

British Microbiology Research Journal 3(4): 501-512, 2013



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In vivo Study of Antiplasmodial Activity of Terminalia avicennioides and Its Effect on Lipid Profile and Oxidative Stress in Mice Infected with Plasmodium berghei

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Author's contribution

The sole author designed and carried out the study. Author OMA wrote the first draft of the manuscript, read and approved the final manuscript.

Research Article

Received 12th March 2013 Accepted 29th May 2013 Published 28th July 2013

ABSTRACT

Aims: To determine the antiplasmodial activity of methanolic extract of *T. avicennioides* and its effect on oxidative stress and the lipid profiles in mice infected with *Plasmodium berghei*.

Study Design: Mice used for this study were grouped into five. The first group was not infected with malaria parasite (normal control), the second group was infected with the parasite but not treated with antimalarial drugs (negative control), the third group was infected with the parasite and treated with 5mg/kg body weight of artesunat (positive control), while the fourth and fifth groups were infected with malaria parasite and treated with 100 and 200mg/kg of *T. avicennioides* respectively.

Methodology: The parasitaemia was monitored for five days. The animals were sacrificed on the fifth day and the blood was collected. The serum was used to assess the biochemical parameters using randox kits.

Results: While parasite density increases in the negative control per day, there was reduction in parasite density in treated groups. The parasite clearance was significantly higher (P = .05) in those treated with 200mg/kg of *T. avicennioides* than those treated with 100mg/kg of *T. avicennioides* and 5mg/kg of artesunat. The malondialdehyde level was significantly higher in the negative control, while superoxide dismutase and catalase levels

were significantly reduced when compared with group treated with 200mg/kgbdwt of T. avicennioides. HDL level was significantly higher (P = .05) in those treated with 200mg/kg than in the normal, negative and positive control. The triglycerides level was significantly higher in the negative control when compared with the group treated with the extract of T. avicennioides.

Conclusion: This study showed that the methanolic extract of *T. avicennioides* display dose-related *in vivo* antiplasmodial and antioxidant activities as well as reduced the serum and liver lipoprotein cholesterol in mice infected with *P. berghei*.

Keywords: Malaria parasite; Terminalia avicennioides; antioxidant; lipid profile; methanolic extract.

1. INTRODUCTION

Malaria remains the worst parasitic diseases in the world. It constitutes one of the major public health problems in the tropic and sub tropic regions of the world. The prevalence of malaria infection has reduced significantly as a result of concerted effort [1]. It is responsible for about 219 million cases, with about 836,000 deaths annually [2]. Increased prevention and control measures have led to a reduction in malaria mortality rates by more than 25% globally and by 33% in the WHO African region since 2000 [2]. Although the incident of malaria infection occur mostly in the tropical regions, but its socio-economic and public health impact are global. Children and pregnant women are more susceptible to malaria infection [3]. Despite all the ongoing efforts to eradicate the infection, it continues to spread across the areas where it had been eradicated before. Therefore, it becomes a major concern and problem to WHO. The emergence and spread of malaria parasite drug resistant strains to most of the first line drugs has caused more threat to the world [4]. This has necessitated the need for more investigation of medicinal plants for other antimalarial agents and optimization of those with existing antimalarial activity when used singly or in combination with orthodox medicine [5].

Malaria control activities in Nigeria are planned and implemented through the Primary Health Care (PHC) system [6]. This method has not really caused any drastic changes in the episode of malaria infection in malaria endemic areas. It has been estimated that only about 20% of episodes of malaria are being treated in health centre in many African countries, including Nigeria [7,8]. Majority of people in developing countries where malaria is endemic prefer to go to non-governmental organizations for the treatment for some reasons which include inaccessibility to health centre, scarcity of antimalarial drugs, deficiencies in the performance of formal health services, including poor clinical skills and cultural beliefs [6]. Because of those shortcomings, many people prefer to treat themselves by self medication with drugs bought from shops and herbal preparations [7].

Herbal medicine has been shown to have genuine utility and about 80% of rural populations depend on it as primary health care [9]. The World Health Organization advocated for countries, especially those in endemic areas to study the efficacy of traditional medicine in their area with the view of identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial original.

In line with the WHO directive, Nigeria government has also embarked on awareness of alternative medicine in the treatment of most of the parasitic diseases in which malaria is one of them. Nigeria government has created a unit in the ministry of health which promotes and

co-ordinates traditional medicine practices in Nigeria. Some of the medicinal plants commonly used traditionally in Nigeria include *Anogeissus leiocarpus, Terminalia, avicennioides, Momordica balsamina, Combretum paniculatum and Trema guineensis*. All these plants have been reported to contain alkaloids, tannins, flavonoids and anthraquinones, but in variable degrees [9]. Although so many of these herbs have been found to be potent in the treatment of malaria infection traditionally, but there are still some gaps that are yet to be filled (such as unpredictable efficacy, short and long term safety, unestablished dosage etc) before the full potential of herbal medicines as a solution to the malaria burden can be adopted.

T. avicennioides is among the medicinal plants commonly used traditionally in Nigeria for the treatment of infection. *Terminalia avicennioides* (*Combretaceae*) is a yellowish brown, hard and durable wood, commonly found in the Savannah region in West Africa [10]. This plant extract is known to be active against trypanosomes [11], against diarrhoea [12], against Candida albicans [13] and malaria parasite [14]. Some studies also showed that the stem bark extract of *T. avicennioides* exhibited both vibrocidal and typhoidal activities [9], and antimicrobial activity [15]. Its efficacy on the healing of ulcer and wound has been also reported [9]. Though many works on the antimicrobial activity of *T. avicennioides* have been carried out, but to the best of my knowledge this is the first study in which the antiplasmodial activity of this plant as well as its effect on oxidative stress and lipid profile are being assessed *in-vivo*. This work studied the *in vivo* antimalarial activity of methanolic extract of *T. avicennioides* and its effect on oxidative stress and lipid profile in mice infected with *P. berghei*.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Malaria Parasite

Adults Swiss albino mice used for this study were obtained from the Animal unit of the Obafemi Awolowo University (OAU), Ile Ife. Nigeria. The animals were kept in well aerated wired cages, fed with standard mouse feed bought from Bendel feed and flour mill, Ewu, Nigeria, and were allowed to drink water freely. The animals were kept for two weeks to be acclimatized to the new environment before they were infected with the malaria parasite. The parasite used (*P. berghei NK 65*) was donated by the laboratory of Dr O. Aina of the Department of Parasitology, National Institute for Medical Research (NIMR), Yaba, Lagos, Nigeria. The parasites were maintained in animals by serial passage of blood collected from a patent donor mouse to a naïve recipient.

2.2 Plant Materials and their Extract

The stem bark of *T. avicennioides* (Locally called *udi*) was harvested at Akungba-Akoko, Ondo state, Nigeria by a laboratory attendant of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo state, Nigeria. It was identified by Dr. A.O. Obembe of the same Department. The Herbarium specimen with voucher number UIH22318 *T. avicennioides* was deposited at the Herbarium unit of the University of Ibadan, Ibadan, Nigeria. The bark of the plant was washed thoroughly and airdried. This was later cut into pieces and then ground. 1000g of powdered back was weighed into 70% methanol (2.7L) and this was left for 72 hours. The extract was filtered and evaporated to dryness in vacuum with a rotary evaporator to concentrate the filtrate and

obtain the methanolic extract of *T. avicennioides*. The concentrated filtrate was put into the dish, covered with a net and left for 24 hours to dry.

2.3 *In-vivo* Antimalarial Assay

A 4-day suppressive test against P. berghei infection in mice was used. Mice weighing between 18-25g were distributed into five groups. Each group comprised five animals. The first group was not infected with the parasite and this was used for normal control, while other four groups were infected intraperitoneally with an aliquot of 0.2ml of standard inoculum (1x10⁷ Plasmodium berghei strain NK 65 parasitized erythrocytes). Among the four groups, the first infected group was not treated with antimalarial drug. This was used as negative control. The second infected group was treated with 5.0mg/kg body weight of artesunat, and this was used as positive control. The fourth and fifth groups were infected and treated with 100mg/kg body weight and 200mg/kg body weight of methanolic extract of T. avicennioides respectively. 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 mice were stunned and the thoracic and abdominal regions were opened to expose the heart, and other organs. The protocol was according to the guidelines of National Institute of Health (NIH) publication 85-23, 1985, for laboratory animal care and use. The study was approved by local Institution Review committee. Blood was collected by heart puncture into ethylene diamine tetraacetic acid (EDTA) and plain bottles. The blood in the plain bottle was centrifuged at 5,000 rpm for 5 minutes to obtain the serum for biochemical parameters, while the blood in EDTA bottle was used to determine haematological parameters. Heart, kidney and liver of the animals were excised and homogenized in ice cold normal saline (1:4w/v), centrifuged at 5,000 rpm for 5 minutes and the supernatant was stored in the freezer until analysis was done.

2.4 Parasitological Study

Thick blood film was prepared from blood collected from each mouse 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-leucocytes/µl of blood as described by Nwagwu, *et al.*, [16].

2.5 Biochemical Parameters Assays

2.5.1 Determination of serum and tissue superoxide dismutase and malondialdehyde

The MDA, SOD and Catalase levels were used as indices of oxidative stress. Lipid peroxidation in serum was assessed by measuring the thiobarbitutric acid reactive substances (TBARS) and expressed in terms of malondialdehyde (MDA) formed per mg protein as described by Varshney and Kale [17]. SOD activities were measured by the method described by Misra and Fridovich [18]. Catalase activity was determined by the method described by Beers and Size [19].

2.6 Determination of Lipid Profile

2.6.1 Total triglycerides assay

Serum total triglycerides concentration was measured by the Tietz [20] method, as described in the manual of the Randox Total triglycerides kit.

2.6.2 Total cholesterol assay

Serum total cholesterol level was measured by the Trinder [21] method, as described in the manual of the Randox Total cholesterol kit.

2.6.3 HDL-cholesterol assay

Serum HDL-cholesterol concentration was measured by the NIHCDS [22] method, as described in the manual of the Randox HDL-cholesterol kit.

2.6.4 LDL-cholesterol assay

Serum LDL-cholesterol level was calculated by the Friedewald *et al.*, [23] method, as described in the manual of the Randox HDL-cholesterol kit.

2.7 Statistical Analysis

The differences among groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 15.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. The results were expressed as mean \pm standard deviation (SD). The level of significance was estimated at P < .05

3. RESULTS

In the negative control, the result showed that the parasite growth rate increased from 9% in day 1 to 72% in day 5 when compared with day 0 which marked the last day before the commencement of the treatment. The parasite growth rate increased in day 1 by 8% in the positive control when compared with day 0. There was a reduction in the parasite growth rate from day 3 by 30% and finally by 72% by day 5 in positive control when compared with day 0. The rate of parasite growth increased by 10% in day 1 in the animal treated with 100mg/kg of *T. avicennioides* and this was followed by reduction in the parasite growth rate from day 3 by 36% and finally by 88% in day five as compared with day 0. While there was a decrease in the parasite density from day 2 by 5% in animal treated with 200mg/kg of *T. avicennioides* to 95% in day 5 as compared to day 0. The rate of clearance was highest in those treated with 200mg/kg of *T. avicennioides* as compared to those treated with 5mg/kg of artesunat and those treated with 100mg/kg of *T. avicennioides* (Table 1).

The serum MDA was significantly higher (P = .05) in the negative when compared with other groups. There was significant increase in MDA level (P = .05) in positive control group when compared with the normal control and the group treated with 100mg/kg of T. avicennioides. The MDA level was also significantly higher (P = .05) in those treated with 200mg/kg when compared with the normal control (Fig. 1).

Fig. 2 shows that the mean serum SOD level was significantly higher (P = .05) in animal treated with 200mg/kg of T. avicennioides than in all other groups. There was also a significant increase (P = .05) in the serum SOD levels in the group treated with 100mg/kg of T. avicennioide as compared to normal, negative and positive controls. In the liver, the mean SOD level was significantly higher (P = .05) in the groups treated with 100 and 200 mg/kg of T. avicennioides than the normal, negative and positive control.

The mean serum Catalase level was significantly lower (P = .05) in the negative control when compared with all other groups. There was a significant increase in the mean serum catalase level in the normal control and the group treated with 200mg/kg of T. avicennioides when compared with all other groups. In the liver the mean serum catalase level was significantly higher (P = .05) in the negative control and group treated with 100 and 200mg/kg of T. avicennioides than the positive control (Fig. 3).

This study also assessed the effect of methanolic extract of T. avicennioides on the lipid profile in mice infected with P. berghei. The result shows that the mean HDL cholesterol level was significantly higher (P = .05) in the groups treated with 100 and 200mg/kg than in the normal control and negative control. The negative control had the lowest HDL level when compared with others (Fig. 4). The mean LDL Cholesterol level was significantly higher (P = .05) in the positive control than in the negative control and the group treated with 100mg/kg of T. avicennioides. The LDL Cholesterol level was also significantly higher in group treated with 200mg/kg of T. avicennioides when compared with the group treated with 100mg/kg (Fig. 4). Fig. 4 shows that the total triglyceride was significantly higher (P = .05) in the positive control group than in the groups treated with methanolic extract of 100 and 200mg/kg of T. avicennioides and normal control. The triglycerides level was also significantly higher in the negative control when compared with normal control and the group treated with 100mg/kg of T. avicennioides. The serum total Cholesterol level was significantly higher in the group treated with 5mg/kg of artesunat than the groups treated with 100 and 200mg/kg of methanolic extract of T. avicennioides (Fig. 4).

Table 1. Effect of treatment of mice infected with *P. berghei* with methanolic extract of stem bark of *T. avicennioides* on parasite

Negative control (MP without treatment) (%)	Positive control (MP + 5mg/kg b.w of artesunat) (%)	MP + 200mg/ /kg b.w of <i>T.</i> avicennioides (%)	MP + 200mg/ kg b.w of <i>T. avicennioid</i> es (%)
109±0.4	108±0.7	110±0.9	108±0.6
119±0.6	101±0.5	100±0.5	95±0.4
136±0.6	70±0.5	64±0.5	55±0.4**
158±0.5	32±0.4	24±0.6	18±0.6**
172±0.4	23±0.5	12±0.2	05±0.2**

*All the figures are in percentages and they are reported as means ± SD.

⁺⁺ Means of all the values were compared with day 0 which was a day before the commencement of the treatment.

⁺⁺⁺ The parasite density in day 0 was considered to be 100%.

^{**} P< 0.05 in comparison with negative control and positive control

^{*}All the figures are in percentages and they are reported as means ± SD.

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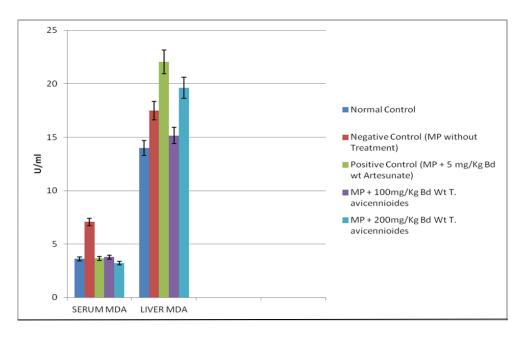


Fig. 1. The effect of methanolic extract of stem bark of *T. avicennioides* on serum and liver MDA levels in mice infected with *P. berghei*

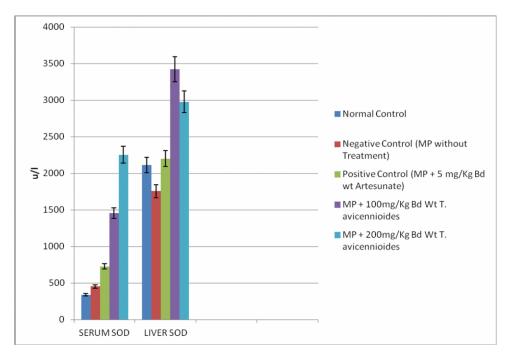


Fig. 2. The effect of methanolic extract of stem bark of *T. avicennioides* on serum and liver SOD levels in mice infected with *P. berghei*

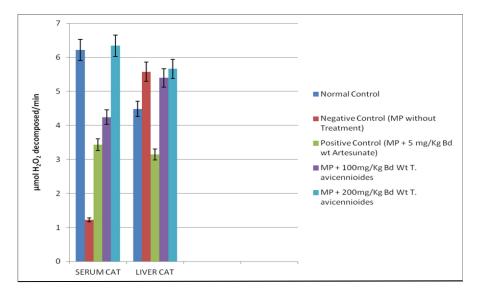


Fig. 3. The effect of methanolic extract of stem bark of *T. avicennioides* on serum and liver Catalase levels in mice infected with *P. berghei*

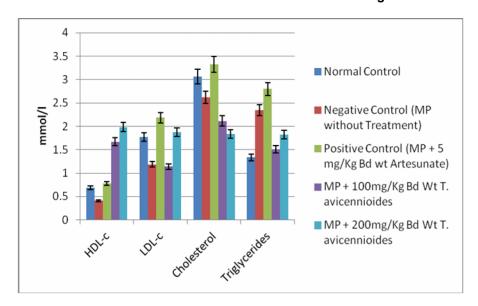


Fig. 4. Effect of methanolic extract of stem bark of *T. avicennioides* on the HDL-c, LDL-c, total cholesterol and Triglyceride levels in mice infected with *P. berghei*

4. DISCUSSION

In this study, the methanolic extract of *T. avicennioides* has proved to have a strong antiplasmodial activity by the role it played in the reduction of the parasite load in the infected mice when compared with the negative control (Table 1). The reduction in the parasiteamia is an indication that the stem bark of *T. avicennioides* contained some secondary metabolites (glycosides, saponins, tannins, phenol and ellagic acid) which could be responsible for the

parasite destruction [11,14]. This study revealed that the rate of parasite growth inhibition was dose related and higher in the group treated with 200mg/kg than in any other groups (Table 1). This shows that methanolic extract of *T. avicennioides* contained more antiplasmodial activity at that dosage and performed better in the clearance of the parasite at that concentration.

The present study has not only established the fact that *T. avicennioides* has antiplasmodial activities, but it also showed that *T. avicennioides* has tendency to boost antioxidant and HDL level in the body. It has been reported that oxidative stress is common among malaria patients because of the activation of the immune responses by malaria parasites, thereby causing release of reactive oxygen species (ROS) [24,25]. Destruction of malaria parasites by the immune system has been related to the increased production of ROS by macrophages and polymorphonuclear neutrophil [26] which serve as effector cells in the elimination of the parasite. Though these changes play a key role in the host defense against malaria parasites, but may also render host tissues such as erythrocytes more vulnerable to oxidative damage [27].

From this study, the serum MDA level was significantly higher in the negative control as compared to other groups, while SOD and catalase levels were significantly higher in the groups treated with artesunat and methanolic extract of T. avicennioides when compared with the negative control (Figs.1-3). The increased in the serum MDA level and decrease in the catalase and SOD levels in the negative control indicates that there was an oxidative environment and stress in the negative control, which could be as a result of high parasite load in this group (Figs. 1-3). The sharp reduction in serum MDA level and increased in the serum SOD, and catalase levels in the positive control and the groups treated with 100 and 200mg/kg indicates that there was no oxidative environment and stress in those groups treated with artesunat and T. avicennioides (Figs. 1-3). This could be because of the reduction in the parasite load in those groups that received treatment when compared with the negative control. It has been reported that malaria parasites can be responsible for the upsurge of oxidative environment and stress in man [26]. The serum and liver SOD and catalase levels were significantly higher in the groups treated with T. avicennioides when compared with other groups (Figs. 2, 3). This increase suggests that T. avicennioides has antioxidant activity and therefore can boost the antioxidant level in an organism. The levels of MDA in the liver were higher in the positive control and the group treated with 100 and 200mg/kg of methanolic extract of *T. avicennioides* than normal and negative control group. This increased in the liver MDA in the positive control and the groups treated with 100 and 200mg/kg of T. avicennioides could be as a result of the accumulation of the merozoites as well as drugs in the liver cells as metabolism of drugs takes place in the liver (Fig. 2).

This work also study the effect of *T. avicennioides* on the lipid profile level in mice infected with *P. berghei*. The study revealed that there was an increase in the HDL-c in the group treated with *T. avicennioides* which showed that *T. avicennioides* is capable of boosting the HDL-c level *in vivo*. The increase in HDL-cholesterol level in the positive control and the groups treated with methanolic extract of *T. avicennioides* (Fig. 4) could be as a result of the decrease in the parasite load in these groups, while the reduction in the HDL-cholesterol level in the negative control could be because of the high level of parasite load in this group. Malaria has been implicated to cause changes in the plasma lipid profile in man, with typical rise in triglyceride concentration and reduction in HDL-cholesterol concentration [3,28]. It has been reported that the HDL-level could be determined by the parasite load in an organism [3]. The significant increase in HDL-cholesterol in the groups treated with the 100 and 200mg/kg of methanolic extract of *T. avicennioide* when compared with other groups also

indicates that *T. avicennioides* has the capacity to boost good cholesterol in man and therefore capable to prevent the malaria infected person from artherosclerosis.

The level of LDL-cholesterol was significantly lower in the negative control when compared with the positive control and the group treated with the 200mg/kg of methanolic extract of *T. avicennioides* (Fig. 4). This reduction might be because of the high parasite load in the negative control (Table I). This agrees with the previous study which showed that the increase in the malaria parasite load can reduce the LDL-cholesterol level in man [28]. The increase in the LDL-cholesterol levels in the group treated with 200mg/kg and the positive control could be because of the decrease in the parasite load in these two groups (Table I).

The serum triglycerides level was higher in both negative and positive controls when compared with the normal control and the groups treated with 100 and 200mg/kg of methanolic extract of *T. avicennioides* (Fig. 4). The increase in the total triglycerides level in the negative control could be as a result of the increase in the parasite load in that group. The reason for increase in the level of total triglycerides in the positive control is not clear because it is expected that the level of the triglycerides should decrease as the parasite load increases [28]. This study also showed that *T. avicennioides* is able to reduce the total cholesterol in the organism (Fig. 4)

4. CONCLUSION

This study showed that the methanolic extract of *T. avicennioides* display dose-related *in vivo* antiplasmodial and antioxidant activities as well as reduced the serum and liver lipoprotein cholesterol in mice infected with *P. berghei*.

ETHICAL APPROVAL

The author hereby declares that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

ACKNOWLEDGEMENTS

I acknowledge Dr Omonkhua, A. and Cyril-Olutayo, M. of Department of Biochemistry and Plant Science, respectively, Adekunle Ajasin University, Akungba-Akoko for the active roles played during this study. Dr. A.O Obembe from the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria who help in identifying the plant and B. F Ologunorisa of the same Department for assisting in harvesting the plant are also appreciated. I also thank Dr. O. Aina, Department of Parasitology, National Institute for Medical Research (NIMR), Yaba, Lagos, Nigeria for donating the *P. berghei*.

COMPETING INTERESTS

The author has declared that no competing interests exist.

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