

Hepatotoxicity Potential of Coartemether on Wistar Albino Rat using Liver Enzyme Assay.

Ugokwe C.U.^{1*}, Asomba H.C.¹ and Onwuzulike I.O.²

¹ Department of Zoology, University of Ibadan, Nigeria.

² Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria.

Abstract: Considering the wide use of Coartemether (COARTEM®) majorly in Africa, due to its high efficacy in the treatment of uncomplicated *P. falciparum* malaria, this study elucidated its hepatotoxicity potentials. Forty-eight (48) male wistar albino rats with weight range of 187-245g divided into four groups with two replicates were allowed to acclimatize to laboratory condition for 14 days. Rats in group A served as the control and were administered with distilled water and rat chow throughout the experimental period, while rats in group B, C and D were administered orally with 28, 42 and 56mg/kg/day of Coartemether respectively for six days. Two animals from each group/replicate were sacrificed on day 2, 4, and 6. Blood samples were collected for analysis of serum ALP, AST, ALT and Bilirubin using standard methods and enzyme kits. The result showed a significant ($P < 0.05$) time-dose dependent increase in ALP, ALT, AST and Bilirubin levels in albino rats treated with different doses of coartemether compared to the control. These results are clear manifestations that Coartemether therapy might pose hepatic injury and endanger liver functions via its enzyme systems dysfunctioning.

Keywords: Bilirubin, Coartemether, Hepatotoxicity, Liver enzyme, Malaria.

I. Introduction

Malaria still remains one of the most deadly infections especially in the tropical and subtropical regions of the world despite various control programmes [1]. The World Health Organization in its 2014 World Malaria Report officially stated that an estimated 3.3 billion people in 97 countries and territories are at risk of being infected with malaria and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year) [2]. The burden is heaviest in the African Region, where an estimated 90% of all malaria deaths occur, and in children aged less than 5 years, who account for 78% of all deaths. Effective treatment of malaria has been a major challenge largely due to resistance of the Plasmodium parasite (the causative organism of the disease) to antimalarial agents [3, 4]. One of the chemotherapeutic drugs recommended by the World Health Organization (WHO) in the management of malaria are artemisinin-based combination therapy (ACT) which is proven to be the most effective first-line strategy for the treatment of uncomplicated *P. falciparum* malaria, presently eliciting a high degree of resistance to conventional antimalarial drugs [5, 6].

Coartemether (Artemether-Lumefantrine) is an effective ACT known popularly under the brand names COARTEM® and is used majorly in Africa, due to its high efficacy against the parasite. Coartemether is a fixed dose preparation of Artemether (20mg) and Lumefantrine (120mg) in the ratio 1:6. Artemether has strong blood schizontocidal and moderate gametocytocidal activities and its activities like other artemisinin derivatives, depends on the intact peroxide bridge of the molecule [7]. Lumefantrine is a class II blood schizontocide which inhibits heme polymerization [8]. The combination of artemether, that rapidly reduces parasite biomass, with longer acting lumefantrine, that eliminates residual parasites, has proven to be highly effective in achieving malaria parasitic cure, symptomatic relief and gametocyte carriage reduction [9]. Most common observed adverse effects of Coartemether include gastrointestinal (abdominal disturbance, nausea, anorexia, vomiting and diarrhea) and central nervous system (dizziness, headache) effects. Most of these reported adverse effects which are also typical of clinical symptomatology of acute malaria were rated mild to moderate [9, 10, and 11].

Liver plays a key role in the metabolism of drugs [12] and their enzymes serve as signals for the detection of clinical abnormalities in the body [13, 14]. Enzymes like alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) are very symbolic in disease diagnosis and drug interactions assessment [12]. Fluctuation in liver enzyme levels indicates damage to the liver organ and impaired physiologic function [13, 14]. Since the liver metabolizes drugs, the negative interactive tendencies of such drugs are possible particularly with those that are frequently used in the treatment of malaria. Thus, the use of liver enzymes as markers in determining the tolerance of anti-malarial drugs is very imperative at this period of drug pressure due to increase malaria attacks and high persistence of drug-resistant *plasmodium falciparum* in Africa.

Considering the high efficiency and wide spread use of Coartemether, especially in Nigeria, the knowledge of its hepatic toxicity will be essential in medical risk assessment and management of malaria patients. Therefore the study aims to evaluate the effects of Coartemether on liver enzymes and serum bilirubin concentration in rats.

II. Materials And Method

2.1 Animals

Forty-eight malewistar albino rats of more than four months old weighing 187-245g were obtained from the Animal House, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The animals were maintained under standard laboratory condition with a standard temperature condition 26 ± 2 °C and a 12:12h natural light: dark cycle. Standard laboratory chow and water were provided ad libitum. Animals were allowed to acclimatize for a period of 14days before the experiment started. All animal experiments were in compliance with the international guidelines for handling experimental animals [15].

2.2 Drug

COARTEM® (artemether-lumefantrine) was procured from the Pharmacy Department, University of Nigeria Teaching Hospital, Enugu, Nigeria. For this study, the drug was prepared with a known mass of the Coartemether (artemether-lumefantrine) powder suspended in a distilled water to yield a suspension of 4mg Artemether/24mg Lumefantrine per ml. the doses selected were based on the recommended dosage (4mg Artemether/24mg Lumefantrine/kg body weight) and (8mg Artemeter/48mg Lumefantrine/kg body weight).

2.3 Experimental Design

The rats were randomly divided into four groups (A-D) of six rats each with two replicates per group and well labelled for easy identification. Group A served as control and was given distilled water. Group B received 28mg/kg/day of Coartemether (4mg Artemether/24mg Lumefantrine/kg body weight). Group C was given 42mg/kg of Coartemether (6mg Artemether/36mg Lumefantrine/kg body weight). Group D received 56mg/kg/day of Coartemether (8mg Artemeter/48mg Lumefantrine/kg body weight). The experimental groups were subjected to treatment orally, once daily for 6days. Two animals from each group and replicate were sacrificed on day 2, 4 and 6. Blood samples were collected, allowed to clot and centrifuged for 15 min at 3,000 rpm. Clear serum was then separated from the cells and assayed for biochemical parameters.

2.4 Biochemical Analysis

The levels of alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) were analysed spectrophotometrically with Randox assay kits using the procedure described by [16]. Also total and direct Bilirubin was also determined using the procedure described by [17].

2.5 Statistical Analysis

Data were analysed using one way analysis of variance (ANOVA) test and presented as mean \pm standard deviation. All statistical analyses were done using SPSS (version 17.0 for Windows).Differences between means were considered significant at $P < 0.05$

III. Result

The results (Tables 1-3) show the effects of Coartemether (Artemether-Lumefantrine) administration on liver function enzymes and bilirubin level in wistar albino rats. The serum levels of ALT, AST, ALP increased significantly ($P < 0.05$) with increase in Coartermeth concentrations in the treatment groups as compared with the controls after day 2 (Table 1). The increase in bilirubin levels after day 2 when compared with the control was not significant. At day 4 of treatment, there was a progressive and dose dependent increase in bilirubin and enzymes levels across coartemether concentration gradient in the test groups when compared with the control (Table 2). However, there was a significant decrease in bilirubin and ALT levels in wistar albino rats in group D treated with 56mg/kg of Coartemether (Table 2) when compared with other test groups. As the duration of treatment increased to day 6, levels of ALT, AST, ALP and bilirubin increased significantly ($P < 0.05$) (Table 3) compared to that of day 2, day 4 and control. More so, decrease in ALT, AST, ALP in group D treated with 56mg/kg of Coartemether at day 6 was observed (Table 3).

Table 1: Effect of coartemether on liver function enzymes and bilirubin level after day 2 treatment

GROUPS	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	TOTAL BILIRUBIN (IU/L)	DIRECT BILIRUBIN (IU/L)
A (control)	26.20±36.49 ^a	24.00±12.73 ^a	22.00±32.53 ^a	2.80±0.85 ^a	1.20±0.00 ^a
B (28mg/kg of Coartemether)	44.56±24.96 ^b	27.50±2.12 ^a	54.00±28.28 ^b	6.65±5.30 ^b	1.70±0.00 ^a
C (42mg/kg of Coartemether)	64.35±17.75 ^c	33.00±18.35 ^{ab}	90.50±20.51 ^{bc}	3.15±0.35 ^a	1.45±0.35 ^a
D (56mg/kg of Coartemether)	77.35±9.55 ^c	63.00±8.49 ^b	121.50±6.36 ^c	6.55±5.16 ^b	4.00±3.96 ^b

Results are expressed as Mean of the samples ± standard deviation. Mean values in same column with different superscript are significantly different (P>0.05)

Table 2: Effect of coartemether on liver function enzymes and bilirubin level on rat after day 4 treatment

GROUPS	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	TOTAL BILIRUBIN (IU/L)	DIRECT BILIRUBIN (IU/L)
A (control)	35.25±19.16 ^a	41.50±9.19 ^a	43.50±10.60 ^a	3.15±0.35 ^a	2.00±0.42 ^a
B (28mg/kg of Coartemether)	66.35±1.91 ^b	57.00±8.49 ^a	83.50±14.85 ^b	11.50±2.40 ^b	4.35±2.05 ^b
C (42mg/kg of Coartemether)	69.10±36.91 ^{ab}	86.50±37.48 ^b	114.00±29.69 ^{bc}	14.50±0.70 ^c	4.90±0.42 ^b
D (56mg/kg of Coartemether)	94.75±0.49 ^c	55.00±7.07 ^a	169.50±27.58 ^c	9.40±1.13 ^b	3.75±1.20 ^{ab}

Results are expressed as Mean of the samples ± standard deviation. Mean values in same column with different superscript are significantly different (P>0.05)

Table 3: Effect of coartemether on liver function enzymes and bilirubin level on rat after day 6 treatment

GROUPS	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	TOTAL BILIRUBIN (IU/L)	DIRECT BILIRUBIN (IU/L)
A (control)	40.00±8.49 ^a	37.00±4.24 ^a	85.00±7.07 ^a	10.85±91.92 ^a	2.30±0.85 ^a
B (28mg/kg of Coartemether)	106.75±2.47 ^c	69.00±4.24 ^b	202.50±17.68 ^c	15.15±1.34 ^b	7.40±0.85 ^{bc}
C (42mg/kg of Coartemether)	95.55±6.71 ^c	66.50±4.95 ^b	162.00±26.87 ^{bc}	11.20±1.13 ^a	5.90±0.57 ^b
D (56mg/kg of Coartemether)	66.90±1.27 ^b	65.00±7.07 ^b	123.00±4.24 ^{ab}	10.35±7.78 ^a	8.95±1.06 ^c

Results are expressed as Mean of the samples ± standard deviation. Mean values in same column with different superscript are significantly different (P>0.05)

IV. Discussion

Drugs produce a wide variety of clinical and pathological hepatic injury. Liver enzyme assay can indicate tissue cellular damage long before structural damage can be picked by conventional histological techniques. Such measurement can also give an insight to the site of cellular tissue damage as a result of assault by chemical agents [18]. Increase (changes) in these biochemical markers such as ALT, ALP, AST and bilirubin are indicators of hepatotoxicity. Generally, hepatotoxicity is defined as rise in either ALT level more than 3x of upper limit of normal (ULN), ALP level more than twice ULN or total bilirubin level more than twice ULN when associated with increase ALT, AST or ALP [19, 20].

From the study, a significant time-dose dependent increase in ALP, ALT, AST and Bilirubin levels was observed in albino rats treated with different doses of coartemether compared to the control. It is clear from this observation that there is interaction between coartemether drug and the liver enzymes. This is expected as the liver serves as the major conduit for drug metabolism [12].

Increased activities of ALT and ALP enzymes show that the integrity of hepatocytes was abnormal. An elevated level of ALP serves as biomarker for hepatic damage to cells that undergo toxic liver injury or liver ischemia [21]. This could result in the release of intracellular enzymes into systemic circulation [22]. More so, AST and ALT are enzymes that catalyze the transfer of alpha-amino groups from aspartate and alanine to the alpha-keto group of ketoglutarate acid to generate oxalacetic and pyruvic acids respectively, which are important contributors to citric acid cycle [21]. AST and ALT activities are also stimulated by induced hepatic cell injuries which normally lead to their leakage into circulation or their increased synthesis by the liver [22].

Furthermore, the treatment groups especially in group D treated with 56mg/kg of Coartemether exhibited high level of AST activity compared to the other treatment groups, indicating the possibility that coartemether drug increased the enzyme levels which may be attributed to liver damage. AST is diffusely represented in the heart, skeletal muscle, kidney, brain and red blood cells, and ALT has low concentrations in skeletal muscle and kidney; an increase in ALT serum levels is, therefore more specific and reliable for liver damage [21]. Also, it is known that reactive oxygen species (ROS) are generated during the process of drug biotransformation and these ROS generated can bind and react with cellular components in the liver to cause hepatic injury, thus impairing liver function [22]. These findings are similar to the findings of other researchers [23, 24, and 25]. Researchers have also shown that other antimalarial drugs such as chloroquine, amodiaquine,

quinine and halofantrine were reported to elevate serum ALT, AST and ALP and may induce hepatic damage [26, 27, and 28].

The total and direct bilirubin content in the plasma was high in the treatment groups compared to the controls. It has been known that increased levels of total and direct bilirubin in systemic circulation is an indication of impairment of liver functions enzymes or an obstruction of the bile duct system that is supposed to eliminate it [21]. This study has shown that the antimalarial Coartemether drug can modulate the activities of liver function enzymes and oxidative stress biomarkers. Therefore, the observed significant increase in serum ALT, AST, ALP and bilirubin in the treated animals when compared with the control suggest that the drug might induce hepatic damage or hepatotoxicity in the albino rats.

V. Conclusion

The outcome of this research suggests a time-dose related increase in ALP, ALT, AST and Bilirubin levels in rats administered with different doses of Coartemether. Thus, it is logical to conclude that the elevation of bilirubin and liver enzymes studied above showed that the administration of Coartemether may induce hepatotoxicity.

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