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Research Article

Toxicological and Biochemical Studies of the Effects of Artesunate Overdose on Albino Rats

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ABSTRACT

The effect of artesunate on the haematological indices, hepatotoxicity and histology of the liver was investigated in 25 male adult albino Wistar rats collected from University College Hospital (UCH) Ibadan. The animals were divided into five groups of five each, Group A serve as a control, where normal clean drinkable water and feed were administered. Groups B,C, D and E were administered intra-peritoneally with 100mg/kg, 200mg/kg, 400mg/kg and 500mg/kg artesunate three times daily for a period of 7days. The animals were humanely sacrificed on the 7th day. The blood cell and serum enzymes were analysed. Haematological indices indicated insignificant (P<0.05) differences in the blood counts (WBC, Lymph, Gran, RBC HGB, and HCT/PCV) of all treated groups as compared with control while a significant dose dependent increase was observed in AST and ALT only at the highest dose of 500mg/kg. However, this was not in the case of ALP. The outcome suggests a compromise of liver function and possible damage to the hepatocytes at the highest dose level tested. Artesunate is thus safe when administered within the therapeutic range (within the given dose.)

Keywords: Haematological parameters, Enzyme assay, *Plasmodium falciparium,* Toxicology, Hemotoxicity, Hepatospecific.

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Introduction

Malaria has indeed become a pandemic disease in most parts of the world and its pandemic nature has been fortified with the advent of mutant strains of malaria parasites which defy chemotherapy, spurring researchers into seeking new chemotherapy or making a modification to pre-existing ones. One of the most recent phytochemical agents isolated and biologically characterized to exhibit anti-malaria potency is artemisinin. Artesunate is a widely used member of artemisinin derivatives. (Woodrow et al., 2005). The generally recommended effective doses in humans with uncomplicated falciparum caused malaria range between

10mg/kg/day for artemisinin and 2-5mg/kg/day for other artemisinin derivatives (White, 1997).

The underlying 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 oxygen species such as oxygen radical that propagated free radical reaction capable of inducing oxidative damage in the parasite (Olliaro *et al*., 2001). Therefore the present study is aimed at investigating the hepatotoxic and heamolytic effect of active acute exposure of rats to artesunate overdose.

Hepatoxicity (from hepatoxic) implies chemical-driven damage. More than 900 drugs have been implicated in causing liver injury (Friedman et al, 2003). Hepatoxicity and drug induced liver injury is responsible for over 50% of all hospital admissions and 50% of all acute liver failure, (Mcnally et al, 2006, Ostapwicz et al, 2002).

Hemotoxicity also known as hemotoxins are toxins that destroyed red blood cells (that is, cause haemolysis), disrupt blood clotting and or cause organ degeneration and generalized tissue damage. Hemotoxins are frequently employed by venomous animals including vipers.

In malaria endemic areas such as Nigeria, self-medication is quite common and purchase of antimalarial drugs in the open market is rampant. The possibility of administering overdose and misappropriation in the usage of antimalarial are very common.

Therefore, the present study is aimed at investigating the possible toxic potentials of the drug on the liver and blood using the hepatospecific and hemospecific enzymes markers.

Materials and Methods

Animals

Twenty five 16 weeks old Wistar albino rats with an average weight of 185g were purchased from University College Hospital (UCH) Ibadan animal house for the experiment. The animals were randomly divided into 5 groups each having 5 rats. The control and the experimental rats were being provided with feed and clean drinkable water. The rats were kept inside the cage in the animal house of Rufus Giwa Polytechnic Owo, Ondo State and were allowed to acclimatize for two weeks and were studied at room temperature. Experiments on animals and protocols conform to the guidelines of the National Institutional Health (NIH), (NIH publication 85-23, 1985) for laboratory animals and use.

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Artesunate Drug
Commercial Artesunate injections (Adamsnate) manufactured by Adams Pharmaceutical Groups Co. (Anhui) Ltd China was purchased from Fegtouchi Pharmacy, Ondo State. An intra-peritoneal method of administration was adopted.

Determination of Weight of Animals
The weights of the animals were determined using an electronic weighing balance every 7 days to verify and quantify the change in weight throughout the period of administration.

Experimental Design
The experimental animals were divided into groups of 5 (five) Albino Wister rats each. Groups B to E were treated, while group A was used as a control group. The drug was administered intra-peritoneally to the group as follows:

Group A: distilled water and feed, Group B:100mg/kg, Group C: 200mg/kg,, Group D: 400mg/kg and Group E: 500mg/kg. The animals were weighed before sacrificed for 24hours after the last dose on the 7th day. 5milimeter of blood samples was collected from the globular vein of the animal, through EDTA bottle which contains an anticoagulant reagent that prevents the blood from clotting, it was then centrifuged for 5 minutes for the separation of the red blood cell and the serum or plasma with the aid of centrifugation machine for 200rpm (revolution per minute). The centrifuge machine was used for the spinning of the blood for the serum to be separated and then used for analysis.

Chemicals and Reagents
All chemicals were of an analytical grade and are supplied from Sigma Chemical Co. USA. Distilled water was used in all biochemical assays.

Collection of Blood Sample
The rats were sacrificed through cervical dislocation and blood samples obtained through cardiac puncture. The blood was collected into appropriately labelled heparinized sample bottles and spinned at 4000 rev/sec for 5 minutes to harvest the serum. The serum was analyzed for various biochemical parameters.

Biochemical Method
Preparation of Liver Homogenate
The livers were excised and homogenized in 0.25M sucrose solution using mortar and pestle.

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Statistical Analysis

The experimental results were expressed as the mean ± S.E.M. Statistical significance of difference in in parameters amongst groups was determined by One way ANOVA followed by Duncan's multiple range test. P <0.05 was considered to be significant.

Results and Discussion

Results

Table 1: Doses (mg/kg)

Weight(g)	Control	100mg/kg	200mg/kg	400mg/kg	500mg/kg
Initial	112±1.39	153.88±6.96	176.78±13.23	196.23±9.49	198.24±9.52
Final	115.52±0.83	158.18±6.35	184.28±13.81	199.99±9.82	200.91±9.88

Values are reported as mean standard deviation from 25 rats

Table 1: The mean values of the body weight of the male adult albino Wistar rat before and after 7days of intra-peritoneal artesunate administration was noted.

Table 2: Toxicological and biochemical effects of artesunate on serum enzymes in rats (Liver function test)

Group	ALT	AST	ALP
1(Control)	23.75±1.10^{a}	23.02±1.47^{a}	30.36±1.59^{ab}
2 (100mg/kg)	130.57±0.47^{ab}	55.64±9.46^{a}	40.48±4.01^{bc}
3(200mg/kg)	140.97±1.66^{ab}	62.59±3.51^{a}	42.32±5.60^{c}
4(400mg/kg)	176.47±1.83^{ab}	89.63±0.90^{a}	31.32±1.16 ^{a}
5(500mg/kg)	180.07±0.84^{b}	96.26±9.09^{a}	34.50±0.80^{bc}

All values are presented in mmol. Values are mean ± S.D., n=5. Means with different superscripts down a column are significantly different at P<0.05. Values are expressed as mean ±SE. Same superscripts indicate values are not significantly different, while different superscript indicate value as significantly different. Administration of different doses of artesunate caused significant (p≤0.05) increase in the activity of liver enzyme compared with corresponding activity in control group.

Table 2 shows the toxicological and biochemical effects of artesunate on serum enzymes in rats (Liver function test).

Table 3: Toxicological and biochemical effect of artesunate on haematological parameters in rats

Group	WBC	Lymph	Gran (%)	RBC	HGB	HCT/PCV
1(Control)	7.43±0.20^{abc***}	42.30±0.98^{a}	42.30±0.98^{c}	6.51±0.32^{a}	10.33±0.57^{a}	30.30±1.3^{ab}
2(100mg/kg)	9.40±1.68^{bc}	63.73±1.71^{d}	63.73±1.71^{a}	6.66±0.64^{a}	12.73±0.90^{b}	34.60±3.6^{b}
3(200mg/kg)	6.00±0.32^{ab}	68.6±1.71^{c}	65.60±1.71^{a}	6.42±0.04^{a}	12.87±0.03^{b}	34.27±0.6^{c}
4(400mg/kg)	3.87±1.76^{a}	75.17±2.22^{cd}	75.17±2.22^{ab}	6.80±0.73^{a}	8.97±0.82^{a}	27.03±2.6^{bc}
5(500mg/kg)	10.37±0.07^{c}	50.23±1.25^{bc}	50.23±1.25^{bc}	7.20±0.47^{a}	13.40±0.98^{b}	38.93±3.7^{a}

*value are mean ± S.D., n=5
***means with different superscripts down a column are significantly different at p<0.05 WBC I(10^3/uL), Lymph (%), Gran (%), RBC (/L), HGB (g/L), HCT (%)
WBC white blood cell
Lymph Lymphocyte
Gran Granulocyte
RBC Red blood cell
HGB Haemoglobin
HCT Haematocrit or PCV (Packed Cell Volume)
Values are expressed as Mean±SE, same superscripts indicate values are not significantly different while different superscripts indicate values are significantly different. Haematological profile concentration of blood count were significantly (P≤0.05) varied in groups given different doses of artesunate when compared with corresponding concentrations in control group.

Table 3 shows the toxicological and biochemical effect of artesunate on haematological parameters in rats.

Discussion

The highest overall concordance of toxicity in animals with humans is with haematological, gastrointestinal and cardiovascular adverse effects (Wilson *et al* 2002), while certain adverse effects in humans, especially hypersensitivity and idiosyneratic reasons, and are poorly correlated with toxicity in animals. Furthermore, it is quite difficult to ascertain certain adverse effects in animals, such as headaches, abdominal pain, dizziness and visual disturbance. In addition, interspecies differences in the pharmacokinetic parameter make it difficult to translate some adverse effects from animals to human.

The liver is a major target organ for toxicity of xenobiotics and drugs, because most orally ingested xenobiotics and drugs pass through the liver and some chemicals are metabolized into toxic intermediates in the liver (Jaeschke *et al* 2002).

The assessment of liver damage by artesunate, is the determination of enzyme activities such as ALT and AST is largely used. In the present study, the increase in liver function test of ALT, AST and ALP in artesunate treated rats had been attributed to the damaged structural integrity of the liver, because these are normally located in the cytoplasm, mitochondria or microsomes and are released into the circulation after cellular damage or due to alterations in the permeability of cell membrane and increased synthesis or decreased catabolism of aminotransferases (Nuduka *et al* 2007).. In the present study, intra-periotoneal administration of artesunate was accompanied by elevation of total blood count when compared with concentrations in control group.

Table 2: It was observed that there is dose dependent increase in the activities of Alkaline phosphatase, Aspartate transaminase and Alanine transaninase which can be traced to possible necrosis where these enzymes are native, since they are not classified among the secretory enzymes. The drug was reported to accumulate significantly in three tissues, liver, erythrocyte and to a lesser extend in the kidney (Li *et al* 2006). The erythrocyte is known to have a low activity of ALT and AST. The kidney would rather play an excretory role than metabolic living in the liver as the potential site of substantial of artesunate and it metabolites which also represent the site of detoxification for most antibiotics chemicals (Amin and Geoffrey *et al* 2007). Artesunate is however known to inhibit protein synthesis in most mammalian system exposing the hepatocyte to unprotectable oxidative injury this accounting for the degree of oxidative damage done by accumulated artesunate and it metabolite.

Table 3: haematological investigations revealed following significant changes in the value of different parameters investigated when compared with those of respective controls. However, the increase or decrease ($p<0.05$) in the values obtained were significant was still within normal biological and laboratory limits or the effect was dose dependent. The increase in RBC was an indication of changes in the rate of the RBCs production. Similarly, the increase in all other blood parameters (WBC, Lymph, Gran, HGB and HCT/PCV) was an indication of the variations in their rate of production. WBC and indices relating to it such as lymphocytes usually show an increase in activity in response to a toxic environment (Robins SL, 1974). The effects of anaemia are greatly influenced by its severity, duration, and rate of development (Taiwo and Anosa, 1995). In this study, it may thus be safe to conclude that significant toxicity is usuallya result of a suicide attempt or inappropriate self-administration for therapeutic purposes (Raffi and Mark, 2009).

Nevertheless, Mulenga (1998) carried out a research study that the use of these drugs should be controlled and restricted to proven multi-drug resistance on severe malaria in order to preserve their efficacy. Also, Menally et al (2006) carried out a research study that drug-induced liver injury is responsible for 50% of all acute liver failures.

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With all these related studies and researches, large gaps both in literature and policy framework are still left uncovered. This means that the metabolic interaction within the body system of human being need close monitoring by health care providers and need to be simplified as well as given concrete interpretation of artesunate intake in order to maintain good health, hence the need for this research.

Conclusion

Conclusively, the research work shows that artesunate is not significantly toxic at the recommended dosage but an overdose may be potentially toxic to the liver and the red blood cell. Therefore artesunate is thus safe, when administered within the therapeutic range.

References

Amin R, Geoffery, T., (2007). Simulation and Prediction of invivo drug metabolism bin au human population from in vitro data. *Nature Reviews Drug Discovery*. 6(2): 140-148.

Andrade, R,J., Robles M., Fernandez-Castaner, A., Lopezortega S., Lopez-Vega MC., Lucena, M.I., (2007): "*Assessment of drug-induced hepatoxicity in clinical practises a challenge for gastroenterologist*". *World J.Gastoenterol* 13(3): 329-40.

Arundel, C., Lewis, J.H., (2007). "Drug induced liver disease "*Curr opin Gastroenterol*. 23(3): 244-54.

Benichou, C., (1990): "*Criteria of Drug induced liver disorder. Report of an international Consenus meeting*" *J. Hepatol;*. 11(2): 272-6

Brewwer. T.G., Pegging J.O., Grate S.J., (1994) Neurotoxicity in Animals due to Arteer and Arteether and Artemether. *Trans Rol. Soc. Trop Med Hyg*. 88 (Suppil); 533-36.

Dondrop, A.L, *et al* (2010): Artesunate Verse guanine in the treatment of severe falciparium malaria: a randomized trial. *The lancet* 376 (9753): 1647-1657

Friedman, Scott, Grendell, James H, M.C Quaid Kenneth r., (2003). Current diagnosis and Treatment in gastronenterology. New York: lang Medical books/McGraw-Hill. Pp. 664-679.

Gardner Groneberg, D. A., Grosse-Siestrup, C., Fischer, A. (2002) In vtro models to study hepatotoxicity. *Toxicol Pathol* 30: 394-399.

ISSN: 1597 – 9928. Science and Education Development Inst., Nigeria

Jaeschke H., Gores G.J., Cederbaum A.I., Himson J.A., Pessayre D., Lemasters J,J (2002): Mechanisms of Hepatoxicity. *Toxicol Sci.* 65(2): 167-76

James, L.P., Mayeux PR, Hinson, J.A., (2003): ''Acetaminopheno induced hepatotoxicity. Drug Metab, Dispos 31(12): 1499-506.

Kuvandik Sahu, S. C., Tuschl, G., Hrach, J., Hewitt, P. G., Muelluer, S.O. (2008).Application of hepatocyte cultures to predict toxicities in hepatotoxicity:from genomics to invitro and in vivo models (ed.) John Wiley and Sons England.

Lehne, R.A., Leanne, C., Diane, H., (1990): Pharmacology for Nursing Care. W.B.Sauders Company, Philadelphia. Pp: 964-965.

Li Q, Xie L.H, Haeberle A, Zhang J., Weina, P., (2006): The evaluation of radio labelled artesunate on tissue distribution in radio labelled artesunate on tissue distribution in rats and protein binding in humans. *Am J. Trop.Med.Hyg*. 75(5): 817-826.

Mcnally, Peter, F., (2006): GL/Liver Secrets with student consult Access Saint Louis . Mosby 1-5-605-3-618-7.

Mulenga, M., (1998): Facing Drug Resistance: Therapeutic Option for treatment of Uncomplicated. Plasmodium falciparium malaria in Adult Zambians *Journal of Medical and Health Science*, 2(1): 11-20

Ngokere, A.A., Ngokere, T.C., Ikwudinma, A,P., (2004). Acute Study of Histomorphological and biochemical changes caused by artesunate in visceral organs of the rabbit, *Journal of Experimental and Clinical Anatomy*, 3(2): 11-16

NIH publication 85-23, 1985

Nuduka Naruse K., Tang W., Makuuchi M. (2007): Artificial and Bioartificial liver support; A review of perfusion treatment for hepatic failure patients. *World J. Gastroenterol 13*: 1516-1521

Olliaro P, Haynes, R.K., Meunier B., Yuthavong Y., (2001): possible modes of action of the artemasinin-type compounds. *Trends in Parasitology*. 17(3): 122-126.

Wilson H, Betton G, Robinson D, Thomas K and Monro A, (2002). Concordance of the toxicity of pharmaceuticals in humans and in animals, *Regul. Toxicol. Pharmacol*., 32: 56-67.

ISSN: 1597 – 9928. Science and Education Development Inst., Nigeria

Ostapowicz, G., Fontana, R.J, Schiodt, F.V., (2002): "results of a prospective study of acute liver failure at 17 tertiary care centres in the United State." *Ann Intern Med*. 137 (12): 947-54.

Patei T., Roberts, L.R., Jones, B.A., Gores, G.J (1998): "Dysregulation of apoptosis as a mechanism of liver disease: an overview" *Semin Liver Dis*: 18(2):105-14.

Pukritayakamee, S., Chotivanich, K., Chantra, A., Clemens, R., Looareesuwan, S., White, N.J., (2004): Activities of Artesuante and Primaquine against Asexual and Sexual Stage Parasites in Falciparium Malaria. Antimicrob. Agents Chemother. 48(4): 1329-1334.

Quighaos, J. (1979). Antimalaria Studies on Quighaosu. *Chinese Medical Journal*. 92: 811-816.

Raffi K and Mark S. (2009): Plant poisoning, glycosides-cardiac. Continually Updated Clinical Reference.

Robins SL (1974). Lymph nodes and spleen: Pathologic basis of disease. WB Saunders Co., Philadelphia. 1050.

Taiwo VO and Anosa VO (1995). Fibrinogen, leukocyte and haematocrit value in cattle with various disease conditions. *Tropical Veterinarian*. 13:51-58

Trail, Seaquamat, (2005):" Artesunate Versus quinine for treatment of severe falciparium malaria: a randomized trial" *The Lancet* 336(948). 717-725.

White, N.J.WH, (1997): Assessment of the pharmacodynamic Properties of Antimalaria Drugs In Vivo. Antimicrobial Agents for *Chemotheraphy*. 41:1413-1422.

WHO (1998): Malaria Chemotheraphy. Tech. Reg. Ser. WHO Geneva

Woodrow et al , C.J., Haynes, R.K., Krishna, S., (2005): Artemisinin Mechanism of Action Post graduate. *Medical Journal,* 81(952): 71-78

ISSN: 1597 – 9928. Science and Education Development Inst., Nigeria