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Some Morphological Findings on the Heart of Adult Wistar Rats Following Experimental Artesunate Administration

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ABSTRACT

There has been increasing reports of ischemic cardiac disease which may be attributed to cardiovascular risk factor as well as adverse effects of some drugs. This study assessed the effect of oral artesunate on the heart morphology. Twenty-four adult wistar rats weighing between 150 and 230 g were separated into four groups, each containing six rats. Group A rats received distilled water while Group C, B and D received 4 mg kg⁻¹ body weight of artesunate orally on day one of the treatment. Group A continued with the same dose for the next 3 days of the treatment. Rats in group B and C received 2 mg kg⁻¹ body weight of artesunate orally for the next 6 and 13 days, respectively. The treated rats in group B, C and D were sacrificed, respectively on 5th, 8th and 15th day of the treatment. The control rats were sacrificed on the 15th day. The heart of each rat was removed and weighed before fixing in 10% formal saline for histological procedures. Results showed normal histological features in control while the treated rats revealed distortions, disaggregation, vacuolation and degenerative changes in cardiac tissue. The heart weight increased significantly ($p < 0.05$) from mean value of 0.41 ± 0.02 in group A to 0.48 ± 0.02 in group B, 0.49 ± 0.03 and 0.57 ± 0.04 g in group C and D, respectively. The observed, distortions, disaggregation, vacuolations and increased heart weight in the treatment groups may impair cardiovascular function and predispose to cardiovascular disorder.

Key words: Artesunate, degenerative changes, heart weight

INTRODUCTION

The problem of cardiotoxicity over the last decade has severally been reported in association with the drugs currently used for the treatment of *Plasmodium falciparum* infection (Touze *et al.*, 2002). Artesunate drug is commonly used as an alternative in chloroquine resistant cases of *Plasmodium falciparum* infection (Nwanjo and Oze, 2007). The effectiveness of artesunate is due to its rapid and complete hydrolysis to form dihydroartemisinin which is three to five fold more active and toxic than the parent compound (Li *et al.*, 2002). The effectiveness of artesunate is indicated in its mechanism of action. The presence of a peroxide bond that breaks in the parasite and free radicals, thereby exerting a direct cytotoxic effect on the cells and cellular membrane explains the efficacy of artesunate. Artesunate is rapidly absorbed after oral administration and it is metabolized in the liver (Ibezim and Odo, 2008). Several studies have shown that high doses

of artesunate can produce neurotoxicity such as selective damage to brainstem centers in mice and rats (Nontprasert *et al.*, 2002). Wesche *et al.* (2000) had reported the cardiotoxicity of halofantrine. Similarly, Ofusori *et al.* (2008) also have reported adverse cardiac effects in mice following treatment with quinine and mefloquine.

Cardiotoxicity of artesunate has been observed following administration of high doses (Hitti, 2004). The heart is a muscular, fist-sized organ that is located superiorly between the lungs in the left side of the anterolateral thoracic cavity. It lies deep to the true ribs, the pectoralis muscles and the mammary glands in women (Toovey, 2006). The heart is a muscular organ that circulates the blood through the vessels to different parts of the body (Burtis and Ashwood, 2003). The apex is directed anteroinferiorly toward the left. Its wall consists largely of cardiac muscle (myocardium), lined and surrounded by membranes-endocardium and pericardium. It is divided by a septum into right and left halves. The endocardium is a thin internal layer (endothelium and sub endothelial connective tissue) or living membrane that cover its valves. The myocardium is a thick middle layer that is composed of cardiac muscle while the epicardium is a thin external layer formed by the visceral layer of serous pericardium (Keith and Arthur, 2006). Reversible reticulocytopenia, drug fever, drug rash, bradycardia and transient first degree heart block have been reported following artesunate administration (Pukrittayakamee *et al.*, 2004). Metabolic reaction caused by toxicants primarily affects the heart, liver, kidney and lungs (Dybing *et al.*, 2002). This study reported the effects of artesunate on the heart weight and histology in adult wistar rats.

MATERIALS AND METHODS

Twenty four wistar rats of both sexes weighting between 150 and 230 g were used for this study that has been conducted in 2009. The rats were maintained under standard laboratory conditions. They were fed daily with rat feeds purchased from Global Farms Ogbomoso and water was given to the rats *ad libitum*. The wistar rats were subjected to a period of three weeks of acclimatization before the treatment. The wistar rats were separated into four groups each contained six rats. Group B, C and D (n = 18) served as treatment groups while group A (n = 6) was the control. The rats in treatment groups B, C and D orally received 4 mg kg⁻¹ body weight of artesunate base dissolved in distilled water through orogastric tube the first day. Wistar rats in the treatment group B continued with this dosage for the next three days while rats in group C and D received 2 mg kg⁻¹ once daily for the next six and thirteen days, respectively. This was done to investigate some effects of acute, therapeutic and chronic treatment of artesunate on the heart. The control group A received equal volume of distilled water as contained in the experimental doses.

The treated rats in group B, C and D were sacrificed by cervical dislocation on the 5, 8 and 15th day of the study, respectively while the control group A was also sacrificed on the 15th day of the study. The thoracic region was then dissected; blood was collected from the apex of the heart for blood platelet count. The heart of each rat was then blotted dry and weighed using a digital sensitive weighing balance before fixing in 10% formal saline for routine histological procedures.

Routine histological procedure was done following the methods of Carleton (1967) Histological slides were prepared and examined under the light microscope at x400 magnification. Digital micrographs of the desired sections were obtained to record the morphologic observations using a research microscope.

Statistical analysis: All data were presented as Mean±SEM. Statistical analysis of the data of comparisons between the control and treated groups in this study were carried out using one-way analysis of variance and significance was tested for using student's t-test. The $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the average body weights of the rats before and after the treatment. The control rats gained weights significantly ($p < 0.05$) from the beginning of the study to the end of the study. The body weights generally decreased in the treated rats as indicated in Table 1. The weights of the rats in group B and C (treated) rats did not decrease significantly from the beginning to the end of the treatment. The weights of the rats in group D (treated) decreased significantly from the beginning to end of the treatment (Table 1).

The mean heart weight of the control rats at end of the study was 0.41 ± 0.02 g which increased significantly ($p < 0.05$) to 0.48 ± 0.02 in group B (treated) rats, 0.49 ± 0.03 g in group C (treated) rats and 0.57 ± 0.04 g in group D treated rats.

The mean heart weights in the treated rats significantly increased compared with the control rats (Table 1). Treatment with a dose of 4 mg kg^{-1} body weight of the drug for four days caused a significant increase in the blood platelet counts of the rats in treatment group B ($27.2 \pm 3.36 \times 10^9 \mu\text{L}^{-1}$), as compared with the control group A rats ($14.4 \pm 1.88 \times 10^9 \mu\text{L}^{-1}$) while treatment with the same dose for the first day and with 2 mg kg^{-1} for the next six days and thirteen days in groups C and D rats, respectively did not significantly affected the platelet count in the two groups as compared with the control group A rats, as shown in the Table 1.

Histological findings: Figure 1 shows the micrograph heart from control (group A) rats. The cardiac histology of the control rats revealed a normal appearance showing normal and centrally arranged nucleus, connective tissue also appeared normal the cardiac muscle fibers are well arranged.

Figure 2 shows the cardiac histological features from artesunate-treated rats. The cardiac histology of the artesunate-treated rats showed enlargement of the connective tissue, vacuolation and deposit of serum in the endomysial capillary in the heart of treated rats.

Figure 3 shows the cardiac histological features from artesunate-treated rats after receiving the treatment for 7 days. The distortion, vacuolations and connective tissue separation or enlargement became more pronounced in this group compared with group B. Loss of nuclear and cellular components were more evident in this group.

Figure 4 shows the cardiac histological features from artesunate-treated rats after receiving the treatment for 14 days. The cardiac muscle fibers appeared diffused and degenerated. The toxic effect of artesunate became pronounced in this section with increased loss of cellular components, nuclei and vacuolations in this cardiac section of artesunate-treated rats.

Table 1: The Mean±SEM of the heart and body weights with blood platelet count after treatments

Groups	Heart weight (g)	% body weight	Initial body weight	Final body weight	Blood platelet count ($\times 10^9 \mu\text{L}^{-1}$)	% body weight
A	0.41 ± 0.02	0.22	156 ± 2.7	190 ± 1.5	14.4 ± 1.88	7.58
B	$0.48 \pm 0.02^*$	0.28	176 ± 0.8	169 ± 4.6	27.2 ± 3.36	16.09
C	$0.49 \pm 0.03^*$	0.25	195 ± 2.5	193 ± 2.1	19.7 ± 1.45	10.21
D	$0.57 \pm 0.04^*$	0.30	218 ± 3.7	189 ± 4.8	12.2 ± 1.40	6.46

*Significant difference between treated and control group ($p < 0.05$)

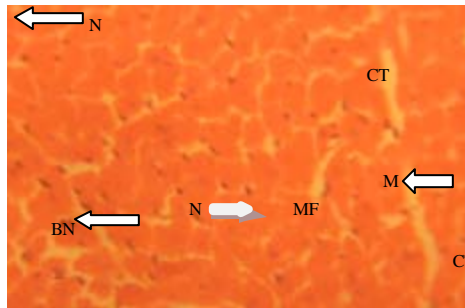


Fig. 1: Photomicrograph of the transverse section of the heart of group A (control) rat. H and E Stain x400. N is the nucleus, MF is muscle fibre, CT is connective tissue, BN bi-nuclei. The nuclei appeared normal, the connective tissue was normal, the nuclei and cardiac muscle fibres were well arranged

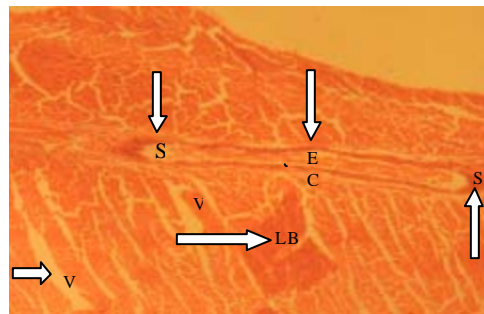


Fig. 2: Photomicrograph of the transverse section of the heart of group B treated rat H and E Stains x400. EC is endomysial capillary, S is serum, V is vacuolation, LB is lump of bacteria. The cells were normal, there was little damage to the cell; such as enlargement of the connective tissue or vacuolation, lump of bacteria and deposit of cirum in the endomysial capillary

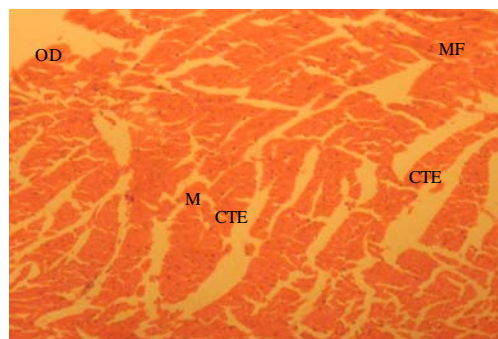


Fig. 3: Photomicrograph of the transverse section of group C treated rat. H and E Stains x400. V is vacuolation, MF is muscle fibre, CTE is connective tissue separation. The muscle fibres were normal, however, the nuclei were not visible and vacuolations were seen on the micrograph

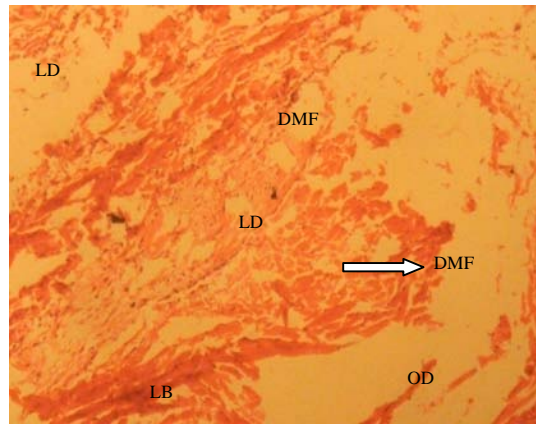


Fig. 4: Photomicrograph of the transverse section of the heart of group D treated rat. H and E Stains x400. LD is lipid deposit, LB is lump of bacteria, OD is oedematous deposit, DMF is diffused muscle fibre. There is great lysis of the cells, the cardiac muscle cells were also diffused with high deposits of lipids and oedematous deposits

DISCUSSION

Morphological changes that were dose dependent were observed in the heart sections from artesunate-treated rats compared with the control rats. These observations are supported by earlier findings of Ogbonnia *et al.* (2010) which reported that degenerative changes were observed in the heart tissue of mice following subchronic administration of high doses of chromolaena odorata administration. Administration of aflatoxin-B1 (AFB1) to rats resulted in cardiac damage (Mohamed and Metwally, 2009). Histopathological changes also have been observed in fetal heart rats that were treated with enalapril maleate; an antihypertensive drug that reduces blood pressure (Khaki *et al.*, 2008). Rodents that were exposed to isoproterenol treatment showed myocardial infarction (Upaganlawar *et al.*, 2011). Similarly, myocardial infarction also has been observed in rats following exposure to isoproterenol (Upaganlawar and Balaraman, 2010). Cardiomyopathy resulting from oxidative stress has been reported in wistar rats following administration of Doxorubicin (DOX) an extremely effective antitumor anthracycline and antibiotic drug (Saalu *et al.*, 2009). The myocardium of rats that were exposed to endosulfan treatment showed different degrees of degeneration, some of the myofibrils were found to be granular with pyknotic nuclei (Jalili *et al.*, 2007). The reported findings of these investigators appeared to be in agreement with the findings from this present study.

Disaggregation of connective tissues, vacuolations and degenerative changes are prominent in the artesunate-treated rats. Chemotherapy drugs are toxins and cause damage to the heart. As a result of this damage the heart is unable to pump enough blood to supply the body with essential oxygen and nutrients. Damage to the heart muscle by toxin is called cardiac toxicity. Cardiac toxicity may cause arrhythmias or it can develop into heart failure. One of the ways that radiation and chemotherapy drugs damage cells is by forming free radicals. Free radicals are unstable molecules which are formed during many normal cellular processes that involve oxygen. Cardiotoxicity is damage to the cardiac muscle inhabiting the normal function of the heart. It is commonly seen with chemotherapy and medications taken to control existing diseases. An overabundance of free radicals leads to oxidative stress causing the death of cardiac muscle cells.

Myocardial damage leads to disintegration of heart cell membrane (Irvine *et al.*, 1980). Myocardial damage has been observed in rats that were subjected to Isoprenaline treatment (Vibha *et al.*, 2011). Damage of the heart muscle cells (Myocytes) due to increased cardiac troponin I had been reported in albino rats following treatment with okposi and uburu salt lakes in Nigeria (Agbafor *et al.*, 2011). This report is consistent with the finding from this study. Konate *et al.* (2011) similarly reported that the heart weights of rats that were treated with aqueous acetone extract of *Cienfuegosia digitata* cav. (Malvaceae) increased significantly which is consistent with the present finding. Similarly, Liu *et al.* (2010) reported that microscopic observation in animal studies showed that high-dose cobalt chloride caused hyperemia, swelling of the heart and spotty necrosis. Histopathology analysis of the heart showed cell swelling, interstitial edema and loss of cellular outline, hyalinization of tissues and atrophy of cells in mice that were exposed to detergents solution (Yahaya *et al.*, 2011). Vaghasiya *et al.* (2011) had reported that the absolute and relative heart weight of female mice increased slightly following administration of methanol extract of *Pluchea arguta* Boiss. This observation is at variance with result of this study. Moreover, no significant microscopic change was observed in the hearts of rats that were treated with *Hymenocardia acida* leaf extract (Obidike *et al.*, 2011).

Heart failure does not mean that your heart has stopped or is about to stop. It means that the heart muscle can't pump with enough force to supply the body with blood containing essential oxygen and nutrients.

Cardiovascular Disease (CVD) has been described as the major cause of morbidity and mortality in adult human being (John *et al.*, 1999). Epidemiological studies have shown that elevated concentrations of serum total cholesterol and LDL-cholesterol, triglycerides, fibrinogen and platelet count are independent risk factors for CVD (John *et al.*, 1999). There has been increasing report of Ischaemic heart disease (Khaw and Barret-Connor, 1987). This increase may be attributed to an increase in the prevalence of known cardiovascular risk factor including drugs and local herbs (Ajani *et al.*, 2008).

Cushma *et al.* (1996) had reported that increase in serum total cholesterol LDL cholesterol and triglyceride level with a reduction in HDL cholesterol value with artesunate administration observed in the study conducted indicated that treatment with artesunate predisposes to cardiovascular disease. Serum fibrinogen is a component of the blood that plays a central role in clotting process. Goodrich *et al.* (1999) also have shown that the level of fibrinogen and platelet count is independent cardiovascular factor. Similarly, Cushma *et al.* (1996) have suggested that higher level of this parameter may predispose the affected individual to develop clots in their arteries and platelet aggregation as increase plasma viscosity, thereby increasing the risk of a heart attack or stroke. Ajani *et al.* (2008) have similarly reported from their study that artesunate administration increased both fibrinogen level and platelet count, indicating evidence of cardiovascular toxicity. They added that the data obtained from their study indicated that both amodiaquine and artesunate predispose to cardiovascular disease but this effect was more pronounced with artesunate than with amodiaquine.

The heart weights of the artesunate-treated rats significantly increased ($p < 0.05$) compared with the control rats. The observed increase in the heart weights of the treated rats is in agreement with the reported findings of Izunya *et al.* (2010) that recorded significant increase in heart weights of the wistar rats following chloroquine administration.

The increased heart weight has been attributed to the fact that poor metabolic product of the extract resulted in poor oxygen carriage. The heart needs to pump more blood leading to increased

metabolic activities of the heart which resulted in enlargement and increased in the heart weight. Similarly, Ajani *et al.* (2008) reported that, the heart weights of the artesunate-treated rats increased significantly ($p < 0.05$). This report is consistent with the finding from this study. The increased in the heart weight of the artesunate-treated rats in this present study might be due to increase metabolic activities of the heart as a result of blood-pumping action of the heart following toxic effect of the metabolic product of artesunate. This might have interfered with oxygen carrying capacity of the blood. The blood platelet count of the treated rats also increased significantly particularly in group B treated rats. The increased heart weights and platelet counts of the treated rats in this study might have resulted from the toxic effect of the artesunate administered to the treated rats.

The distortions, disaggregation of the connective tissues, vacuolations and degenerative changes in the cardiac muscle of the artesunate treated rats may be due to the toxic effect of the drug on the cardiac morphology.

CONCLUSION

This study concluded that the increased heart weights and platelet counts coupled with loss of cellular components and distortions of the cardiac morphology may adversely affect cardiac functions and consequently predispose to cardiovascular disease. Further studies that could corroborate these observations may be necessary.

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