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HEPATO-PROTECTIVE POTENTIALS OF TURMERIC AGAINST DOXORUBICIN INDUCE OXIDATIVE STRESS LIVER DAMAGE IN WISTAR RATS

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ABSTRACT

Doxorubicin is an anthracycline used in the treatment of several cancers, not without severe adverse effects which hampers its efficiency, thus the need to reduce its toxicity in clinical conditions. The current study investigated turmeric root extract for its protective action against doxorubicin-induced liver histo-pathological damage in Wistar rats. 54 adult Wistar rats were divided into 9 groups of six animals each. Group 1 animals received normal saline, group 2 animals received doxorubicin, group 3 animals were given doxorubicin and turmeric, group 4 animals received doxorubicin and vitamin C, group 5 animals received doxorubicin and vitamin E, group 6 animals received doxorubicin, vitamins C and turmeric, group 7 animals received doxorubicin, vitamin E and turmeric, while group 8 animals received doxorubicin, vitamins C and E and, group 9 animals receive doxorubicin, vitamins C and E and turmeric. The study lasted for 28 days and liver were harvested and processed for histological assessment. Results revealed that doxorubicin caused destroyed hepatic tissues morphology after 14 days and necrosis after 28 days of administration, while liver from the control animals and those that received turmeric alone or in combination with either vitamins C or E or both with doxorubicin for 14 and 28 days showed normal histo-morphological features. On the contrary, the liver of animals that received vitamins C and E alone showed normal morphological features but with congested veins and sinusoids and high concentration

of Kuffer cells. Thus turmeric root extract protected the liver against the damaging effect of doxorubicin-induced toxicity.

KEYWORDS: Turmeric, Doxorubicin, Hepato-protective oxidative stress, Wistar rats.

INTRODUCTION

Conventional anthracycline–containing regimens are considered a mainstay of therapy for several decades, in the management of cancer and they have demonstrated benefits in terms of response rate, time to disease progression, and overall survival (Winer et al., 2001). The first two anthracyclines were isolated from the pigment-producing *Streptomyces peucetius* early in the 1960s and were named doxorubicin (DOX) and daunorubicin (DNR) (Minotti et al., 2004), both drugs possessing aglyconic and sugar moieties. The aglycone consists of a tetracyclic ring with adjacent quinone-hydroquinone groups, a methoxy substituent and a short side chain with a carbonyl group. The sugar, called daunosamine, is attached by a glycosidic bond to one of the rings and consists of a 3-amino-2,3,6- trideoxy-L-fucosyl moiety. The only difference between these two molecules is the fact that the side chain of DOX terminates with a primary alcohol, whereas that of DNR terminates with a methyl group (Minotti et al., 2004). Despite its widespread use, the cytotoxic effects of anthracyclines are multidirectional. Doxorubicin has been reported to cause hepatic damage found histo-pathologically to be a higher tendency for liver fibrosis manifested by the presence of many spots of focal collected cellular granulomatous lesions (spots of densely collected inflammatory cells composed mainly of macrophages and lymphocytes at the center and a number of fibrocytes at the periphery); with collagenous fibrils markedly distributed in the necrotic foci (Hassan et al., 2009). Other researchers have also reported that DOX also causes pathological changes in hepatocytes such as an increase in mitochondrial vacuolization, swelling, weak pyknotic nucleus and dilatation of the intercellular space (Kalendera et al., 2005; Injac et al., 2008 and Zeidan et al., 2002). Studies attempt to attribute the toxicity of DOX in the liver to be related an increase in oxidative stress. In the liver, oxidative stress caused by increased ROS production can result from two different ways, the most common occurring when the semiquinone form of DOX reacts with O^2 producing O^{2*-} and H_2O_2, and an alternative way occur through NADPH oxidases, the principal extra-mitochondrial producers of ROS in hepatocytes (Kassner et al., 2008). NADPH-oxidases are present in small levels but their levels increase in response to extracellular stimulus, such as DOX treatment (Kassner et al., 2008). The literature shows that DOX-induced ROS

production leads to an increase in lipid peroxidation and a reduction in the activities of SOD, CAT and glutathione peroxidase (GPx), as well as DNA damage, and a decrease in GSH levels, which confirm DOX hepatotoxicity (Odom et al., 1992; Ortiz et al., 2008 and Kalendera et al., 2005). Literatures have also reported that there is an impaired mitochondrial function observed with DOX treatment which has been attributed to alterations in the typical markers of oxidative stress observed in damaged liver tissue (Djordjevie et al., 2006 and Injac et al., 2008). It was also reported that ROS production could also lead to the activation of IkB kinase (IKK) that phosphorylates IkB inhibitors activating IB that, in turn, activates NF-kB that leads to the expression of proinflammatory cytokines culminating in cell death (Lu et al., 2005). Thus, because of their potency in cancer treatment, it is essential to mitigate the toxicity of DOX associated with the liver, which is mainly due to an increase in oxidative stress, hence the importance of this study to evaluate the hepato-protective potentials of turmeric in Wistar rats.

Turmeric is a golden spice derived from the rhizome of the *Curcuma longa* plant, which belongs to the Zingiberaceae family (Gupta et al., 2013). Dry turmeric contains 69.43% carbohydrates, 6.3% proteins, 5.1% oils, 3.5% minerals, and other elements (Islam et al., 2002). The bioactive chemical constituents in turmeric have been extensively investigated. Currently, 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 (Yuan et al., 2011). Curcuminoids (mostly curcumin) and essential oils (primarily monoterpenes) are the major bioactive constituents showing different bioactivities. Curcumin possesses anti-inflammatory, immunomodulatory, and antiatherogenic activities and is a potent inhibitor of various reactive oxygen-generating enzymes (Ara´ujo et al., 2001 and Chainani-Wu 2003). It has been used in indigenous herbal medicine for the treatment of inflammatory and liver disorders. Antioxidative properties of curcumin are well documented. Curcumin is a potent scavenger of reactive oxygen species including superoxide anion radicals and hydroxyl radicals. It has also been reported to inhibit erythrocyte lipid peroxidation (Borra et al., 2013). Curcumin administration attenuated the arsenic, gentamicin, and acetaminophen-induced oxidative stress in rats (El-Demerdash et al., 2009 and Cekmen et al., 2009). Curcumin also prevented free radical formation-induced myocardial ischemia and paraquat induced lung injury in rats (Manikandan et al., 2004).

Additionally, curcumin protected against diazinon-induced toxicity in blood, liver, and erythrocyte of male Wistar rats (Messarah et al., 2013). Curcumin a component in turmeric has been found to be a potent anti-oxidant and free radical scavenger (Fujisawa et al., 2004). It inhibits lipid peroxidation (Sreejayan-Rao 1994) and also inhibits Nitric Oxide Synthase (NOS) over-expression (Spinas 1999 and Pan et al., 2000). Also, Isirima and Christian (2021), had reported the anti-oxidant potentials of turmeric. In their study, they found that turmeric demonstrated anti-oxidant properties my reversing the significant reduction in the serum concentration of SOD, GPx, CAT, GSH and TAS as well as an increase in the serum level of MDA, caused by doxorubicin.

METHODS

Animals

27 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). Approval of the use of the animals for the study was received from the University of Port Harcourt Ethical committee.

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, (2013) 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

27 adult Wistar rats were divided into nine groups of three 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. Lungs tissues were dissected and collected for histological studies. 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

Histopathology Studies

The animals were anaesthetized with diethyl ether, dissected aseptically to remove the liver which were then transferred into 10% chloroform, and it was later trimmed down to a size of 2mm to 4mm thickness. This was done 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.

RESULTS AND DISCUSSION

Plate 1 presents the liver histology of normal control of Rats showing normal histo-architecture of the liver with conspicuous central vein and sinusoids with Kuffer cells. The effect of doxorubicin toxicity on liver of Wistar rats exposed for 14 days is presented in plate 2. It shows destroyed hepatic tissues architecture. The effect of 14 days turmeric treatment simultaneously with DOX on the liver in Wistar rat is presented in plate 3, showing normal histology of the hepatocytes. Plate 4 presents the effects vitamin C on the liver in doxorubicin induced toxicity in Wistar rats after 14 days of concomitant drug treatment, showing normal morphological features but with congested sinusoids and high concentration of Kuffer cells, while plate 5 shows the effects of vitamin E on the liver in doxorubicin induced toxicity after 14 days of simultaneous drug treatment. It reveals normal histological appearance of liver cells, but with sinusoids having high concentration of Kuffer cells as well as congested veins. The combined effect of simultaneous drug treatment with turmeric and vitamin C of Wistar rats exposed to doxorubicin for a period of 14 days is presented in plate 6, showing normal liver histology. Plate 7 shows the effects of Turmeric and Vitamin E on the liver in doxorubicin induced toxicity in Wistar rats treated for 14 days, showing normal histology of the liver. The effect of vitamin C and E combined treatment of Wistar rats exposed to doxorubicin for 14 days is presented in plate 8, showing normal histology of the liver. Plate 9 presents the effects of combined simultaneous treatment of Wistar rat exposed to doxorubicin for 14 days with turmeric and Vitamins C and E. It shows normal histology of the liver. The effect of doxorubicin exposure to Wistar rats for 28 days is presented in plate 10. It reveals necrosis of the hepatocytes. Plate 11 shows the effects of turmeric concomitant treatment with doxorubicin for 28 days on the liver in Wistar rats. It shows normal histology of the hepatoctes. The effect of vitamin C on the liver in animals given doxorubicin simultaneously for 28 days is shown in plate 12, also revealing normal histological features of the liver. Similarly, the effect of vitamin E on the liver in animals treated simultaneously with doxorubicin for 28 days is presented in plate 13, showing normal liver histology. In a similar manner, the liver histology of animals treated with a combination of turmeric and vitamin C after 28 days of exposure to doxorubicin is shown in plate 14, revealing normal histology of the liver. The effect of the combined treatment of turmeric and vitamin E on the liver after 28

days of exposure to doxorubicin is presented in plate 15, also showing normal liver histology. The effect of the combination of vitamins C and E on the liver in animals treated with doxorubicin concomitantly for a period of 28 days is presented in plate 16, showing normal liver histology, while plate 17 presents the effects of turmeric and Vitamins C and E on the liver in doxorubicin induced toxicity in Wistar rats. It shows normal histology of the liver after 28 days of treatment.

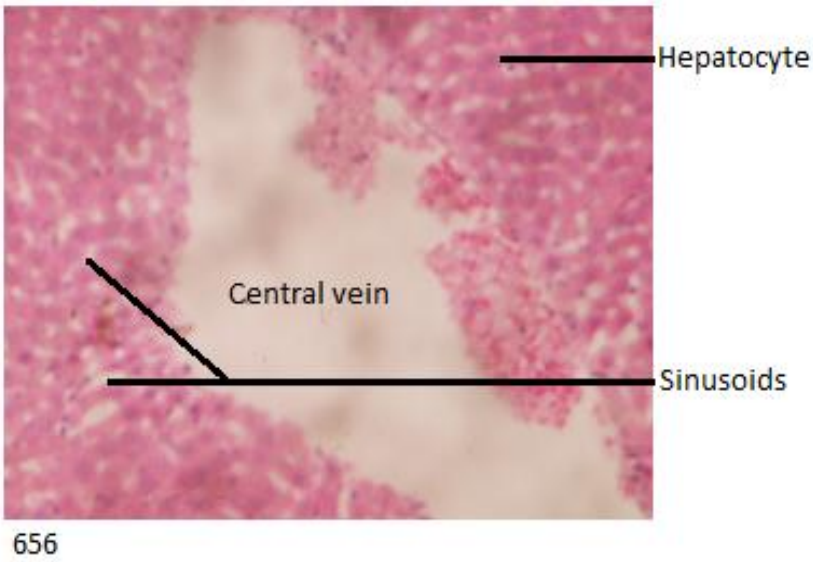

Plate 1: Histology of normal control rat. It shows normal histo-architecture of the liver with conspicuous central vein and sinusoids with Kuffer cells.

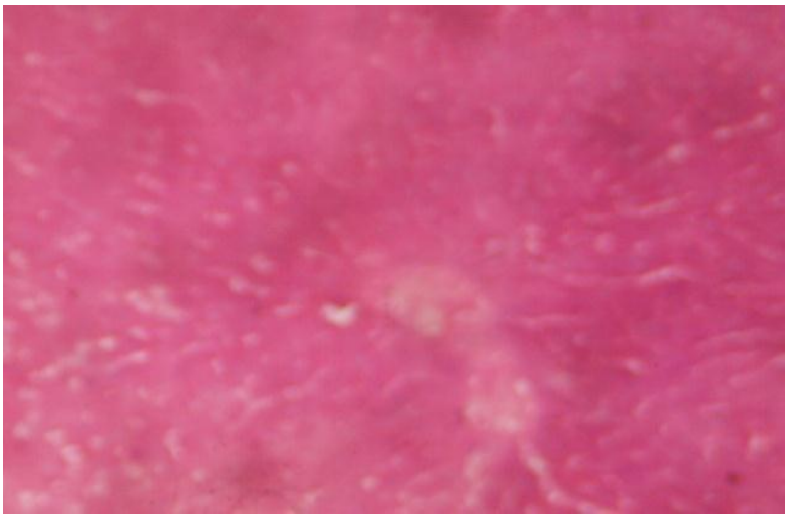

Plate 2: Effects of doxorubicin toxicity on liver in Wistar rats (Day 14), showing destroyed hepatic tissues architecture.

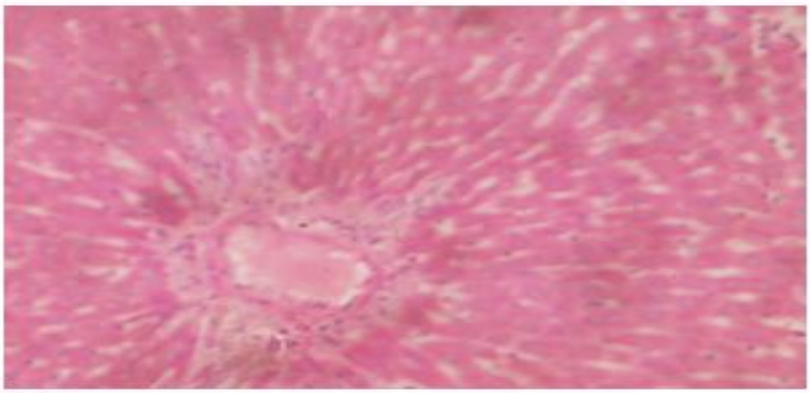

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Plate 3: Effects turmeric on the liver in doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the liver.

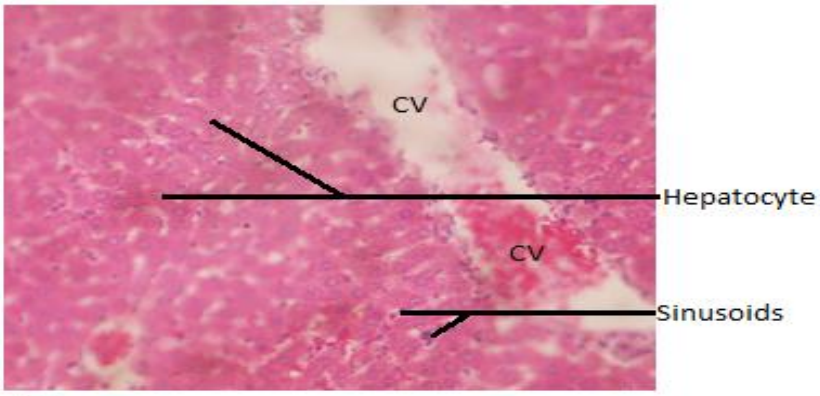

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Plate 4: Effects vitamin C on the liver in doxorubicin induced toxicity in Wistar rats (Day 14), showing normal morphological features with congested sinusoids and high concentration of Kuffer cells.

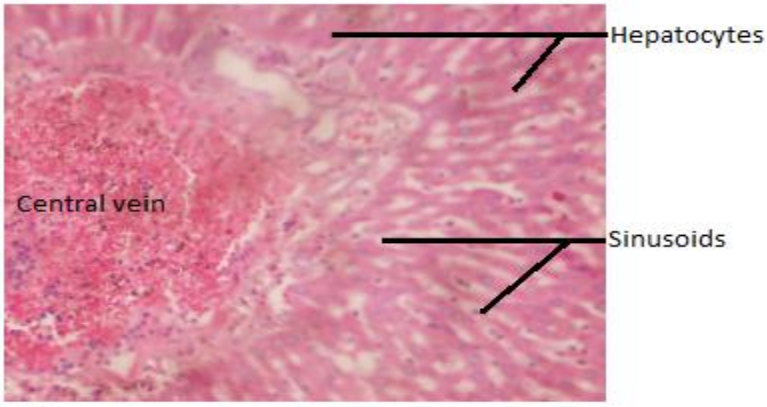

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Plate 5: Effects of vitamin E on the liver in doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histological appearance of liver cells, but with sinusoids having high concentration of Kuffer cells as well as congested veins.

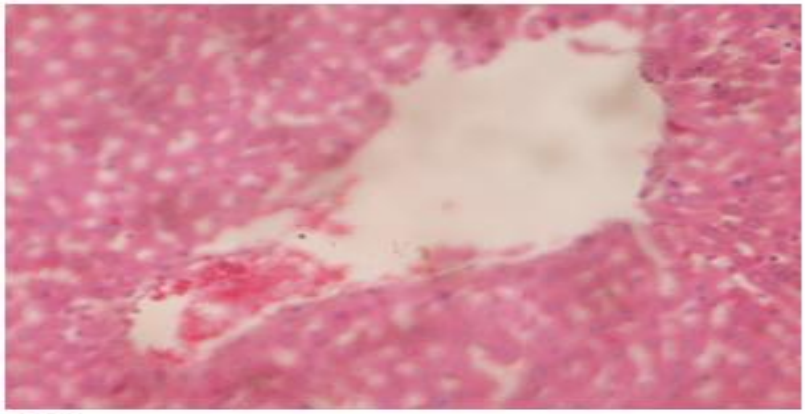

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Plate 6: Effects of Turmeric and Vitamin C on the liver in doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the liver.

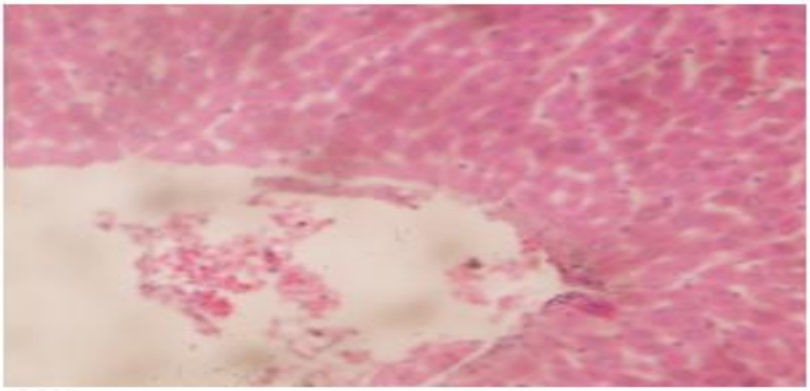

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Plate 7: Effects of Turmeric and Vitamin E on the liver in doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the liver.

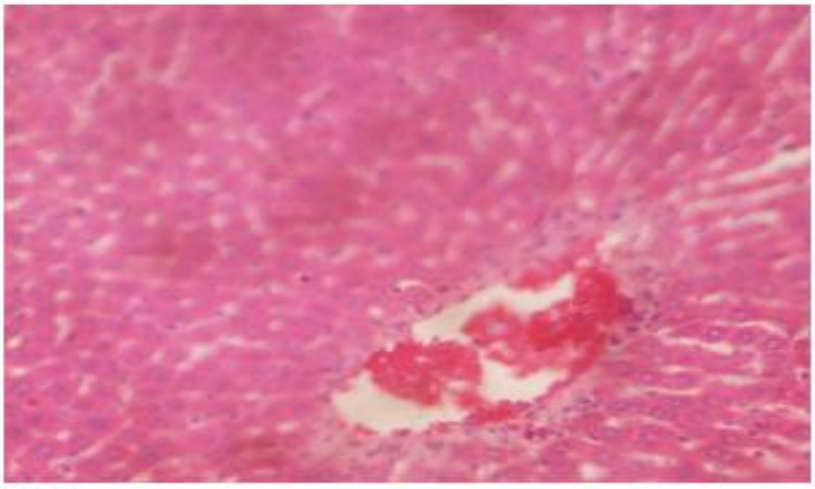

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Plate 8: Effects of Vitamins C and E on the liver in doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the liver.

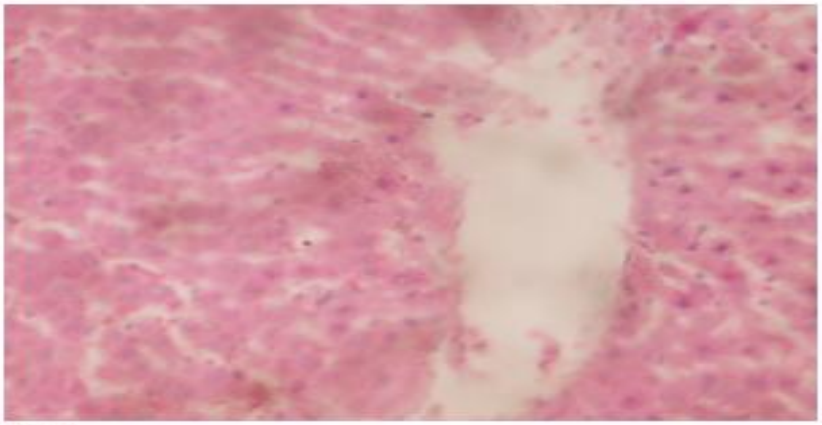

Plate 9: Effects of Turmeric and Vitamins C and E on the liver in doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the liver.

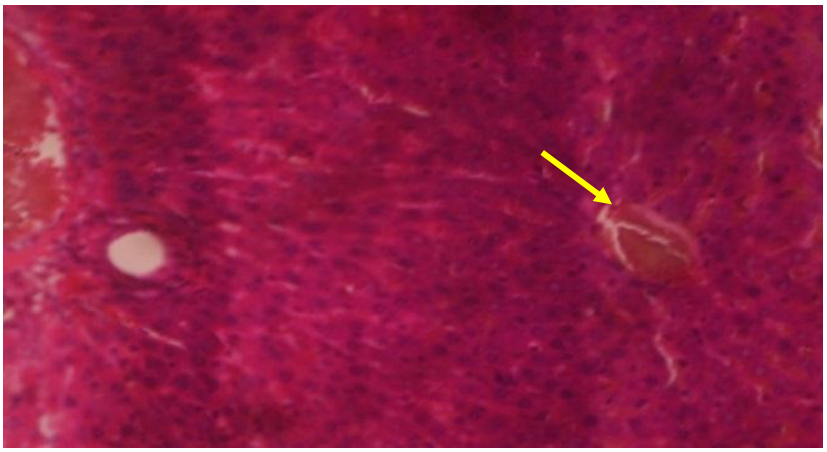

Plate 10: Effects of doxorubicin toxicity on liver in Wistar rats (Day 28), showing tissue necrosis.

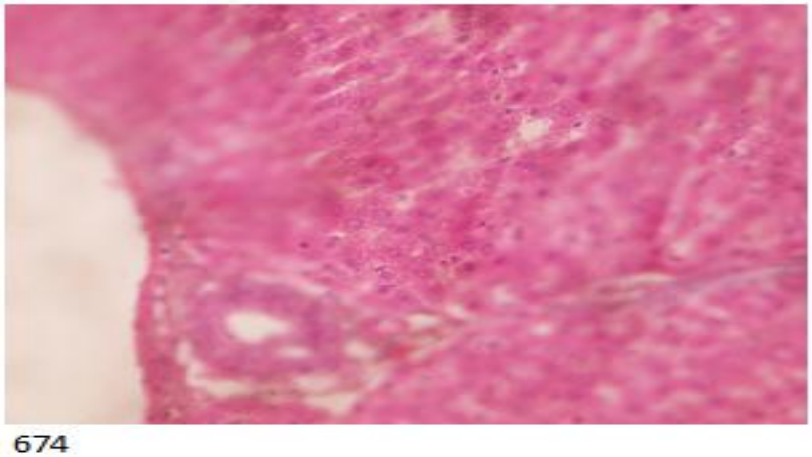

Plate 11: Effects of Turmeric on the liver in doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the liver.

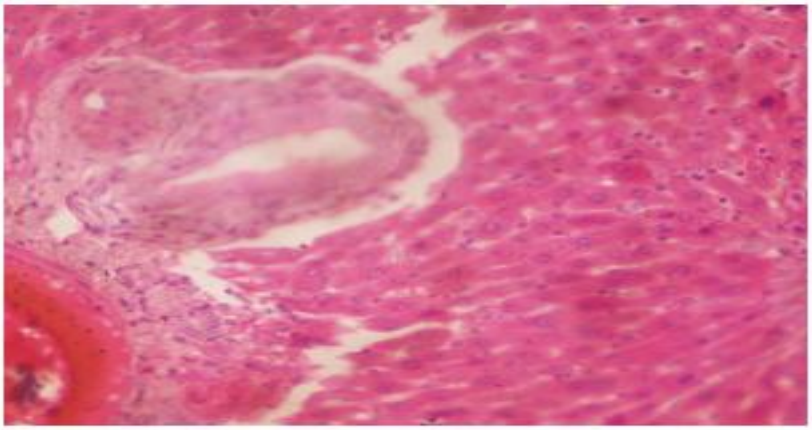

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Plate 12: Effects of Vitamin C on the liver in doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the liver.

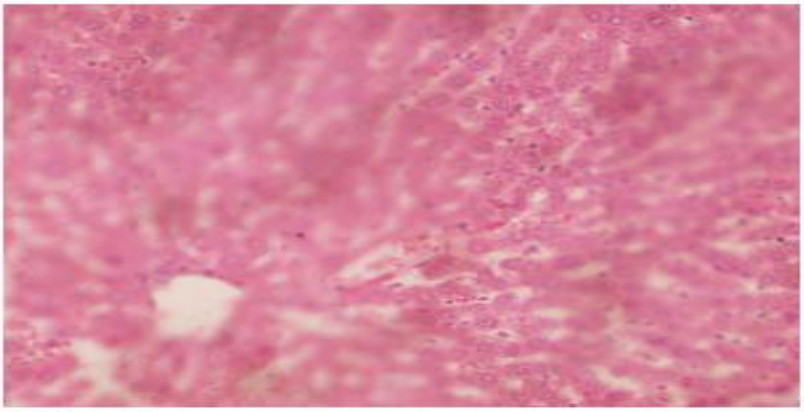

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Plate 13: Effects of Vitamin E on the liver in doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the liver.

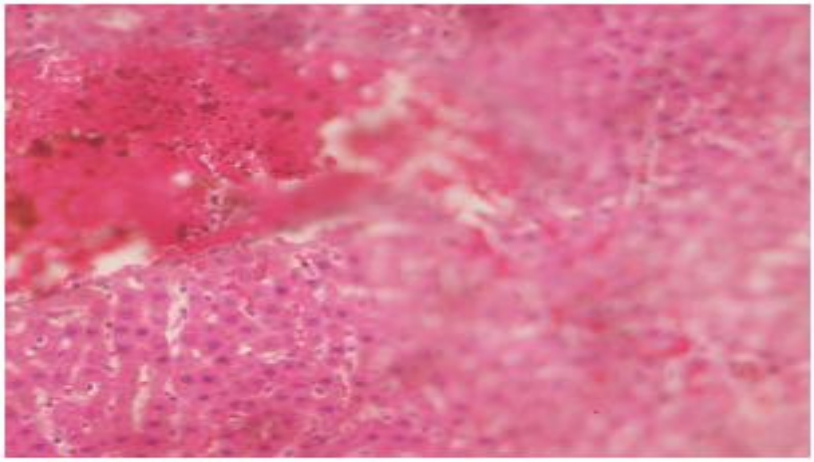

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Plate 14: Effects of Turmeric and Vitamin C on the liver in doxorubicin induced toxicity in Wistar rats. It shows normal histology of the liver (Day 28).

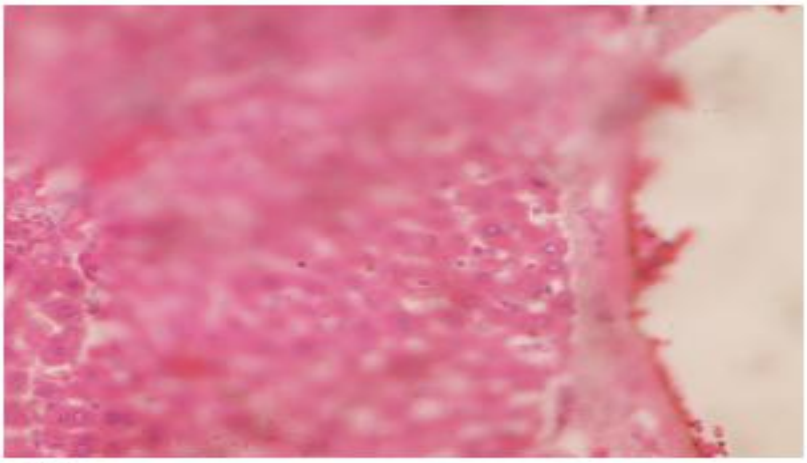

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Plate 15: Effects of Turmeric and Vitamin E on the liver in doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the liver.

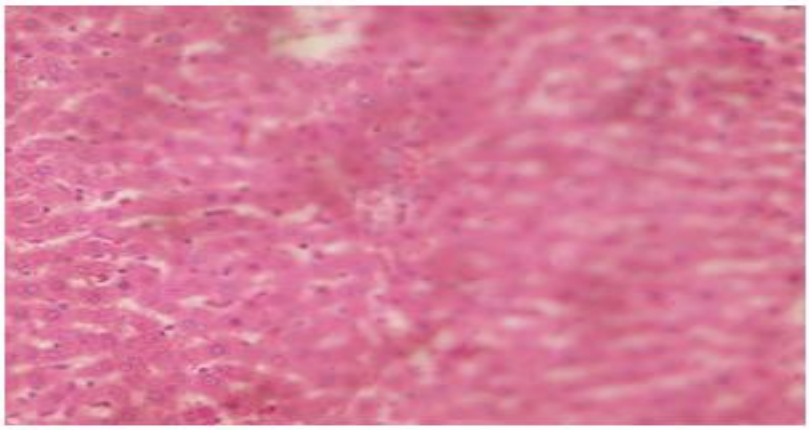

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Plate 16: Effects of Vitamins C and E on the liver in doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the liver.

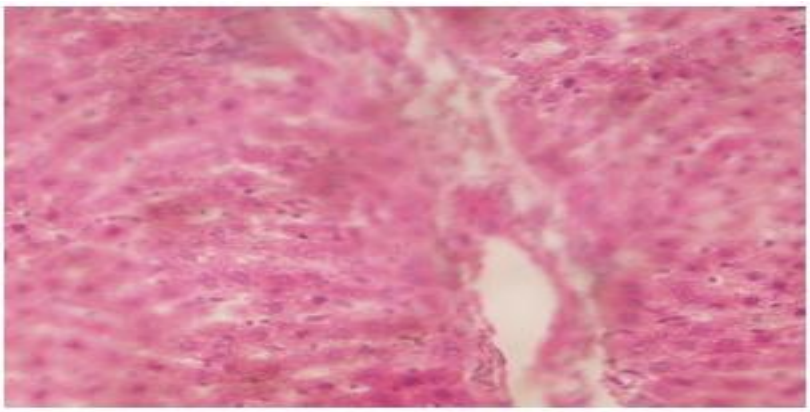

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Plate 17: Effects of Turmeric and Vitamins C and E on the liver in doxorubicin induced toxicity in Wistar rats . It shows normal histology of the liver (Day 28).

Our study revealed that administration of doxorubicin caused destroyed hepatic tissues architecture after 14 days and necrosis after 28 days of administration, while liver from the control animals showed normal histo-morphological features. It was also found that the liver from all animals that received turmeric alone or in combination with either vitamin C or vitamin E or both with doxorubicin for 14 and 28 days showed normal histo-morphological features, while those that received vitamin C alone showed normal morphological features but with congested sinusoids and high concentration of Kuffer cells and those that received vitamin E alone reveals normal histological appearance of liver cells, but with sinusoids having high concentration of Kuffer cells as well as congested veins. These findings evidently reveal that turmeric alone or in combination with vitamin C and E can protect the liver against the damaging effect of doxorubicin-induced toxicity. Several authors have reported that the main mechanism of doxorubicin induced toxicity is production of oxygen free radicals in the mitochondria of cells and the presence of iron further increase this effect and thus increase in oxidative stress (Tacar et al., 2013; Ichikawa et al., 2014; Tokarska-Schlattner et al., 2006; Suliman et al., 2007 etc.), it therefore implies that the protective effect of turmeric against doxorubicin toxicity must be related with anti-oxidant scavenging activities. This assertion may be very correct because it has be reported that curcumin a major constituent of turmeric is a potent inhibitor of various reactive oxygen-generating enzymes (Ara´ujo et al., 2001 and Chainani-Wu 2003) and a potent anti-oxidant and free radical scavenger (Fujisawa et al., 2004). It inhibits lipid peroxidation (Sreejayan-Rao 1994) and also inhibits Nitric Oxide Synthase (NOS) over-expression (Spinas 1999 and Pan et al., 2000). Thus our findings are in agreement with these reports. Again, the fact that different degrees of distortions were observed with vitamin C and vitamin E, but normal tissues with turmeric implies that the anti-oxidant properties of turmeric may be greater than those of vitamins C and E.

CONCLUSION

Turmeric root extract prevented the liver histo-pathological changes associated with the doxorubicin induced oxidative stress damage. These effects are attributed to its' anti-oxidant properties which is found in curcumin, a major constituent in turmeric. This protective effect of turmeric was found to be greater than those of vitamin C and vitamin E.

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