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HEPATOTOXIC EFFECT OF ARTESUNATE, AN ANTIMALARIAL DRUG IN WISTAR ALBINO RATS

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ABSTRACT

Objective: To study the effect of artesunate, the antimalarial drug on liver in Wistar albino rats. Methods: Artesunate was administered at a dose of 36, 72 and 110mg/kg body weight intraperitonially respectively for 14 days in Wistar albino rats. The effect of artesunate on liver was assessed based on biochemical and histopathological analysis. Results: The results disclosed that on artesunate administration at a dose of 110mg/kg body weight for 14 days caused a significant increase ($p<0.001$) in the levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma glutamyltransferase in serum. Histopathological analysis showed a pattern of hepatocellular necrosis at the above dose. The levels of superoxide dismutase and catalase in liver homogenate were also decreased significantly ($p<0.01$) in artesunate administered animals. Conclusions: The present study proves that artesunate has a potential to cause hepatic damage.

KEYWORDS: Artesunate, Liver injury, Wistar albino rats.

INTRODUCTION

1. Malaria

Malaria is caused protozoan parasites belonging to the genus *Plasmodium* transmitted by female *Anopheles* species mosquitoes. Four species of *Plasmodium* commonly infect humans. The infection can develop suddenly and produce several life-threatening complications. There are about 100 countries or territories in the world considered as malaria endemic and more than 2400 million of the world's population are still at risk. [1-6]

India has the largest malarial population in the world. With prompt, effective treatment, however, it is almost always curable. However, increase in the incidence of drug resistance malaria is an alarming signal. In India chloroquine resistance was first detected in 1973 in Karbi-Anglong district in Assam. Gradually it has spread to the entire country. In our state, Karnataka, Chloroquine resistant malaria cases are observed in Kolar, Raichur, Bellary, Mandya, Bagalkot, Dakshina Kannada, Chamarajanagar, Gadag and Chitradurga. [6,7]

Treatment of drug resistant malaria is a challenge faced by physicians. Combination therapy is employed to prevent the incidence of chloroquine resistant malaria. Antimalarial combination therapy is the simultaneous use of two or more blood schizontocidal medicines with independent modes of action and, thus, different biochemical targets in the parasite. Artemesinin derivatives based combination therapy is one of the important strategy for treating drug resistant malaria. [6,8]

2. Artesunate

Artemisinin is a sesquiterpene lactone produced from a plant called *Artemisia annua* which is a chinese medicinal herb which has been used as treatment for fevers in China for more than 1500 years in the form of antipyretic tea. Artemisinin is a highly crystalline compound which does not dissolve in oil and water and can only be given by enteral route. Semi - synthetic derivatives of artemisinin was mainly formulated to overcome the poor bioavailability of artemisinin which reduce its effectiveness. Artemisinin undergo semi-synthetic process to produce dihydroartemisinin then the process continue to produce a water soluble ester called artesunate and two oil soluble preparations called artemether and arteether. Artesunate is available in oral, rectal, intramuscular injection, or intravenous injection form. The artemisinin derivatives are active against all *Plasmodium* species especially *P. falciparum and P. vivax*. These derivatives are able to rapidly destroy all the blood stages of the parasite and hence results in the shortest fever clearances times of all antimalarials. In combination with other drugs, artemisinin combination therapy are the first-line treatment recommended by the World Health Organization for Plasmodium falciparum infections. [6, 8-10]

3. Rationale

There are few case reports of clinical hepatitis and few published animal data revealing hepatotoxicity of artemisinin derivatives. [10-12] Drug induced liver injury is serious adverse event which may interfere with malaria management and may add on as a risk factor for

mortality. Ours being a malaria endemic region, artesunate based combination therapy is widely used as first line therapy. [7] Hence we have taken up this research work to study the serious adverse effects related to artesunate.

MATERIALS AND METHODS

2.1 Drugs and Chemicals

Artesunate (Zydus Cadilla, Himachal Pradesh), was obtained from our hospital pharmacy. The chemicals used for biochemical analysis were of analytical grade and was procured from Himedia, India.

2.2 Instruments

Autoanalyser, UV Spectrophotometer.

2.3 Animals

Adult Wistar albino rats of either sex weighing 175-200 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (IAEC), Yenepoya University. The rats were maintained under standard conditions in the animal House (CPCSEA approved, Reg No: 347) under Department of Pharmacology, Yenepoya University, Mangalore. The rats were kept in polypropylene cages (U.N.Shah manufacturers, Mumbai) under standard housing conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water *ad libitum*. The rats were maintained on a 12:12 hour light-dark cycle.

2.6 Experimental Protocol

The rats were divided into 4 groups with 12 animals in each group. Group I received normal saline intraperitonially (I.P) and served as a normal control. Group II, III and IV received artesunate 36, 72 and 110mg/kg body weight for 14 days I.P in normal saline. The dosage of artesunate was extrapolated from the human dose using the conversion table.[13] On 15^{th} day blood samples of the animals were taken by cardiac puncture under ketamine anesthesia (150mg/kg bodyweight, I.P). Once the blood was withdrawn, the animals were sacrificed by high dose of ketamine (300mg/kg bodyweight I.P). The liver was dissected out for preparing homogenate.

2.7 Assessment of Hepatoprotective Activity

The blood which was drawn under ketamine anesthesia was allowed to clot and the serum was separated at 3000 rpm for 10minutes. The serum was used for the assay of alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], gamma glutamyltransferase [GGT], bilirubin [BIL] and albumin [ALB].

2.8 Assessment of Antioxidant Activity

The liver which was separated out was washed instantly with distilled water to remove blood. It was used for making homogenate for the assessment of superoxide dismutase [SOD] and catalase [CAT].

All biochemical techniques were carried out based on previous published methods.[14]

2.9 Statistical Analysis

One way analysis of variance (ANOVA) and multiple group comparisons were made by Tukey Kramer test using Graph pad Prism software. The values were expressed as mean ± S.D for 12 samples in each group. *P* value < 0.05 was considered as significant.

RESULTS

3.1 Effect on LFT

The results (Table:1) showed that there is a significant increase ($p<0.01$) in the levels of ALT, AST, ALP, and GGT in serum of artesunate administered (110 mg/kg bodyweight I.P) rats (Group IV) on comparing with the normal rats (Group I). At the same time, the serum levels of BIL and ALB were not significantly ($p>0.01$) altered in artesunate administered rats (Group II, III, IV) on comparing with the normal rats (Group I).

3.2 Effect of Liver Histopathology

The results (Table:2) showed that a pattern of necrosis in rats which received artesunate at a dose of (110 mg/kg bodyweight I.P) rats (Group IV).

3.2 Effect on Antioxidant Enzymes

The results (Table:3) showed that consumption of artesunate at a dose of (110 mg/kg bodyweight I.P) rats (Group IV) for 14 days caused a significant decrease in the levels of SOD and CAT in liver homogenate on comparing with the normal rats (Group I).

Table:1 Effect on LFT

GROUP	AST	ALT	ALP	GGT	BIL	ALB
I. NORMAL	244.37 ± 12.68	61.62 ±17.08	160.78 ± 7.49	3.10± 6.75	0.37± 0.04	3.3± 0.094
II. ARTESUNATE 36mg/kg b.w	245.01 ± 6.68 [a]	52.27 ± 38.51 [a]	182.2 ± 8.52 [a]	3.262 ± 3.061 [a]	0.40 ± 0.01 [a]	3.217 ± 0.86 [a]
III. ARTESUNATE 72mg/kg b.w	255.67 ± 5.28 [a]	67.34 ± 6.76 [a]	185.01 ± 5.85 [a]	3.561 ± 0.2 [a]	0.44 ± 0.01 [a]	3.73 ± 1.64[a]
IV. ARTESUNATE 110mg/kg b.w	1490.63 ± 6.20[b]	480.21 ± 5.7 [b]	615.42 ± 11.5 [b]	11.34 ± 2.17 [b]	0.40 ± 0.02[a]	3.2 ± 0.23[a]

Values are expressed as Mean ± Standard Deviation; n=12

a : $p>0.05$; not significant on comparing artesunate (Group II,III) with normal group (Group I); considered not significant

b : $p<0.001$ on comparing artesunate (Group IV) with normal group (Group I); considered extremely significant

Table:2 Effect of Artesunate on the histology of liver

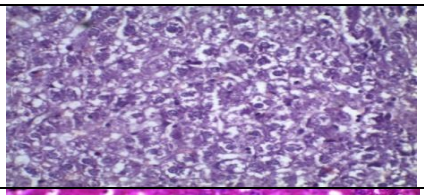
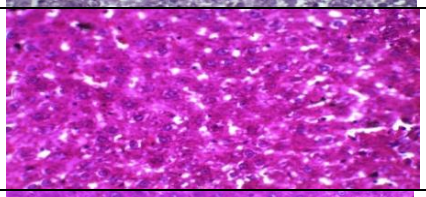
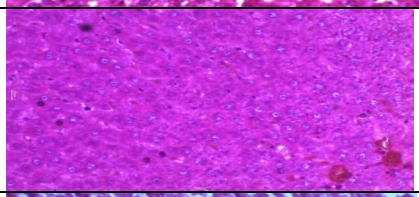
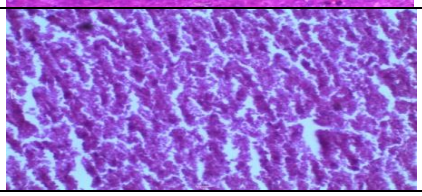
Groups	Histology	
	Photo	Inference
I. NORMAL		Normal
II. ARTESUNATE 36mg/kg b.w		Normal
III. ARTESUNATE 72mg/kg b.w		Normal
IV. ARTESUNATE 110mg/kg b.w		Massive necrosis

Table:3 Effect on antioxidant enzymes

GROUP	SOD	CAT
I. NORMAL	534.31 ± 11.95	17.28±1.24
II. ARTESUNATE 36mg/kg b.w	547.27 ± 4.77 [a]	14.12 ± 1.38 [a]
III. ARTESUNATE 72mg/kg b.w	502.98 ± 4.1[a]	12.28 ± 1.59 [a]
IV. ARTESUNATE 110mg/kg b.w	49.3 ± 3.20[b]	1.62±0.01 [b]
Values are expressed as Mean ± Standard Deviation; n=12 a : p>0.05; not significant on comparing artesunate (Group II,III) with normal group (Group I); considered not significant b :p<0.001 on comparing artesunate (Group IV) with normal group (Group I); considered extremely significant		

DISCUSSION

Malaria is one of the causes of death and illness in children and adults, mainly in tropical countries. Integrate approach is required for control of malaria, including prevention by primarily vector control and need prompt treatment with effective antimalarials. Due to incresing parasite resistance and failure of single drug treatment of malaria in many endemic countries has led to a widespread promotion of Artemisinin-based combination therapy. [1-10]

In our study the extent of hepatic damage was assessed by estimating the levels of markers like ALT, AST, ALP, GGT, BIL and ALB in serum. It was noted, after artesunate administration the elevated levels of ALT, AST, ALP, GGT were seen, thus endorsing its hepatotoxic potential. Histopathological analysis also showed a pattern of hepatocellular necrosis.

In this work, liver homogenate of the rats administered with artesunate exhibited a decreased activity of SOD & CAT. This gives a hint about the role of oxidative stress induced by artesunate. We know that anti-malaria property of artimisinin derivates lies in its endo-peroxide bridge which can generate free radicals [8] which may be responsible for its hepatotoxic potential.

Our results are matching up with the previous findings of artesunate induced liver injury.[9,11,12] Based on the above facts and findings it is concluded that artesunate produces hepatic damage by interrupting the hepatocellular integrity and by the release of reactive oxygen species.

The role of artenimol, the metabolite of artesunate cannot be ruled out. This compound may be a part of many hepatocellular reactions. These reactions will lead to the covalent binding of drug to the macromolecules, leading to the formation of non-functioning adducts. Once adducts are formed, they travel to cell surface of the hepatocyte, where they serve as a target for cytolytic attack by T cells, leading to liver injury.

CONCLUSION

The antimalarial drug artesunate has a potential to cause liver injury. It should be cautiously used in malaria patients who have previous history of liver disease.

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