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Effect of Artemisinin with Folic Acid on the Activities of Aspartate Amino Transferase, Alanine Amino Transferase and Alkaline Phosphatase in Rat

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Abstract: Studies on the effect of artemisinin alone and artemisinin with folic acid on the activities of aspartate amino transferase (ASAT), alanine amino transferase (ALAT) and alkaline phosphatase (ALP) in the serum of male Wistar rats were carried out. Different groups of rats (8 group⁻¹) were orally given 0.75, 1.50, 3.00 and 6.00 mg kg⁻¹ b.wt. of artemisinin. Each of these doses was also administered concurrently with 1.50 mg kg⁻¹ of folic acid, respectively. Artemisinin only elevated the activities of serum ASAT, ALAT and ALP significantly at the four dose levels. When 1.50 mg kg⁻¹ of folic acid was concurrently administered with artemisinin, the elevated serum level of ASAT, ALAT and ALP was significantly reversed almost completely at low dose of 0.75 and 1.50 mg kg⁻¹ artemisinin. Folic acid only reversed the elevated activity of ASAT, ALAT and ALP by artemisinin partially when the dose of artemisinin was high (i.e., 3.00 and 6.00 mg kg⁻¹ of artemisinin). These results suggest that folic acid offers complete relief to metabolic disorders at low artemisinin concentration while the relief is partial at high concentrations.

Key words: Enzyme activity, folic acid, artemisinin, antimalarials

INTRODUCTION

Malaria is transmitted to human by plasmodium through mosquito bite. Despite increasing research and control efforts malaria still remains major health problem malaria is the most important vector born disease (Curtis, 1993). About 400 million people experience clinical episodes and 2 million deaths occur annually with about 90% mortality found in tropical Africa and Southeast Asia where the disease is prevalent (WHO, 1993; Coker *et al.*, 2001).

These regions of the world are characterized by optimum conditions of temperature, heavy rainfall and dense vegetation which promote the survival of the *Plasmodium* species (Curtis, 1993).

The characteristics of malaria disease include shaking, chills and relapsing fever (Maegraith and Fletcher, 1992). It is associated with pulmonary edema renal failure, tropical splenomegaly syndrome and nephritic syndrome (Guyton and Hall, 1996).

During the First World War, the first antimalarial drug, Dichloro-Diphenyl Trichlorethane (DDT) was used (Alvin *et al.*, 1978). It was latter discovered to be a major environmental pollutants and was later banned (Peters, 1990). Chloroquine is the cheapest and the most widely available first line drug. The spread and intensity of resistance by the malarial parasite to chloroquine and other antimalarials such as mefloquine including combination drugs (Molta *et al.*, 1993; Olliaro and Trigg, 1995; Sowunmi *et al.*, 1996) necessitated the search for new drugs. This efforts led to the choice of artesunate which is obtained from the plant, *Artemisia annua* (Woedenbag *et al.*, 1994) and from which the antimalarial principal (artemisinin) was discovered in 1971 (Liy and Wu, 1998).

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Artemisinin works by destroying the cell membrane of the parasite slows down protein synthesis disorganizes the ribosome, dilate the nuclear envelope and disintegrate the food vacuoles. In the mosquito life cycle it inhibits the development of the trophozoites, kills the schizont of the parasite and prevents progression of the disease (Meshnik *et al.*, 1996). Clinical findings up to date have not revealed any pattern of resistance to artemisinin. It is considered as an effective alternative drug for a more virulent falciparum malaria (Baradell and Fitton, 1995; Karbwang *et al.*, 1994).

Artemisinin is commonly administered with folic acid. The objective of this study is to evaluate the effect of artemisinin and the role of folic acid on the activities of alanine aminotransferases, aspartate aminotransferases and alkaline phosphatases.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing between 150 and 180 g were used. They were fed with grower's mash (Vital Feeds Limited, Lagos) and water *ad libitum* and kept in the animal house of the Faculty of Pharmacy, University of Uyo under standard laboratory conditions.

Drugs/Chemicals

Artemisinin (manufactured by Mekopharm Chemical Company, Vietnam but purchased in Uyo), folic acid (Unique Pharmaceuticals, Lagos, Nigeria) and freshly prepared physiological saline.

Administration of Drugs/Chemicals on the Animals

Seventy overnight fasted rats were divided into 9 equal groups (each containing 8 rats). The drugs were administered orally to the rats once daily for 5 days as shown below:

- **Group A:** Received 0.75 mg kg⁻¹ artemisinin only
- **Group B:** Received 0.75 mg kg⁻¹ artemisinin and 1.50 mg kg⁻¹ folic acid
- **Group C:** Received 1.50 mg kg⁻¹ artemisinin only
- **Group D:** Received 0.50 mg kg⁻¹ artemisinin and 1.50 mg kg⁻¹ folic acid
- **Group E:** Received 3.00 mg kg⁻¹ artemisinin only
- **Group F:** Received 3.00 mg kg⁻¹ artemisinin and 1.50 mg kg⁻¹ folic acid
- **Group G:** Received 6.00 mg kg⁻¹ artemisinin only
- **Group H:** Received 6.00 mg kg⁻¹ artemisinin and 1.50 mg kg⁻¹ folic acid
- **Group I:** Saline water only (control)

Collection of Blood and Preparation of Serum

Overnight fasted rats were sacrificed on the 6th day and blood collected by passing a needle on syringe through the ventricle of the heart.

The serum was prepared from clotted blood by gently decanting the serum from the cells into iron-free centrifuge tubes and the tubes were spun at 10,000 rpm for 5 min in a centrifuge machine (MSE England). The serum was then decanted into another set of clean tubes and stored at 15°C for a period not exceeding 24 h.

Determination of Enzymes

The prepared serum samples obtained from the blood of the sacrificed animals were analyzed. Aspartate amino transferase and alanine amino transferase were estimated using end point calorimetric method as explained in Randox enzyme kits (Reitman and Frankel, 1957). The absorbance of the test solution was measured using an UV-Vis-NIR spectrophotometer with 540 nm at 37°C.

The activity of alkaline phosphatase in the serum was determined using phenolphthalein monophosphate method (Nashar, 1974; Ngaha and Plumer, 1977). The absorbance of the test solution was measured with 540 nm at 37°C. Enzyme activity is given in IU L⁻¹.

Statistical Analysis

The data obtained were expressed as Mean±SD. Student's t-test was used for the analysis the level of significance and p<0.05 was taken to be statistically significant.

RESULTS AND DISCUSSION

The results in Table 1 shows that the activity of ASAT in the serum increases as the dose of the drug increases. At a dose of 1.5 mg kg⁻¹ artemisinin, the activity of ASAT (51.26±2.79) doubled that of the corresponding control value (20.75±4.10). As the dose was increased to 3.0 and 6.0 mg kg⁻¹ artemisinin, the ASAT correspondingly tripled (75.36±5.75) and quadrupled (86.00±1.31) that of the corresponding control value, respectively. This increase was statistically significant (p<0.05). This elevated activity is an indication of liver dysfunction (Murray *et al.*, 1990). Concurrent administration of 0.75 mg kg⁻¹ artemisinin and 1.50 mg kg⁻¹ folic acid reduced the ASAT activity from 46.56±3.52 to 34.50±4.84. This combination did not reduce the ASAT activity to the control value of (20.75±4.10). This shows that folic acid can reverse metabolic disorder associated with low concentration of artemisinin. Concurrent administration of 6.0 mg kg⁻¹ artemisinin with 1.50 mg kg⁻¹ of folic acid and that of 3.00 mg kg⁻¹ artemisinin with 1.50 mg kg⁻¹ folic acid did not reduce the ASAT level significantly. It is probable that the 1.50 mg kg⁻¹ of folic acid administered was inadequate and perhaps a higher dose could have reversed this metabolic disorder.

Alanine amino transferase (ALAT) in the serum decreased with increasing dose of artemisinin. At a dose of 0.75, 1.50, 3.0 and 6.0 mg kg⁻¹ artemisinin the serum ALAT was 24.67±8.39, 22.69, 22.33 and 18.40 IU L⁻¹. The decrease in serum ALAT was dose dependent. Concurrent administration of these doses with 1.50 mg kg⁻¹ of folic acid increased ALAT level to control levels in the first three doses (i.e., 0.75, 1.50 and 3.0 mg kg⁻¹ of artemisinin). However, at the highest dose of 6.0 mg kg⁻¹ of artemisinin the increase in the level of ALAT was lowest. The results of this study showed that artemisinin might cause some metabolic disorders and that folic acid could reverse, partially or completely, this effect.

The Mean serum alkaline phosphatase (ALP) activity of samples treated with 0.75, 1.50, 3.0 and 6.0 mg of artemisinin was 170.23±22.15, 181.01±13.59, 189.76±6.27 and 200.40±16.77 IU L⁻¹, respectively. These values showed a significant dose dependent increase of ALP activity over the control (121.33±20.91). While the concurrent administration of 1.50 mg kg⁻¹ folic acid with 0.75 or 1.50 mg kg⁻¹ artemisinin significantly reduced the elevated value of ALP that of 1.50 mg kg⁻¹ of folic acid with 3.0 and 6.0 mg kg⁻¹ artemisinin did not significantly reduce the elevated ALP activity.

Table 1: The effect of different doses of artemisinin (artesunate) alone and artemisinin with folic acid on serum aspartate amino transferase (ASAT), alanine amino-transferase (ALAT) and alkaline phosphatase (ALP)

Groups	Treatment (mg kg ⁻¹ b.wt.)	ASAT activity (U L ⁻¹)	ALAT activity (IU L ⁻¹)	ALP activity (IU L ⁻¹)
A	0.750 mg ART	46.56±3.52*	24.670±8.39	170.23±22.15*
B	1.50 mg ART+0.750 mg FA	34.50±4.84*	27.090±0.71	158.49±26.79*
C	1.500 mg ART	51.26±2.79*	22.690±4.93*	181.01±13.59*
D	1.500 mg ART+1.500 mg FA	49.50±6.36*	25.000±8.49*	171.68±10.19*
E	3.000 mg ART	75.36±5.75*	320.330±7.09*	189.76±6.27*
F	3.000 mg ART+1.500 mg FA	54.00±4.24*	24.500±0.71*	183.83±16.61*
G	6.000 mg ART	86.00±131*	18.400±2.73*	200.40±16.77*
H	6.000 mg ART+1.500 mg FA	80.61±5.42*	21.000±4.24*	191.23±13.40*
I	Normal saline (control)	20.75±4.10	26.650±3.93	121.33±20.91

ART: Artemisinin, FA: Folic acid, Control: Physiological saline. The results are expressed as Mean±SD. n = 8, *Statistical significance (p<0.05)

Some enzymes act as markers and indicators of disease states. Enzyme levels in the intracellular fluids such as blood form an integral part of diagnosis. Increase in the activities of enzymes in serum is indicative of cell damage. Some disease states also decrease enzyme synthesis leading to reduced enzyme concentration in the serum. ALAT, ASAT and ALP are often present in high concentrations in the liver. ASAT (EC 2.6.1.1) is found primarily in heart, liver, skeletal muscle and kidney whereas ALAT (EC 2.6.1.2) is found primarily in liver and kidney, with lesser amounts in heart and skeletal muscle (Giannini *et al.*, 2005). Liver disease is the most important cause of increased ALAT activity and a common cause of increased ASAT activity. In hepatocellular injury or necrosis they leak into the circulation and raise the serum level of the enzymes. In most types of liver disease, ALAT activity is higher than that of ASAT. Progressive increase in the ASAT/ALAT ratio is correlated with a concomitant decrease in liver function. An ASAT/ALAT ratio of 1 or higher has a good specificity for the diagnosis of cirrhosis (Giannini *et al.*, 2003). The major diagnostic use of ASAT is in myocardial infarction and ALAT in viral hepatitis and acute pancreatitis (Ellis *et al.*, 1978; Reichling and Kaplan, 1988). In inflammatory conditions, there is usually a leakage of cytoplasmic enzymes into the circulation. As such, ALAT is observed to be higher than that of ASAT. In gross necrosis, the level of ASAT may rise above that of ALAT (Giannini *et al.*, 2005).

From the results obtained in this study, it can be inferred that the administration of different doses and most especially, 6.00 mg kg⁻¹ artemisinin to male wistar rats caused significant increase in serum aspartate amino transferase, alkaline phosphatase and alanine amino transferase activities. These effects are indicative of liver problems. Folic acid gave complete relief to the metabolic disorders only at low doses of artemisinin while it effected partial or no relief at much higher doses. The administration of folic acid alongside that of artemisinin in the treatment of malaria is therefore beneficial and advisable.

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