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Histological Studies of the Cardiotoxicity of Artesunate in Wistar Rats

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ABSTRACT

Artesunate is a semi-synthetic derivative of artemisinin (Qinghaosu), an ancient Chinese fever medicine, which is now widely used as an antimalaria. The histological effects of normal and double normal dose of artesunate on the heart in Wistar rats were studied. The rats were divided into three groups (A, B and C) of five rats each. A and B served as the treatment groups, while C served as the control group. Group A rats were given 4mg/kg body weight (b.w) of artesunate daily for 3 days followed by 2mg/kg b.w daily for next 4 days. Group B rats were given 8mg/kg/kg b.w of artesunate daily for 3 days followed by 4mg/kg b.w daily for next 4 days, while group C rats were given only distilled water. On day eight of the experiment, the rats were weighed and sacrificed by cervical dislocation. The hearts were carefully dissected out and quickly fixed in 10% formal saline for histological studies. The histological findings after H and E method showed normal histological features in all the groups. Our study therefore suggests that artesunate at normal and double normal dose has no effects on the histology of the heart in wistar rats.

Key Words: antimalarial, artesunate, heart, histology, toxicity.

INTRODUCTION

Cardiotoxicity has become a major concern during treatment with antimalarial drugs [1]. For example, quinidine and halofantrine, have been reported to cause clinically significant delays in ventricular repolarization, resulting in a prolongation of the electrocardiographic QT interval on the electrocardiogram (ECG) [2]. Moreover, the use of chloroquine has been associated with toxic cardiovascular effects, including a fall in blood pressure [3], rhythm abnormalities [4,5], cardiomegaly and cardiac failure [6,7] and electrocardiographic changes, including T-wave depression or inversion, and prolonged QRS and QTc intervals [8,9]. However, the belated discovery that the antimalarial halofantrine causes marked QT prolongation and sudden death

[10] well after its registration by several regulatory authorities has focused attention on the potential cardiotoxicity of the antimalarial drugs [11].

Artesunate (AS), the most widely available of the artemisinin-related compounds, is a semi-synthetic hemisuccinate derivative of dihydroartemisinin (DHA) [12]. It is a new antimalarial drug, characterized by an immediate and rapid reduction of parasitemia with high efficacy in resistant parasites strains [13, 14]. AS may be given parenterally, intravenously, intramuscularly, orally, or rectally [15]. Oral artesunate is used either alone or in combination, usually with mefloquine or amodiaquine [16].

The artemisinins (ARS) are potent antimalarial drugs that are remarkably well tolerated [11]. However, high intramuscular doses of the oil-based artemether and artemotil have been associated with significant QT prolongation in toxicologic studies conducted in beagle dogs, raising the possibility of cardiotoxicity with this class of drugs [17]. A report has also shown that intravenous AS does not have significant cardiovascular effects in patients with severe falciparum malaria and high intravenous AS does not prolong the QT interval [11]. There were no reports in literatures documenting the effect of ARS on the histology of the heart. In view of this, this study was undertaken to investigate the effects of AS on the histology of the heart in wistar rats.

MATERIALS AND METHODS

Location and Duration of Study

This study was conducted at the histology laboratory of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The preliminary studies, animal acclimatization, drug procurement, actual animal experiment and evaluation of results, lasted for a period of one month (January, 2010). However, the actual administration of the drug to the test animals lasted for one week (15th, January to 21st, January 2010).

Animals

Fifteen adult wistar rats weighing between 100-150g were used for this experiment. They were obtained and maintained in the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State. They were divided into three groups A, B, and C of five rats each. Groups A and B were the treatment groups, while Group C served as the control. They were kept in each group per cage and fed with grower's mash produced by Bendel Feeds and Flour Mills Limited, Ewu, Nigeria. Water was given ad libitum. They were allowed to acclimatize for one week before commencement of the study. Ethical approval was sought and received from the Department of Anatomy, College of Medicine, Ambrose Alli University, Ekpoma, Edo State on the need to observe completely the rules guiding the employment of rats for scientific studies.

Drug Administration

The AS tablets used for this experiment were manufactured by Mekophar Chemical Pharmaceutical Join-Stock Company, Ho Chi Minh City, Vietnam and purchased from Irrua Specialist Hospital, Irrua, Edo State. The drug solution was made with distilled water (1mg/ml) and administered to the animals by orogastric tube for a period of seven days. The dosage of AS was as per WHO recommendation of 4mg/kg body weight daily for 3 days followed by 2mg/kg body weight daily for the remaining 4 days. All the animals were weighed before the experiment.

The drugs were administered to the groups as follows:

Group A: 4mg/kg body weight of AS daily for 3 days followed by 2mg/kg body weight daily for the remaining 4 days.
Group B: 8mg/kg body weight of AS daily for 3 days followed by 4mg/kg body weight daily for the remaining 4 days.
Group C (Control): Distilled water.

The animals were sacrificed by cervical dislocation 24 h after the last dose on the 8th day of the respective treatment and the hearts were harvested.

Histological study
The heart tissue was immediately fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin and serial sections were cut and each section was stained with hematoxylin and eosin [18]. The slides were examined under light microscope and photographs were taken.

RESULTS

Histological analyses of the hearts in all the groups (A, B and C) showed normal morphological appearances (Plates 1 and 2). The myocardium in all the groups showed well organized myofibrils with long cylindrical mononucleated cells. In histological anomalies of the heart associated with antimalarial toxicity, certain features are recognized. These include hypertrophy of myocardiocytes with heavily vacuolated cytoplasm, disorganisation of the myofibrils and scanty cellular components [19, 20, 7]. Of interest however, is that none of these histological anormalies were observed in the tissue micrographs.

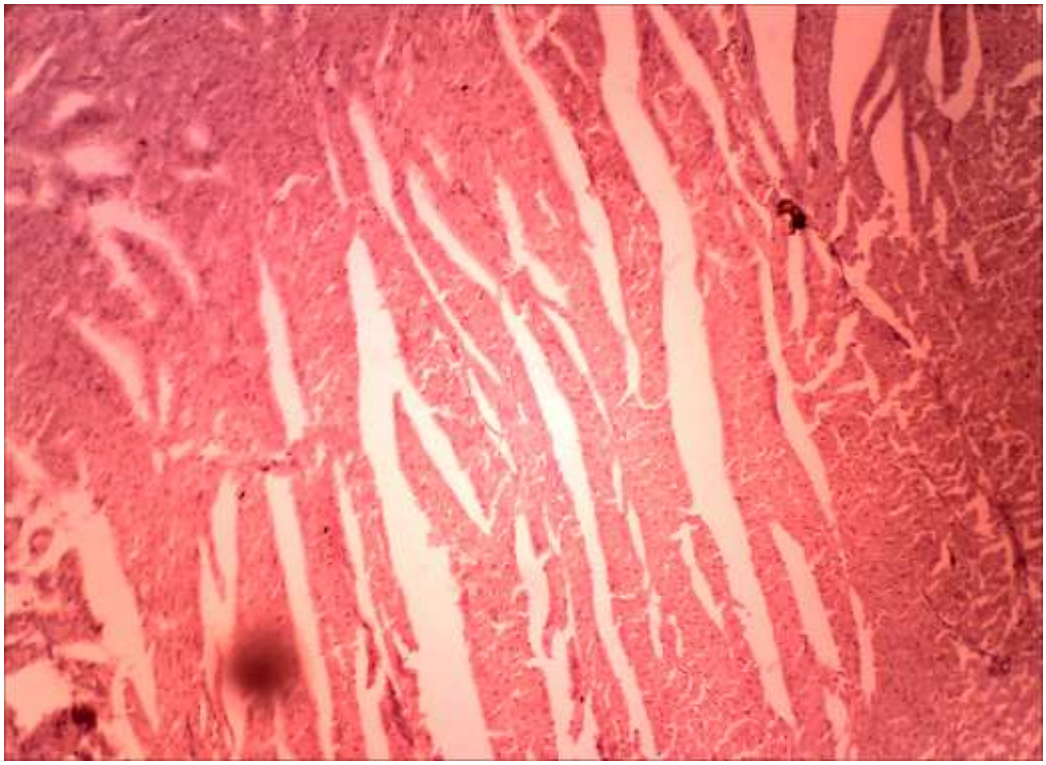

Plate 1: Photomicrograph of sections of heart tissues from groups A, B, and C showing normal histological features (H & E stain, X100).

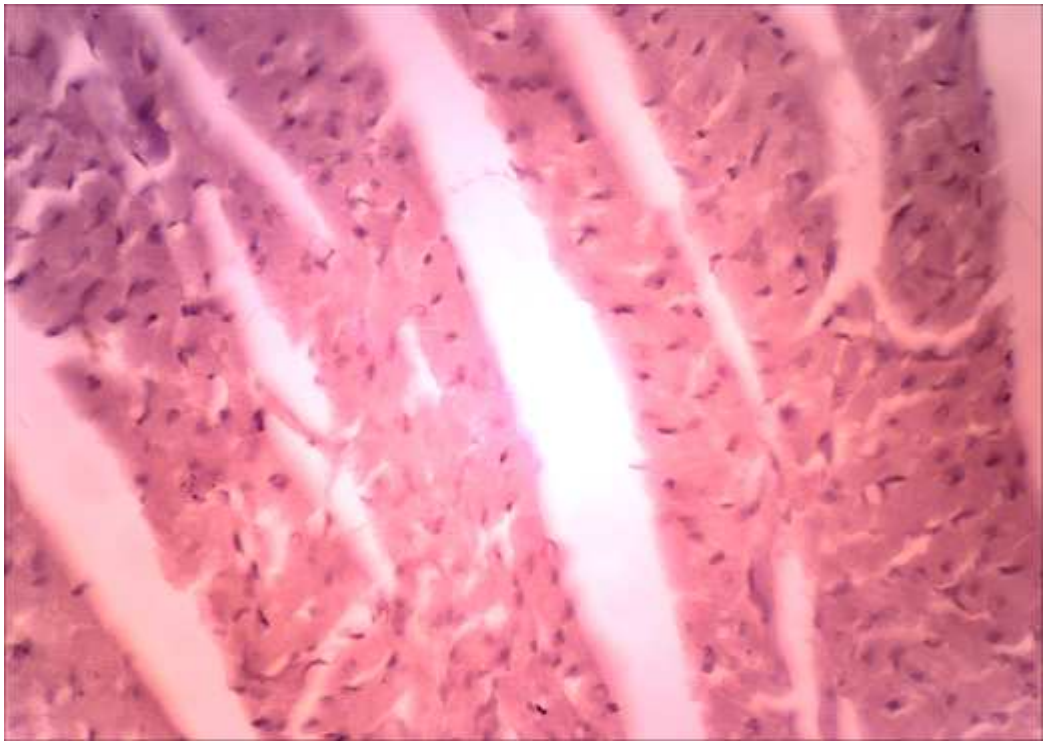

Plate 2: Photomicrograph of sections of heart tissues from groups A, B, and C showing normal histological features (H & E stain, X400).

DISCUSSION

AS is a water-soluble ARS derivative extracted from the plant *Artemesia annua* (quinghao). In this study, the effects of AS on the histology of the heart in wistar rats were investigated. ARS is a sesquiterpene lactone containing an endoperoxide bridge representing the active moiety of the molecule. In addition to their antimalarial activity, artemisinin and its derivatives are also active against cancer cells [21, 22, 23, 24, 25].

ARS exerts its anti-malarial activity by generation of free radicals or reactive oxygen species (ROS), through iron-catalyzed cleavage of the endoperoxide bridge [26]. The ROS cause macromolecular damage by alkylating heme and and several other proteins, such as translationally controlled tumor protein, histidine-rich protein and pfATP6, the sarco/endoplasmatic reticulum calcium ATPase [27, 28, 29, 30].

Of interest, is the fact that ROS have been implicated in a lot of adverse effects in different parts of the body [31, 32, 33]. With respect to the cardiovascular system these include atherosclerosis, ischaemic heart disease, hypertension, cardiac hypertrophy and cardiac failure [34]. Normally these ROS are effectively kept in check by the various levels of antioxidant defenses. Imbalance of this reaction either due to excess ROS formation or insufficient removal by antioxidants leads to oxidative stress (OS) [35]. ROS may cause cellular alteration by inhibiting protein synthesis, inactivating enzymes, cross linking of proteins and DNA, thereby leading to loss of membrane functions [36] and myocyte architecture.

Large clinical studies with malaria patients have shown that AS is well tolerated, with a few and insignificant side effects [37, 38, 39]. However, several studies have showed evidence of toxicity on the brainstem [40, 41, 42], superior colliculus [43], stomach [44], testis [45, 46] and the liver [47] in artesunate treated rats. Moreover, AS has been reported to destroy cancer cells [48], and

also reduces proliferation, interferes in DNA replication and cell cycle and, enhances apoptosis through the intrinsic death pathway by ROS generation [30].

The results of the present study, however, suggest that artesunate at normal and double normal dose has no effects on the histology of the heart in Wistar rats. There are reports that chronic oral administrations of quinine and chloroquine have effects on the histology of the heart in Wistar rats [7, 19].

CONCLUSION

This study suggests that oral administration of artesunate at normal and double normal dose has no effects on the histology of the heart in wistar rats.

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