Asian Journal of Medical Sciences 3(4): 176-179, 2011
ISSN: 2040-8773

Received: June 27, 2011 Accepted: August 27, 2011 Published: August 30, 2011

Acute Exposure to Artesunate and its Effect on the Hematological Indices, Hepatotoxicity and Histology of the Liver of Adult Wistar Rats

[1]Wilson O. Hamman, [1]Sunday A. Musa, [1]Fareha Muzaffar, [1]Sabdat O. Sani, [1]Uduak E. Umana, [1]James A. Timbuak, [2]Andrew J. Nok and [3]Samuel A. Ojo
[1]Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria-Nigeria
[2]Department of Biochemistry, Faculty of Science, Ahmadu Bello University Zaria-Nigeria
[3]Department of Veterinary Anatomy, Ahmadu Bello University Zaria-Nigeria

Abstract: The effect of artesunate on the hematological indices, hepatotoxicity and histology of liver was investigated in 20 adult male wistar rats. The animals were divided into 4 groups of 5 each and group 1 which served as control were administered normal saline while groups 2, 3 and 4 were administered 1, 2 and 5 mg/kg/day respectively for a period of 5 days. The animals were humanely sacrificed on the sixth day and blood samples were obtained for hematological indices and serum enzyme analysis. The liver were excised and processed for light microscopy using the H & E stain. Hematological indices indicated insignificant difference in the RBC, WBC and DC counts, while a significant dose dependent increase in PCV and hemoglobin were observed ($p<0.05$). No changes were observed in the serum levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) among the groups. Histological examination of the liver revealed points of focal necrosis among the treated groups. The mild liver tissue damage was more evident among the over dosed group. Artesunate is thus safe, when administered within the therapeutic range.

Key words: Artesunate, hepatotoxicity, hematological indices, histology, liver

INTRODUCTION

Artesunate is a potent blood schizoriticide agent for *Plasmodium falciparum*. It is effectively used against *P. falciparum* resistant to all other malaria drugs (Larinate, 2006). It is a water-soluble hemi-succinate derivative of dihydroartemisinin. Artemisinin is sequiterpene lactone isolated from *Artemisia annua*, a herb traditionally used in China for the treatment of malaria (Hien and White, 1993). Artesunate is the most widely available Artemisinin related compound and may be given intravenously, intramuscularly, orally or rectally (Nasten *et al*., 1994)

The underlying mechanism for the parasiticidal activity of this drug is free radical reaction. This may include free radical production in the parasite's food vacuole (Eckstein-Ludwig *et al*., 2003) and inhibition of parasitic calcium ATPase (Artemisinin derivative, 2006). The target tissue for Artesunate are red blood cells, with preferential accumulation in *P. falciparum* infected erythrocytes (Campbell *et al*., 1968). This study was carried out to evaluate the effect of oral administration of Artesunate on the hematological indices, hepatotoxicity and the histology of the liver of adult wistar rats.

MATERIALS AND METHODS

Xenobiotic: Fifty (50) mg tablets Artesunate® (Greenfield Pharmaceuticals Co. Ltd. China), packed in aluminium foil and plastic blister packs were purchased in Zaria-Nigeria. Artesunate solution was made by dissolving the tablets in normal saline.

Animal treatment: 20 apparently healthy male wistar rats weighing between 180 and 200 g were obtained from the animal house of the department of Human Anatomy, Ahmadu Bello University Zaria-Nigeria. The animals were kept in a cross-ventilated room (temperature 30.0±2.00ºC, 12 h light/12 h dark cycle) and fed with livestock growers mash, mixed with groundnut cake powder and maize chaff. Clean drinking water was liberally provided.

Experimental design: The 20 male Wistar rats were obtained from the animal house of the Department of Human Anatomy, Ahmadu Bello University Zaria-Nigeria in June, 2010 and randomly divided into 4 group of 5 rats each. Group 1 which served as a control were orally administered normal saline. Groups 2, 3 and 4

Corresponding Author: Wilson O. Hamman, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria-Nigeria, Tel.: 234-08062303007

Table 1: Effect of acute exposure to Artesunate on the hematological indices of adult male Wistar rats

Hematological indices	Group 1	Group 2	Group 3	Group 4	p-value
RBC (1012/L)	5.10±0.31	4.56±0.12	5.12±0.45	5.48±0.21	0.236
PCV (%)	43.40±0.51	30.20±1.99*	37.80±1.16*	42.60±0.75*	0.001
Hemoglobin (g/dL)	14.40±0.25	10.10±0.73*	12.60±0.51*	14.20±0.37*	0.001
WBC (109/L)	11.36±0.40	13.66±1.08	12.82±0.61	12.42±0.81	0.245

p<0.05; Mean±SEM

Table 2: Effect of acute exposure to Artesunate on the differential white blood cell counts of adult male Wistar rats

Variable (%)	Group 1	Group 2	Group 3	Group 4	p-value
Neutrophils	29.00±0.90	25.60±3.50	24.80±1.74	21.60±2.16	0.192
Lympocytes	67.00±1.52	69.80±2.49	72.80±2.49	75.40±2.27	0.081
Eosinophils	1.80±0.66	2.00±0.32	1.00±0.45	1.60±0.25	0.447
Monocytes	2.20±0.49	2.80±0.74	1.40±0.51	1.40±0.75	0.356
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.000

Mean±SEM

Table 3: Effect of acute exposure to Artesunate on the serum level of AST, ALT and ALP in adult Wistar rats

Serum enzyme	Group 1	Group 2	Group 3	Group 4
AST (M/L)	27.00±1.594	28.80±2.154	25.00±1.000	28.80±1.463
ALT (μ/L)	38.00±2.223	39.00±1.732	40.00±1.517	41.80±1.530
ALP (μ/L	10.28±0.576	10.44±0.982	11.42±0.922	11.40±1 068

Mean±SEM

respectively received 2, 4 and 10 mg/kg/day of Artesunate in normal saline on the first day (normal start dose) and 1, 2 and 5 mg/kg/day (subnormal, normal and overdose, respectively) for 4 days. On the sixth day, the animals were humanely sacrificed and blood samples collected through cardiac puncture from the base of the heart. For hematological indices, blood samples were placed in EDTA containers, while those for serum enzyme analysis were placed in plain serum bottles. Liver tissues were excised, fixed in 10% formalin, paraffin sections prepared and processed for light microscopy using the H & E staining technique.

Statistical analysis: One way analysis of variance (ANOVA) was use to compare the data obtained within groups and Student's t-test was used to compare data obtained between groups. Differences were considered significant with p<0.05.

RESULTS

A significant decrease in the Packed Cell Volume (PCV) and hemoglobin concentration was observed (p<0.05) among the Artesunate treated groups as compared to the control, while the other hematological indices measured were not significantly affected by the 5-day oral administration of Artesunate as shown in Table 1 and 2.

Serum concentration of ALT showed a slight dose dependent increase among the Artesunate treated groups. However, the observed increase in concentration of ALT is not significant as compared to the control group. The

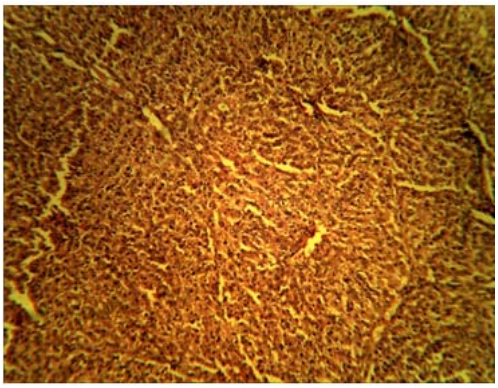

Fig. 1a: Light micrograph of the liver of male Wistar rat of the control group showing normal portal triad (PT), hepatocytes (H) and sinusoids (S). H&E, X 100

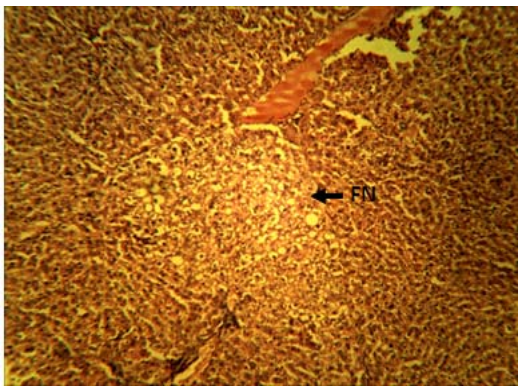

Fig. 1b: Light micrograph of the liver of male Wistar rat treated with 1 mg/kg Artesunate showing an area of Focal Necroses (FN). H&E, X100

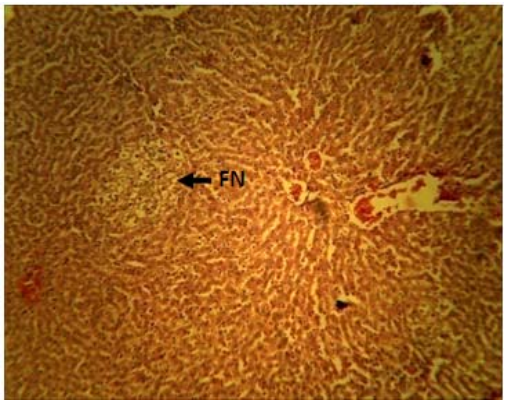

Fig. 1c: Light micrograph of the liver of male Wistar rat treated with 2 mg/kg Artesunate showing an area of focal necroses (FN). H&E, X100

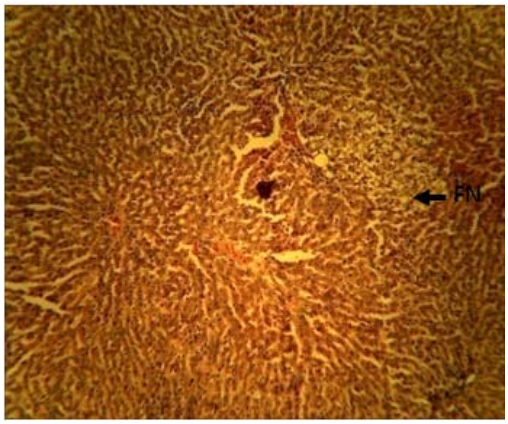

Fig. 1d: Light micrograph of the liver of male Wistar rat treated with 5 mg/kg Artesunate showing an area of focal necroses (FN). H&E, X100

result obtained forthe serumconcentrations of AST and ALP showed no significant difference between the Artesunate treated groups and the control as shown in Table 3.

Histology of the liver: Light microscopic examination of the Liver sections of the control group showed a normal histology with clearly outlined figs of anastomosing hepatocytes along with adjacent sinusoids radiating from the central veins towards the periphery of the liver lobules. Present at the portal areas are connective tissues within which are portal veins and hepatic arteries as shown in Fig 1a. Similar histological features was observed in the sections obtained from the subnormal, normal and overdosed Artesunate treated groups except for the distinctive presence of areas of focal necrosis shown in Fig. 1b, c and d, respectively.

DISCUSSION

Packed cell volume and hemoglobin concentration showed significant difference amongst the hematological indices measured. This may not be unconnected with artesunate's ability to accumulate in the red blood cells being the primary site of action. The RBC counts show statistically insignificant difference, thus the decrease in PCV may have occurred due to increase in plasma volume or alteration of the size and shape of the erythrocytes (Widman, 1980). Since the hemoglobin concentration alters in the same pattern as the PCV, the most likely alteration may have occurred in the size and shape of the erythrocytes. This could be as a result of Artesunate adding on the plasma membrane of the erythrocytes, as it is the target site of action. Analysis of the serum level of enzymes AST and ALT did not show any significant difference, though a dose dependent increase in ALT was observed. This suggests tonicity of the liver tissue in the treatment groups upon Aretesunate administration; since elevation of the serum enzymes indigenous to liver is an indicator of hepatotonicity. Transaminases are important useful markers of hepatocellular toxicity and damage (Faber *et al*., 1981). Points of focal necrosis were observed in the treatment groups though the extent of tissue damage amongst the various treated groups seemed almost the same. This can be expected as well since the hepato-specific markers just showed slight increases. The level of serum hepato-specific markers is a function of the extent of tissue damage. Thus, acute exposure to all doses of Artesunate may cause hepatic damage, though the effect of various doses may be more prominent over chronic exposure.

CONCLUSION

The study revealed Artesunate had insignificant effect on the hematological indices except for PCV and Hb concentration, on acute exposure. Since the effect on PCV and Hb concentration was not dose-dependent, the observed changes may have been caused by other factors, thus its effect can be considered safe. Although mild level of toxicity and liver tissue damage was observed, the liver is one of the few glands with a remarkable ability to regenerate. Artesunate can thus be said to be safe when administered within therapeutic range.

REFERENCES

Artemisinin derivative., 2006. Coarsucam's properties, Sanoti Pharmaceuticals. Retrieved from: http://en.impactmalaria.com/iml/cx/medias/Coarsucam. (Accessed on: 12 June, 2010).

Campbell, E.J.M., C.J. Dickinson and J.D.H. Slater, 1968. Clinical Physiology. 3rd Edn., Blackwell, Oxford, pp: 27-28.

Eckstein-Ludwig, U., R.J. Webb, I.D.A., van Goethem, J.M. East, A.G. Lee, M. Kimura, P.M. O' Niell, P. Bray, S. Ward and S. Krishna, 2003. Artemisinins target the SERCA of *Plasmodiumalciparum*. Nature, 424: 957-961.

Faber, J.L., K.R. Chein and S. Mitlnacht, 1981. The Pathogenesis of Irreversible cell injury in ischemia. Am. J. Pathol., 102: 273.

Hien, T.T and N.J. White, 1993. Qinghaosu. Lancet, 341: 603-608.

Larinate, 2006. Artesunate Injection and Tablets, Crusade Against Malaria. Retrieved from: www-malaria-ipca.com.

Nasten, F.C., F.O. Lunemburger, K. Ter, C.J. Woodrow, T. Chong-Suphajaisiddhi and N.J. White, 1994. Treatment of Multi-drug resistant falciparum malaria with a 3-day Artesunate-Mefloquine combination. J. Infect. Dis., 170: 971-977.

Widman, F.K., 1980. Clinical Interpretation of Laboratory Test. 9th Edn., American Association of Publishers, Washinton DC, pp: 293-295.