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Toxicological Evaluation of Some Artemisinin Combination Therapies (ACTs) on the Kidney and Liver of Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OEE designed the study, wrote the protocol and supervised the work. Authors EES and GEC carried out all laboratories work and performed the statistical analysis. Author UEB managed the analyses of the study and wrote the first draft of the manuscript. Author EJA managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

World Health Organisation (WHO) recommends the use of artemisinin-based combination therapies (ACTs) as drug of choice for the treatment of malaria in endemic regions of the world. This study was designed to evaluate the effects of therapeutic doses of ACTs: Artesunate (Artesunat®), Artesunate-Mefloquine (Artequin®), Artemether-Lumefantrin (Coartem®) and Dihydroartesiminin-Piperaquine (P-Alaxin®) on the integrity of the liver and kidneys of albino Wistar rats. Thirty (30) albino Wistar rats weighing between 200 g – 280 g were randomly divided into 5 groups with 6 animals per group. Group 1 served as control (CTR) while Group 2 received artesunate (AS) for 5 days. Groups 3, 4 and 5 received therapeutic doses of artequine (AQ), coartem (CT) and p-alaxin (PA) respectively for 3 days. The animals were sacrificed under chloroform anaesthesia and blood samples obtained through cardiac puncture for biochemical investigations. Serum ALT activity of

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Groups 2 and 3 were significantly elevated ($p<0.05$) while Groups 4 and 5 experienced marginal increased. Group 4 also showed significant increase in total bilirubin though it was marginally increased in all other groups when compared to the control. Creatinine levels were marginally increased in all groups while the urea levels were relatively stable across all groups except in Group 3 where significant increase was observed. The results therefore indicate possible hepatic injury and renal toxicity in albino Wistar rats hence the need for caution and proper attention while undergoing malaria treatment with ACTs.

Keywords: Malaria; Artemisinin-based Combination Therapies (ACTs); liver; kidney and toxicity.

1. INTRODUCTION

Malaria remains one of the greatest causes of mortality and morbidity in the world. Globally, there are between 124 – 283 million cases of clinical malaria every year. Currently, estimated 367,000 to 755,000 deaths are attributed to malaria annually, 90% of them in Africa [1]. In Nigeria, malaria is holoendemic hence clinical cases of the disease are seen throughout the year. It is the commonest cause of out-patient hospital attendance in all age groups in the country. Studies have revealed that the readily available and cheap antimalarial drugs: chloroquine and sulphadoxine-pyrimethamine are no longer efficacious in the treatment of malaria in Nigeria due to the development of resistant to these agents [2,3]. Prompt and adequate treatment with an effective antimalarial drug is an essential strategy to reduce the morbidity and mortality resulting from malaria infections. Presently, artemisinin-based combination therapy is the drug of choice for the treatment of malaria in the country and this has proven to be effective in combating the disease. The rationale for the use of ACTs is to reduce the probability of resistant developing simultaneously to the two drugs with independent mechanism of action [3].

The artemisinins are developed from the Chinese wormwood (*Artemisia annua*) and the derivatives namely; artemether, artesunate and dihydroartemisinin have now gained popularity as short acting drugs which could be used in combination with drugs which have longer half-life [4]. Artemether-lumefantrine, artesunate-mefloquin, dihydroartemisinin-piperaquin alongside artesunate are among the commercially available artemisinin-based antimalaria therapies. ACTs have been reported to be safe and efficacious in the treatment of uncomplicated malaria in Nigeria and other countries [1,5-7].

Artemisinin and its derivatives have a short half life while the other drugs in ACTs are often with longer half life hence the risk of drug resistant is substantially reduced. Artemisinins are considered to have high safety margins, however, they may be toxic under certain conditions [8] and when used in combination as ACTs on renal function [9]. This study was designed to evaluate and compare the toxicities of ACTs; artemether-lumefantrine, artesunate-mefloquin, dihydroartemisinin-piperaquin and artesunate on the liver and kidney of albino Wistar rats. These organs are involved in the metabolism and elimination of drugs as such they are susceptible to drug induced injuries.

2. MATERIALS AND METHODS

2.1 Materials

Artemether-lumefantrin (Coartem 80 mg/480 mg), Artesunate-Mefloquin (Atequin 600 mg/750 mg), Dihydroartemisinin-piperaquin (P-Alaxin 40 mg/ 320 mg) and Artesunate (Artesunat 50 mg) were purchased from Tonay Pharmacy, Itu Road, Uyo, Akwa Ibom State, Nigeria. Reagents used for analysis were gotten from Randox Laboratories Ltd, UK. All reagents and chemicals were of analytical grades.

2.2 Experimental Design and Treatment of Animals

Thirty (30) albino Wistar rats weighing between 200g – 280 g were randomly divided into 5 groups with 6 animals per group. The rats were kept in standard cages and laboratory conditions, and were fed with normal rat pellets and water *ad libitum*. Group 1 served as control while Groups 2 – 5 were administered therapeutic doses of ACTs as shown in Table 1.

24 hours after the last dosage, the animals were sacrificed under chloroform anaesthesia. Blood samples were removed through cardiac puncture into a plane sample bottle. Serum was gotten from the blood by centrifugation and then preserved for biochemical investigations.

Table 1. Administration of ACTs to groups 2–5

Groups	ACT brand	Dosage	Duration
2. AS	Artesunat®	1.43 mg of artesunate per kg b.w of rat.	Twice on day 1 followed by once daily from day 2 to 5
3. AQ	Artequin®	2.86 mg of artesunate and 3.57 mg of mefloquine per kg b.w of rat.	Daily for 3days
4. CT	Coartem®	1.14 mg of artemether and 6.86 mg of lumefantrine per kg b.w of rat.	Twice daily for 3days
5. PA	P-Alaxin®	1.71 mg of dihydroartemisinin and 13.71mg of piperaquine per b.w of rat.	Once daily for 2 days followed by two third of the dosage on day 3

2.3 Estimation of Biochemical Parameters

Serum ALT activity was assayed based on the method developed by Wroblewski and Ladue [10], while the method of Doumas and Briggs [11] was used to assay AST activity. The method of Bowers and McComb [12] was used to estimate ALP activity in the serum. Determination of Total Bilirubin (TB) concentration was carried out according to the method of Jendrassik and Grof, [13]. Serum urea and creatinine concentration were estimated based on the methods of Tobacco et al. [14] and Narayanan and Appleton [15] respectively.

2.4 Statistical Analysis

The data from biochemical analysis were subject to one way analysis of variance (ANOVA) and Student's T-test using SPSS package. Values (P<0.05) were considered significant against the control.

3. RESULTS AND DISCUSSION

3.1 Results

The results of liver enzyme activities and evaluation of kidney function following administration of some ACTs are presented in Tables 2 and 3 respectively.

Table 2. Serum enzyme activities of albino wistar rats administered with different ACTS

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
1. CTR	16.04±3.20	16.36±4.31	36.99±1.68
2. AS	20.29±3.54*	13.93±4.66	24.67±3.03*
3. AQ	20.32±3.09*	12.40±1.87*	35.32±2.31
4. CT	18.90±4.70	12.00±3.68*	37.37±3.84
5. PA	18.90±5.57	15.26±3.99	28.86±5.77*

*Values are presented as mean ± standard deviation. * = significantly different from control (p<0.05). Number of rats (n) = 6*

Table 3. Effect of administration of ACTs on urea, creatinine and bilirubin concentration in albino wistar rats

Groups	Creatinine (µmol/l)	Urea (mmol/l)	Total bilirubin (µmol/l)	CONJ. bilirubin (µmol/l)
1. CTR	84.21±20.39	3.01±0.36	22.90±4.30	6.10±2.10
2. AS	87.42±18.05	2.51±0.77	23.38±4.00	5.17±1.77
3. AQ	89.84±28.92	3.76±0.40*	23.60±4.57	3.53±1.75*
4. CT	106.82±30.75	2.75±2.31	29.40±3.21*	6.52±2.70
5. PA	101.82±32.25	3.03±0.74	24.79±4.00	5.12±2.09

*Values are presented as mean ± standard deviation. * = significantly different from control (p<0.05). Number of rats (n) = 6*

3.2 Discussion

Hepatocyte membrane distortion is associated with membrane leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of serum/plasma enzymes namely; ALT, AST and ALP [16]. These enzymes serve as biomarkers for evaluation of hepatocellular damage, however, ALT is the most reliable. AST is known to be abundant in the cardiac muscle, skeletal muscle, kidney and testes thus any disease affecting any of these extra hepatic tissues significantly elevates the serum level of the enzyme [17]. The observed significant increase in ALT activity in Groups 2 and 3 indicates possible drug induced hepatic damage or hepatotoxicity in the albino Wistar rats. This is in collaboration with other studies where the effects of P-Alaxin and Coartem on pregnant albino Wistar rats were evaluated [18,19,20].

Furthermore, a study by Ofem et al. [21] reported that activities of serum ALT, AST and ALP increased significantly upon the administration of Coartem and P-Alaxin on albino Wistar rats. Elevation of serum enzyme activities can also be attributed to causes other than liver injury such as muscle damage. These enzymes exist as isoenzymes which originates from other tissues hence the need for evaluation of other biomarkers of liver function. Total bilirubin was found to be significantly elevated in coartem treated group, suggestive of hepatobillary injury. There were non-significant increases in total bilirubin in other groups. Intravascular haemolysis of red blood cells due to drug treatment has been reported. In particular, quinine based drug, when used for chemoprophylaxis has been shown to stimulate the production of drug dependent complement fixing capable of causing intravascular red cell lyses [22].

Elevated levels of creatinine and urea in serum are an indication of poorly functioning kidneys and malfunctioning such as impairment of glomerular filtration. A rise in blood creatinine is observed only with marked damage to functioning nephrons [23]. This study revealed a non-significant increase in the levels of creatinine in all groups while urea was significantly increased in artequin treated group. However, a better evaluation of kidney function would be through a creatinine clearance test as suggested by American Diabetes Association [24].

4. CONCLUSION

Summarily, it can be concluded from this study that ACT therapies can have deleterious effect on the kidney and liver. Furthermore, dihydroartemisinin-piperaquin has the best safety profile among the ACTs studied while artemether-lumefantrin and artesunate-mefloquin are more likely to induce significant toxicological effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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