International Journal of TROPICAL DISEASE & Health

25(2): 1-9, 2017; Article no.IJTDH.35021
ISSN: 2278–1005, NLM ID: 101632866

Combination Therapy of Methanolic Leaf Extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* on Malaria Parasite Count and Its Effect on Some Biochemical Parameters in Mice Infected with *Plasmodium berghei*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2017/35021
Editor(s):
(1) Ranthilaka R. Ranawaka, Department of Dermatology, General Hospital Kalutara, Sri Lanka.
Reviewers:
(1) Mostafa Abbas Shalaby, Cairo University, Egypt.
(2) Aina, Oluwagbemiga Olanrewaju, Nigerian Institute of Medical Research, Nigeria.
(3) Leonardo Basco, Aix-Marseille Université, Marseille, France.
Complete Peer review History: http://www.sciencedomain.org/review-history/20664

Original Research Article

Received 24th June 2017
Accepted 25th July 2017
Published 25th August 2017

ABSTRACT

Background: The introduction of Artemisinin Combination Therapy by WHO was done to prevent drug resistance to malaria. Unfortunately, these drugs are unaffordable to most of the people living in malaria endemic areas; therefore the use of medicinal plants is common among the people living in malaria endemic areas. Among the medicinal plants used for the treatment of malaria are *Anogeissus leiocarpus* and *Terminalia avicennioides*.
Aim: This work studied the combination therapy of methanolic leaf extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* on parasitemia count and its effects on the liver function,

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body weight and lipid profiles in mice infected with *Plasmodium berghei*.
Methodology: Mice used for this study were divided into six groups. The first group was neither infected with *Plasmodium berghei* nor treated with any drugs (normal control). The second group was infected with *Plasmodium berghei* but not treated (negative control), the third group was infected and treated with artemether-lumefantrine at 5 mg/kg body weight (positive control). The fourth, fifth and sixth groups were infected and treated with 100, 200 and 400 mg/kg body weight of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* respectively. The parasitemia was monitored for four days and parasite was counted using microscope. All biochemical parameters were determined.
Results: There was significant increase ($P<0.05$) in the parasite density in negative group when compared with other groups. Parasitaemia counts were significantly reduced ($P<0.05$) in the mice treated with 400 mg/kgbdwt when compared with other infected groups. HDL and body weight of experimental animal used, were significantly higher ($P<0.05$) in the group treated with 100 mg/kgbdwt when compared with the group treated with 400 mg/kg, while liver enzymes activities were significantly higher ($P<0.05$) in the group treated with 400 mg/kg.
Conclusion: Although the antiplasmodial activity of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* was higher at 400 mg/kg body weight but its effect on liver function, body weight and lipid profile was best at 100 mg/kgbdwt.

Keywords: Malaria; combination therapy; medicinal plant; Anogeissus leiocarpus and Terminalia avicennioides; liver function; lipid profile.

1. INTRODUCTION

Malaria infection has been one of the major infectious diseases that painstakingly affect people, especially in the tropical and sub-tropical regions of the world [1]. The historical background of malaria springs from its ancient origin as a zoonotic disease in the primates of Africa through to the 21st century [2]. It is estimated that malaria is responsible for nearly 367,000 deaths each year, mostly of African children aged below 5 years [3]. The remedy against malaria infection has been sought for, for over a decade and through concerted efforts its effect on human has been drastically reduced [4]. The most important aspect in the reduction of malaria infection is the quick diagnosis of it. Although diagnosis with polymerase chain reaction is the most sensitive and effective method, this laboratory tool is commonly used in research laboratories and cannot be found in the rural areas where malaria is endemic.

The discovery of chloroquine as the main drug to treat malaria infection in the sixties brought hope to the world before the report of drug resistance was confirmed [5]. The resistance then called for more search of drugs that are potent against malaria parasite. The introduction of Artemisinin Combination Therapy (ACTs), by WHO was done to prevent drug resistance by the malaria parasite against Artemisinin, its derivatives and their drugs partners [6,7]. Unfortunately, ACT treatment failures have been reported especially in some malaria endemic countries, and this is not unconnected with drug abuse which is very common in the malaria endemic countries [3]. In addition, these drugs are expensive, limiting their use in a population with average annual income around $100 [7]. Therefore the use of traditional and less expensive preparations is common among people living in malaria endemic regions [8].

Historically, many drugs that are effective against parasitic diseases stem from traditional medicine, such as quinine and artemisinin [9]. Today, 30% of drugs on the pharmaceutical market or companies came from nature, and medicinal plants constitute a popular source of potential antimalarial agents [10,11]. It has been estimated that 25% of the modern medicines are made from plants, first used traditionally [12]. WHO has supported the use of the medicinal herbs for the treatment of malaria provided they are potent [8].

Among the medicinal plants used traditionally to treat malaria, *Anogeissus leiocarpus* and *Terminalia avicennioides* have been proved to be potent and they have been used for the treatment of other parasitic diseases in the tropics, especially Africa [13,14,15]. The mono-therapy efficacy of methanolic crude extract of these two plants against malaria infection and their adverse effects had been extensively studied in our previous studies. These studies have confirmed that these two plants have

antiplasmodial activities when used singly [8,16,17,18]. It has been reported that malaria infection could be responsible for kidney and liver dysfunction and sometimes it affects lipid metabolism in the body as well [18,19]. Apart from malaria infection, the dysfunction of some organs in the body could also be affected by the drugs used during the treatment of malaria infection. It has been reported that the dosage used could have effect on the function of liver and kidney and in some other organs in the body [6,18,20,21]. Renal dysfunction in malaria infection could be determined by the measurement of plasma creatinine level, while the level of AST and ALT could be used as indication of liver dysfunction. Though, some studies and our previous studies have shown antiplasmodial activity of *Anogeissus leiocarpus* and *Terminalia avicennioide* as monotherapy [8,13,16], but there is no report of any study on antiplasmodial activities of the combination therapy of methanolic leaf extracts of *Anogeissus leiocarpus* and *Terminalia avicennioide*. With the advocacy of WHO for combination therapy, this work therefore studied the antiplasmodial activity of combination therapy of methanolic leaf extracts of *Anogeissus leiocarpus* and *Terminalia avicennioide* in mice infected with *P. berghei* and its effect on lipid profile and liver and kidney dysfunction.

2. MATERIALS AND METHODS

2.1 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 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.

2.2 Parasite Acquisition

The parasite used (*Plasmodium berghei* NK 65) was donated by Professor O. G. Ademowo from Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria. The parasites were maintained in the animals by serial passage of blood collection from a patent donor to naïve recipient.

2.3 Plant Materials

The leaf of *Anogeissus leiocarpus* (locally called *Ayin*) and the leaf of *T. avicennioides* (locally called Udi) were 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 UIH22318 *Anogeissus leiocarpus* and UIH22319 *T. avicennioides* were deposited at the Herbarium unit of the University of Ibadan, Ibadan, Nigeria.

2.4 Plant Extracts

Anogeissus leiocarpus leaves and *Terminalia avicennioides* leaves were plucked and air dried at room temperature and was later ground into powder. 300 g of both leaves powder was soaked into 1000 ml of methanol separately for 72 hours. The extract was filtered and evaporated to dryness with a rotary evaporator. 1.17 g of each plant methanolic leaf extracts was diluted into 65 ml of distilled water to make the solution for treatment. The calculation was based on the quantity of solution that was needed to treat the entire experimental animals used.

2.5 *In-vivo* Antimalarial Assay

Thirty six Swiss albino mice weighing from 18 – 21 g were distributed into six groups, each group comprised six animals. The first group (normal control) was not infected with *Plasmodium berghei,* while other groups were infected intraperitoneally with an aliquot of 0.2 ml of standard inoculum ($1x10^7$ *Plasmodium berghei strain* NK 65 parasitized erythrocytes). The second group was not treated (negative control). The third group was infected with the parasite and treated with 5 mg/kg body weight of artemether-lumefantrine (positive control), the fourth, fifth, and sixth groups were infected and treated with 100, 200, and 400 mg/kg body weight of combined methanolic leaf extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus* respectively. All treatments were orally administered once daily with the intubator for four consecutive days. Blood was taken from the tail vein of the mice before treatment once daily to assess the parasitaemia levels. 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. On the fifth day of treatment, mice were dazed using chloroform. Blood was collected into EDTA and plain bottles by heart puncture; part of the blood in the EDTA bottles was used to determine the final parasitaemia levels. 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 carried out. All the biochemical parameters were carried out from the serum collected in the plain bottles.

2.6 Parasitological Study

On each consecutive days of treatment, blood was collected from the tail vein of the infected mice and both thick and thin smears were prepared on a microscope slide and were labelled accordingly. The thin film was fixed with 30% methanol. Staining was done with 10% Giemsa stain and the slides were viewed under light microscope with X100 magnification, malaria parasites were counted. The number of parasite counted per 200 white blood cells was recorded and used to calculate parasite density assuming 8000 leucocytes/µl of blood.

2.7 Determination of Weight Gain

The initial weights of mice were taken before the animals were infected with the parasite. The weight was taken on each day before the treatment and the final weight was taken on the last day before the animals were sacrificed. Weight gained was calculated by deducting initial weight from the final weight.

2.8 Biochemical Assay

Serum total triglycerides concentration was measured by the Tietz [22] method, as described in the manual of the Randox Total triglycerides kit. Serum total cholesterol level was measured by the Trinder [23] method, as described in the manual of the Randox Total cholesterol kit. Serum HDL-cholesterol concentration was measured by the NIHCDS [24] method, as described in the manual of the Randox HDL-cholesterol kit. Serum LDL- cholesterol level was calculated using Friedewald formula: LDL= (TC – HDL) – (TG/5.0) [25]. The creatinine level was measured by the method described by Narayanan and Appleton [26] Alanine aminotransferase (ALT) and Aspartate aminotransferase were determined by the Christen et al. [27] methods as described in the manual of commercial randox test kits specific for the test. All these biochemical parameters were estimated using semiautoanalyzer (Photometer 5010V5+, Germany.

2.9 Statistical Analysis

The differences among the groups were analysed by the one-way analysis of variance and the significant test was done using Microsoft excel 2007 and SPSS 17.0 software and descriptive and inferential statistics for this analysis. The results were expressed as Mean± Standard Error (SE), where the ANOVA level of significance was considered as $P<0.05$.

3. RESULTS

Table 1 shows the effect of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicenniodes* on parasitaemia levels in mice infected with *P. berghei*. The increase and suppression in parasitaemia levels were compared with the initial parasite density counted in day 1 before the commencement of the treatment. Among the negative control group, the mean parasitaemia level was significantly increased from 980.0±32.02 in day 1 to 1568.0±26.0 in day 5. While the mean parasitaemia levels in the positive control was significantly reduced from 1401.5±34.0 in day 1 to 252.27±21.0 in day 5. The parasitaemia levels was significantly reduced from 1328.0±23.0 in day 1 to 239.04±20.0 in day 5 in the group treated with 100mg/kg body weight of combined extracts of *A. leiocarpus* and *T. avicenniodes.* There was also a significant reduction ($P<0.05$) in mean parasitaemia levels in the group treated with 200 mg/kg body weight from 1143.0 ± 24.0 in day 1 to 102.87±21.0 in day 5, while the parasitaemia levels was reduced to 00.00 in day 5 in the group treated with 400 mg/kg body weight.

Fig. 1 shows the weight gained by the experimental animal. The study showed that the mean body weight gain by the negative control group was significantly lower than the mean body weight gain by the normal control group and the group treated with 100 mg/kg body weight of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicenniodes*. Among the treated groups, the mean body weight gain in group treated with 100 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicenniodes* was significantly higher ($P<0.05$) than those treated with 200 mg/kg and 400 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicenniodes*.

Table 2 shows the effect of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* on some lipid profile. The study shows that the HDL levels was significantly higher (P<0.05) in the normal control group than in all other groups, while it was significantly reduced in the group treated with 400 mg/kg of combined *A. leiocarpus* and *T. avicennoides* when compared with other groups. Among the groups treated with combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennoides*, the HDL level was significantly reduced (P<0.05) in group treated with 400 mg/kg when compared with the groups treated with 100 and 200 mg/kg of *A. leiocarpus* and *T. avicennoides*. The HDL level was not significantly higher in the group treated with 100 mg/kg than in the group treated with 200 mg/kg. The level of triglyceride was significantly lower in the group treated with 400 mg/kg than in all other groups studied. There was significant increase in the triglyceride level in the group treated with 200 mg/kg of *A. leiocarpus* and *T. avicennoides* when compared with other groups treated with combined extracts of *A. leiocarpus* and *T. avicennoides*. The LDL level was significantly higher in the negative control than in all other groups, while it was significantly reduced in the group treated with 400 mg/kg when compared with other groups studied. The total cholesterol was also significantly reduced in the group treated with 400 mg/kg when compared with all other groups, while it was highest in the group treated with 200 mg/kg combined extracts of *A. leiocarpus* and *T. avicennoides*.

Table 3 shows that creatinine level was significantly higher (P<0.05) in the groups treated with 100, 200 and 400 mg/kg combined extracts of *A.leiocarpus* and *T. avicennioides* than in the normal control. There was significant increase in the creatinine levels in the groups treated with 200 and 400 mg/kgbdwt when compared with the group treated with 100 mg/kg of *A. leiocarpus* and *T. avicennioides*, The level of AST and ALT was also significantly higher in the group treated with 100, 200 and 400 mg/kg of *A. leiocarpus* and *T. avicennioides* when compared with the normal control, but among the groups treated with combined extracts of *A. leiocarpus* and *T. avicennioides*, the AST and ALT levels were significantly higher in the group treated with 200 and 400 mg/kg when compared with the group treated with 100 mg/kg of combined methalonic leaf extracts of *A. leiocarpus* and *T. avicennioides*.

4. DISCUSSION

Drug resistance by malaria parasites has made the eradication of malaria infection more difficult in the regions where it is endemic. Several methods had been adopted by WHO such as vaccination, using of treated mosquito net, introduction of presumptive treatment among the most susceptible groups etc. All these were done purposely to minimize the infection to nothing, though there is a reduction in the prevalence of malaria infection [5], but malaria is still posing a serious threat in some regions where it is endemic [6]. WHO at a time encouraged the use of local medicinal plant for the treatment of malaria infection and this was a laudable idea which really helps to combat malaria infection locally at a very cheap or no cost. While this is adopted there is a need to monitor the efficacy of those medicinal plants used locally and also to know its toxicological effect on people. Our group had conducted a research on antiplasmodial activities of two plants (*A. leiocarpus* and *T. avicenniodes*) which are traditionally used to treat malaria infection. In our study, it was confirmed that these plants have antiplasmodial activity and its efficacy was encouraging at higher dosage but the level of toxicity was also very high at the higher dosage [8,13,14,18].

Table 1. Effect of treatment with combined methanolic leaf extracts of *A. leiocarpus* and *T. avicenniodes* on parasitaemia levels in mice infected with *P. berghei*

Day	Negative control	Positive control	MP+100 mg/kg	MP+200 mg/kg	MP+400 mg/kg
1	980.0± 32.0	1401.5±34.0	1328.0±23.0	1143.0±24	1332.66±32.0
2	1136.8±44.0	1121.2±20.0	1142.02±21.0	891.54±25.0	933.1±14.0
3	1097.6±23.5	910.98±23.0	1029.28±20.0	537.21±24.0	479.88±24.0
4	1274.0±22.0	574.62±22.0	783.52±21.0	228.60±21.0	199.95±32.0
5	1568.0±26.0	252.27±21.0	239.04±20.0	102.87±21.0	00

**Treatment started in day 1, therefore the parasitaemia levels recorded on day 2 to day 5 were compared with day1; **MP represents Malaria parasite*

!Negative control was infected but not treated; Positive Control was infected and treated with 5 mg/kg of artemether-lumefantrine; All the test groups were infected and treated with combined leaf extracts of A. leiocarpus and T. avicenniode

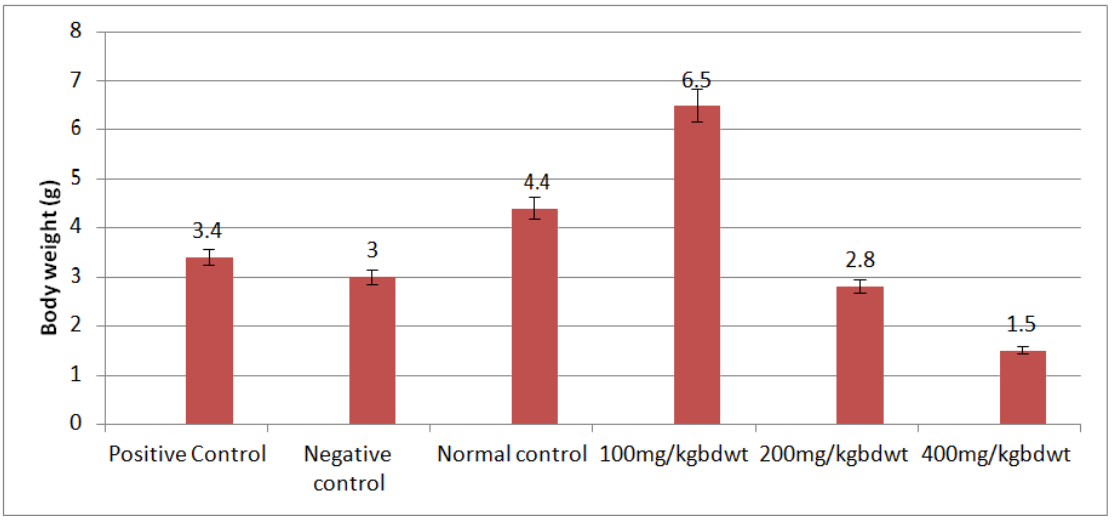

Fig. 1. Effect of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* on the body weight gained by mice infected with *Plasmodium berghei*

**bars represents standard error means*

***kgbdwt means kilogram body weight*

Table 2. Effect of treatment of combined methanolic leaf extracts of *A. leiocarpus and T. avicennioides* on lipid profile in mice infected with *P. berghei*

Treatment	HDL	Triglycerides	Lipid profile LDL	Total cholesterol
Normal control	1.34±0.11^{a}	1.47±0.07^{a}	0.95±0.05^{a}	2.97±0.38^{a}
Positive control	0.79±0.10^{b}	1.50±0.19^{a}	1.11±0.01^{a}	2.58±0.00^{a}
Negative control	0.53±0.13^{bc}	1.23±0.17^{a}	1.90±0.02^{b}	2.61±0.13^{a}
100 mg/kgbdwt	0.95±0.10^{b}	1.30±0.04^{a}	1.22±0.26^{a}	2.64±0.10^{a}
200 mg/kgbdwt	0.92±0.12^{b}	1.49±0.02^{a}	1.12±0.17^{a}	3.01±0.47^{a}
400 mg/kgbdwt	0.34±0.12^{bc}	0.68±0.24^{b}	0.57±0.62^{a}	1.22±1.34^{a}

**Normal control group was not treated and infected with P.berghei*

**Positive control was infected with P.berghei and treated with 5mg/kgbdwt (kilogram/body weight) of artemether-lumefantrine*

***Test groups were infected with P.berghei and treated with combined methanolic leaf extracts of A.leiocarpus and T. avicennioides.*

**** All parameters were measured in mmol/l*

Table 3. Effect of treatment of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* on Kidney and liver function in mice infected with *P. berghei*

Treatment	Creatinine (µmol/L)	AST (µL)	ALT (µL)
Normal control	44.9±0.03a	102.9±0.03a	43.9±0.18a
100 mg/kgbdwt	44.1±0.04a	119.5±0.21bc	77.9±0.11b
200 mg/kgbdwt	44.0±0.14a	498.6±0.17bd	97.3±0.27bc
400 mg/kgbdwt	49.0±0.24b	560.6±0.62be	124.3±1.3bd

**Normal control group was not treated and infected with P.berghei*

**Test groups were infected with P.berghei and treated with combined methanolic leaf extracts of A.leiocarpus and T. avicennioides*

Traditionally, extract of leaves of different medicinal plants are usually combined together for the treatment of malaria infection. This act is also in line with the recommendation of WHO which supports combination therapy of antimalarial drugs in order to prevent insurgence of drugs resistance by the malaria parasites. Therefore, this work studied the efficacy of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennoides* in mice infected

with *P. berghei* to prove their potency against mono-therapy of different medicinal plants.

The result showed that there was a drastical suppression of the parasitaemia levels in day 5 in all the groups treated with combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennoides* when compared with the level of parasitaemia in day 1 (Table 1). The level of suppression in this study was not different from the previous studies where efficacy of each of the plants was considered separately [8,13,16]. This showed that the two plants may possibly consist of the same phytochemical such as tannin, alkaloid, saponins, flavonoid and anthraquinones. The rate of inhibition was higher in the group treated with 400 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennoides* (Table 1). The study also revealed that the rate of parasite growth inhibition is dosage dependent. This agrees with previous study [10,27,28], The increase in parasite inhibition rate in the group treated with 200 mg/kg and 400 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennoides* when compared to the positive control group may be that the groups treated with 200 mg/kg and 400 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennoides* contained higher secondary metabolites at that higher concentration which could be responsible for the destruction of malaria parasites [29].

The effect of treatment at different dosage on the body weight of mice used was also studied. Though the results showed that the parasite chemo-suppression was lower in the group treated with 100 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* when compared with those treated with 200 and 400 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennoides,* but the body weight gain was significantly higher in the infected mice treated with 100 mg/kg than in all other groups (Fig. 1). The increase in the weight gain in this group could be because of the reduction of oxidative damage which has been reported to be dose related [8]. The weight gain was least in the group treated with 400 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennoides*.

The role of malaria infection and effect of drugs used on the changes in lipid metabolism cannot be overemphasized. It has been reported that malaria parasite induces changes in the serum lipid profile [30]. Our study showed a significant reduction in the HDL level in the negative control when compared with other groups except in the group treated with 400 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* (Table 2). This shows that the level of malaria parasite could affect the HDL level in the body [31,32]. Though the parasitaemia was significantly reduced in the group treated with 400 mg/kg in this study, but there was a significant reduction in HDL level also in this group than in other groups treated with combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* (Table 2). The high parasitaemia and increase in HDL levels in the group treated with 100 mg/kg of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* shows that the reduction in HDL level may not be determined by the level of parasitaemia alone, but other factors such as dosage of drugs taken for the treatment may also be responsible for it (Table 2). This result was in agreement with other studies which showed that high dosage of drugs could cause damage to lipid metabolism in the body [31,33]. The significant reduction in triglyceride, LDL and total cholesterol levels in the group treated 400 mg/kg in this study showed that taken the combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* at the high dosage could have serious effect on the lipid metabolism in the body.

While advocating for the use of medicinal plants to treat malaria infection, it is also necessary to consider the toxic effect of such medicinal plants at different dosage. Therefore this study also considered the effect of combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* on liver and kidney functions. Hepatic dysfunction is one of the complications characterized by malaria infection. The sudden increase in liver enzyme activities such as ALT and AST in the serum of malaria patients could be used to diagnose hepatic dysfunction [32]. Our study revealed that there was significant increase in the serum AST and ALT levels in the groups treated with combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* when compared with the normal control (Table 3). This showed that combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* has a negative effect on the liver of the experimental animal and this can lead to liver dysfunction. The significant increase in the AST and ALT in the groups treated with 200 and 400 mg/kg in this study when compared with the group treated with 100 mg/kg showed that effect of combined methanolic leaf extracts of

A. leiocarpus and *T. avicennioides* on liver dysfunction is dose related. The increase in the creatinine level among the treated group when compared with normal control is also an indication that the combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* is also capable of causing kidney dysfunction.

5. CONCLUSION

Though combined methanolic leaf extracts of *A. leiocarpus* and *T. avicennioides* antiplasmodial activity was higher at very high dosage but its effect at that dosage could lead to liver and kidney dysfunction.

CONSENT

It is not applicable to this study.

ETHICAL APPROVAL

All authors hereby declare 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.

ACKNOWLEDGEMENT

The authors acknowledge the contribution of Professor O.G. Ademowo, Institute for Advanced Medical Research and training, College of Medicine, University of Ibadan, Ibadan, Nigeria, for donating parasite during this study. The effort of Dr. Obembe of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo state who helped us to identify the medicinal plants used in this study is appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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