Evaluation of turmeric's protective potential of the kidneys against Doxorubicin induced oxidative stress damage in Wistar rats.

Isirima, Joshua Charles and Okoroafor, Dorcas Okayo

Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Choba-Port Harcourt, 500272, Nigeria

***Abstract*: Doxorubicin is an effective anthracycline used in the treatment of various cancers, but with severe a adverse effect which affects its potency, thus there is a need to reduce its toxicity in clinical setting in the management of cancers. The current study investigated turmeric root extract for its protective action against doxorubicin-induced kidney histo-pathological damage in Wistar rats. 27 adult Wistar rats were divided into 9 groups of three 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 kidneys were harvested and processed for histological assessment. Results revealed that doxorubicin caused formation and accumulation of inflammatory cells and edematous tubules (nephritis) and necrosis after 14 and 28 days of administration in the kidneys, while kidneys 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. This was also true for, the kidneys of animals that received vitamins C and E alone or in combination simultaneously with doxorubicin. Thus turmeric root extract protected the kidneys against the damaging effect of doxorubicin-induced toxicity.**

Keywords: **Turmeric, Doxorubicin, kidneys, oxidative stress, protective potential, Wistar rats**

I. INTRODUCTION

Doxorubicin is an anthracycline (with a trade name of Adriamycin) that has been used in the treatment of cancer; (solid malignancies and lymphomas) although, not without severe side effects (Minami et al., 2010), such as cardiac, renal and haematological toxicities (Fadillioglu et al., 2003). Doxorubicin is used to treat solid tumors like carcinomas of the uterus, breasts, thyroid, esophagus, ovaries and lungs, acute leukemia, Hodgkin's and non-Hodgkin's lymphomas and soft tissue sarcomas (Gokcimen et al., 2007 and Wang et al., 2014). Doxorubicin has been shown to cause renal toxicity due to oxidative stress in various experimental animal models and human studies (Injac et al., 2008 and Saenko et al., 2005). As a result of oxygen use in aerobic organisms, reactive oxygen metabolites (ROM) occur naturally. In a study by Liu et al., (2007), GSH, GST and SOD levels decreased in doxorubicin-administered rats. While in another study by (Yagmurca et al., 2004) on the protective effects of caffeic acid phenethyl ester (CAPE) against doxorubicin-induced kidney injury, it was revealed that histological examination showed glomerular vacuolization, tubular desquamation, adhesion, and thickening in Bowman's capsule and damage in the tubular and glomerular capillary basal membranes in doxorubicin group. Still in another study by (Yagmurca et al., 2015), it was revealed that administration of a single dose of doxorubicin of 20 mg/kg into the peritoneum of Wistar rats resulted in similar tubular and glomerular damage, while in another study by (Jadhav et al., 2013), it was revealed that administration of doxorubicin resulted in nephrotoxicity. In a study to investigate the toxic effects of doxorubicin on some organs in Wistar rats indicated that injected with 1 mg / kg dose once a week for four weeks results in congestion, hemorrhage, and thrombosis, (Shivakumar et al., 2012) indicating severe congestion and hemorrhage among renal tubules. Several studies revealed that doxorubicin interferes with glomerular podocytes leading to their injury and, consequently, to nephropathy (Park, et al., 2003; Wang et al., 2000 and Rook et al., 2005). The most common event documented in all the studies is the presence of a severe proteinuria. Exposure of renal tissue to the local passage of leaked proteins may evoke structural changes in the nephron leading to focal glomerulosclerosis (Okuda et al., 1986; Wang et al., 2000 and Lebrecht et al., 2004). a glomerular disease characterized by marked proteinuria, steroid resistance, hypertension, and a high incidence of progression to renal failure (Tong et al., 2008). Proteinuria is also related with focal fusion of podocytes foot processes and swelling, extensive glomerular vacuolization and quick and progressive renal failure (Park, et al., 2003; Wang et al., 2000 and Rook et al., 2005). Other important effects, although present in a smaller scale of intensity, are the presence of extensive glomerular lesions, tubular dilatation, interstitial fibrosis and inflammation (Okuda et al., 1986; Bertani et al., 1986 and Fajardo et al., 1980), an increase in plasma creatinine levels and hipoalbuminemia (Park, et al., 2003; Wang et al., 2000 and Rook et al., 2005), dyslipidemia (Keane et al., 1987), hypercoagulability, increase in kidneys size with

a granular pale color surface (Okuda et al., 1986; Wang et al., 2000) and glomerular capillary permeability (Saad et al., 2001).

Doxorubicin-induced changes in the kidneys of rats include increased glomerular capillary permeability and tubular atrophy (Wapstra et al., 1999). Doxorubicin causes an imbalance between free oxygen radicals and antioxidants. Oxygen free radicals are produced in larger amounts due to the use of agents like doxorubicin, and this leads to oxidative damage in cellular membranes, organelles and even in genetic material (Funk et al., 2014). The most important indicators of this damage are lipid peroxidation (LPO) and protein oxidation (Karaman et al., 2006), which results in tissue injury (Karaman et al., 2006). Although the exact mechanism of doxorubicin-induced nephrotoxicity remains unknown, it is believed that the toxicity may be mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules, membrane LPO, and protein oxidation (Liu et al., 2007). The oxidant-antioxidant system has different sources, one of which is nitric oxide (NO). Nitric oxide (NO) is a free radical gas which acts as a cytoprotective or a cytotoxic agent, i. e. both a scavenger and a producer of free oxygen radicals. NO is generated by either endothelial nitric oxide synthase (eNOS) or inducible nitric oxide synthase (iNOS) (Nathan and Xie 1994). NO reacts with superoxide anions and is converted into peroxynitrite. Peroxynitrite both removes superoxide anion and becomes a damage-producing radical. Possible role of doxorubicin in NOS metabolism occurs via direct or indirect stimulation of NO production, and this might be a consequence of increased free radical generation. Free radical production and/or NO release induced by doxorubicin is entirely responsible for the doxorubicin-induced toxicity (Radi et al., 1991). Another source of the oxidant system is malondialdehyde (MDA), an indicator of lipid peroxidation. Other sources of radicals include neutrophils and myeloperoxidase (MPO) system. MPO production increases in inflammatory responses and produces hypochlorite radicals leading to cellular damage (Gurel et al., 2004). An injury to the renal system particularly kidney can lead to high morbidity and mortality rate because of its significant role in detoxification and excretion of drug and toxic material, as well as regulation function, and elimination of urine and creatinine (Vaidya et al., 2008). Because of the usefulness of doxorubicin in the treatment of cancer, it is essential to investigate on a possible treatment regimen or strategy to mitigate the adverse effect of doxorubicin on the tissues, thus the essence of this study. Thus in this study, we investigated the effect possible antioxidant potentials of turmeric root extract against doxorubicin induced oxidative stress kidney damage 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. 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 (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). 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). Furthermore, Canales-Aguirre and coworkers (Canales-Aguirre et al., 2012) 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 (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).

II. 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, and 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. Kidney 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 kidneys 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.

III. RESULTS AND DISCUSSION

Plate 1, is kidney histology of normal control of rats showing normal histo-morphology. Plate 2 shows the effects of Doxorubicin toxicity on the kidneys in Wistar rats after 14 days of treatment. It shows inflammatory cells and edematous tubules (nephritis) and necrosis. Plate 3 shows the effects of Turmeric on the kidneys in Doxorubicin induced toxicity in Wistar rats after 14 days of treatment, showing normal histology of the kidney. Plate 4 shows the effects of Vitamin C on the lungs in Doxorubicin induced toxicity in Wistar rats after 14 days of treatment. It shows normal histology. Plate 5 shows the effects of Vitamin E on the kidney in Doxorubicin induced toxicity in Wistar rats after 14 days of treatment. It shows normal histology of the kidney. Plate 6 shows the effects of Turmeric and Vitamin C on the kidney in Doxorubicin induced toxicity in Wistar rats after 14 days of administration. It shows normal histo-morphology. Plate 7 shows the effects of Turmeric and Vitamin E on the kidney in Doxorubicin induced toxicity in Wistar rats after 14 days of treatment. It shows normal histo-morphology of the kidney. Plate 8 shows the effects of Vitamins C and E on the lungs in Doxorubicin induced toxicity in Wistar rats after 14 days of treatment. It shows normal histology of the kidney. Plate 9 shows the effects of Turmeric and Vitamins C and E on the kidney in Doxorubicin induced toxicity in Wistar rats after 14 days of administration. It shows normal histo-morphology. Plate 10 shows the effects of Doxorubicin toxicity on the kidney in Wistar rats 28 days treatment. It shows inflammatory cells and edematous tubules (nephritis) and necrosis. Plate 11 shows the effects of Turmeric on the kidney in Doxorubicin induced toxicity in Wistar rats after 28 days of treatment. It shows normal histo-morphology of the kidney. Plate 12 shows the effects of Vitamin C on the kidney in Doxorubicin induced toxicity in Wistar rats after 28 of treatment. It shows normal histo-morphology of the kidney. Plate 13 shows the effects of Vitamin E on the kidney in Doxorubicin induced toxicity in Wistar rats after 28 days of treatment. It shows normal histo-morphology of the kidney. Plate 14 shows the effects of Turmeric and Vitamin C on the kidney in Doxorubicin induced toxicity in Wistar rats after 28 days of treatment. It shows normal histo-morphology of the kidney. Plate 15 shows the effects of Turmeric and Vitamin E on the kidney in Doxorubicin induced toxicity in Wistar rats after 28 days of treatment. It shows normal histo-morphology

of the kidney. Plate 16 shows the effects of Vitamins C and E on the kidney in Doxorubicin induced toxicity in Wistar rats after 28 days of treatment. It shows normal histo-morphology of the kidney. Plate 17 show effects of Turmeric and Vitamins C and E on the kidney in Doxorubicin induced toxicity in Wistar rats after 28 days of treatment. It shows normal histo-morphology of the kidney.

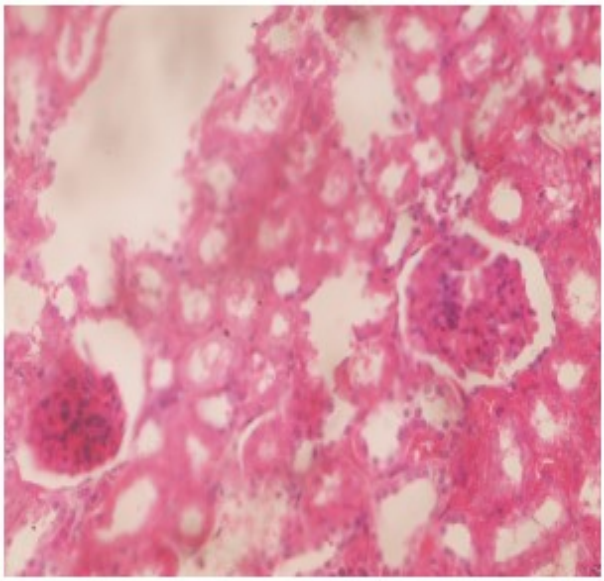


Plate 1: Kidney histology of normal control of Rats showing normal histo-morphology of the kidney

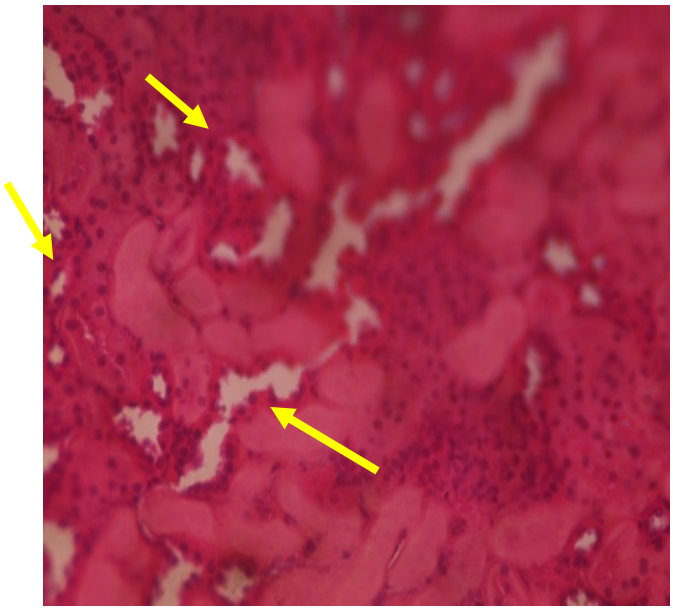


Plate 2: Effects of Doxorubicin toxicity on kidney in Wistar rats (Day 14). Showing inflammatory cells and edematous tubules (nephritis) and necrosis

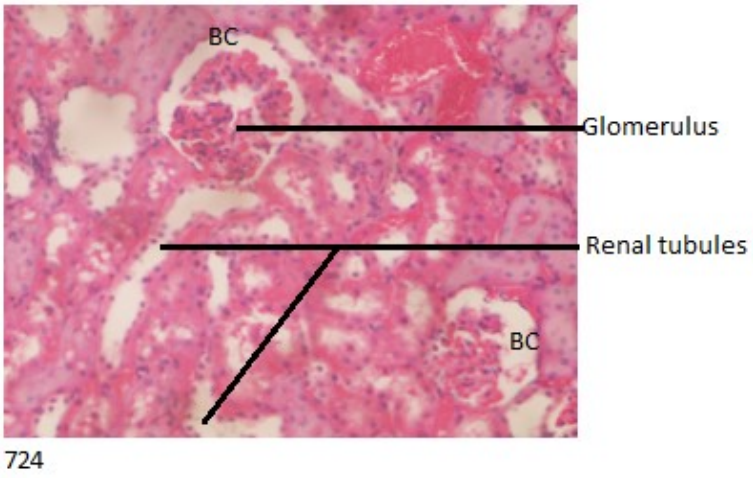


Plate 3: Effects of Turmeric on the kidney in Doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the kidney

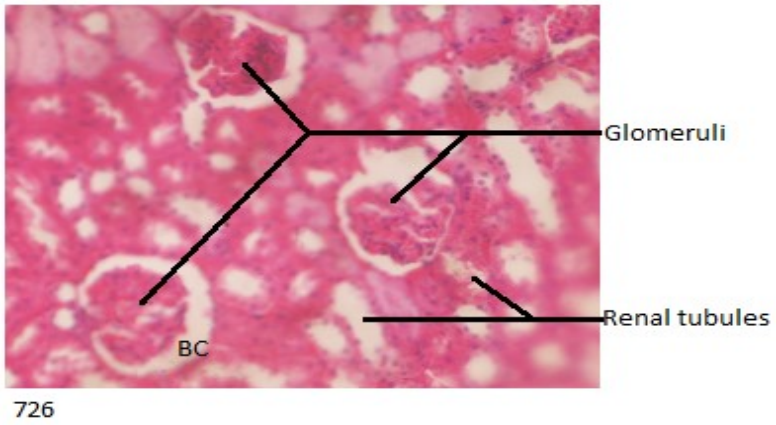


Plate 4: Effects of Vitamin C on the kidney in Doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the kidney

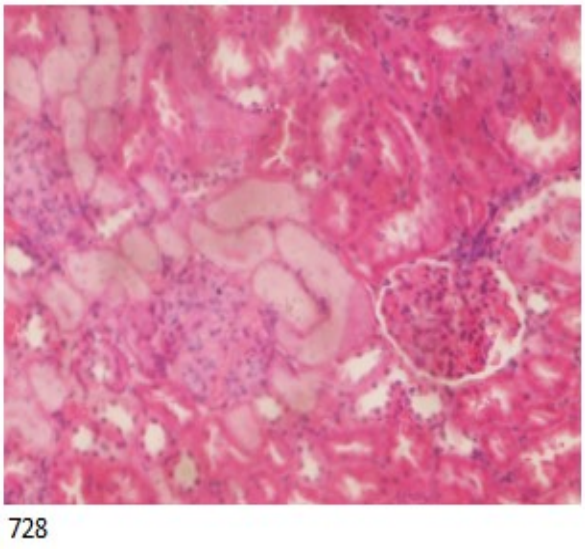
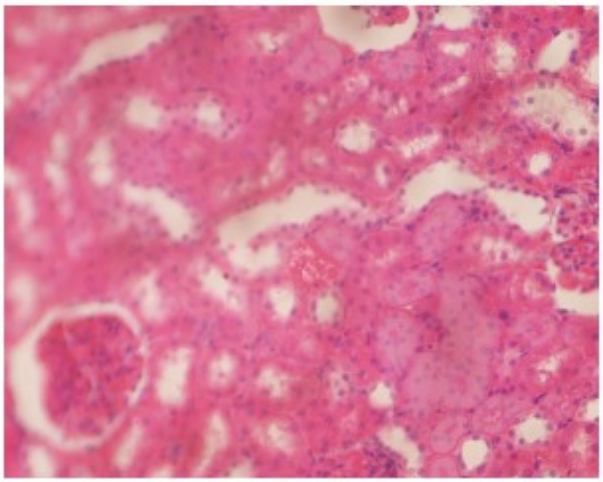
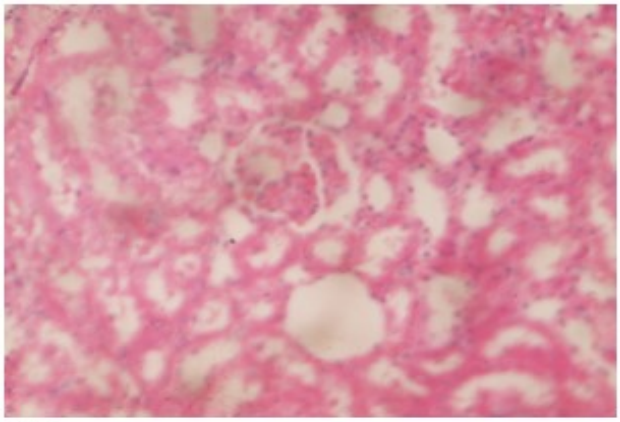


Plate 5: Effects of Vitamin E on the kidney in Doxorubicin induced toxicity in Wistar rats (Day 14). It shows normal histology of the kidney



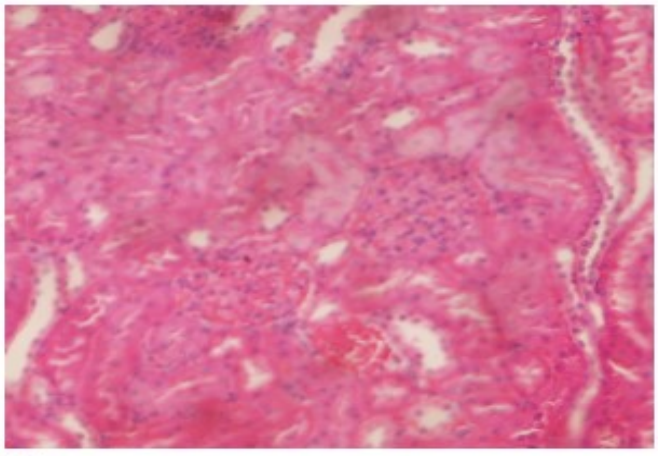
730

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



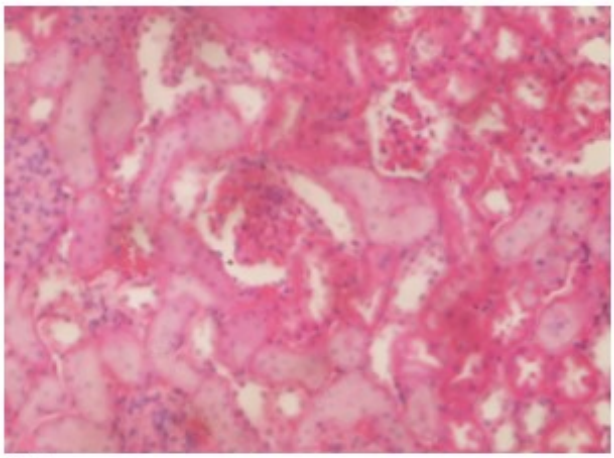
732

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



734

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



736

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

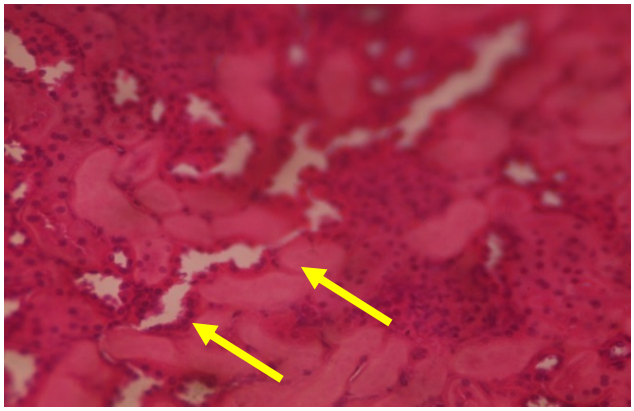
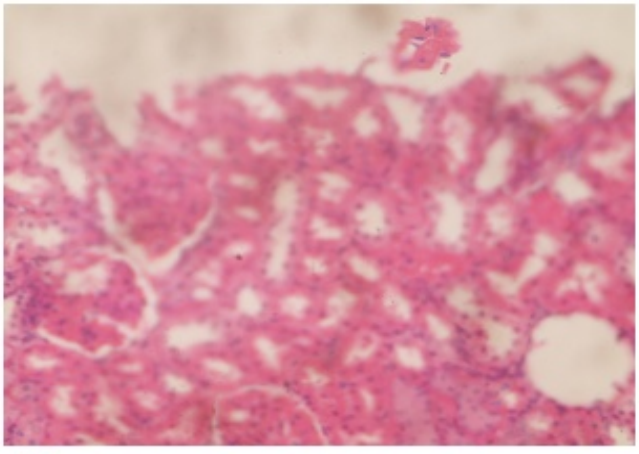
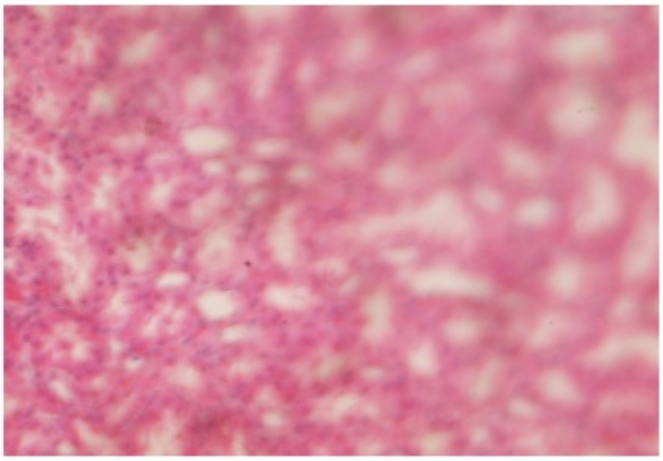


Plate 10: Effects of Doxorubicin toxicity on the kidney in Wistar rats (Day 28), showing inflammatory cells and edematous tubules (nephritis) and necrosis



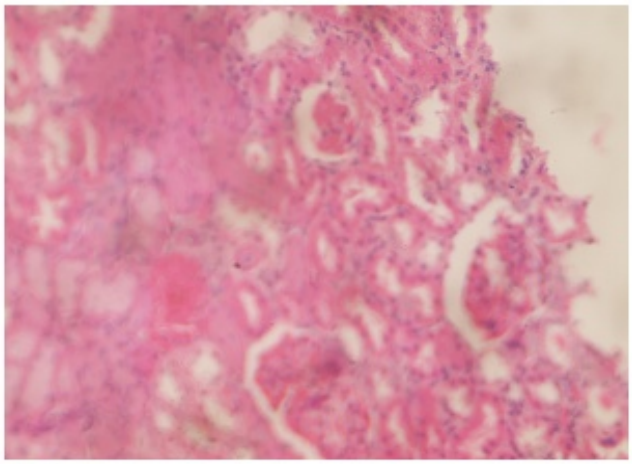
740

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



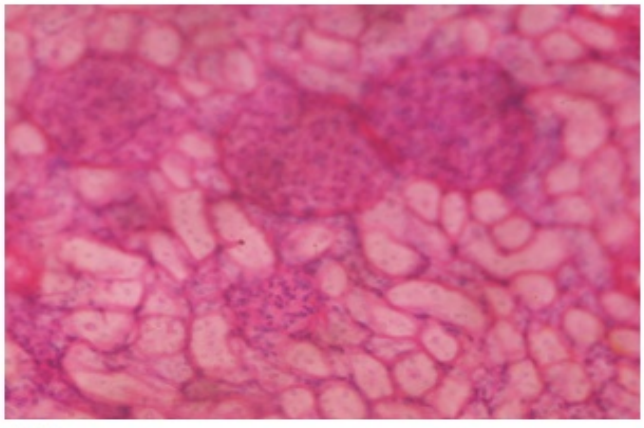
742

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



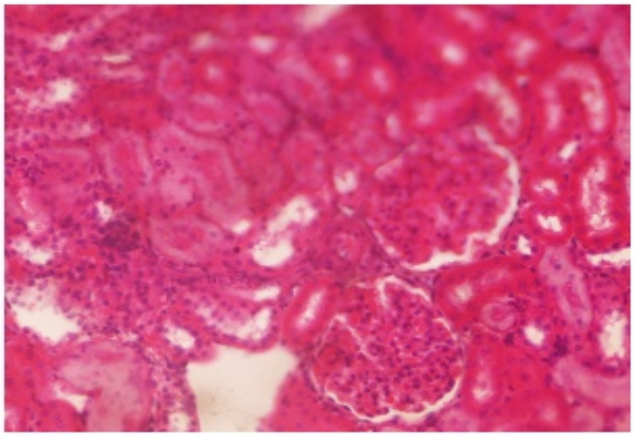
744

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



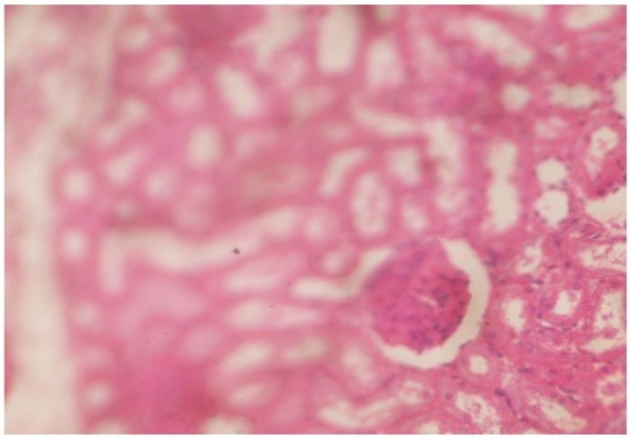
746

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



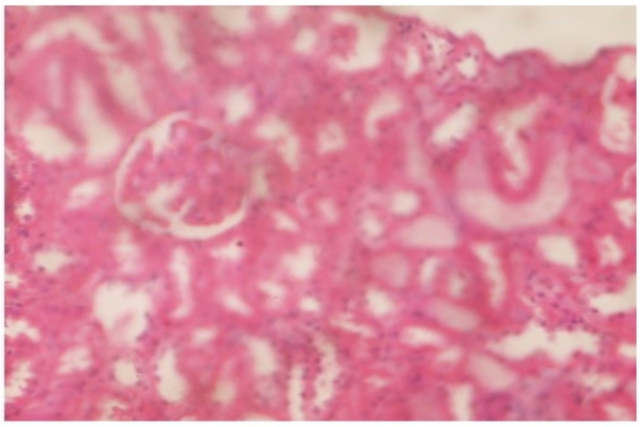
748

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



750

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



752

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

A proper examination of the kidney results revealed that doxorubicin administration for 14 and 28 days caused formation of inflammatory cells and edematous tubules (nephritis) and necrosis, which agrees with the results (Yagmurca et al., 2004) who reported that doxorubicin-induced kidney injury, causing glomerular vacuolization, tubular desquamation, adhesion, and thickening in Bowman's capsule and damage in the tubular and glomerular capillary basal membranes and the report of Yagmurca et al., (2015), who also reported tubular and glomerular damage due to doxorubicin toxicity. On the contrary, the kidneys from the control animals showed normal renal histology. The renal histology of animals that received turmeric and vitamin C independently and in combination for 14 and 28 days were all normal, this was also true for animals that received vitamins C and E alone or in combination. The acceptable inference about these adverse effects of doxorubicin remains that doxorubicin caused free oxygen radical formation as reported by Tokarska-Schlattner et al., (2006) and an increase in oxidative stress (Injac et al., 2008 and Saenko et al., 2005) or hydrogen peroxide and superoxide radical proliferation which reduce the levels of the endogenous enzyme (Superoxide dismutases, Reduced glutathione, catalase, and glutathione peroxidase) Liu et al., (2007) which are responsible for scavenging free radicals. The most important indicators of this damage are lipid peroxidation (LPO) and protein oxidation (Karaman et al., 2006), which results in tissue injury (Karaman et al., 2006). Although the exact mechanism of doxorubicin-induced nephrotoxicity remains unknown, it is believed that the toxicity may be mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules, membrane LPO, and protein oxidation (Liu et al., 2007). 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 (Yadav et al., 2009; Maheshwari and Singh 2009; El-Demerdash et al., 2009; Cekmen et al., 2009 and Fujisawa et al., 2004). This is intriguing, but the most astounding finding is the proof that turmeric actually blocked the toxic effects of doxorubicin in the kidney, implying that, if this is established turmeric can actually become a supplement in treatment of cancer without fear of renal toxicity.

IV. CONCLUSION

The study revealed that, DOX causes an increase in oxidative stress by the production of free radicals or ROS which in turn reduces the activities of endogenous anti-oxidants, thus leading to a decrease in the total anti-oxidant status of the system and thereby causing accumulation of inflammatory cells and edematous tubules (nephritis) and necrosis. All these are reversed on administration of turmeric root extract, vitamins C and E individually and collectively.

REFERENCES

[1] Ara´ujo C. C. Ara´ujo and L. L. Leon, (2001). "Biological activities of Curcuma longa L," *Mem´orias do Instituto Oswaldo Cruz*, vol. 96, no. 5, pp. 723–728,.

[2] Baker, J. R. (1945). Cytological Technique. (2nd ed.), (p. 211). London: Methuen.

[3] Bertani, T.; Cutillo, F.; Zoja, C.; Broggini, M.; Remuzzi, G. (1986). Tubulointerstitial lesions mediate renal damage in adriamycin glomerulopathy. *Kidney. Int., 30*, 488-496.

[4] Borra S. K., P. Gurumurthy, M. Mahendra, K. M. Jayamathi, C. N. Cherian, and C. Ram, (2013). "Antioxidant and free radical scavenging activity of curcumin determined by using different in vitro and ex vivo models," *Journal of Medicinal Plants Research*, vol. 7, no. 36, pp. 2680–2690,.

[5] Canales-Aguirre A. A. Canales-Aguirre, U. A. Gomez-Pinedo, S. Luquin, M. A. Ram´ırez-Herrera, M. L. Mendoza-Maga˜na, and A. Feria- Velasco, (2012)."Curcumin protects against the oxidative damageinduced by the pesticide parathion in the hippocampus of the rat brain," *Nutritional Neuroscience*, vol. 15, no. 2, pp. 62–69,.

[6] Cekmen M., Y. O. Ilbey, E. Ozbek, A. Simsek, A. Somay, and C. Ersoz, (2009) "Curcumin prevents oxidative renal damage induced by acetaminophen in rats," *Food and Chemical Toxicology*, vol. 47, no. 7, pp. 1480–1484,.

[7] Chainani-Wu N. (2003). "Safety and anti-inflammatory activity of curcumin: a component of tumeric (Curcuma longa)," *Journal ofAlternative and ComplementaryMedicine*, vol. 9, no. 1,pp. 161–168,.

[8] El-Demerdash, F. M., M. I. Yousef, and F. M. E. Radwan, (2009). "Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs," *Food and Chemical Toxicology*, vol. 47, no. 1, pp. 249–254,.

[9] Fadillioglu E., Erdogan H., Sogut S (2003). Protective effect of erdosteine against doxorubicin-induced cardiacmyopathy in rats. Journal of Applied Toxicology. 23 (1), 71-74.

[10] Fajardo, L.; Eltringham, J.; Stewart, J.; Klauber, M. (1980). Adriamycin nephrotoxicity. *Lab. Invest. 43*, 242-253.

[11] Fujisawa, S., T. Atsumi, M. Ishihara and Y. Kadoma (2004). Cytotoxicity, reactive oxygen species-generating activity and radical scavenging activity of curcumin and other related compounds. Anti-Cancer Res., 24: 563-570

[12] Funk F, Krüger K, Henninger C, Wätjen W, Proksch P, Thomale J, Fritz G. (2014). Spongean alkaloids protect rat kidney cells against cisplatin-induced cytotoxicity. Anticancer Drugs. doi:10.1097/CAD.0000000000000119.

[13] Gupta, S. C., B. Sung, J. H. Kim, S. Prasad, S. Li, and B. B. Aggarwal, (2013). "Multitargeting by turmeric, the golden spice: From kitchen to clinic," *Molecular Nutrition and Food Research*, vol. 57, no. 9, pp. 1510–1528, 2013.

[14] Gokcimen A, Cim A, Tola HT, Bayram D, Kocak A, Ozgüner F, Ayata A. Protective Effect of N-Acetylcysteine, (2007). Cafeic Acid and Vitamin E on Doxorubicin Hepatotoxicity. Hum Exp Toxicol 26: 519–525.

[15] Gurel A, Armutcu F, Sahin S, Sogut S, Ozyurt H, Gulec M, Kutlu NO, Akyol O. (2004). Protective role of alpha-tocopherol and caffeic acid phenethyl ester on ischemia-reperfusion injury via

nitric oxide and myeloperoxidase in rat kidneys. Clin Chim Acta 339 (1–2): 33–41.

[16] Hanan, B., Akram, H., Hassan, R., Ali, H., Zeinab, S., & Bassam, B. (2013). Techniques for the Extraction of Bioactive Compounds from Lebanese *Urtica dioica.* Maceration method. *American Journal of Phytomedicine and Clinical Therapeutics*, 1(6), 507-513

[17] Injac R, Boskovic M, Perse M, Koprivec-Furlan E, Cerar A, Djordjevic A, Strukelj B. (2008). Acute Doxorubicin Nephrotoxicity in Rats with Malignant Neoplasm can be Successfully Treated with Fullerenol C60 (OH)24 via Suppression of Oxidative Stress. Pharmacol Rep 60 (5): 742–749.

[18] Islam, F., M. Karim, M. Shahjahan, M. Hoque, M. R. Alam, and M. A. Hossain, (2002). "Study on the effect of plant spacing on the production of turmeric at farmers field," *Asian Journal of Plant Sciences*, vol. 1, no. 6, pp. 616-617, 2002.

[19] Jadhav VB, Thakare VN, Suralkar AA, Naik SR. (2013). Ameliorative effect of Luffa acutangula Roxb. on doxorubicin induced cardiac and nephrotoxicity in mice. Indian J Exp Biol; 51 (2): 149–156.

[20] Karaman A., E. Fadillioglu, E. Turkmen, E. Tas, and Z. Yilmaz, (2006). "Protective effects of leflunomide against ischemiareperfusion injury of the rat liver," *Pediatric Surgery International*, vol. 22, no. 5, pp. 428–434.

[21] Keane, W.; Kasiske, B.; O'Donnell, M. (1987). The role of lipids in progressive glomerular disease. *Adv. Exp. Med. Biol., 223*, 81-87.

[22] Lebrecht, D.; Setzer, B.; Rohrbach, R.; Walker, U. (2004). Mitochondrial DNA and its respiratory chain products are defective in doxorubicin nephrosis. *Nephrol. Dial. Transplant*, *19*, 329-336.

[23] Liu LL, Li QX, Xia L, Li J, Shao L. (2007). Differential Effects of Dihydropyridine Calcium Antagonists on Doxorubicin-Induced Nephrotoxicity in Rats. Toxicology; 231 (1): 81–90.

[24] Maheshwari RK, Singh AK (2006) Life Science 78: 2081—2087.

[25] Manikandan P., M. Sumitra, S. Aishwarya, B. M. Manohar, B. Lokanadam, and R. Puvanakrishnan, (2004). "Curcumin modulates free radical quenching in myocardial ischaemia in rats," *The International Journal of Biochemistry & Cell Biology*, vol. 36, no. 10, pp. 1967–1980,.

[26] Messarah M., W. Amamra, A. Boumendjel (2013). "Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythrocytes," *Toxicology and Industrial Health*, vol. 29, no. 1, pp. 77–88,.

[27] Minami M, Matsumoto S, and Horiuchi H. (2010), Cardiovascular side-effects of modern cancer therap. Circ. J. 74 (9): 1779-1786.

[28] Nathan, C. and Q.W. Xie, (1994). "Regulation of biosynthesis of nitric oxide," *Journal of Biological Chemistry*, vol. 269, no. 19, pp. 13725–13728.

[29] National Institute of Health (2002). Public health service policy on humane care and use of laboratory animals; *Office of the laboratory animal welfare, USA,* p. 1-19.

[30] Okuda S.; Oh, Y.; Tsuruda, H.; Onoyama, K.; Fujimi, S.; Fujishima, M. (1986). Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. *Kidney Int*., *29*, 502—510.

[31] Park, E.; Kim, S.; Lee, M.; Lee, H.; Lee, I.; Sung, J.; Yoon, Y. (2003). Protective effects of N-acetylcysteine and selenium against doxorubicin toxicity in rats. *J. Vet. Sci., 4*, 129-136.

[32] Pan, M. H., S. Y. Lin-Shiiau and J. K. Lin (2000). Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of Ikappa Bkinas and NFkappaB activation in macrophages. Biochem. Pharmacol., 60: 1665-1676

[33] Radi R., J. S. Beckman, K. M. Bush, and B. A. Freeman, (1991). "Peroxynitrite oxidation of sulfhydryls: the cytotoxic potential of superoxide and nitric oxide," *Journal of Biological Chemistry*, vol. 266, no. 7, pp. 4244–4250.

[34] Rook, M.; Lely, A.; Kramer, A.; Goor, H.; Navis, G. (2005). Individual differences in renal ACE activity in healthy rats predict susceptibility to adriamycin-induced renal damage. *Nephrol. Dial. Transplant*, *20*, 59-64

[35] Saad, S.; Najjar, T.; Al-Rikabi, A. (2001). The preventive role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. *Pharmacol. Res.*, *43*, 211-218.

[36] Saenko IuV, Shutov AM, Napalkova SM, Selivanova OS, Evstegneeva EIu. (2005). The Effect of Erythropoetin on Doxorubicin-Induced Oxidative Stressin rat Kidney. Eksp Klin Farmakol; 68 (6): 52–54

[37] Shivakumar P, Usha Rani M, Gopalo Reddy A, Anjaneyula A. (2012).A study on the toxic effect of doxorubicin on the histology of certain organs. Toxiccol Int., 19 (3):241-244.

[38] Spina, G. A. (1999). The dual role of nitric oxide i islet B-cells. News Physiol. Sci., 14:49-54

[39] Sreejayan-Rao, M. . (1994). Curcuinoids as potent inhibitors of lipid peroxidation. J. Pharm. Pharmacol., 46: 1013-1016

[40] Tokarska-Schlattner M, Zaugg M, Zuppinger C, (2006). New insights into doxorubicin-induced cardiotoxicity: the critical role of cellular energetics. J Mol Cell Cardiol; 41: 389-405.

[41] Tong, Y.; Wang, Q.; Hou, H. (2008). Protection by chinese herbs against doxorubicin-induced focal and segmental glomerulosclerosis in rats. *Drug Dev. Ind. Pharm., 34*, 663-667.

[42] Vaidya,V. Ferguson,M. Bonventre,J. (2008). Biomarkers of acute kidney injury, Annu, Rev,Pharmacol, Toxicol. 48, 463-393

[43] Wang, Y.; Wang, Y.; Tay, Y.; Harris, D. (200). Progressive adriamycin nephropathy in mice: Sequence of histologic and immunohistochemical events. *Kidney Int.*, *58*, 1797-1804.

[44] Wang X, Teng Z, Wang H, Wang C, Liu Y, Tang Y, Wu J, Sun J, Wang H, Wang J, Lu G. (2014). Increasing the cytotoxicity of doxorubicin in breast cancer MCF-7 cells with multidrug resistance using a mesoporous silica nanoparticle drug delivery system. Int J Clin Exp Pathol 7 (4): 1337–1347.

[45] Wapstra F. H., H. Van Goor, P. E. De Jong, G. Navis, and D. De Zeeuw, (1999). "Dose of doxorubicin determines severity of renal damage and responsiveness to ACE-inhibition in experimental nephrosis," *Journal of Pharmacological and ToxicologicalMethods*, vol. 41, no. 2-3, pp. 69–73.

[46] Yadav VR, Suresh S, Devi K, Yadav S (2009) Journal of the American Association of Pharmacetical Scientists 10: 752—762.

[47] Yagmurca M, Erdogan H, Iraz M, Songur A, Ucar M, Fadillioglu E. (2004). Caffeic Acid Phenethyl Ester as a Protective Agent against Doxorubicin Nephrotoxicity in Rats. Clin Chim Acta; 348: 27–34.

[48] Yagmurca M1, Yasar Z2, Bas O3 (2015). Effects of quercetin on kidney injury induced by doxorubicin. *Bratisl Med J 2015; 116 (8),* 486 – 489

[49] Yuan, S. Li, W., G. Deng, P. Wang, P. Yang, and B. Aggarwal, (2011). "Chemical composition and product quality control of turmeric (*curcuma longa* L.)," *Pharmaceutical Crops*, vol. 2, pp. 28–54, 2011.