International Journal of Medicine and Medical Sciences ISSN 2167-0404 Vol. 10 (8), pp. 001-004, August, 2020.
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*Full Length Research Paper*

# Hepatotoxic and hemolytic effects of acute exposure of rats to artesunate overdose

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Accepted 13 April, 2020

**Hepatotoxic and hemolytic effects of artesunate overdose were examined in rats. Forty (40) rats were grouped randomly into four designated as A, B, C, and D. and were given oral administration of artesunate as follows: 0 mg/kg (control), 1 mg/kg (Under-dose), 2 mg/kg (Normal dose) and 4 mg/kg (Overdose) respectively. The administration was continued for 5 days. Hepatotoxicity was monitored in the rats as a function of changes in serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total serum albumin and malondiadehyde (MDA) level. The hemolytic effect of this drug was monitored by changes in the packed cell volume (PCV), total bilirubin, conjugated bilirubin and malondiadehyde (MDA) levels of erythrocyte. For group D (overdose group) subjects when compared with the control (group A), there was significant ($p<0.05$) decrease in serum albumin and hematocrit, but significant increase in serum levels of total bilirubin, and conjugated bilirubin. An increased hepatocyte and erythrocyte malondiadehyde level was also observed in group D. The result also shows increased activities for the serum enzymes in all the groups when compared with control group but significant increase was recorded for groups C and D. There is a clear indication that hepatotoxicity and hemotoxicity are associated with artesunate administration at both required and overdose conditions however these effects are magnified in overdose conditions.**

**Key words:** Artesunate, hepatotoxicity, hemotoxicity, serum enzymes, bilirubin, malondialdehyde.

## INTRODUCTION

Malaria has indeed become a pandemic disease in most part of the world, and its pandemic nature has been fortified with the advent of mutant strains of malaria parasite which defy chemotherapy, spurring researchers into seeking new chemotherapy or making modification to the pre-existing ones. One of the most recent phytoche-mical agents isolated and biologically characterized to exhibit anti-malaria potency is artemisinin; this compound was isolated from *Artemisia annua.* Recently, there have been other synthetic derivatives of artemisinin which includes: artemether, arteether (artemotil), artesunate and artenimol (â-dihydroartemisinin, DHA). Artemisinin and its derivatives are known to exhibit potency against the asexual, and erythrocytic forms of *Plasmodium falci-parum* and *Plasmodium vivax* Pukrittayakamee et al., 2004).The generally recommended effective doses in humans with uncomplicated falciparum-caused malaria range between 10 mg/kg/day for artemisinin and 2 - 5 mg/kg/day for other artemisinin derivatives. The under-lying mechanism for the parasiticidal activity of these agents is a free radical reaction (Olliaro et al., 2001). This reaction is initiated with the reaction of artemisinin with iron present in the heam prosthetic group of hemoglobin, leading to the generation of activated oxygen species, such as oxygen radicals and superoxide radicals. These radicals then propagate free radical reaction capable of inducing oxidative damage in the parasite (Meshnick et al., 1996; Olliaro et al., 2001). The mechanism of action of these drugs and their ability to accumulate in the red blood cell being its primary site of action, had raised questions regarding the safety of artemisinin and its derivatives, especially artesunate which is fast gaining wide-spread acceptance in most malaria endemic regions of the world. This research evaluated the toxicological influence of different artesunate dosage administration on

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on the biochemical integrity of the liver and the red blood cells (erythrocyte is the primary site of action, the site of metabolism is the liver). The parameters used in the evaluation of hepatotoxicity include serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total serum albumin and malondiadehyde (MDA) level. The parameters estimated to determine the hematotoxicity are the packed cell volume (PCV), total bilirubin, conjugated bilirubin and MDA levels of erythrocyte.

## MATERIALS AND METHODS

### Chemicals and assay kits

Artesunate$^{R}$ (Tianji Pharma and Chem- China). Copper sulphate pentahydrate, di-sodium potassium phosphate anhydrous and sodium dihydrogen phosphate (anhydrous) were products of British Drug House (Poole, England). Malondialdehyde (MDA), Trichloroacetic acid (TCA), thiobarbituric acid (TBA), and ferrous sulfate hexahydrate, were products of Sigma Chemicals Co.(St Louis, Mo, USA). ALT, AST and ALP assay kits (Randox), Quantichrom$^{TM}$ BCG Albumin assay kit, Follin-ciocaltaeu, sodium-potassium tartrate were products of Patel industries (Munbai). All others chemicals used were of analytical grade.

### Experimental animal

Experimental animals (*Rattus norvegicus*) weighing 80 - 100g used for this research were obtained from the animal house of the college of Health Sciences, Igbinedion University, Okada. They were housed in wooden cages [Protected with wire mesh]. They were fed and given water *ad libitum* on normal rat cube, the room temperature was maintained at 25 ± 2$^{O}$C throughout the experiment.

### Experimental design

The animals were assigned randomly to four groups, each with ten (10) rats. Group A: The control, the animals were placed on placebo (normal saline, 0.85% sodium chloride solution). Group B: These animals were administered with oral doses of 1 mg/kg/day of artesunate in normal saline (Under dose). Group C: These animals were administered with oral doses of 2 mg/kg/day of artesunate in normal saline (Normal dose). Group D: These animals were administered with oral doses of 5 mg/kg/day of artesunate in normal saline (Over dose). The administration was continued for 5 days (normal duration of treatment in humans). After the experiment, the animals were sacrificed after cervical dislocation method; the thoracic region was opened to expose the heart. Blood samples were drawn from the heart via cardiac puncture. The liver was decapsulated and perfused in physiological saline, the organ was homogenized in 0.1 M phosphate buffer (pH 7.2)

### Hematological assay

### The hematocrit (packed cell volume)

Non-heparinized capillary tubes (75mm long with a bore of 1.155 ± 0.085 mm) sealed at one end was filled up to 2/3 length with whole blood, was spun at 12,000 x g for five minutes in a micro-hematoctit centrifuge. The packed cell volume was determined using the graduation in the standard hematocrit chart and recorded as % total blood

### Biochemical parameters

### Alkaline phosphatase activity (ALP)

This enzyme acts on esters of phosphoric acid and catalyse hydrolysis and phosphate group release. For enzymatic assay, P-nitrophenol phosphate was used as the substrate, a colorless solution which turns yellow upon hydrolysis. The yellow coloured complex can be measured spectrophotometrically at 405 nm.

Activity [U/L] 3300 x change in absorbance at 405nm/change in time [min].

### Alanine transaminase (ALT)

ALT catalyses the transamination of alanine to alpha-keto glutarate to form glutamate and pyruvic acid, which then reacts with 2,4-dinitro-phenylhydrazine to form the hydrazone derivative of pyruvate, a coloured complex which can be measured at 546 nm. The activity (U/L) was determined from the standard calibration graph.

### Aspartate transaminase (AST)

ALT catalyses the transamination of aspartate to alpha-keto glutarate to form glutamate and oxaloacetate, which then reacts with 2,4-dinitro-phenylhydrazine to form the hydrazone derivative of oxaloacetate, a coloured complex which can be measured at 546 nm. The activity (U/L) was determined from the standard calibration graph.

### Determination of total serum albumin (BCG)

Bromcresol green forms a colored complex specifically with albumin. The intensity of the color, measured at 620nm, is directly proportional to the albumin concentration in the sample. The concentration of total serum protein (g/dl) is determined from the calibration graph.

### Malondialdehyde assay

### Principle of assay

Lipid peroxidation generates peroxide intermediates which upon cleavage release malondialdehyde, a product which reacts with thiobarbituric acid. The product of the reaction is a coloured complex which absorbs light at 532 nm. The MDA levels of samples were determined from the calibration graph.

### Procedure of assay

1 ml aliquot of each homogenate supernatant was incubated for 2 h at 37°C with 3 ml of ferrous sulphate/ phosphate buffer solution. 1 ml of each mixture was added to a tube containing 2ml trichloroacetic acid (TCA). After centrifugation at 700 × g for 10 min, 2 ml of each supernatant was added to 1 ml of 2-thiobarbituric acid (TBA) solution. The tubes were boiled for 10 min and allowed to cool, and the absorbance at 532 nm was measured

### Calculation

Thiobarbituric Acid Reactive Substance of the samples were calcu-

**Table 1.** The effect of artesunate administration on the total serum albumin (g/dL), serum enzyme activity (U/L) and Malondialdehyde level of the liver (nmol/L).

| Plasma enzymes | Alkaline phosphatase (U/L) | Aspartate transaminase (U/L) | Alanine transaminase (U/L) | Total serum albumin (g/dL) |
|---|---|---|---|---|
| Group A | 4.83 ± 1.09 | 1.30 ± 0.02 | 1.40 ± 0.98 | 1.72 ± 1.90 |
| Group B | 6.28 ± 1.76 | 2.68 ± 0.94 | 1.73 ± 0.43 | 1.89 ± 0.78 |
| Group C | 15.87 ± 3.54[a] | 3.20 ± 1.02 | 2.83 ± 0.02 | 0.88 ± 0.54 [a] |
| Group D | 26.22 ± 3.59 [a] | 4.80 ± 1.47 [a] | 4.63 ± 1.13 [a] | 0.29 ± 0.21[a] |

Values are reported as mean +/- standard deviation from 10 rats. [a]Values differ significantly when compared with the control ($p < 0.05$).

lated as malondialdehyde (MDA) equivalents, using serial dilutions of Malondialdehyde standard (10 nmol/ml).

#### Assay for total bilirubin

#### Principle

Conjugated bilirubin reacts with diazitized sulphanilic acid in alkaline medium to form a blue complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

#### Calculation

Total bilirubin [mg/dl] = 10.8 x A[578 nm]
Conjugated bilirubin [mg/dl] = 14.4 x A[546 nm]

#### Statistical analysis

The results are expressed as mean ± standard deviation. The difference between mean values was assessed for significance by student T-test (SPC-XL, 2000) and considered significant at 0.05.

## RESULTS AND DISCUSSION

Table 1 shows the effect of artesunate administration on the total serum albumin, serum enzyme activities and Malondialdehyde level of the liver. Alkaline phosphatase, aspartate transaminase, alanine transaminase, and liver MDA levels showed a dose-dependent increase. On the contrary, serum albumin level was decreased in a dose-dependent manner.

Albumin is a protein synthesized in the liver; it is the most abundant plasma protein in the plasma accounting for 60% of the total serum protein. The functions of albumin include: maintenance of colloid osmotic pressure, binding of key substances such as long-chain fatty acids, bile acids, bilirubin, haematin, calcium and magnesium, carrier for nutritional factors and drugs, effective plasma acid/base homeostasis (Kim et al., 1998). Evidently, these functional roles of serum albumin make it a reliable marker for diagnosis of liver disease Benoît et al. (2000), nephritic syndrome, malnutrition and protein-losing enteropathies (Goldwasser and Feldman, 1997). The dose-dependent decrease in the albumin could be a result of decrease in the number of cells responsible for albumin synthesis-the liver through necrosis (Goldwasser and Feldman, 1997) or a direct interference with the albumin-synthesizing mechanism in the liver, artesunate has been reported to inhibit protein synthesis in mammalian cells (Xu et al., 2007) or an additive influence of both factors.

The observed dose-dependent increase in the activities of alkaline phosphatase, aspartate transaminase, and alanine transaminase, can be traced to possible necrosis of the where these enzymes are native, since they are not classified among the secretory enzymes. Isoenzyme analysis of these enzymes would have given a clearer detail of their tissue origin. Since the assay was not conducted, the reported tissue distribution of the drugs would be correlated with the likely tissue origin of these enzymes. The drug was reported to accumulate signify-cantly in three tissues; liver, erythrocyte and to a lesser extent the kidney (Li et al., 2006). And the erythrocyte is known to have a low activity of ALT and AST, the kidney would rather play an excretory role than metabolic leaving the liver as the potential site of substantial of artesunate and its metabolites which also represent the site of detoxification for most xenobiotic chemicals (Amin and Geoffrey, 2007). These enzymes can therefore be assumed to have originated from the liver, although with little contribution from extrahepatic tissues.

Meanwhile the proposed necrotic effect of artesunate on the liver was demonstrated by the observed dose-dependent increase in malondialdehyde level of the liver. The increased MDA level of the liver is possibly due to lipid peroxidation. The source of biological oxidant which could induce this peroxidation of lipid is central to the mechanism of action of the drug; it is known to generates reactive oxygen species (Olliaro et al., 2001). The normal response of hepatocyte to lipid peroxidation would have been the induction of antioxidant enzymes, artesunate is however known to inhibit protein synthesis in most mam-malian system (Xu et al., 2007) exposing the heap-tocyte to unprotectable oxidative injury, thus accounting for the degree of oxidative damage done by the accumulated artesunate and its metabolites and the reduction in the amount of the serum albumin under this condition.

Table 2 shows the effect of artesunate administration on the total serum bilirubin, serum conjugated bilirubin, packed cell volume (PCV) and Malondialdehyde level of

**Table 2.** The effect of artesunate administration on the total serum bilirubin (mg/dL), serum conjugated bilirubin (mg/dL) packed cell volume and Malondialdehyde level of the red blood cells (nmol/L).

| Hematologic parameters | Total bilirubin (mg/dL) | conjugated bilirubin (mg/dl) | (%)Packed cell volume | MDA (RBC) (nmol/L) |
|---|---|---|---|---|
| Group A | 0.14 ± 0.01 | 0.03 ± 0.00 | 43.3 ± 1.98 | 12.43 ± 3.12 |
| Group B | 9.27 ± 1.08 [a] | 0.07 ± 0.01 | 40.8 ± 2.86 | 16.38 ± 7.43 |
| Group C | 0.70 ± 0.02 | 0.19 ± 0.02 [a] | 38.5 ± 7.42 | 25.57 ± 2.12[a] |
| Group D | 1.22 ± 0.03 [a] | 0.17 ± 0.03 [a] | 32.3 ± 1.74 [a] | 36.32 ± 2.77 [a] |

Values are reported as mean +/- standard deviation from 10 rats. [a] Values differ significantly when compared with the control ($p < 0.05$).

of the red blood cells. Total bilirubin increased significantly in groups B and D, while conjugated bilirubin levels increased significantly. The PCV levels of overdose decreased significantly, while MDA levels increased significantly in normal and overdose groups. The decreased PCV observed for overdose group agrees with the published work of (Xie et al., 2005). The increased level of bilirubin also confirms the increased level of red blood cell destruction associated with artesunate administration, while the increase in the total bilirubin can be accounted for by the heam degradation that follows red blood cell destruction (Krücken et al., 2005).

## Conclusion

From the data available from this research, artesunate is not significantly toxic at the recommended dosage but an overdose may be potentially toxic to the liver and the Red blood cell.

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