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Research Paper

Cardio-protective effect of turmeric against Doxorubicin induced-oxidative stress in Wistar rats

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ABSTRACT: *Doxorubicin is one of the effective antineoplastic drugs, commonly used against breast, ovarian, testicular, thyroid, lung cancers and haematological cancers including Hodgkin Lymphoma and prevalent non-Hodgkin lymphomas, although, not without severe adverse effect including cardio-toxicity, potentially due to induction of oxidative stress. Thus, it is our aim in this study to evaluate the potential of turmeric root extract, and vitamis C and E to mitigate this cardio-toxicity in Wistar rats. In this study, 54 adult Wistar rats were divided into 9 groups of six animals each. Group 1 animals served as control (normal saline), group 2 animals served as negative control, and received Doxorubicin (DOX), group 3 animals were given DOX and turmeric, group 4 animals received DOX and vitamin C, group 5 animals received DOX and vitamin E, group 6 animals received DOX, vitamins C and turmeric, group 7 animals received DOX, vitamin E and turmeric, while group 8 animals received DOX, vitamin C and vitamin E and finally, group 9 animals receive DOX, vitamin C, vitamin E and turmeric. The experiment lasted for 28 days and heart harvested and processed for histological assessment. The histological study revealed that DOX caused distorted cardiac muscle with fused nuclei, while turmeric root extract as well as vitamin C and the combination of turmeric and the other two vitamins prevented these pathological changes, implying an exhibition of anti-oxidant potentials.*
Keywords: *Cardio-protective, turmeric, Doxorubicin, oxidative stress*

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I. INTRODUCTION

Anthracyclines are widely recognized as a class of effective chemotherapeutic agents to treat different types of cancer since their discovery in the 1960s [1, 2, 3, 4, 5, 6, and 7]. Doxorubicin (DOX) is one of the effective antineoplastic drugs, commonly used against breast, ovarian, testicular, thyroid, lung cancers and haematological cancers including Hodgkin Lymphoma and prevalent non-Hodgkin lymphomas [4 and 8]. Doxorubicin is a product of *Streptomyces peucetius var. caesius*, a prototype agent of anthracycline antibiotics [9]. Despite its widespread use, DOX therapy demonstrated dose-limiting effects owing to its acute and chronic cardiac toxicity [10 and 11. The mechanism of this cardiotoxicity was researched by many studies and today it is thought that the major cause of this effect is the tissue damage induced by free oxygen radicals [11]. During the doxorubicin metabolism, reduction of the kinone group by cytochrome P-450 reductase and xanthine oxidase into the semikinone radical [12] and the capture of the electrons released during this process by oxidative agents like oxygen, initiates a reaction chain that forms free oxygen radicals and causes cardiomyocytes cell death [13 and 14] i.e., the hydrogen peroxide and superoxide radical reduce the levels of the endogenous enzyme (glutathion peroxidise) that is responsible for scavenging free radicals, thus increase oxidative stress which results in cardiomyopathy [15 and 16]. When it was discovered that the formation of free oxygen radicals had a significant effect in the cardiotoxic mechanism of doxorubicin, because of the reduction of endogenous antioxidant systems in the body, exogenous antioxidants were then introduced along with DOX treatment regimen [14 and 17]. Many drugs, especially those with free radical scavengers and antioxidant effects, were used with DOX combinations [18 and 19]. Melatonin, that is physiologically present in the body and secreted by the pineal gland, is among the antioxidant agents that were used to protect against the oxidative damage of DOX. Both in vitro and in vivo studies show that melatonin and its metabolites are strong free oxygen radical scavengers [20 and 21]. A cumulative treatment dose of.350 mg/m^2 free DOX shows a dose-dependent decrease

in the left ventricular ejection fraction (LVEF) LVEF, and, at a cumulative dose of 550 mg/m^2, a sharp increase in the prevalence of HF is reported [22 and 23]. It is reported that the percentage of patients with DOX related congestive HF (CHF) increases with an increase in the patients' cumulative dose of DOX therapy.

Turmeric is a golden spice derived from the rhizome of the *Curcuma longa* plant, which belongs to the Zingiberaceae family [24]. Dry turmeric contains 69.43% carbohydrates, 6.3% proteins, 5.1% oils, 3.5% minerals, and other elements [25]. The bioactive chemical constituents in turmeric have been extensively investigated. To date, approximately 235 compounds, primarily phenolics and terpenoids, have been identified from various species of turmeric, including twenty two diarylheptanoids and diarylpentanoids, eight phenylpropenes as well as other phenolics, sixty-eight monoterpenes, 109 sesquiterpenes, five diterpenes, three triterpenoids, four sterols, two alkaloids, and fourteen other compounds [26]. Curcuminoids (mostly curcumin) and essential oils (primarily monoterpenes) are the major bioactive constituents showing different bioactivities. Calebin-A, vanillic acid, vanillin, quercetin, and other phenolic compounds have also previously been identified from turmeric [27and 24]. Curcumin possesses anti-inflammatory, immunomodulatory, and antiatherogenic activities and is a potent inhibitor of various reactive oxygen-generating enzymes [28 and 29]. The major secondary metabolites of turmeric, the curcuminoid pigment(s) and volatile oils have been shown to be largely responsible for the pharmacological activities of turmeric powder, extracts and oleoresins [30]. Their scavenging activities against a variety of reactive oxygen species including superoxide anion radicals and nitrogen dioxide radicals are predominant [31]. They are also inhibitors of lipid peroxidation in different animal models [32]. It has also been reported to inhibit erythrocyte lipid peroxidation [33]. Curcumin administration attenuated the arsenic, gentamicin, and acetaminophen-induced oxidative stress in rats [34 and 35]. Curcumin also prevented free radical formation-induced myocardial ischemia and paraquat induced lung injury in rats [36). Furthermore, Canales-Aguirre and co-workers [37] had also reported the protective effects of curcumin against the oxidative damage in the hippocampus of rats after exposure to parathion. Curcumin a component in turmeric has been found to be a potent anti-oxidant and free radical scavenger [38]. It inhibits lipid peroxidation [39] and also inhibits Nitric Oxide Synthase (NOS) over-expression [40]. In this study, the anti-oxidant potential of turmeric is evaluate in combination with vitamins C and E, to unravel their possible combine potential against DOX toxicity, knowing that DOX is a very effective and important anticancer drug.

II. METHODS

Animals

54 adult Wistar rats of either sex weighing 200g to 300g were obtained from animal house of Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria. All animals were allowed two weeks acclimatization in the same facility before the study commenced. They were all allowed free access food and tap water and were exposed to natural light-dark cycle and room temperature. All animals were handled according to standard protocols for the use of laboratory animals (National Institute of Health 2002).

Sample collection

The root of turmeric plant was obtained from fruit garden within PH metropolis and was thoroughly washed to remove all dust particles, identified and authenticated at herbarium unit, by Dr. Ekeke Chimezie (Ph.D.) in the department of plant science and biotechnology, Faculty of Sciences, University of Port Harcourt, River State.

Extraction Method

The root of the plant was left to dry at room temperature between 32 – 35^o C after collection and cleaning until they attained a constant weight. The extraction method that was used was adopted from Hanan et al, [41] which is the cold maceration extraction protocol, with minute adjustments. The powdered turmeric root bark of about 50g was soaked in 70% ethanol of about 1000ml in a 2 litre flask and mixed forcefully at 1hr intermission, for 12 hrs and allowed to settle over-night (35^{o}C) to allow for adequate extraction. Subsequently, the concoction was filtered by means of a filter paper with pore size of 0.45milli-pore. The concentration of the extract was increase using rotary evaporation process at 40^{o}C and 200 rpm. The final semi-solid extract was obtained by drying the content of the rotary evaporator over a steam bath at 40^{o}C. The resultant extract obtained 23% yield, was kept safe at room temperature in desiccators, until it was needed for the study.

Experimental Design

54 adult Wistar rats were divided into nine groups of six animals each. Group 1 animals served as control (normal saline 0.2ml), group 2 animals served as negative control, and received Doxorubicin (DOX), group 3 animals were given DOX and turmeric, group 4 animals received DOX and vitamin C, group 5 animals received DOX and vitamin E, group 6 animals received DOX, vitamins C and turmeric, group 7 animals

received DOX, vitamin E and turmeric, while group 8 animals received DOX, vitamin C and vitamin E and finally, group 9 animals receive DOX, vitamin C, vitamin E and turmeric. The animals were administered the following doses of the drugs and extract; vitamin C was given at a dose of 90mg/70kg/day, Vitamin E was give at a dose of 22.4 IU /70kg/day, DOX was administered at a dose of 10-20mg/m^2 once a week, while turmeric was administered at a dose of 500mg/kg/day. The sequence of administration of these drugs as describe above continued for a period of 28 days, but the animals were sacrificed under diethyl ether anesthesia, on day 14 and day 28th. Blood samples were collected from each animal from the various groups for oxidative stress analysis. The animals were grouped as shown below;

Group 1 = Control
Group 2 = Doxorubicin (DOX)
Group 3 = DOX + Turmeric (T)
Group 4 = DOX + Vitamin C (C)
Group 5= DOX + Vitamin E (E)
Group 6 = DOX + C +T
Group 7 = DOX + E+T
Group 8 = DOX + C+E
Group 9 = DOX + C+E+T

Measurement of serum antioxidants activities and oxidative stress

Plasma levels of total antioxidant status (TAS) were determined using DPPH Method (1, 1 diphenyl 2, picryl hydrazyl) [42], Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation [43], GSH was measured by the method of Beutler et al. [44], Plasma activities of superoxide dismutase (SOD) were determined using the method of Misra and Fridovich, [45] and measured at 480nm. Glutathione peroxidase (GPX) was assayed by the method proposed by Reddy et al. [46]. In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts H_2O_2 to H_2O and O_2. The coloured product formed was measured colorimetrically at 430nm. Catalase (CAT) was determined using direct colorimetric method of Sinha [47]. The method is based on the fact that dichromate is reduced to chromic acetate when heated in the presence of H_2O_2. The chromic acetate produced was measured colorimetrically at 570nm.

Histopathology studies

The animals were anaesthetized with diethyl ether, dissected aseptically to remove the liver which was then transferred into 10% chloroform and later trimmed down to a size between 2mm to 4mm thickness, to allow the fixative to readily penetrate the tissue. The tissues were exposed to different stages of processing by standard methods as described by Baker (1945), including, fixation, dehydration, clearing, impregnation, embedding, sectioning and staining with hematoxylin and eosin (H&E) and finally mounting.

III. RESULTS

Plate 1 presents the heart histology of Wistar Rats of the normal control group showing normal cardiac myofibrils branch, weaved and merged forming a multinucleated network (syncytium) and centrally place nuclei. Plate 2 is the histology of animals treated with Doxorubicin aloe for 14 days. They are Group 2 animals. The effect of doxorubicin o the heart was revealed as distorted cardiac muscle with fused nuclei resulting from disrupted sarcolemma and a heterogeneous cardiac myofibril diameter. Plate 3 presents the heart histology of animals treated with doxorubicin and turmeric concomitantly for 14 days. It shows normal cardiac muscles with clearly seen central nuclei and a homogeneous fibre size cardiac myofibril. Plate 4 reveals the effect of vitamin C on the histology of the heart in Wistar rats concomitantly treated with Doxorubicin for 14 days. It shows normal cardiac muscles with clearly seen central nuclei and cardiac myofibril with homogeneous fibre diameter. Plate 5 presents the heart histology of Wistar rat which simultaneously vitamin E and Doxorubicin for 14 days. It shows distorted cardiac muscles with heterogeneous size cardiac fibres and peripheral nuclei. Plate 6 shows the effects of turmeric and vitamin C on the heart in Doxorubicin induced toxicity in Wistar rats after 14 days of treatment. It shows normal cardiac muscles with conspicuous central nuclei and homogeneous fibre diameter. Plate 7 is showing the cardiac histological of the combined effects of turmeric and vitamin E concomitantly administered with Doxorubicin in Wistar rats for 14 days. It shows normal cardiac muscles with central nuclei and homogeneous fibre diameter. Plate 8 reveals the cardiac histology of Wistar rat administered with vitamins C and E simultaneously with Doxorubicin for 14 days. It shows distorted cardiac muscle with fused nuclei (arrowed). Plate 9 presents the heart histology of Wistar rat given turmeric, vitamins C and E simultaneously with Doxorubicin for 14 days. It shows distorted cardiac muscle with fused nuclei. Plate 10 shows the heart histology of Wistar rat exposed to Doxorubicin with intervention for a period of 28 days. It shows distorted cardiac muscles with peripheral nuclei that are fused as a result of distorted sarcolemma. Plate 11 presents the

cardiac histology of Wistar rat that was simultaneously receiving turmeric and Doxorubicin for a period of 28 days. It shows normal cardiac muscles with visible central nuclei, cardiac myofibril with homogeneous fibre diameter. Plate 12 is showing the histology of the heart in wistar rat that received vitamin C along with Doxorubicin concomitantly for 28 days. It shows normal cardiac muscles with cardiac myofibril of homogeneous diameter branched, weaved and merged, and a visible central nuclei. Plate 13 shows the cardiac histology of wistar rat given vitamin E simultaneously with Doxorubicin for a period of 28 days. It shows distorted cardiac muscles with fused nuclei resulting from distorted sarcolemma. Plate 14 presents the cardiac histology of Wistar rat given turmeric and vitamin C concomitantly with Doxorubicin for a period of 28 days. It shows normal cardiac muscles. Plate 15 shows the cardiac histological effects of turmeric and vitamin E co-administration in Wistar rats receiving Doxorubicin after 28 days of drug treatment. It shows normal cardiac muscles. Plate 16 presents the heart histology of Wistar rat receiving a combination of vitamins C and E along with Doxorubicin for a period of 28 days. It shows normal cardiac muscles. Plate 17 shows the cardiac histology of Wistar rat receiving a combination of turmeric and vitamins C and E along with Doxorubicin for a period of 28 days. It shows normal cardiac muscles

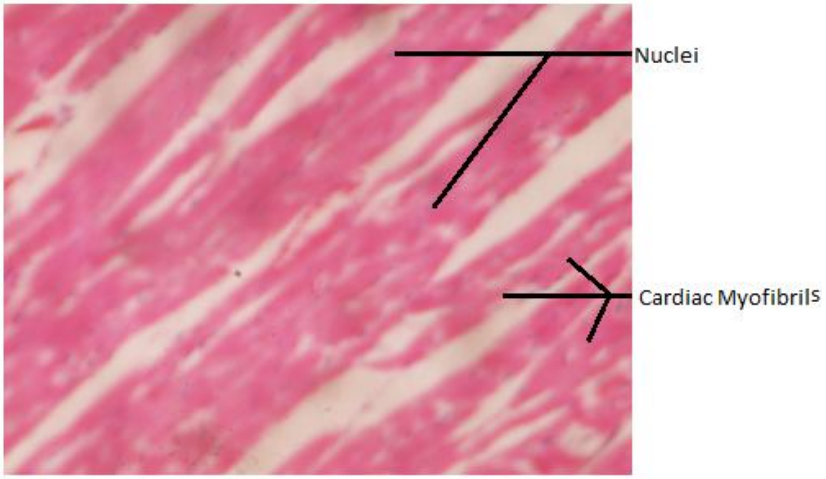

Plate 1: Heart histology of normal control group Wistar Rats showing normal cardiac myofibrils branch, weaved and merged forming a multinucleated network (syncytium) and centrally place nuclei

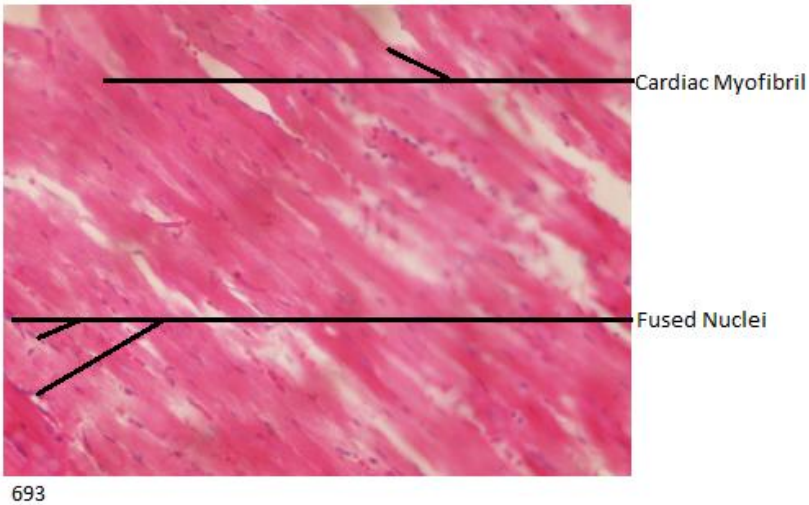

Plate 2: Effects of Doxorubicin toxicity on Heart in Wistar rats treated for 14 days (Group 2). It shows distorted cardiac muscle with fused nuclei resulting from disrupted sarcolemma and a heterogeneous cardiac myofibril diameter.

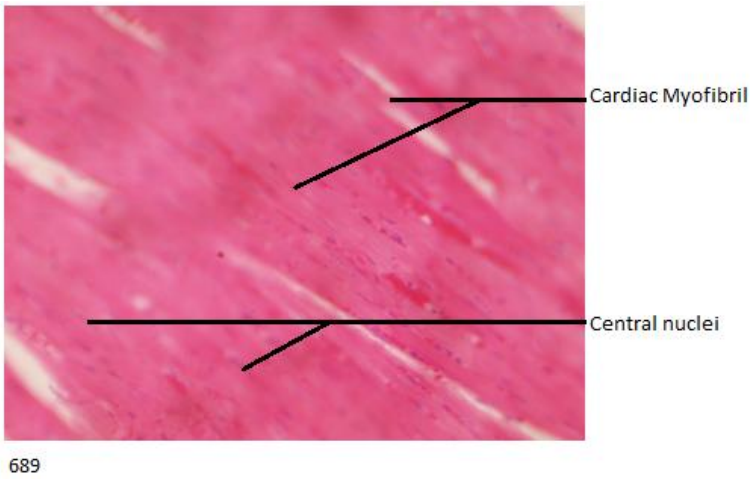

Plate 3: Effects of Turmeric on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 14) (group 3). It shows normal cardiac muscles with clearly seen central nuclei and a homogeneous fibre size cardiac myofibril.

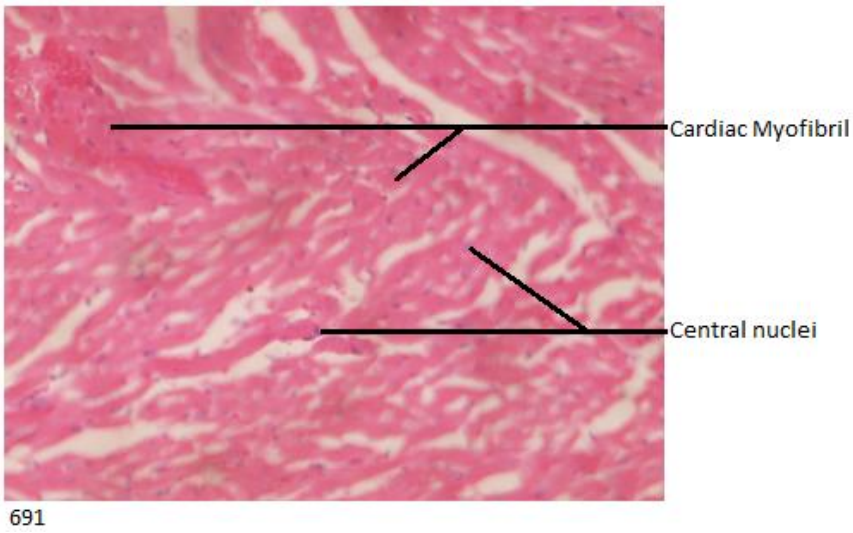

Plate 4: Effects of Vitamin C on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 14) (group 4). It shows normal cardiac muscles with clearly seen central nuclei and cardiac myofibril with homogeneous fibre diameter.

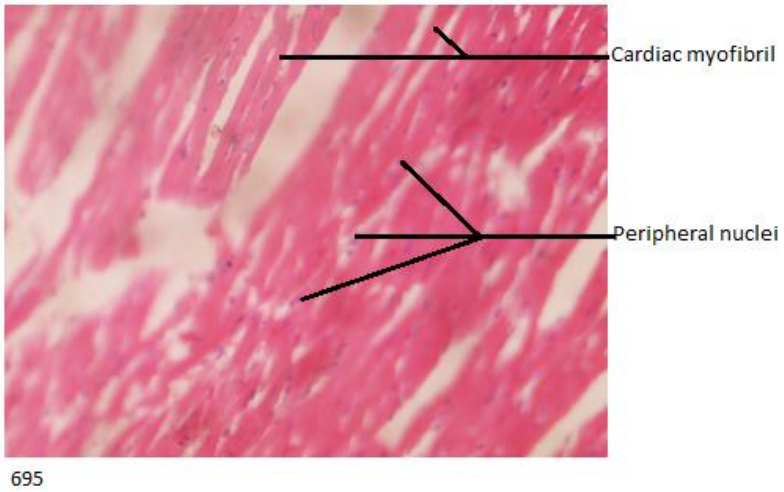

Plate 5: Effects of Vitamin E on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 14) (Group 5). It shows distorted cardiac muscles with heterogeneous size cardiac fibres and peripheral nuclei.

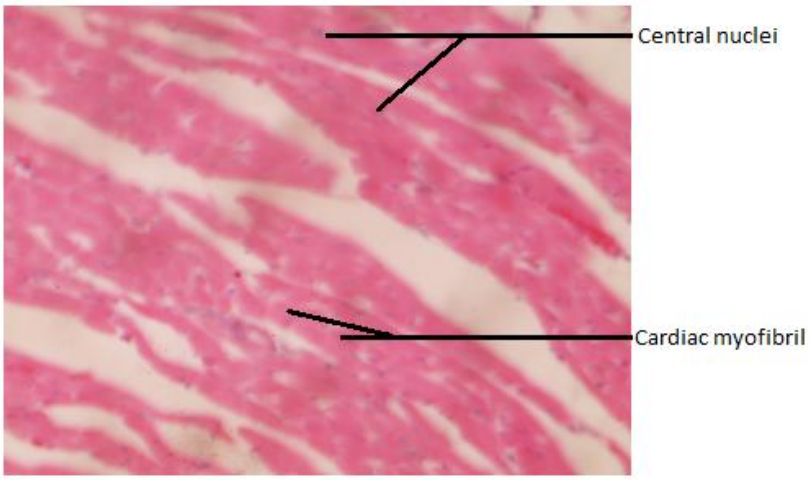

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Plate 6: Effects of Turmeric and Vitamin C on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 14) (Group 6). It shows normal cardiac muscles with conspicuous central nuclei and homogeneous fibre diameter.

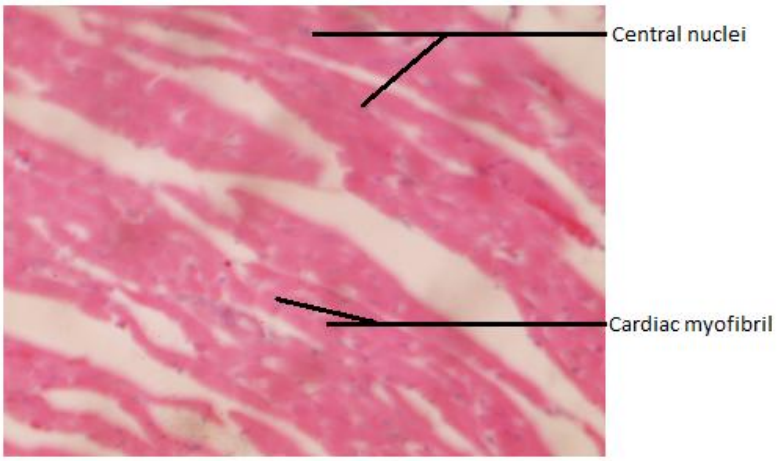

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Plate 7: Effects of Turmeric and Vitamin E on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 14) (Group 7). It shows normal cardiac muscles with central nuclei and homogeneous fibre diameter

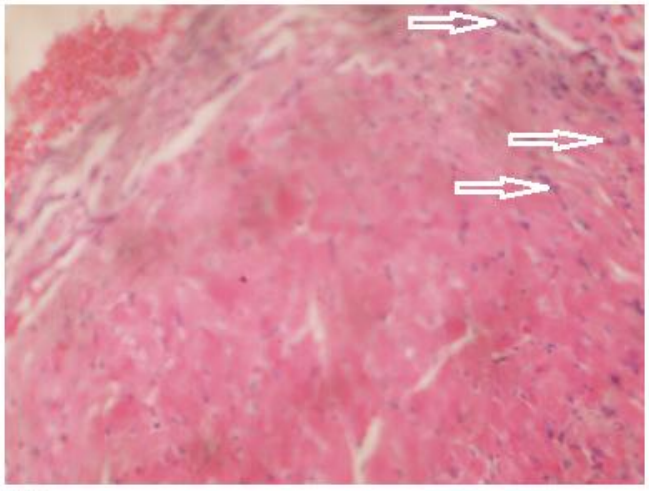

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Plate 8: Effects of Vitamins C and E on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 14) (Group 8). It shows distorted cardiac muscle with fused nuclei (arrowed).

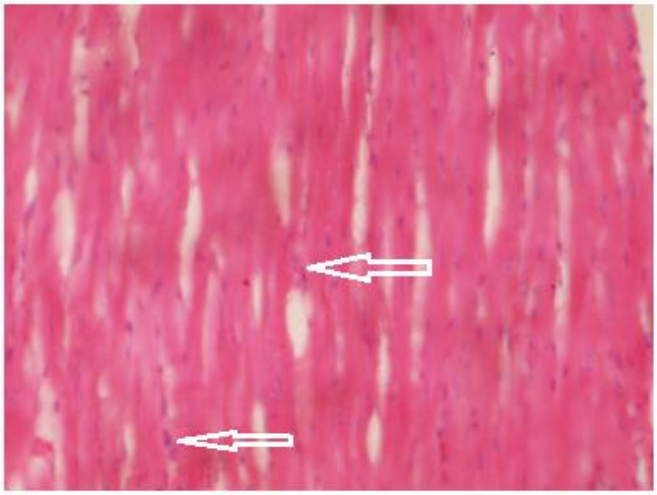

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Plate 9: Effects of Turmeric and Vitamins C and E on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 14)(Group 9). It shows distorted cardiac muscle with fused nuclei (arrowed)

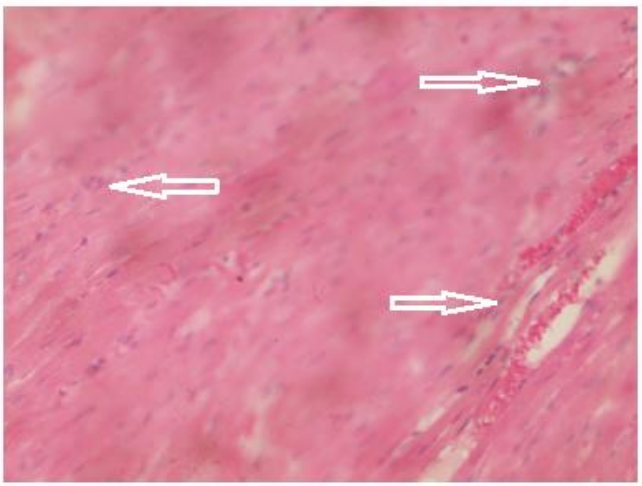

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Plate 10: Effects of Doxorubicin toxicity on the Heart in Wistar rats (Day 28) (Group 2). It shows distorted cardiac muscles with peripheral nuclei that are fused as a result of distorted sarcolemma (arrowed)

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Plate 11: Effects of Turmeric on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 28) (Group 3). It shows normal cardiac muscles with visible central nuclei, cardiac myofibril with homogeneous fibre diameter

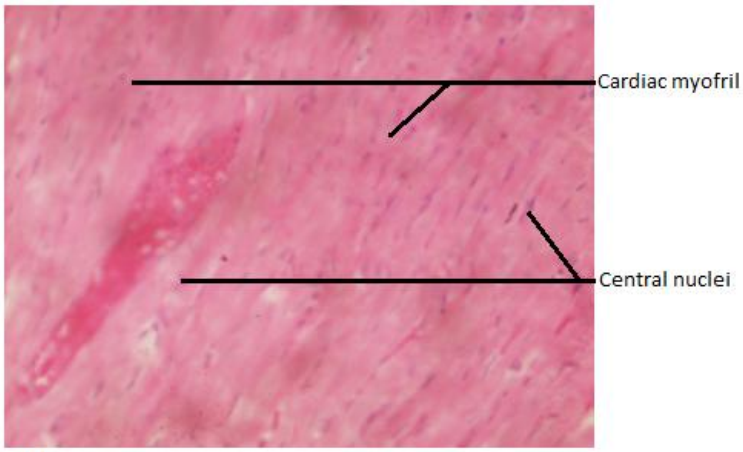

Plate 12: Effects of Vitamin C on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 28) (Group 4). It shows normal cardiac muscles with cardiac myofibril of homogeneous diameter branched, weaved and merged and a visible central nuclei.

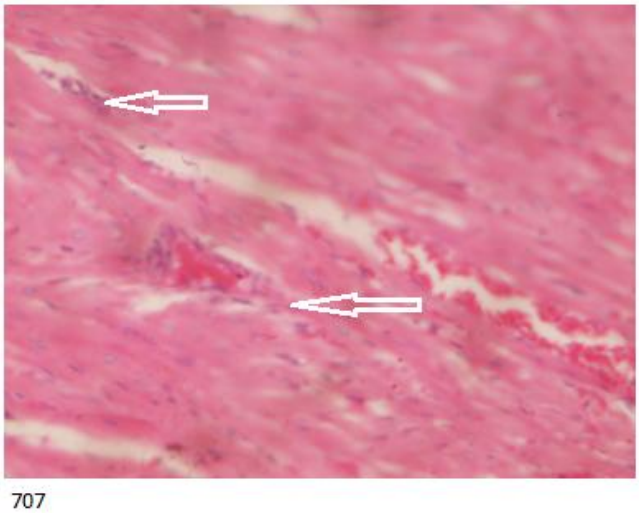

Plate 13: Effects of Vitamin E on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 28)(Group 6). It shows distorted cardiac muscles with fused nuclei resulting from distorted sarcolemma.

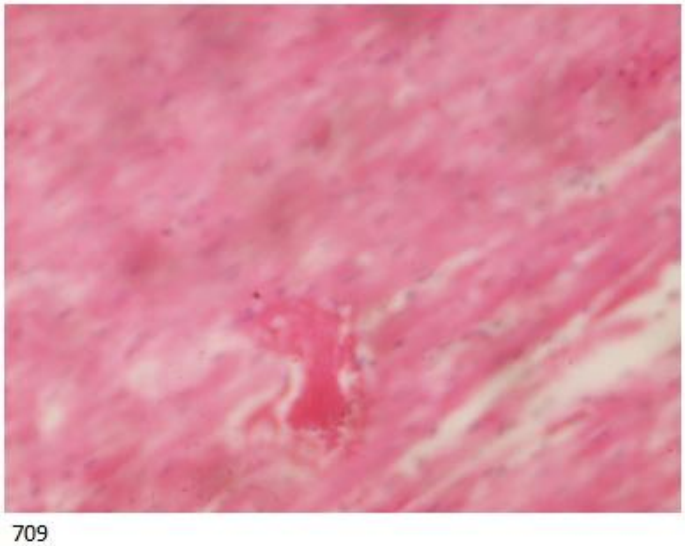

Plate 14: Effects of Turmeric and Vitamin C on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 28) (Group 7). It shows normal cardiac muscles.

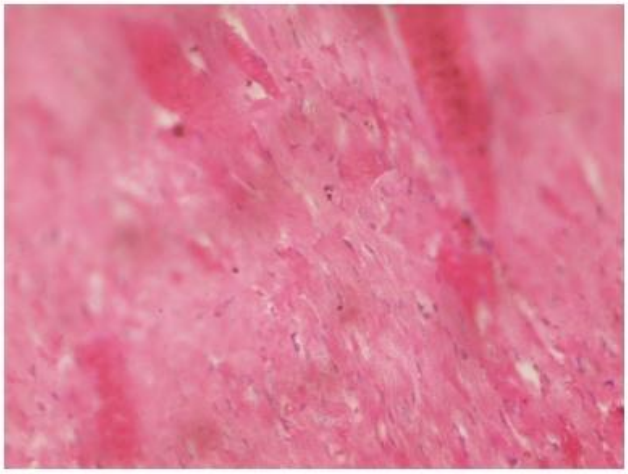

Plate 15: Effects of Turmeric and Vitamin E on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 28) (Group 8). It shows normal cardiac muscles.

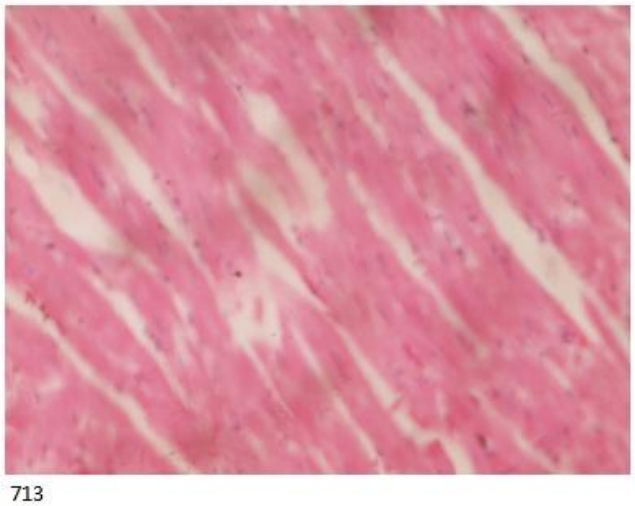

Plate 16: Effects of Vitamins C and E on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 28) (Group 9). It shows normal cardiac muscles.

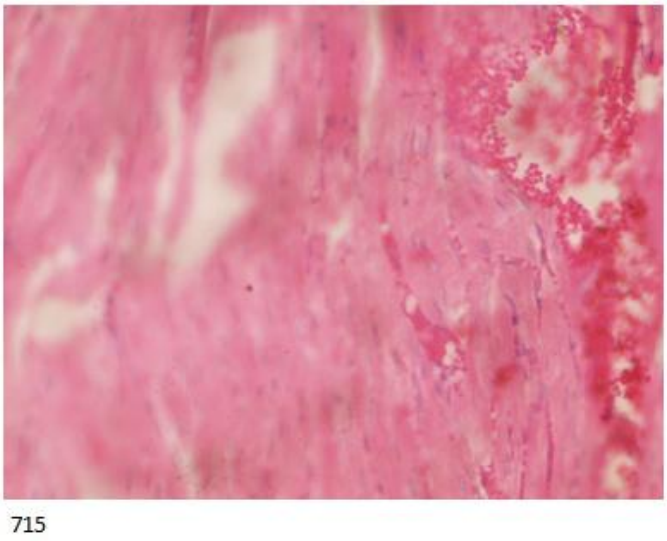

Plate 17: Effects of Turmeric and Vitamins C and E on the Heart in Doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal cardiac muscles.

IV. DISCUSSION

A thorough analysis of the results revealed that doxorubicin administration for 14 and 28 days caused distorted cardiac muscle with fused nuclei resulting from disrupted sarcolemma and a heterogeneous cardiac myofibril diameter, while that of the control was showing normal cardiac myofibrils branch, weaved and merged forming a multinucleated network (syncytium) and centrally place nuclei. The cardiac histology of animals that received turmeric and vitamin C independently and in combination for 14 and 28 days were all normal as well while those that received vitamin E and in combination with vitamin C were distorted on the 14th day but eventually became normal after 28 days. The logical explanation about the adverse effects of doxorubicin remains that, DOX caused free oxygen radical formation as reported by Tokarska-Schlattner et al., [11] or hydrogen peroxide and superoxide radical proliferation which reduce the levels of the endogenous enzyme (glutathion peroxidise) that is responsible for scavenging free radicals, thus increase oxidative stress which resulted in cardiomyopathy as reported by Danelisen and Singal [15 and 16]. Thus the histological distortions observed with the heart, would have occurred as a result of free oxygen radicals formation which had a significant effect in the cardiotoxic mechanism of doxorubicin, because of the reduction of endogenous antioxidant systems. If this is a logical assertion, and which we know it is, then, it is obvious that turmeric root extract, vitamins C and E all possess anti-oxidant properties as revealed by the normal histological tissues in animals concomitantly treated with Doxorubicin, turmeric and vitamins C and E. Off course, vitamins C and E are already established anti-oxidant drugs available, but turmeric is still at the level of speculations and therefore scientific studies are the way forward to upgrade from speculations to clinical application, thus, this study is also relevant to fast-track this transformation. From our study, it is obvious that turmeric possess anti-oxidant properties, when administered alone or in combination of either vitamin C or E or both. Also the fact that turmeric has anti-oxidant properties is not new because several authors have arrived at this conclusion before as revealed by [31, 32, 34, 35 and 38]. This is fascinating, but the most amazing finding is the proof that turmeric actually blocked the toxic effects of doxorubicin in the heart, implying that, if this is established turmeric can actually become a supplement in treatment of cancer without fear of cardiac toxicity.

V. CONCLUSION

Administration of doxorubicin to Wistar rats caused distorted cardiac muscle with fused nuclei due to an increase in oxidative stress. On the contrary, co-administration of turmeric root extracts, vitamins C and E, individually and collectively with doxorubicin concomitantly, prevented or mitigated the cardio-toxic effects of doxorubicin in Wistar rats.

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