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Anti-malarial Activity of Total Saponins from *Terminalia avicennioides* and Its Effect on Liver and Haematological of Infected Mice

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Abstract

Background: *Terminalia avicennioides* is one of the medicinal plants commonly use traditionally, hence this study examined the effect of total saponins from *Terminalia avicennioides* leaves on malaria parasite, haematological and liver in mice infected with Plasmodium berghei.

Methodology: Fifty Swiss albino mice (n=10) were divided into five groups. Groups 1, 2 and 3 were infected with malaria parasite and treated with 100, 200 mg/kg of saponins, and 5 mg/kg artemether-lumefantrine respectively (positive control). Group 4 was infected but not treated (negative control), group 5 was neither infected nor treated (normal control). The treatment was administered orally for four days.

Results: The parasite clearance was higher in the positive control group (80%) than in the group treated with 100 and 200 mg/kg of saponins (43.5% and 56.95%) respectively. White blood cell and lymphocyte count were higher in the group treated with 100 and 200 mg/kg of saponins. The red blood cell and haemoglobin levels were significantly reduced ($p<0.05$) in the group treated with 200 mg/kg of saponins than in all other groups. The platelet count (PLT) was lowest in the group treated with 100 mg/kg body weight of saponin. There was a significant reduction in ($p<0.05$) in the Aspartate transaminase (AST) and Alanine transaminase (ALT) levels in the normal control than in other groups.

Conclusion: The study concluded that the antiplamodial activity of total saponin of *T. avicenniodes* and its effects on haematology and liver dysfunction are dose related. It is therefore recommended that low dosage of saponin from *T. avicennioides* should be used for the treatment of malaria.

Keywords: Malaria; Saponin; Medicinal plant; *Terminalia avicenniodes*; Liver dysfunction; Haematology

Introduction

Malaria has been a menace to the health conditions of both rural and urban populations in Nigeria [1]. Although, it is a global epidemic, but the incidence and severity are higher in the tropics especially in the sub-Saharan Africa, where pregnant women and children are the most susceptible groups [2]. In 2013, it caused an estimated 198 million clinical cases and 584,000 deaths [3]. The disease is characterized by fever, chills, myalgia, headache, nausea, and vomiting [4]. In *P. falciparum* infection, several haematological changes such as anaemia, thrombocytopenia and leukocytosis have been reported [5]. Because of the problem facing the production of vaccine against plasmodium species, the adopted strategy of malaria control is based on chemotherapy and breaking of chain of transmission of the parasites between humans and mosquitoes [6]. Since 2008, Nigeria has changed its treatment guidelines for management of malaria and now use Artemisinin-based combination therapy (ACT) in treating uncomplicated malaria and Quinine for management of acute or complicated malaria [7]. As a result of resistance of malaria parasite to malaria drugs many people in malaria endemic region including Nigerian has resorted into traditional medicine. *T. avicenioides* has been reported as one of the plants that are rich sources of phytochemical such as saponins, steriods alkaloids [8]. The efficacy of *Terminalia avicennioides* for the treatment of, antihelmintic, cough, body pain and asthma has been reported [1].

Saponins, are naturally occurring plant glycosides which possess soap-like qualities and produce lather when mix with water [9]. Over one hundred families of plants contain saponins and there are more than eleven classes of saponins, including; damaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasternes and steroids [9]. Saponins offer tremendous health benefits and studies have shown that they may support the immune system, promote normal cholesterol levels and support overall wellness [8]. They have been found useful in resisting microbial pathogen such as fungi, bacteria, parasite and virus [10]. When saponins are consumed by human it promote normal cholesterol bind with bile and prevent cholesterol from being reabsorbed back into blood stream [9]. The unique chemical of saponins allow them to offer a number of prospective health benefits. Saponins have an antioxidant effect and may even support bone strength [8] and have been proven beneficial in liver, kidney and urinary infections [8].

Many works have been done on the efficacy of methanolic extract of its stem bark against malaria parasite [11-13]. Though there is report on efficacy of methanolic leaf extract of *T avicennioides* for the treatment of malaria [11], but there is dearth of information on the use of saponins from *T avicennioides* as one of the active compound of *T. avicennioides* for the treatment of malaria infection, hence, this study investigated the efficacy of saponins extracted from *T. avicennioides* and its effect on haematological parameters and liver function in mice infected with *Plasmodium kerghei*.

Materials and Methods

Collection and identification of plant materials

Matured leaves of *Terminalia avicennioides* were collected from Arigidi Akoko, Ondo State, Nigeria, and identified in Plant Science and Biotechnology Department of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

Preparation of plant samples

The fresh leaf of *T. avicennioides* was air dried for 4-6weeks then, it was ground to powder form.

Extraction and isolation of saponins from *T. avicennioides* leaves

The method used was according to as described by and as modified by Adanlawo and Elekofehinti [14,15]. A hundred gram (100 g) of ground sample was extracted with 500 ml of petroleum ether (40-60°C) in a soxhlet extractor for 12 hours and was air-dried. The air-dried, defatted sample was extracted with methanol (500 ml) for 12 hour. The methanolic extract was partitioned with n-butanol and water (1:1, v/v) using separating funnel. After a thorough shaking, the mixture was allowed to stand overnight and the n-butanol layer was separated in the next day. The aqueous layer was washed five times with aliquots of n-butanol until it became colourless. The pooled butanolic layer was evaporated under reduce pressure to give a residue which was dissolved in 100 ml methanol and precipitated by adding a large amount of diethyl ether to obtain a solid crystalline dark brown compound [14]. The crude saponin fraction was spotted onto pre-coated silica gel Thin Layer Chromatography (TLC) plate (Merck, Kleselgel 60F-254). The plates were developed with n-butanol:acetic acid:water (60:10:30 v/v/v). The spots on the chromatograms which were due to saponins were identified by spraying with Lieberman–Burchard reagent (methanol:sulfuric acid:acetic acid (50:5:5 v/v/v)). Saponin extract was spotted alongside a standard solution (5 g/l) of saponin white. Concentrated crude saponin extract was applied to a silica gel column of 60-120 mesh. The impurities were washed away with n-hexane through a 2.4 × 50 cm bed of silica gel. The column was eluted with n-butanol:acetic acid:water (1:1:1 v/v/v). The fractions were collected and aliquots applied as a series of spots to a strip of TLC plate, dried, sprayed with Lieberman–Burchard reagent and heated. Positive fractions were pooled together and used for the experiment. The compounds on the plates were detected with ceric sulfate reagent followed by gradual heating. The fractions showing similar TLC profiles were mixed and these collective fractions were individually subjected to repeated column chromatography to furnish the saponins.

Experimental animals

A total of fifty mice weighing between 18-22 g were distributed into five groups. Each group comprised of ten mice. Four of the groups were infected intraperitoneally with an aliquot of 0.2 ml of standard inoculums (1 × 10 *Plasmodium berghei*) strain NK 65 parasitized erythrocytes. The fifth group was not infected with the parasite and was used for normal control. Among the four infected groups, group 1 and group 2 were treated with 100 mg/kg body weight and 200 mg/kg body weight of saponin extracted from *Terminalia avicennioides*, respectively. Group three was used as positive control while group four was not treated at all (negative control). All the treatments were administered once daily by gavage using intubator for four consecutive days. Blood was taken daily from the tail vein of the mice before treatment for the assessment of parasitaemia. The animals were sacrificed twenty four hours after the last treatment and whole blood and liver were collected for biochemical assays. The protocol was according to the guidelines of National Institute of Health (NIH) publication 85-23, for laboratory animal care and use. Liver of the animals were excised and homogenized in ice cold normal saline (1:4 w/v), centrifuged at 5,000 rpm for 5 minutes to obtain supernatant which was used in this study

Parasitological study

Thick blood film was prepared from the whole blood collected from each mouse for four days, and slides were screened for malaria parasite using Giemsa's stain. The number of parasite counted per 200 white blood cells was recorded and used to calculate parasite density.

Haematological assay

The blood collected into the EDTA bottle was used to determine haematological parameters using automated analyzer to analyze the blood sample. The haematological parameters investigated include the white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HGB), lymphocyte (LYM) and platelet.

Biochemical assay

Liver homogenates obtained from mice were used to assay for the liver function using spectrophotometric method with Randox test kits. Para-Nitrophenyl Phosphate (PNPP) was used to determine the ALP activity as described by Cathala and Brunel [16]. ALT and AST levels were measured by the pyruvate and oxaloacetate methods respectively as described by Christen and Metzier [17].

Statistical Analysis

The difference among the group was analyzed using the one-way ANOVA test and the significant test was done using SPSS 17.0 software for this analysis. The results were expressed as Mean ± Standard mean error (SEM), where the ANOVA level of significance was considered at $P<0.05$.

Results

The effect of treatment of infected mice with saponins from *T. avicennioides* on the parasitaemia count is presented in Table 1. The level of inhibition of the parasitaemia count was determined by comparing the changes in parasitaemia count in day 1 to the subsequent days after the commencement of the treatment. In the

negative control there was significantly increase in parasitaemia counts from day 3 to day 4 (573.33 ± 58.10 and 646.70 ± 59.06 respectively) when compared with day 1 (453.33 ± 58.12). The rate of increase in parasitaemia from day 2 to day 4 were 112%, 126% and 142% respectively when compared with day 1 which was 100%. Among the group treated with 100 mg/kg saponins, the parasitaemia count was significantly reduced from 400.00 ± 61.10 in day 1 to 306.00 ± 26.70, 260.00 ± 13.33 and 226.70 ± 35.33 in day 2, 3 and 4 respectively. The rate of parasite suppressions in this group when compared with day 1 which was 100% were 23.5%, 35% and 43.5% in day 2, 3 and 4 respectively. The parasitaemia counts was reduced from 386.00 ± 35.30 in day 1 to 166.70 ± 13.33 in day 4 in the group treated with 200 mg/kg saponins, while the rate of parasite count suppressions from day 2 to 4 when compared to day 1 which was 100% were 6.7%, 55.19% and 56.95% respectively. In the positive control the parasitaemia was significantly reduced from 520.00 ± 46.20 in day 1 to 106.70 ± 13.33 in day 5 and the rate of parasitaemia suppressions from day 2 to day 4 were 28.3%, 71.93% and 80% respectively when compared with day 1 which was 100%.

Treatment	Day 1	Day 2	Day 3	Day 4
Negative control	453.33 ± 58.12^a	513.33 ± 54.60^a	573.33 ± 58.10^c	646.70 ± 59.06^c
100 mg/kg	400.00 ± 61.10^a	306.00 ± 26.70^b	260.00 ± 13.33^c	226.70 ± 35.33^d
200 mg/kg	386.00 ± 35.30^a	360.00 ± 40.00^a	173.33 ± 35.33^b	166.70 ± 13.33^b
Positive Control	520.00 ± 46.20^a	373.33 ± 26.70^b	146.70 ± 26.70^c	106.70 ± 13.33^d

Table 1: Effect of saponins from *T. avicennioides* on parasite density in mice infected with *P. beghei*, Keys: Data presented as Means ± SEM; Mean value with the same superscript along the same column are not significantly different from each other.

The effect of saponins from *T. avicennioides* on the haematological parameters of the Swiss albino mice infected with Plasmodium berghei was also studied and the result showed that white blood cell was slightly higher in the group treated with 100 and 200 mg/kg saponins than in the all other groups in this study (Figure 1).

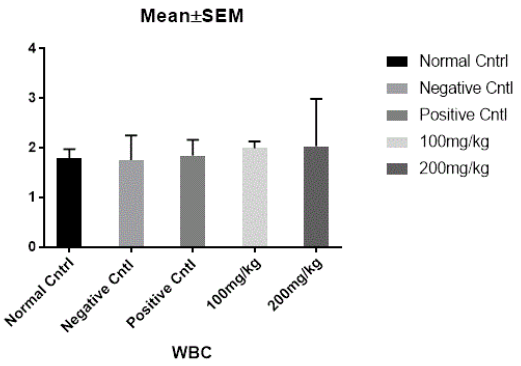

Figure 1: Effect of saponins from *T. avicennioides* on white blood cell of *P. berghei* infected mice.

The mean value of lymphocyte count (LYM) was slightly reduced in the positive control as compared with the group treated with 100 and 200 mg/kg body weight of saponins and normal control (Figure 2). The red blood cell was significantly higher in the normal control group when compared with positive control and the groups treated with 100 and 200 mg/kg of saponin. Among the treated groups, the level of red blood cell was lowest in the group treated with 200 mg/kg of saponin (Figure 3). The haemoglobin level was also significantly higher (P<0.05) in the normal control than in the group treated with 200 mg/kg of saponin. The level of haemoglobin was significantly (P<0.05) reduced in the group treated with 200 mg/kg of saponins than in all other groups (Figure 4). The mean values of the platelet count (PLT) was reduced in group treated with 100 mg/kg body weight of saponin when compared with positive control group and the group treated with 200 mg/kg saponin of *T. avicenniodes* (Figure 5).

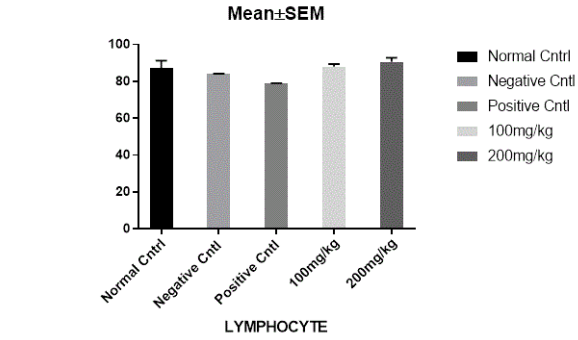

Figure 2: Effect of saponins from *T. avicennioides* on Lymphocyte count of *P. berghei* infected mice.

Table 2 showed the effect of saponins from *T. avicennioides* on liver enzymes. The results showed that the AST level was significantly higher in the negative control group than in the normal control (18.07 ± 2.90) positive control (27.60 ± 1.60) and groups treated with 100 and 200 mg/kg of saponin (21.60 ± 2.10 and 19.70 ± 1.20) respectively. There was significant increase (p<0.05) in the AST levels in the positive control when compared with normal control and the groups treated with 100 and 200 mg/kg of saponin, while the level of AST was slightly higher in the groups treated 100 mg/kg of saponin when compared with group treated with 200 mg/kg of saponin. ALT level was significantly lower (p<0.05) in the normal control (102.22 ± 2.99) than in all other groups in this study, while its level was significantly higher in the negative control (120.17 ± 7.17) than in all other groups. The ALT was slightly higher in the group treated with 100 mg/kg (113.00 ± 3.11) of saponin than in the positive control group (107.37 ± 1.32) and the group treated with 200 mg/kg (106.37 ± 2.37) of saponin. The ALP

level was significantly higher in the negative control group (17.13 ± 1.80) than in all other groups in this study, but its level was slightly higher in the group treated with 100 mg/kg (14.52 ± 1.84) of saponin than in the positive control (13.80 ± 1.59) and the group treated with 200 mg/kg (13.70 ± 2.80) of saponin.

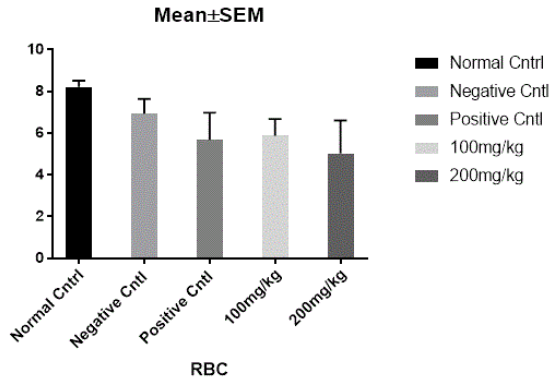

Figure 3: Effect of saponins from *T. avicennioides* on red blood cell count of *P berghei* infected mice.

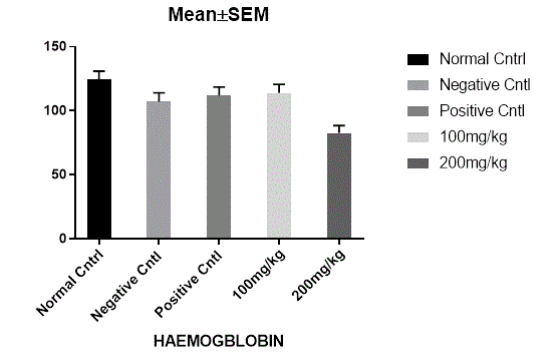

Figure 4: Effect of saponins from *T. avicennioides* on haemoglobin (HGB) of *P berghei* infected mice.

Discussion

Despite decades of research, malaria infection remains a major global health problem with high mortality and morbidity than any other infectious disease [18]. This work studied the efficacy of saponins from *Terminalia avicennioides* against parasitaemia count and their effect on haematological parameter and liver enzymes in mice infected with Plasmodium berghei. This study showed that there was steady increase in the parasitaemia counts from day 1 to the day when the animal was sacrificed in the negative control group. This was in agreement with the previous study which reported that parasitaemia increases progressively after inoculation until the point of death in the absence of suitable treatment [19,20].

The study also showed that there was a significant reduction in the positive control when compared with other treated groups. The level of parasitaemia counts suppression was significantly higher in the group treated with 200 mg/kg of saponin extract of *T. avicennioides* when compared with the group treated with 100 mg/kg body weight. This showed that saponin from *T. avicennioides* is effective in the destruction of the parasitaemia, especially at the higher dosage (Table 1). This concurs with the previous study on the effect of methanolic crude extract of *T. avicennioides* on parasitaemia counts as reported by Akanbi and Omonkhua [11,12].

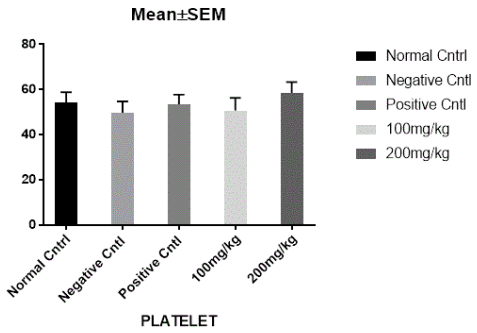

Figure 5: Effect of saponins from *T. avicennioides* on Platelet count of *P. berghei* infected mice.

Treatment	AST	ALT	ALP
Normal control	18.07 ± 2.90^{a}	102.22 ± 2.99^{a}	14.73 ± 2.43^{a}
Negative control	30.60 ± 1.37^{b}	120.17 ± 7.17^{c}	17.13 ± 1.80^{b}
100 mg/kg	21.60 ± 2.10^{a}	113.00 ± 3.11^{b}	14.52 ± 1.84^{a}
200 mg/kg	19.70 ± 1.20^{a}	106.37 ± 2.37^{b}	13.70 ± 2.80^{a}
Positive control	27.60 ± 1.60^{c}	107.37 ± 1.32^{b}	13.80 ± 1.59^{a}

Table 2: Effect of saponins from *T. avicennioides* on AST, ALT and ALP level in liver homogenate in mice infected with *P. berghei*, Key: Data are presented as means and SEM; Mean value with the same superscript along the same column are not significantly different from each other.

The slight increase in white blood cell and lymphocyte among the groups treated with 100 and 200 mg/kg of saponin is an indication that saponin may be able to increase the level of immunity against malaria infection. This could have been responsible for the antiplasmodial activity displayed in this study by the saponin, especially at the dosage of 200 mg/kg. This study agrees with the previous study which showed that saponin has the unique ability to stimulate lymphocyte [21,22].

Anaemia is a common feature in the tropical regions where malaria infection is prevalent. Some parameters used in this study to assess anaemia includes haemoglobin, red blood cell and packed cell volume. In our study, the mean haemoglobin and red blood cell were significantly higher in the normal control group than in the groups that were infected and treated with 100 and 200 mg/kg body weight of saponin. The significant reduction in haemoglobin and red blood cell in these groups shows that saponin may be responsible for this reduction. This agrees with the previous study which reported that saponin has haemolytic properties and is capable to lyse haemoglobin

[23]. The significant reduction in haemoglobin and red blood cell level in the group treated with 200 mg/kg of saponin when compared with the group treated with 100 mg/kg shows that the level of haemolysis caused by saponin could be dose related just as its antiplasmodial effect is dose related. The slight increase in platelet count in the group treated with 200 mg/kg body weight of saponin when compared with other groups in this study indicate that saponin may cause thrombocytopenia at higher concentration.

The significant increase in AST and ALT levels in the negative control when compared to other groups in this study supported the previous reports that malaria parasite could enhance the increase jn the liver enzymes levels in the organism [22,24]. This increase could be as a result of leakage from hepatic cell that were destroyed by the immune response during the infection [25]. There was significant increase in AST level in the positive control group when compared with other groups. This could be linked with the rate of parasite destruction which was higher in the positive control than other treated groups. Among the treated groups there were increase in ALT and ALP levels in the group treated with 100 mg/kg. The increase in the ALT and ALP levels in the group treated with 100 mg/kg could be a function of the level of parasitaemia counts which was higher than in all other treated groups. This supported the previous study that showed that malaria infection is capable of increasing the liver enzymes in the body.

Conclusion

The study concluded that the antiplamodial activity of total saponin of *T. avicenniodes* and its effects on haematology and liver dysfunction are dose related. It is therefore recommended that low dosage of saponin from *T. avicennioides* should be used for the treatment of malaria.

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