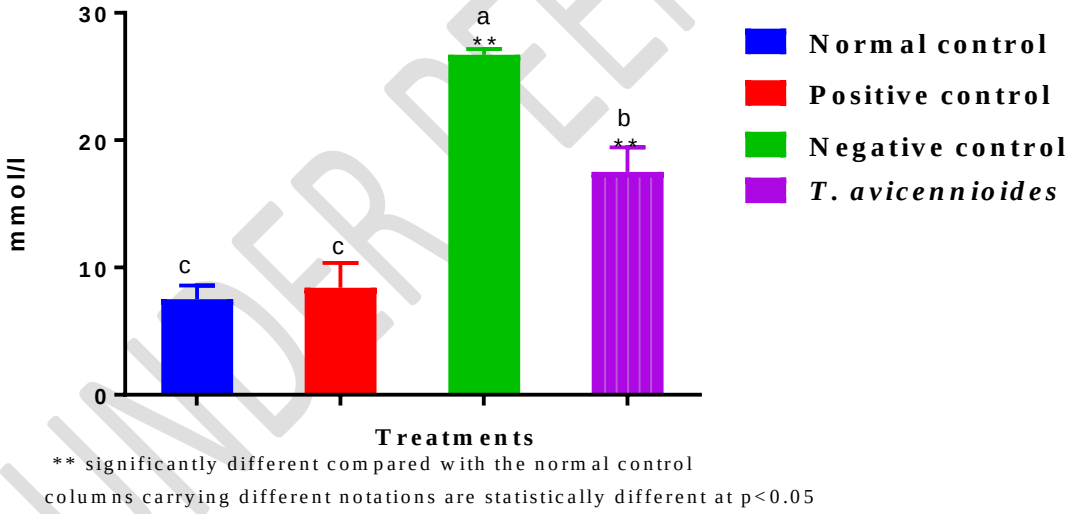

Figure 4: Effect of powdered stem bark of *T. avicennioides* fed to mice infected with *P. berghei* on serum Aspartate Aminotransferase

Figure 4 showed that the mean serum AST level was significantly higher in negative control group (26.71±1.95) when compared with the other groups. There was no significant different in positive control group (8.42±1.10) when compared with the normal control group in normal control l (7.50±0.45).

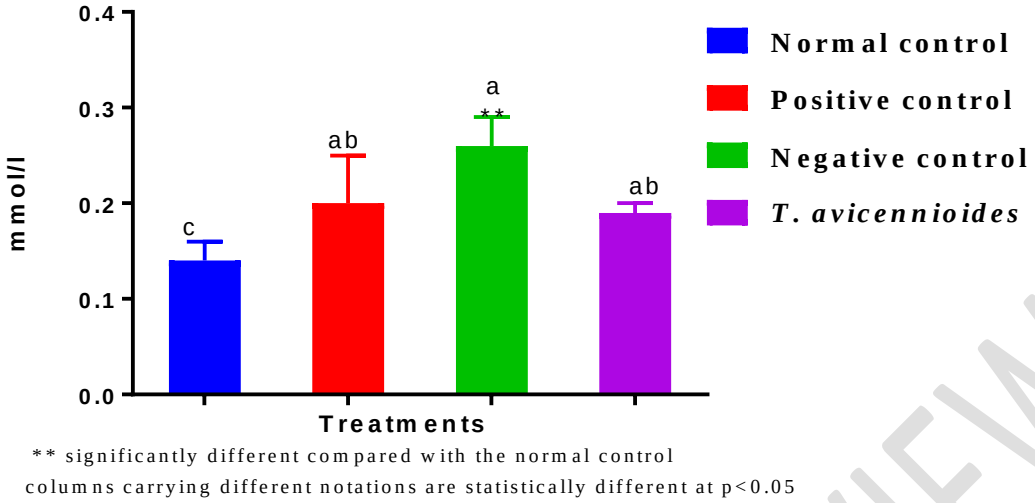

Figure 5: Effect of powdered stem bark of *T. avicennioides* fed to mice infected with *P. berghei* on serum Bilirubin.

Figure 5 showed that the mean bilirubin level in the negative control (0.26±0.03) showed significant difference when compared with the normal control group (0.14±0.02) and group fed with *T. avicennioides* (0.19±0.01).

IV. DISCUSSION

Malaria infection has been a serious problem to those that live in malaria endemic areas. Several factors have been reported to be responsible for this which includes the development of drug resistance to the potent and affordable drugs by the parasite [29]. Many cultures have replaced conventional medicine with traditional herbal remedy and presently traditional herbal medicine has become a comprehensive system of health care delivery in some community including Nigeria [30]

Treatment of *P. berghei*-infected mice with Artemether-Lumefantrine and *T. avicennioides* significantly reduced the parasite density of infected mice after two days and further obvious reduction occurred subsequently. This study showed that there was significant decrease in parasitaemia from Day 2 to Day 5 in the treated groups, while there was increase in parasitaemia from Day 2 to Day 5 when compared with Day 0 in the negative control. In the positive control, there was significant reduction of parasite from Day 2 to day 4. In day 5 the parasite inhibition rate in the positive control compared with *T. avicennioides* may be as a result the mice fed with *T. avicennioides* were allowed feed at free will while Artemether-Lumefantrine was administered orally to the positive control group. The reduction in parasitemia in *T. avicennioides* treated group showed that the plant has antiplasmodial effects. Akanbi [12] reported similar case in *in vivo* study of antiplasmodial activity of *T. avicennioides* and Its effect on lipid profile and oxidative stress in mice infected with *P. berghei.*

This work also investigated the effect of *T. avicennioides* on the lipid profile level in mice infected with *P. berghei.* The study showed that there was an increase in the HDL level in the group fed with *T. avicennioides* which showed that the plant is capable of boosting the HDL. The increase in HDL level in the group treated with artemether lumefantrine and the group fed with *T. avicennioides* (Fig. 1) could be resulted from the decrease in the parasitaemia in these groups, while the reduction in the HDL level in the negative control as compared to other groups indicated

that malaria parasite may be accountable for the reduction in the HDL level. It has been reported that malaria infection produces moderate changes in lipid profile in man, with typical decline in HDL concentration [31].

In this study, elevated serum alkaline phosphatase in the infected groups were observed when compared with the uninfected group (normal control). This showed that malaria parasite may be responsible for the increase in the ALP level. The observed elevated serum level corroborates with the earlier findings. White and Ho [32] observed higher serum level of alkaline phosphatase in the presence of malaria parasites. The observed elevation in serum alkaline phosphatase is an indication of the hepatic stage of the malaria parasite's life cycle in its host and is accompanied by significant perturbation of the hepatocyte membrane leading to leakage of this enzyme out of the liver cells [33]. This finding is in agreement with earlier findings [32], that centrilobular liver damage is one of the factors involved in liver dysfunction in acute malaria infection, which is a direct consequence of the impaired drainage capacity of the hepatocyte.

A significant increase in the activities of ALT and AST in the negative control as compared with all the other groups were observed. The observed significant increase in enzyme activities may be as a result of liver injury and altered hepatocytes caused by the malaria parasite and the consequent leakage of the enzymes into the blood stream. Application of 5mg/kg Artemether and *T avicennioides* tends to cause a decrease in the ALT and AST levels in the mice. In view of the increase in serum AST activities, the etiology of the increased activity of the enzymes are claimed to be due to the malaria infection. These changes, which show an increase of liver enzymes according to the increase in serum AST, ALP and AST levels, are a direct indicator of hepatocyte dysfunction associated with *Plasmodium*. Premaratna *et al.* [34] reported similar result severe hepatic dysfunction associated with *falciparum* malaria. Increase in AST, ALT and ALP liver enzymes have also been reported as a rough proportionality of the extent of liver damage [35].

Malarial hepatitis is a term frequently used to define hepatocytic dysfunction in *Plasmodium* infection. Malarial hepatitis is characterized by a rise in serum bilirubin along with the increase in the serum AST and ALT levels to more than two times the upper limit of normal [35]. In this study, the bilirubin level was higher in the negative control group when compared with all the treated groups. The observed increase in the serum bilirubin level in the negative control could be due to haemolysis that occurred during the erythrocytic stage of the life cycle of malaria infection, Akanbi *et al*., [18] reported similar result.

Hepatic dysfunction in severe malaria is multifactorial; intravascular haemolysis of parasitized erythrocytes, haemolysis of non-parasitized erythrocyte, associated haemoglobinopathies as well as drug induced haemolysis [36]. Disseminated intravascular congealing may also contribute to hepatocellular dysfunction seen in severe *Plasmodium* infection [37]. However, in acute malaria, hepatic dysfunction is reversible in all the patients developing malarial hepatopathy who respond favourably to antimalarial therapy and no residual effects have been documented in survivors Bilirubin normally recedes by 72 hours of starting treatment but it may be delayed in patients [38]. having coexisting renal dysfunction [39]. In this study, we noted significant improvement in liver function test in treated groups which showed that bilirubin and ALT may reduce to reference range upon parasite clearance

V. CONCLUSION

In conclusion, we observed that powdered stem bark of *T. avicennioides* made as dietary feed has antiplasmodial activity. The parasite inhibition rate is comparable with artemether lumefantrine. Furthermore, mild hepatocellular dysfunction was observed which can be equated to that of artemether lumefantrine.

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