Turmeric Protection against Doxorubicin-Induced Lungs Histo-Pathological Damage in Wistar Rats

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Abstract
Doxorubicin is an anthracycline group of antibiotics, which finds it use in the treatment of several malignancies. The use of doxorubicin not only kills neoplastic cells but also affects normal cells, which hampers its efficient use in patients. Therefore, any agent that can reduce the toxicity of doxorubicin will be useful in clinical conditions. The current study investigated turmeric root extract for its protective action against doxorubicin-induced lungs histo-pathological damage in albino 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, vitamins C, and E and turmeric. The experiment lasted for 28 days and lungs were harvested and processed for histological assessment. It was found that DOX caused distorted lungs with thickened inter alveolar septa and distorted alveolar sacs and bronchioles which were reversed by turmeric root extract, thus preventing the lungs histo-pathological changes associated with the doxorubicin induced oxidative stress damage.

Keywords: *Turmeric, Doxorubicin, lungs histo-pathology, Wistar rats*

Introduction

Doxorubicin is a product of *Streptomyces peucetius var. caesius*, a prototype agent of anthracycline antibiotics (Blum and Carter 1974). It is one of the most effective antineoplastic drugs, commonly used against breast, ovarian, testicular, thyroid, lung cancers and haematological cancers including Hodgkin Lymphoma and prevalent non-Hodgkin lymphomas (Octavia et al., 2012 and Vejpongsa et al., 2014). One of the major drawbacks of clinical use of doxorubicin is the development of life threatening cardiomyopathy both in adults as well as children who receive it as a part of cancer treatment regimen (.Carvalho et al., 2014). Multiple mechanisms are responsible for the development of cardiotoxicity in the patients receiving doxorubicin for cancer treatment. One of the most important and predominant mechanisms by which doxorubicin induces cardiomyopathy is the production of

oxygen free radicals in the mitochondria of cells and the presence of iron further increase this effect (Tacar et al., 2013 and Ichikawa et al., 2014). The doxorubicin generates redox active quinone-hydroquinone- containing anthracyclines, that undergo one-electron and two-electron reduction by a wide variety of reducing agents which may be chemical and/or enzymatic (Octavia et al., 2012). This process leads to the formation of semiquinone free radicals and/or other reactive species that trigger antitumor activity either by alkylating DNA, or by causing breaks in the DNA strand or by initiating lipid peroxidation (Gutteridge and Halliwell 1989). Also several other researchers have reported that, the major cause of this effect is the tissue damage induced by free oxygen radicals (Tokarska-Schlattner et al., 2006). During the doxorubicin metabolism, reduction of the kinone group by cytochrome P-450 reductase and xanthine oxidase into the semikinone radical (Menna et al., 2010) 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 (Simunek et al., 2009 and Bates et al., 2006) 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 (Danelisen and Singal 2002 and Suliman et al., 2007). Some of the endogenous antioxidants responsible for detoxification in the body is discussed below. Glutathione or γ-glutamylcysteinylglycine (GSH) is a tripeptide thiol which is ubiquitously synthesized by all eukaryotic cells as a protective measure against the oxygen radical induced oxidative stress (Schumacker 2015 and Lushchak 2012). It acts as a strong antioxidant and its depletion is associated with structural and functional disturbances in cells and rise in the oxidative stress (Schumacker 2015 and Lushchak 2012). The main function of catalase enzymes is to neutralize hydrogen peroxide produced during respiration. These enzymes convert H_2O_2 into water and molecular oxygen (Kodydková et al., 2014). Their reduced activity increases accumulation of H_2O_2 and oxidative stress. The doxorubicin administration reduced the activity of catalase enzyme (Ganesh and Lalrinpuii 2018) Doxorubicin treatment has been found to reduce catalase activity in the heart and liver of rats (Jagetia and Lalnuntluangi 2016 and Kwatra et al., 2016). Likewise, doxorubicin treatment has been reported to reduce catalase activity in bone marrow cells, heart and liver of mice (Jagetia and Lalrinengi 2017 and Jagetia and Lalrinengi 2015). Superoxide dismutase enzymes (SOD) are synthesized by eukaryotic cells and are present inside the cell as well in the extracellular matrix (Carillon et al., 2013). The SODs also remove H_2O_2 generated during respiration and convert it into less harmful products like water and molecular oxygen (Perry et al., 2010). Doxorubicin treatment reduced the SOD activity in the rat lung in the present study (Ganesh and Lalrinpuii 2018). Doxorubicin induces cardiotoxicity, nephrotoxicity and hepatotoxicity by interacting with eNOS and subsequently elevates the superoxide radical production that in turn induces toxic effects (Ganesh and Lalrinpuii 2018). The doxorubicin has been reported to deplete SOD activity in mice bone marrow, liver and heart earlier (Jagetia and Lalrinengi 2017 and Jagetia and Lalrinengi 2015). Doxorubicin has also been found to reduce SOD activity in the rat liver (Jagetia and Lalnuntluangi 2016). Anticancer agents for lung cancer have been implicated in lung injury and interstitial lung disease (ILD). ILD comprises histopathological patterns of acute lung injury (ALI), nonspecific interstitial pneumonitis (NSIP), diffuse alveolar damage (DAD), bronchiolitis obliterans with organizing pneumonia, eosinophilic pneumonia, and pulmonary hemorrhage (Camus et al., 2001). Drug-associated lung injury results either from cellular dysfunction generating the cell-death mechanism (apoptosis) or by impairing the cell and tissue repair sequence. The pattern of pulmonary toxicity by these agents is usually a non-specific interstitial pneumonitis, diffuse alveolar damage and pleural effusion (Dimopoulou et al., 2006). Pulmonary edema, pneumonitis or interstitial lung disease or lung fibrosis has been reported as some of the adverse side effects in cancer patients receiving

doxorubicin alone or in combination with other chemotherapy drugs (Mazzotta et al., 2016 and Irfan et al., 2017).

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).

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, (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

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. 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 lungs 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

The histology of the lungs in normal control Wistar rats is presented in Plate 1. It shows normal morphological features of alveolar sacs, bronchioles and inter-alveolar septa, while the histology of the lungs in animals exposed to doxorubicin for 14 days is presented in plate 2. A close observation revealed distorted lungs with thickened inter alveolar septa and distorted alveolar sacs and bronchioles. Plate 3 shows the histological features of the lungs in animals exposed to doxorubicin and simultaneously treated with turmeric for 14 days. It shows normal lungs histology, while plate 4 presents the histological features of the lungs in animals exposed to doxorubicin and simultaneously treated with vitamin C for 14 days. It

shows thickened inter-alveolar septa. Plate 5 shows the lung's histo-morphology of Wistar rats exposed to doxorubicin and concomitantly treated with vitamin E. A close observation revealed thickened inter alveolar septa and distorted alveolar sacs. The histological features of lungs in animals exposed to doxorubicin and simultaneously treated with a combination of turmeric and vitamin C for a period of 14 days is presented in plate 6. It reveals normal histology of the lungs. Plate 7 shows the histo-morphology of Wistar rats exposed to doxorubicin and concomitantly treated with a combination of turmeric and vitamin E for a period of 14 days. A close observation also reveals normal alveolar sacs, bronchiole, and blood vessels and inter alveolar septa. The effect of vitamin C and vitamin E combination against the adverse effects of doxorubicin on the lungs treated for a period of 14 days is presented in plate 8. It shows distorted lungs with thickened inter alveolar septa. The combine effect of turmeric, vitamins C and E on the toxicity of doxorubicin on the lungs in Wistar rat treated for 14 days is presented in plate 9 and it shows normal lungs histology. Plate 10 reveals the pathological changes observed on the lungs morphology in animals exposed to doxorubicin for 28 days, a closer observation revealed distorted lungs and thickened inter alveolar septa and distorted alveolar sacs. The effect of turmeric against the adverse pathological changes caused by doxorubicin administration for a period of 28 days is presented in plate 11. It was observed that the lung presented normal histo-morphology. Plate 12 presents the effects of vitamin C on the lungs in doxorubicin induced toxicity in Wistar rats treated for 28 days, a closer observation revealed distorted lungs, with deposits on the alveolar sacs and thickened inter alveolar sacs. The effect of concomitant administration vitamin E and doxorubicin for a period of 28 days is presented in plate and it revealed distorted lungs, with and thickened inter alveolar septa. The effect of turmeric and vitamin C simultaneous administration against the adverse effects of exposure to doxorubicin for a period of 28 days is presented in plate 14. A closer observation revealed normal histology of the lungs with normal alveolar sacs, bronchiole and inter alveolar septa. The effect of combined treatment with turmeric and vitamin E against the toxic effects of doxorubicin exposure for a period of 28 days is presented in plate 15. It revealed normal histo-morphology. Plate 16 presents the effect of vitamins C and E concomitant administration against the toxic effects of doxorubicin exposure to Wistar rats for a period of 28 days. A closer look revealed thickened inter alveolar septa. The combined effect of turmeric, vitamins C and E against the toxicity of doxorubicin exposure for 28 days is presented in plate 17. It was observed that the lungs presented normal histo-morphology.

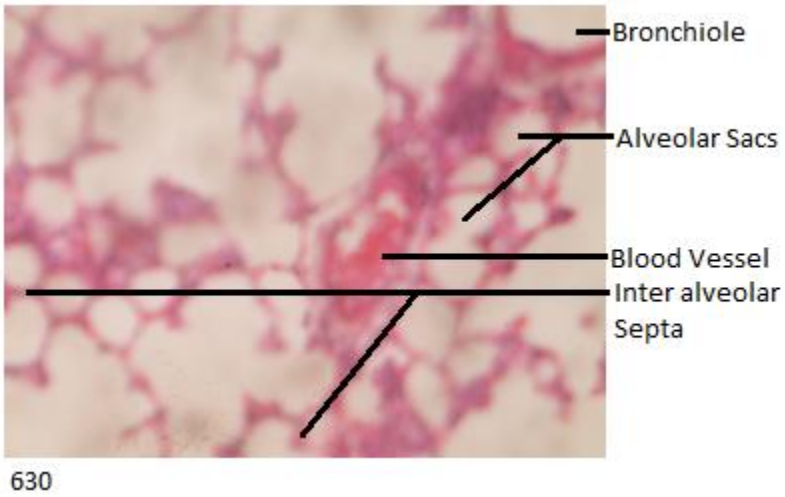

Plate 1: Lungs histology of normal control of Rats showing normal morphological features of alveolar sacs, bronchioles and inter alveolar septa

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Plate 2: Effects of Vitamins C and E on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 14). It shows distorted lungs with thickened inter alveolar septa and distorted alveolar sacs and bronchioles

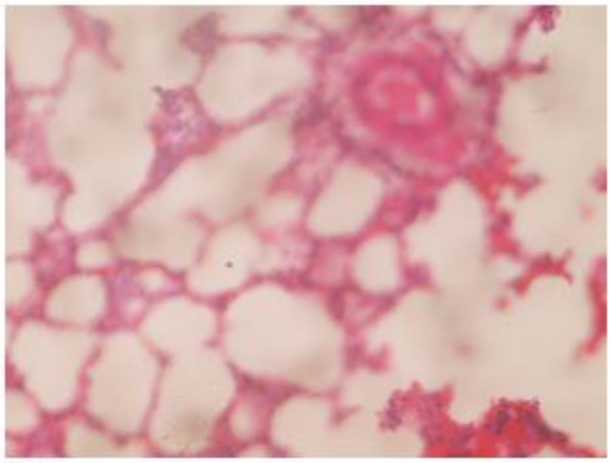

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Plate 3: Effects of Turmeric on the lungs in Doxorubicin toxicity in Wistar rats (Day 14). It shows normal histology of the lungs.

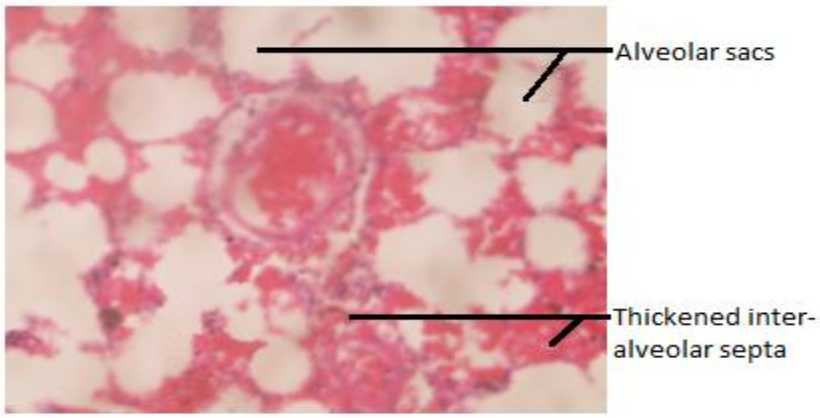

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Plate 4: Effects of Vitamin C on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 14), showing thickened inter alveolar septa

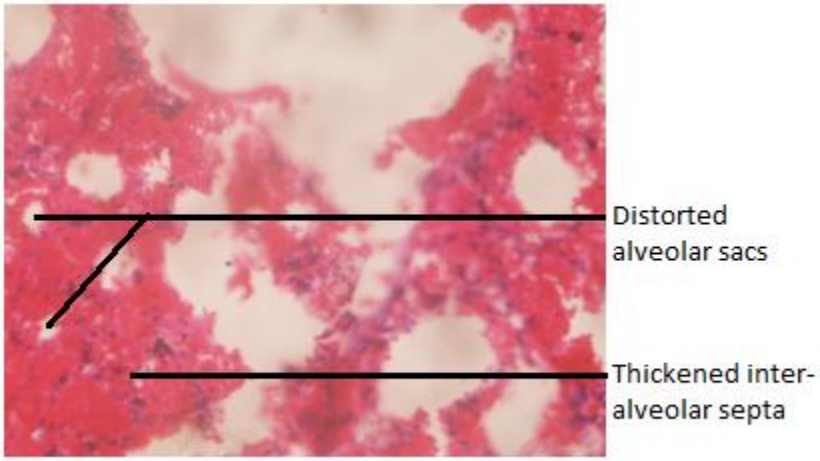

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Plate 5: Effects of Vitamin E on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 14). It shows thickened inter alveolar septa and distorted alveolar sacs

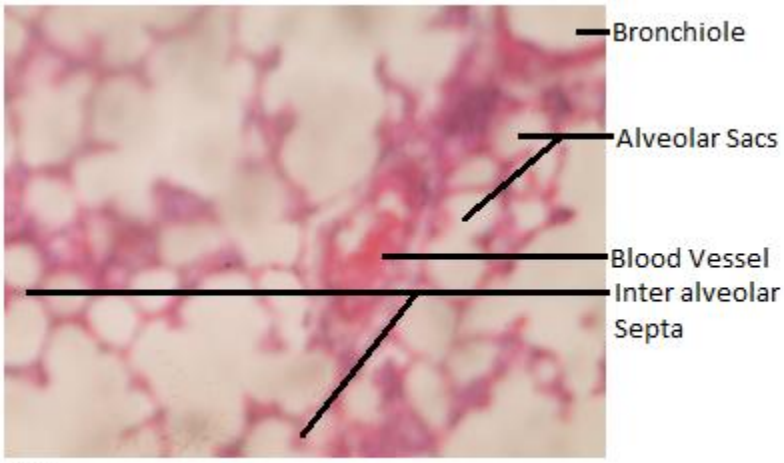

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Plate 6: Effects of Turmeric and Vitamin C on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the lungs

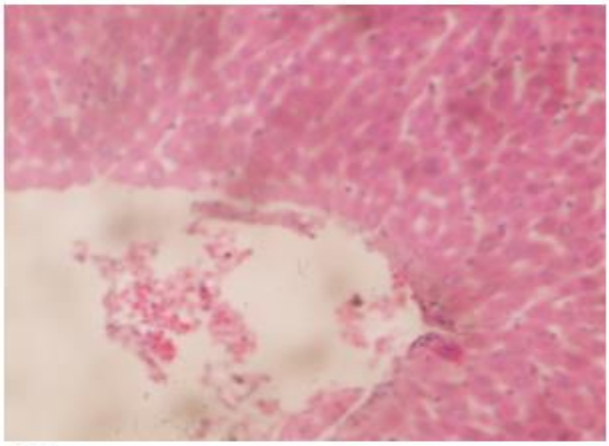

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Plate 7: Effects of Turmeric and Vitamin E on the liver in Doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the liver. It also shows normal alveolar sacs, bronchiole, blood vessels and inter alveolar septa

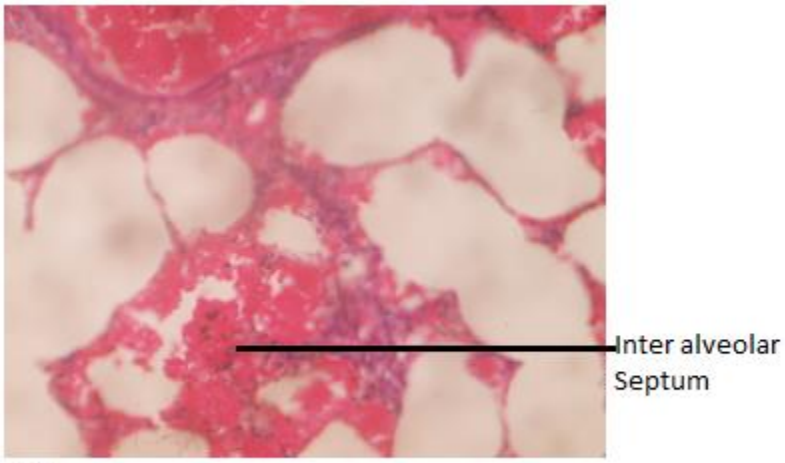

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Plate 8: Effects of Doxorubicin toxicity on the lungs in Wistar rats (Day 14). It shows distorted lungs with thickened inter alveolar septa

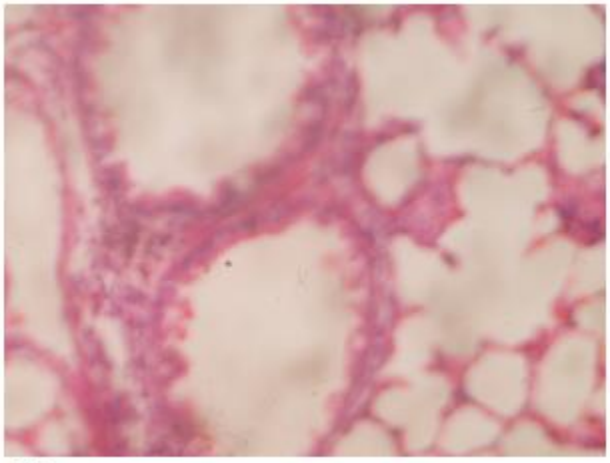

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Plate 9: Effects of Turmeric and Vitamins C and E on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the lungs.

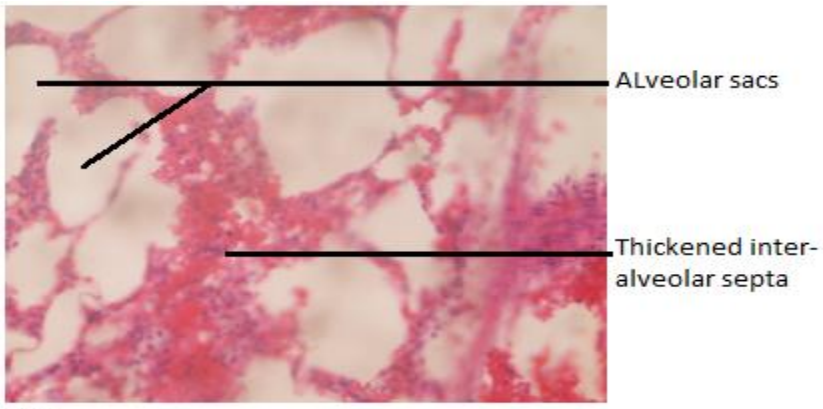

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Plate 10: Effects of Doxorubicin toxicity on the lungs in Wistar rats treated with doxorubicin for 28 days. It shows distorted lungs and thickened inter alveolar septa and distorted alveolar sacs.

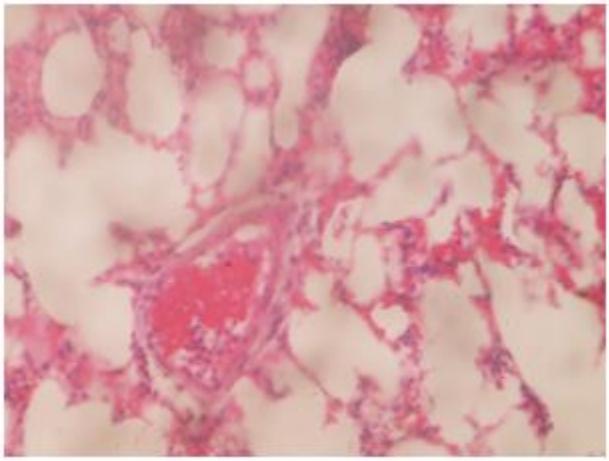

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Plate 11: Effects of Turmeric on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the lungs

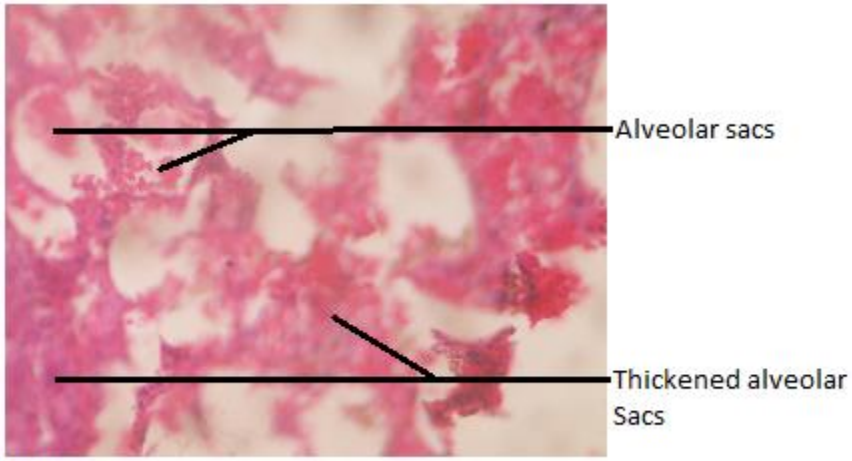

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Plate 12: Effects of Vitamin C on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 28). It shows distorted lungs, with deposits on the alveolar sacs and thickened inter alveolar sacs

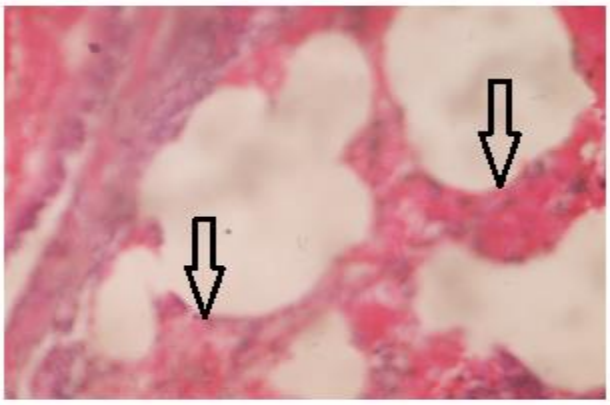

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Plate 13: Effects of Vitamin E on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 28). It shows distorted lungs, with and thickened inter alveolar septa

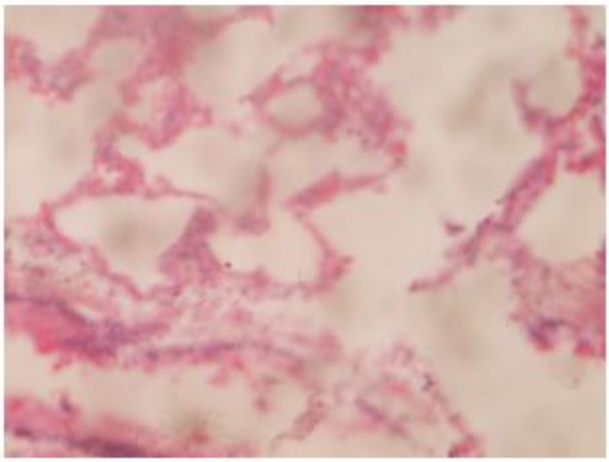

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Plate 14: Effects of Turmeric and Vitamin C on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the lungs with normal alveolar sacs, bronchiole and inter alveolar septa

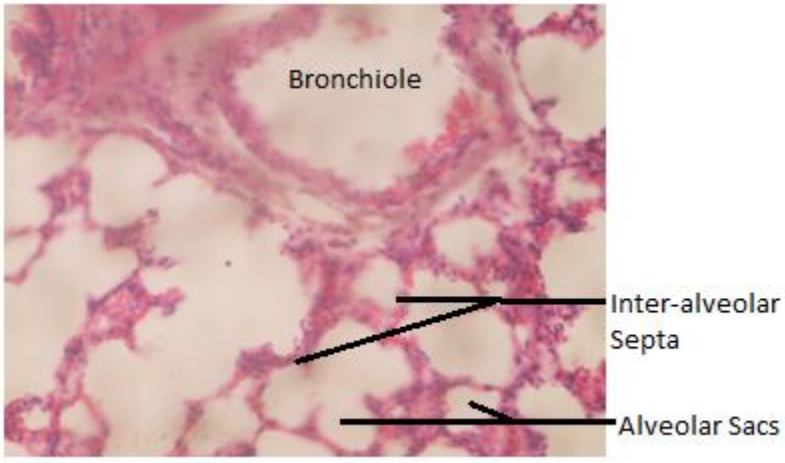

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Plate 15: Effects of Turmeric and Vitamin E on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the lungs.

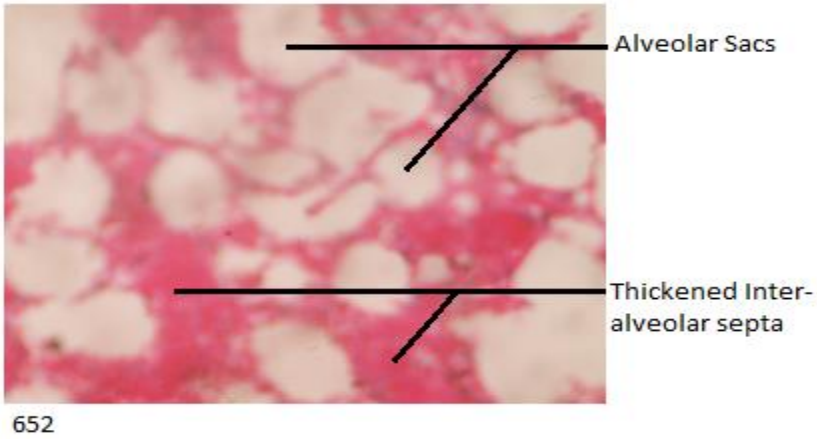

Plate 16: Effects of Vitamins C and E on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 28). It shows thickened inter alveolar septa.

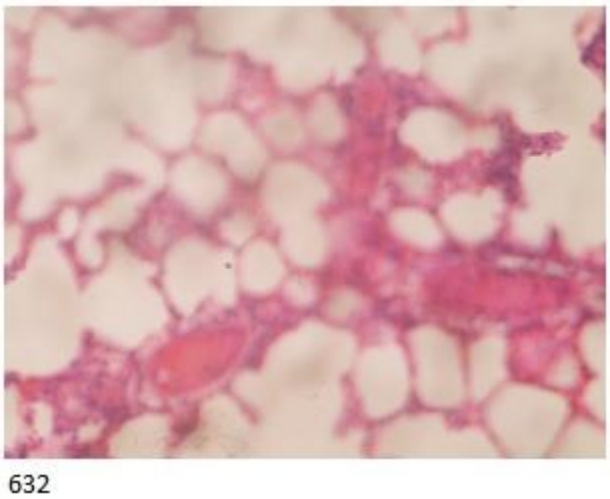

Plate 17: Effects of Turmeric, Vitamins C and E on the lungs in Doxorubicin induced toxicity in Wistar rats (Day 28). It shows normal histology of the lungs.

Discussion

In our study, it was found that administration of doxorubicin for 14 and 28 days caused distorted lungs with thickened inter alveolar septa and distorted alveolar sacs and bronchioles, while the lungs from the control animals showed normal histo-morphological features. It was also found that the lungs 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 or in combination with vitamin E and doxorubicin for 14 and 28 days showed thickened inter-alveolar septa and distorted lungs, with deposits on the alveolar sacs and thickened inter alveolar sacs, Similarly, the lungs from animals that received vitamin E alone or in combination with vitamin C along with doxorubicin for 14 and 28 days showed thickened inter alveolar septa and distorted alveolar sacs and distorted lungs, with and thickened inter alveolar septa respectively. These findings evidently reveal that turmeric alone or in combination with vitamin C and E can protect the lungs 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 imply 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 lungs 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 were found to be greater than those of vitamin C and vitamin E.

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