Journal of Medicine and Medical Sciences Vol. 5(3) pp. 52-60, March 2014
DOI: http:/dx.doi.org/10.14303/jmms.2012.213
Available online http://www.interesjournals.org/JMMS

Full Length Research Paper

Cytoarchitectural comparism of the left ventricle and ascending aorta of antimalarial treated rats

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Abstract

Some of the effects of therapeutic, acute, and overdose treatment of Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy on the left ventricle and ascending aorta of adult Wistar rats were studied using the routine Haematoxylin and Eosin (H and E) and Orcein stains. The drugs were dissolved in distilled water and administered orally using an orogastric tube. The animals were sacrificed on days 3 and 5 after which the excised tissues were subjected to histological analysis. Appearance of autophagic vacuoles were evident in the Chloroquine treated group and the sections revealed some varying degrees of disorientation and disarrangement of cardiac cells, with less staining intensity of elastic fibres occurring in the Orcein stained sections indicating breakdown of the cellular architecture of the left ventricle as a result of acute overdose administration of the drugs with the most profound effect being evident in the Chloroquine treated group. It is concluded that acute overdose treatment of the drugs will have deleterious effects on the metabolic activities of the left ventricle and ascending aorta of adult wistar rats.

Keywords: Chloroquine, Artesunate, Amodiaquine- Artesunate combined therapy, Left ventricle.

INTRODUCTION

Antimalarial drugs are designed to prevent or cure Malaria (Cox, 1992). However, nearly all medicines have the potential to cause harm when they are misused or abused, and the consequences can be dangerous and even fatal (Hollinger and Dabney, 2002).

People that deliberately misuse therapeutic antimalarial drugs may do so for a number of reasons. Some may want to obtain quicker results within the shortest possible time, especially as regards the intake of artesunate whose dose duration is between 5- 7 days (Merimekwu *et al*., 2000). Some may doubt the efficacy of the drugs, and an example is the intake of Chloroquine whose efficacy is now in doubt due to its resistance (Berman, 2004). Some people fear that the doses might not produce the anticipated results; hence they prefer to increase the dose in order to compensate for this presumed inadequacy. In some cases of prophylactic treatment, high doses of antimalarial drugs are administered. An example is chloroquine (300-600mg daily) (Adjene and Caxton-Martins, 2006).

Some other individuals may take these antimalarial drugs at higher doses for the purpose of treating other diseases. Examples are Chloroquine taken at a dose as high as 200- 400mg daily to treat discoid lupus erythematosis, hepatic amoebiasis, and rheumatoid arthritis (Teixeira et al., 2002). Artemisinin is also taken at doses as high as 200-1000mg daily to treat Cancer (Singh and Lai, 2001). However, a relatively small increase in these designed doses may lead to fatal outcomes (Nwanjo and Oze, 2007).

In Malaria endemic areas such as Nigeria, self medication is quite common (Nwanjo and Oze, 2007). Hence, the possibility of administering overdose and incidences of misappropriation are quite common (Udonan, 2000).

Hence, there is a great need to investigate some of the effects of these antimalarial drugs on vital organs of the body.

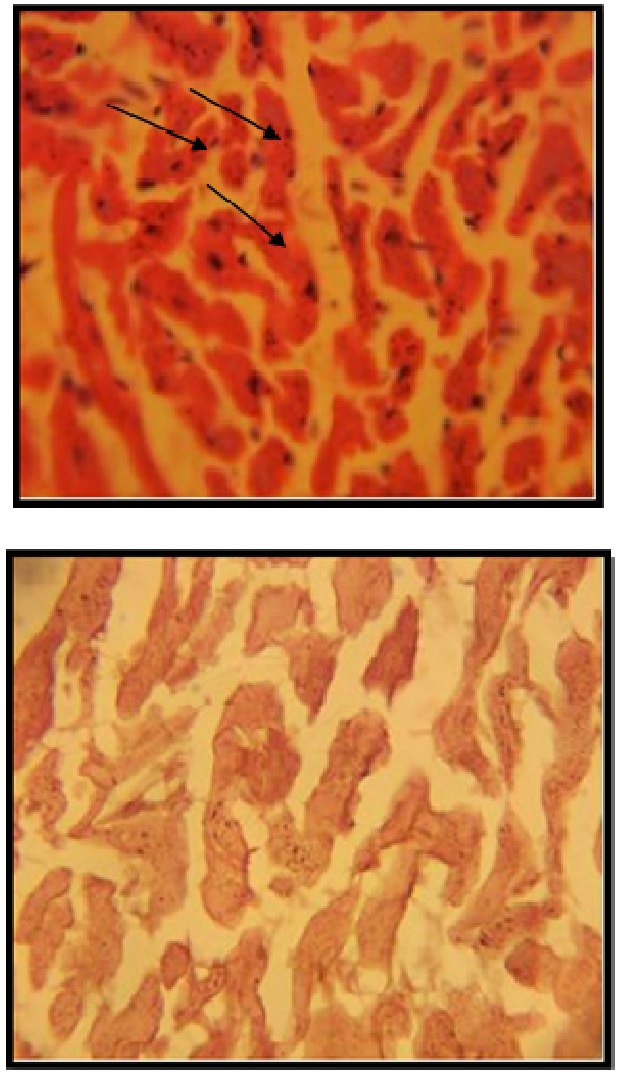
MATERIALS AND METHODS

Drugs and Administration

Chloroquine (Chloroquine phosphate BP of Evans pharmaceutical Co., Lagos. Artesunate (G- Sunate Forte

Group 1

Left Ventricle



H&E and Orcein sections reveal disorientation and disarrangement cardiac cells treated with Chloroquine. The cells reveal less intense staining of the elastic fibres by Orcein indicating some level of disintegration has occurred. Arrows reveal appearance of autophagic vacoulations in the cytoplasm of the cardiac cells. (X400)

of Bliss GVS Pharmaceutcal., India), and Amodiaquine-Artesunate combined therapy (Amodiaquine- Artesunate combined therapy of Plethico Pharmaceutical Ltd, India), was purchased from Fiolu Pharmacy, Gambari, Ilorin, Kwara - State.

1 tablet of each drug was dissolved in 100ml of distilled water, and administered with an oro- gastric tube.

The average weight of rats in each treatment group was determined in order to obtain the various dose of administration.

The therapeutic dose was determined by obtaining the therapeutic dose of a 70kg man for each drug, in order to determine the equivalent dose for the specific average weight of the each treatment group.

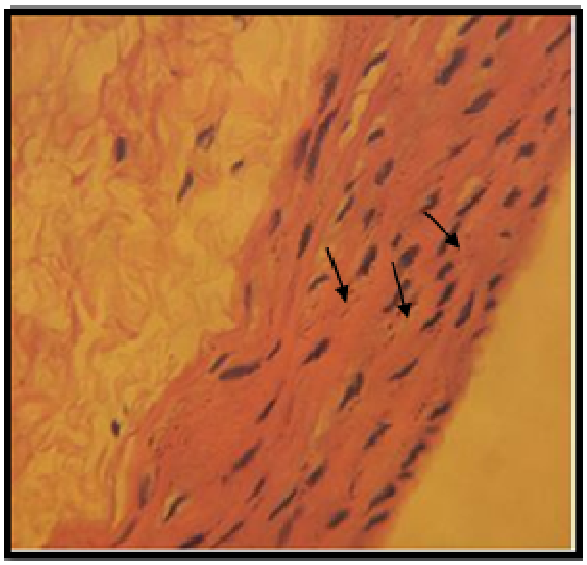
The acute dose was determined by reducing the duration of administration of the therapeutic dose to a 7-hourly period.

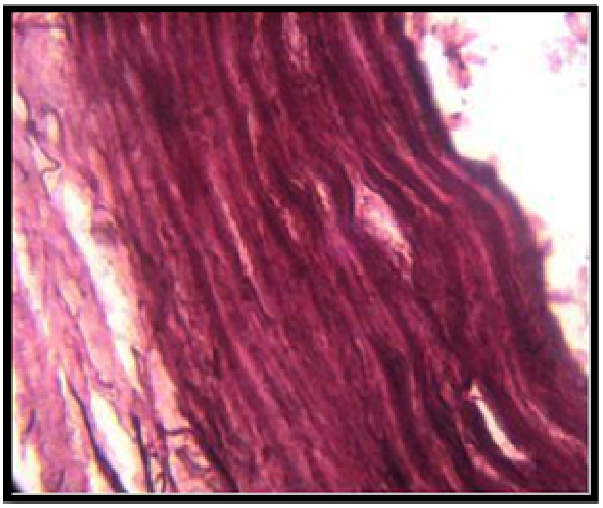
The dose for overdose group was determined by administration of a double therapeutic dose following the same duration of administration as the therapeutic dose.

Hence, the doses are as follows:

Group 1 received 0.53ml and 1.07ml of Chloroquine for

Ascending Aorta





H&E and Orcein sections reveal some level of distortion and disorientation, indicating gradual breakdown of the cellular architecture. The fibres appear less tortuous suggesting some degree of disorientation has occurred also, the arrows indicate portions on the elastic fibres which reveal some degree of disintegration (X400).

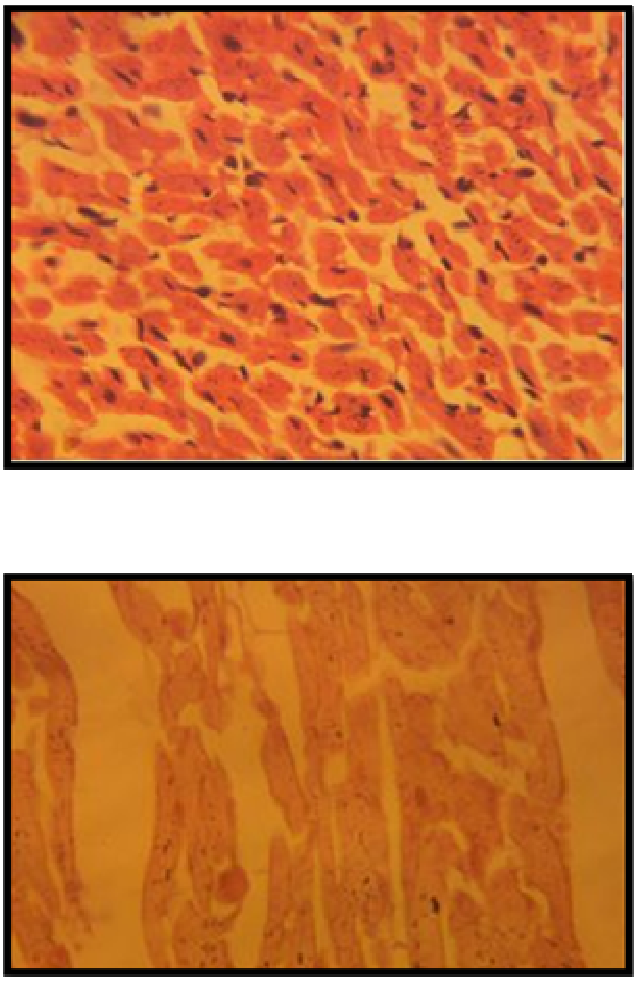
3days.
Group 2 received 0.26ml and 0.52ml of Artesunate for 5days
Group 3 received 1.14ml of Amodiaquine- Artesunate combined therapy for 3days.

Animals and Treatment

20 Wistar rats weighing between 200-300g were used. The animals were kept in Iron- meshed cages and maintained in the Animal holdings of Human Anatomy Department, University of Ilorin, Ilorin, Kwara-State and all the animals were subjected according to ethical treatment according to Guide for Care and Use of Laboratory animals after approval by the ethical committee, University of Ilorin, department of Anatomy. The animals were fed with rat pellets (Bendel feeds, Ilorin, Kwara-State) and liberally supplied with water. The animals were divided into four groups and administered

Group 2

Left Ventricle



H&E and Orcein sections reveal varying levels of disorientation and disarrangement of cardiac cells. The Orcein sections reveal less intense staining of elastic fibres by Orcein indicating some level of disintegration has occurred. (X400)

Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy respectively.

Sacrifice of Animals

Sacrifice of animals was by cervical dislocation. Abdominal incision was made and the thoraco-abdominal region was exposed through the midline.

Collection of Tissue Samples

The left ventricle and ascending aorta were excised and fixed in 10% formal saline for histological examination.

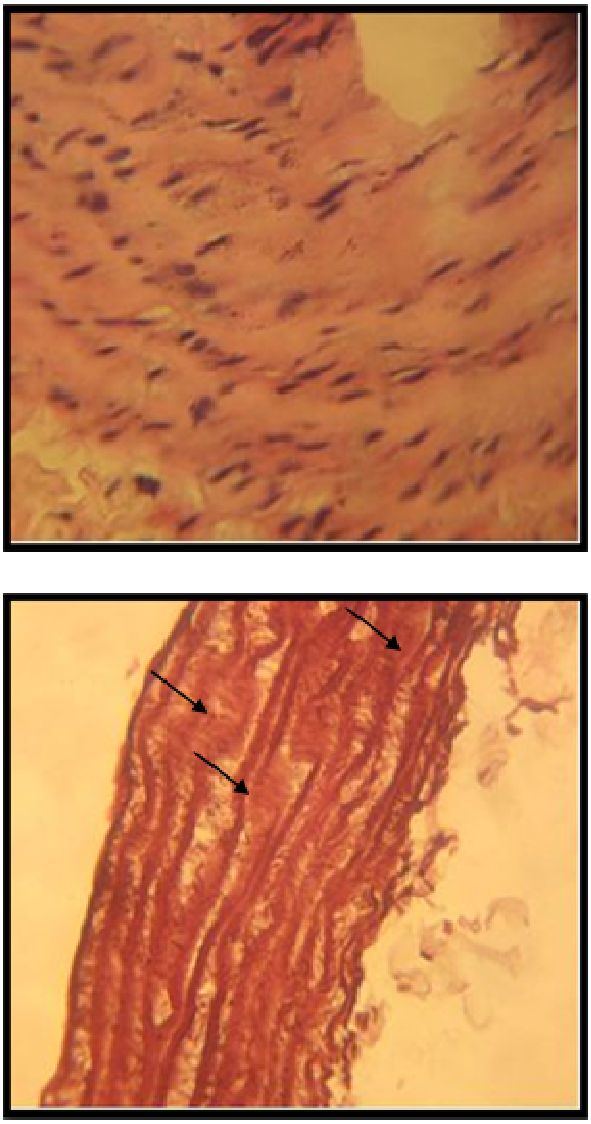
Histological analysis

This was done using Haematoxylin and Eosin (Pearse, 1980) and Orcein – Modified Taezer- Unna (1891) methods respectively.

RESULTS AND DISCUSSION

The present study confirms that Chloroquine, Artesunate, and Amodiaqine- Artesunate combined therapy alters the cellular integrity of the left ventricle and ascending aorta of the rats given acute overdose treatment of antimalarial drugs. Evidence is shown by the appearance of autophagic vacoulations in the cytoplasm of the cells in

Ascending Aorta



Section reveals some level of distortion and disorientation, indicating some degree of breakdown of the cellular architecture, showing the elastic fibres of the ascending aorta treated with Artesunate. The arrows indicate portions on the elastic fibres which reveal some degree of disintegration (X400).

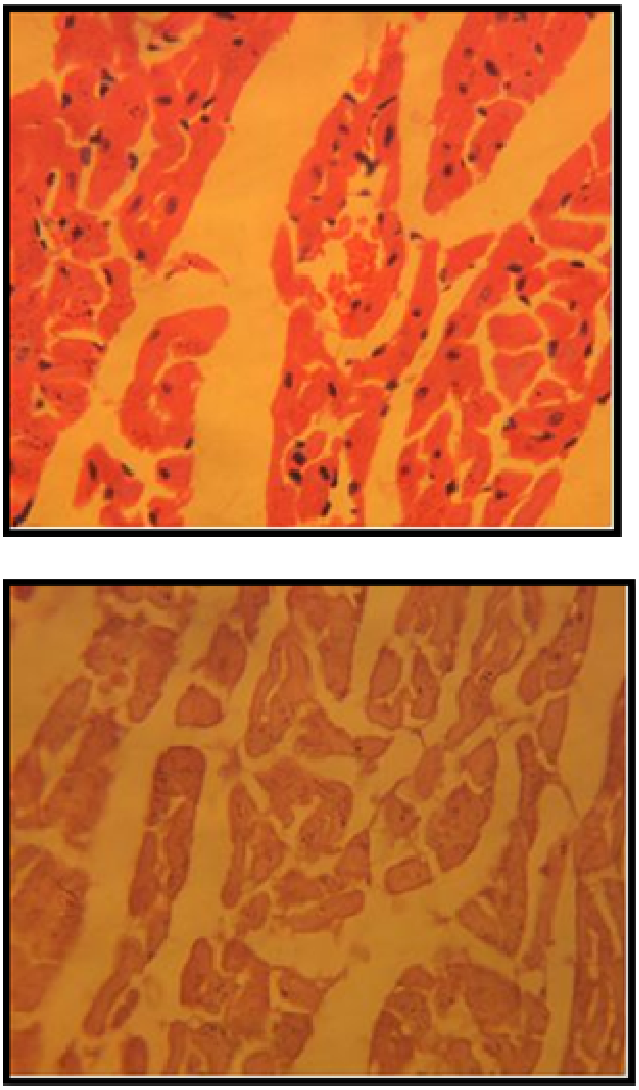
the treatment group.

The integrity of the elastic fibres in the treatment groups appeared distorted; this agrees with the work of Chung *et al.,* (2007) indicating some amount of weakening and degradation of elastic fibres and which can compromise the integrity of the cardiac tissues. Also, some elastic fibres appeared less tortuous compared to the control, indicating some degree of loss of integrity in the fibres.

Many of the known biological effects of Chloroquine are thought to be directly related to its Lysosomotrophism (Christian *et al.,* 1974) which may lead to cell necrosis (Steven *et al.,* 2006). Also, Chloroquine is said to induce lysosomal destabilization or LMP (lysosomal membrane permeability) as a result of increased oxidative stress (Ogunbayo *et al.,* 2006). Lysosomal destabilization occurs as an early event in apoptosis (Kurz *et al.,* 2004). (The discovery of lysosomal destabilization was a result of experiments on the induction of apoptosis in cultured cells by moderate oxidative stress (Kurz *et al.,* 2008). Lysosomal membrane permeability was found to be a response to such stress, which was then apparently fol-

Group 3

Left Ventricle



H&E and Orcein sections reveal some amount of disorientation and disarrangement of cardiac cells, with the less intense staining of elastic fibres by Orcein indicating breakdown of the cellular architecture of the left ventricle (X400).

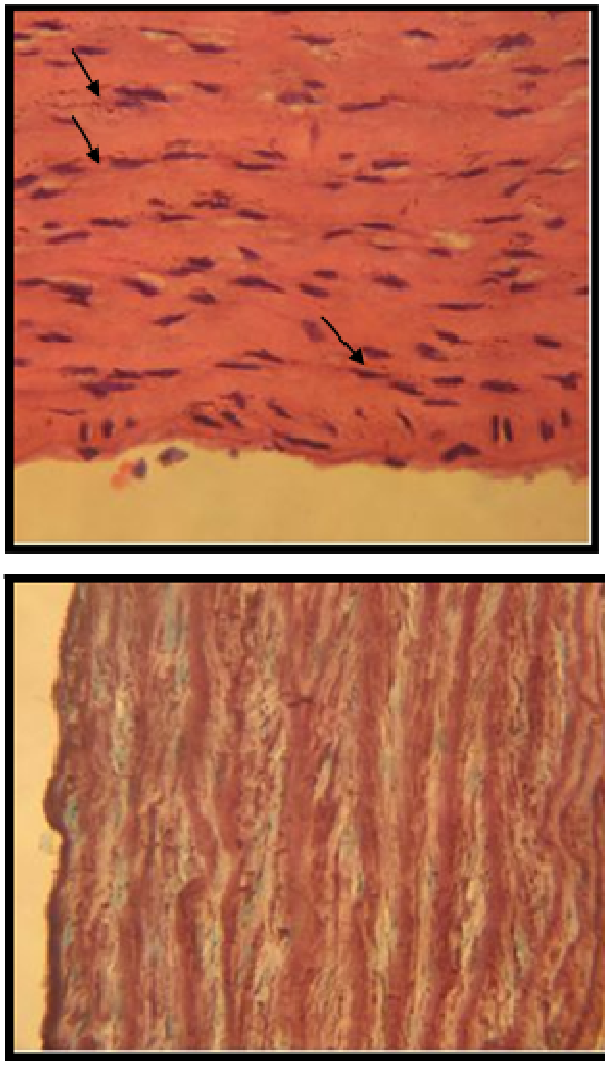
lowed by mitochondrial membrane permeability and classical apoptosis (Brunk *et al.,* 2001).

During schizogony, Plasmodium subsists on amino acids derived from hemoglobin, resulting in production of the prooxidant heme iron complex ferriprotoporphyrin IX (Ginsburg *et al.,* 2003). This heme iron complex is detoxified in Plasmodium through a number of glutathione-dependent pathways (including incorporation into hemozoin) (Ginsburg *et al.,* 2003).

Due to the weak base properties of Chloroquine, it accumulates in the vacuole where it exerts antimalarial properties by raising the internal pH (De Slauriers *et al.,* 1987). It also appears to inhibit the detoxification of ferriprotoporphyrin IX, in part by diminishing the availability of reduced glutathione (Deslauriers *et al.,* 1987). Excess iron and ferriprotoporphyrin IX, in the absence of sufficient glutathione, leads to autooxidation and parasite death (Ginsburg *and Golenser,* 2003). Furthermore, chloroquine appears to interact directly with free ferriprotoporphyrin IX in an electron transfer reaction that produces highly reactive radicals (Ginsburg and Golenser, 2003).

Amodiaquine like Chloroquine also accumulates in the lysosomes and brings about loss of function. The parasite

Ascending Aorta



H&E section reveals a more intense staining of the nuclei suggesting some amount of recovery after assault from overdose intake of the drug**.** Orcein section shows the elastic fibres of the ascending aorta treated with Amodiaquine Artesunate combined therapy. The arrows indicate portions on the elastic fibres which appear disintegrated; also, the fibres appear less tortuous(X400).

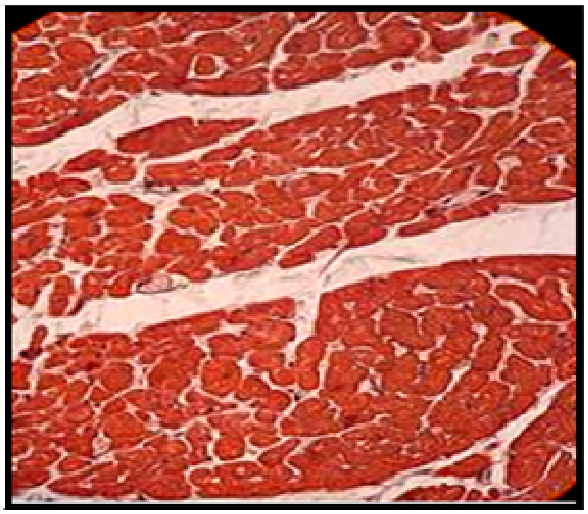
is unable to digest hemoglobin on which it depends for its energy. It also appears to bind to nucleoproteins and inhibits DNA and RNA polymerase (Farombi, 2000).

Artesunate contains two oxygen atoms linked together to form an endoperoxide bridge, this reacts with iron atoms to form free radicals (Ridley and Hudson, 1998). Lysosomes are sensitive to stress, especially to oxidants (Brunk and Svensson 1999; Kurz *et al*. 2004, 2006; Persson *et al.* 2003). This could be the reason for the presence of autophagic vacuoles of the cells in the treatment group. It was reported that a number of acidic hydrolases are capable of degrading most complex organic molecules, such as proteins, carbohydrates, lipids and nucleotides (Yu *et al.,* 2003b).

Lysosomes are observed to be a heterogeneous group of vacuoles of different sizes, form, and density. In the transmission electron microscope, some lysosomes are seen to contain still recognizable extra- and/or intracellular material under degradation, while others look homogeneous or contain electron-dense whorls and clumps. Lysosomes are also described as suicide bags' (de Duve, 1959), and it is suggested that these membranes are in need of antioxidant protection, (Rupar *et al*., 1992). This hypothesis is evident from the results obtained from this investigation; in the histological sections the cells of the treatment groups revealed the

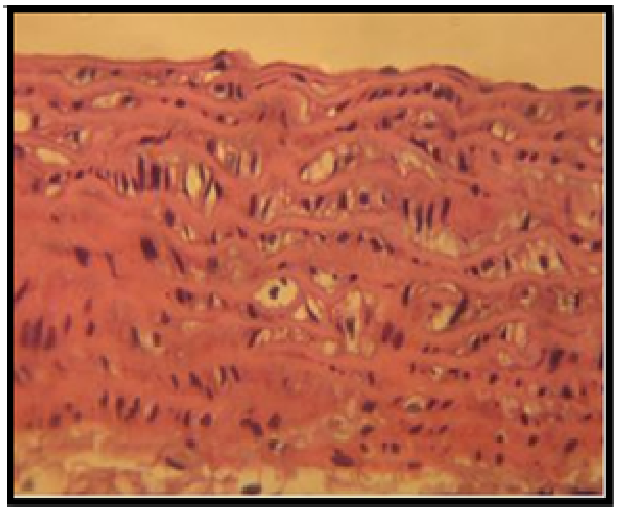
Control

Left Ventricle



Transverse section of cardiac muscle showing normal cell morphology and intact cell distribution of the cardiac cells x400 (Ross *et al.,* 2003)

Ascending Aorta



Section of the ascending aorta revealing intact fibres and deep staining intact nuclei x400

presence of autophagic vacuoles.

CONCLUSION

The purpose of chemotherapy is to cure the Medical condition being treated. However, in a bid to accomplish this purpose, cases that involve drug misuse and abuse occurs. There is therefore a great need to apply caution while taking these drugs. From this study, it has been demonstrated that acute, and overdose treatment of Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy have deleterious effects on the histological integrity (elastic fibres, and lysosomes) of the left ventricle and ascending aorta of adult wistar rats when assaulted with Chloroquine, Artesunate, and Amodiaquine- Artesunate combined therapy.

Hence, antimalarial drugs should be taken with a great

deal of caution as there is a high level of vulnerability towards antimalarial drug toxicity arising as a result of abuse and also misuse.

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How to cite this article: Akinrinade I. D., Memudu A.E., Dare B.J. and Ogundele O. M. (2014). Cytoarchitectural comparism of the left ventricle and ascending aorta of antimalarial treated rats. J. Med. Med. Sci. 5(3):52-60