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The assessment of serum liver enzymes in malaria induced wistar rats treated with the crude extract of *Artemisia annua* and Artemisinin combination therapy

Ekam VS[1*], Ukpanukpong RU[2], Ihe SO[3], Odio SB[4], Ekam NV[5]
[1-5] Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria

Abstract
In this study, serum liver enzymes were assessed in malaria induced wistar rats treated with the crude extract of *Artemisia annua* and artemisinin combination therapy (ACT). Twenty four (24) rats used were divided into four (4) groups of six rats each weighing between 180 – 220g. Group 1 (control) and 2 (malaria untreated) received 1ml of distilled water while group 3 (ACT treated) and 4 (*A. annua treated*) received 1.43mg/kg and 300mg/kg of ACT and *A. annua* extracts respectively. Treatment was administered twice a day for three consecutive days, after which blood was taken through cardiac puncture into treated sample bottles for analysis. The results for the liver enzymes showed that there was a significant increase ($P<0.05$) in AST levels of all the experimental groups when compared with the control, however ALT levels showed no significant changes ($P>0.05$) when compared with the control. The serum ALT levels of both the malaria untreated (86.83±1.42) and *A. annua* treated group (91.67±1.43) were significantly lower ($P<0.05$) when compared with the control group (107.00±1.53), while that of the ACT treated group (127.33±2.04) was significantly higher ($P<0.05$) than the control.

Keywords: Liver enzymes, *Plasmodium*, aspartate aminotranferase, alkaline phosphatase, alanine aminotranferase, *A. annua*

Introduction
Malaria an infectious disease of mankind and other animal is now a global burden. It has been reported that malaria has one of the greatest morbidity and mortality rate when compared with other infectious diseases (World malaria report, 2005; WHO, 2000) [11, 14]. Malaria is caused by a single celled protozoon called *Plasmodium* which develops sexually and asexually in mosquito. The implicated species are *P. falciparum, P. ovale, P. vivax, P. malaria* and *P. knowlesi*. However, *P. knowlesi* rarely causes disease in humans. Most fatalities caused by malaria are credited to *P. falciparum.* Among many conditions, malaria contributes to poverty and results in poor pregnancy outcomes. The asexual stages of development in *Plasmodium* are sporozoites, merozoites, trophozoites and schizonts. The parasite is basically transmitted through the bite of an infected female anopheles mosquito. On biting a healthy individual, the symptoms usually follow about 8 to 25 days and they include fever, headache, dizziness, nausea, vomiting, joint pains, dry cough, etc (Fairhust *et al., 20*10).
Artemisia annua commonly known as sweet wormwood is a popular plant used in the treatment of various ailments. The leaves are widely used for the treatment of malaria (Rezelman and Gloris, 2008) [7]. *A. annua* can also be used as an immune system booster because of the antiviral and anti-fungi properties it possesses. Artemisinin the active ingredient of *A. annua* has been used in the treatment of several diseases such as malaria, circulatory disorders, inflammatory conditions, common cold, fever, stomach upset, pains, constipation, cancer, fungal infection, bacterial infections and even viral infections. Artemisinin and its derivatives are important anti-malarial agents which are effective against nearly all the developmental stages of the parasite. Artemisinin is presently the last line of defense against malaria parasite. Serum liver enzymes are essential in amino acid metabolism and other essential metabolic reactions processes. Their presence or absence in some organs and / or tissues and the increase or decrease of the levels of serum liver enzymes in tissues serves as indicators of certain prevailing medical conditions. Knowing that malaria affects both the red blood cells and the liver cells, the effect on liver cells could affect the levels of liver enzymes and there are scarcity of information on the effect of anti-malarial drugs on the serum liver enzymes. As a result of the paucity of such information, it therefore became necessary to investigate the effect of the crude extract of *A. annua* and artemisinin combination therapy (ACT) on serum liver enzymes in malaria induced wistar rats.

Materials and methods
Collection and preparation of materials
ACTs, Dextrose and Distil Water: The anti malarial drug zymal® (Artemether Tablet 80mg + Lumenfantrine Tablets 480mg) manufactured by Innova CapTab, Pharmaceutical Co Ltd, 81-B EPIP, Phase-I, Jharmajri, Baddi (H.P) India. Manufacturing Lic. No.: MNB/06/394, Distilled water and 5% dextrose water was bought from Turtle Bay pharmacy in Calabar.
Plant material: The leaves of *Artemisia annua* was collected from the biotechnology farm owned and operated by Prof. Ebiamadon Andi Brisibe, of the Department of Genetics and Biotechnology, Faculty of Science, University of Calabar. It was taken to the Botany Department of the University for Identification and specimens were deposited at the department's herbarium.
Parasites: The strain of *Plasmodium falciparum* used for this study was obtained and authenticated by the Calabar office of Roll Back Malaria.
Laboratory animals: A total of 24 Inbreed adult male and

female wistar albino rats weighing between 180 - 220g were used for this study, they were purchased from the animal house unit of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, and were housed in a well ventilated wooden cages in the animal house, and were fed with rat pellets (growers' marsh manufactured by Vital feeds Ltd, Lagos) and tap water *ad libitum.* The animals were acclimatized for three weeks and their body weights noted before and after the commencement of the experiment. The animals were divided into four groups, based on their weights as shown in table 1.

Innoculation: The infection of the recipient rats was initiated by injection of the parasites preparation authenticated by the Calabar office of Roll Back Malaria to healthy test rats via intramuscular route as described by David *et al* (2004) [1] and Peter and Anatoli (1998) [5]. 2ml of *Plasmodium falciparum* sample with a parasite load of 161.5 was diluted with 5% dextrose water using a dilution factor of 1:4 (Shakya *et al.*, 2012) [8]. 0.5ml per kilogram of body weight of the diluted plasmodium base solution was subsequently injected into the animals in group 2, 3 and 4 via intramuscular method (David *et al.*, 2004) [1].

Determination of Degree of Parasitaemia: The CareStart™ Malaria HRP2 Pf (Cat #: G0141) test kit, manufactured by Access Bio, Inc. 65 Clyde Road, Somerset, NJ, 08873, USA, was used to investigate the level of infection in the groups of rats that were inoculated with the malaria parasites. 48 hours after inoculation, a drop of blood was collect from the tails of the infected rats and tested for the presence of *plasmodium* according to the method describe by the manufacturer.

Administration of Drug: The antimalarial drug, zymal® (Artemether 80mg + Lumenfantrine 480mg) tablet was used as the artemisinin combination therapy. It was powdered in a mortar, mixed with 50ml distilled water and administered as aqueous suspension by oral gavage at a dose of 1.143mg/kg body weight twice a day for three consecutive days.

Administration of Extract: 40g of the powdered *Artemisia annua* leaves was socked in ethanol for 12 hours and filtered thereafter. The filtrate was further filtered using a watman filter paper and then concentrated by evaporation using a water bath at 40^{o}C. The 40g of powdered *A. annua* leaves yielded 2.9g of extract. The crude extract was administered to group 4 animals at a dose of 300mg/kg body weight twice a day for three consecutive days.

Collection and preparation of tissue for analysis: After 3 days of treatment, the rats were weighed and fasted overnight. Blood samples were collected from the untreated, treated and control groups for investigation of lipid profile. The animals were anaesthetized with trichloromethane (chloroform). They were then dissected and blood samples were collected through cardiac puncture using sterile syringes into screw cap sterile test tubes.

Estimation of serum liver enzymes: the aspartate aminotransfearse (AST) and alanine aminotransferase (ALT) were estimated using randox kit as described by the manufacturer (Reitman and Frankel, 1957) [6]. AST level is measured by monitoring the concentration of oxaloacetate hydrozone formed with 2, 4-dinitrophenylhydrazine.

α–oxoglutarate + L-aspartate $\xrightarrow{GOT}$ L-glutamate + oxaloacetate.

The ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

α-oxaloacetate + L-aspartate $\xrightarrow{GOT}$ L-glutamate + oxaloacetate.

While alkaline phosphatase (ALT) activity was measured using randox kit colorimetric method as described by the manufacturer (DGKC, 1972) [2].

P-nitrophenylphosphate $+H_2O \xrightarrow{ALP}$ PHOSPHATE + P-nitrophenol.

This method is based on the interaction of P-nitrophenylphosphate with water to form a coloured complex whose absorbance is read at 405nm.

Statistical analysis: Data was expressed as mean ± standard error of mean. The data obtained were analyzed statistically using one-way analysis of variance (ANOVA) at a 95% (0.05) probability level.

Results

Table 2 shows the baseline values of serum liver enzyme, AST (iu/l), ALT (iu/l), ALP (iu/l).

Effects of treatment on AST activities: the result presented in table 2 showed that the AST activities of the experimental animals in the malaria untreated group (160.17±5.71), ACT treated group (158.67±2.49) and *A. annua* treated group (193.00±4.08) were all significantly higher ($P<0.05$) when compared with the control (132.17±8.64). However, the *A. annua* treated group vary significantly from both the malaria untreated and ACT treated groups at $P<0.05$.

Effects of treatment on ALT: the result also showed that the ALT activities on the experimental animals in both the malaria untreated group (83.67±4.29), ACT treated, group (87.17±6.33) and *A. annua* treated group (78.33±4.18) showed no significant difference ($P>0.05$) when compared with the control.

Effects of treatment on ALP: the result in table 2 further showed that the ALP activities in the malaria untreated group (86.83±1.42) and *A. annua* treated group (91.67±1.43) were significantly lower ($P<0.05$) when compared with the control (107.00±1.53) while the ACT treated group (127.33±2.04) was significantly higher ($P<0.05$) when compared with the control. More so, the *A. annua* treated group is significantly higher ($P<0.05$) than the malaria untreated group but lower that the ACT treated group, while the ACT treated group is significantly higher ($P<0.05$) than the malaria untreated group.

Discussions

There was a significant increase in the AST levels in all the experimental groups when compared to the control. The ALT levels of group 2, 3 and 4 showed no significant difference when compared with the control. Also, a decrease in the ALP levels were observed in the malaria untreated and *A. annua* treated groups while that of the ACT treated group showed an increase in the serum ALP and AST levels which suggest some levels of hepatoxicity by the drugs. This is because hepatotoxicity is often associated with elevated levels of AST, ALT and ALP in blood (Goodman and Gilman, 1990) [4]. Vasudevan and Skrecumari (2007) [9] also reported that increased serum levels of AST, ALT and ALP have been noticed among other liver diseases like cirrhosis and hepatitis following an exposure to a toxic substance such as anti-malarial drugs and drinks. On the other hand, a significant decrease in the serum ALP levels

was noticed in the *A. annua* treated group which suggest that the increased AST may not depict a liver damage or disease but could come from other organs were AST can be found.

Table 1: Experimental protocol

Groups	Numbers of rats	Treatments
1	6	Normal control
2	6	Malaria infected and untreated
3	6	Malaria infected and ACT treated
4	6	Malaria infected and *Artemisia annua* treated

Experimental group distribution of wistar albino rats during treatment with antimalarial drug (ACT) and extract from *Artemisia annua* leaves.

Table 2: Baseline values of serum liver enzymes Effects of the crude extract of *A. annua* and Artemisinin Combination Therapy (ACT) on serum liver enzymes in malaria induced wistar rats

Treatment groups	AST (M/1)	ALT (iuA)	ALP (iu/1)
1.(Control)	132.17±8.64	82.83±1.96	107.00±1.53
2.(Malaria untreated)	160.17±5.7 P	83.67±4.29	86.83±1.42a
3.(ACT treated)	158.67±2.49a	87.17±6.33	127.33±2.040
4.(A. annua treated)	193.00±4.08aAc	78.33±4.18	91.67±1.43aAc

Values expressed as Mean±SEM, n=6, P<0.05
a significantly different from control (group 1) at P<0.05
b significantly different from group 2 at P<0.05.
c significantly different from group 3 at P<0.05.

Conclusions

At the end of the study, the *A. annua* treated group showed a general decrease in serum enzyme levels relative to the control group except for AST which implies that *A. annua* extract may not really have any hepatotoxic effect. Furthermore, no liver damage could be suspected from the use of the crude extract of *A. annua* and its derivatives as judge from the levels of the serum liver enzymes. Therefore, the use of the leaves in areas where the ACTs are not readily unaccessible should be encouraged.

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