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Prevention of doxorubicin-induced renal function abnormalities by turmeric in Wistar rats

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Abstract

Doxorubicin is an anthracycline antibiotic with anti-neoplastic potentials mediated mainly by affecting the DNA synthetic machinery of the cell. It is found to be effective against a wide range of cancers including the ovary, uterus, lung, breast etc. Doxorubicin exerts its nephrotoxicity on the kidney by inducing inflammatory changes leading to increased capillary porousness and glomerular shrinking. This study investigated the effect of turmeric on the renal function biomarkers in doxorubicin-induced oxidative stress in Wistar rats. 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, 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, vitamin C and vitamin E and finally, group 9 animals receive doxorubicin, vitamin C, vitamin E and turmeric. The experiment lasted for 28 days and blood samples were collected from each animal from the various groups for renal function assay. Doxorubicin caused significant increase in the serum levels of urea, creatinine, sodium (Na⁺), calcium (Ca²⁺), and decrease serum levels of Chloride (Cl⁻), magnesium (Mg²⁺) and potassium (K⁺). These abnormal electrolyte imbalances were prevented by administration of turmeric alone or in combination with vitamins C and or E along with doxorubicin concomitantly.

Keywords: Turmeric; Renal Function Abnormalities; Doxorubicin; Wistar rats

1. Introduction

Doxorubicin has been used as an effective anti-cancer treatment for over five decades. It is an anthracycline antibiotic with anti-neoplastic potentials mediated mainly by affecting the DNA synthetic machinery of the cell [1]. It has been found to be effective against a wide range of tumours such as cancers of the ovary, uterus, lung, breast, uterine cervix in addition to haematological malignancies [2, 3, and 4]. Presently, the clinical usefulness of doxorubicin is limited owing to its serious adverse effects including hepatotoxicity, cardiotoxicity, pulmonary toxicity, myelosuppression, and nephrotoxicity. Doxorubicin induced nephrotoxicity comprises a state of progressive glomerular damage associated with atrophic tubular lesion [5]. The mechanism of doxorubicin nephrotoxicity was reported to be correlated with its cyto-toxicity, manifested by the destruction of cell membranes and other cellular components as a result of DNA intercalation [6]. This cyto-toxicity is assumed to be facilitated by oxidative stress induced by excess reactive oxygen species (ROS). ROS activity alters specific intracellular structures such as proteins, lipids and nuclear DNA [1]. Mitochondrial DNA is another target for ROS activity, resulting in mutations, rearrangements, and transcriptional

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errors. These alterations affect not only mitochondrial functions but overall cellular activity, threatening its survival and ending with cell death [1]. Furthermore, doxorubicin toxicity was found to disrupt kidney function and could have a profound effect on total body metabolism [7]. Biochemical and physiological alterations of kidney tissue could affect the extracellular space due to the intense relationship between the cell environment and the extracellular space [8]. Doxorubicin has been reported by several researchers to cause alterations in the kidney function parameters. In one of such studies Manal et al., [9], in an investigation to unravel Boswellic acids' ability to ameliorate doxorubicin-induced nephrotoxicity in mice revealed that doxorubicin caused significant increases in serum markers of kidney damage, creatinine and urea. Doxorubicin-induced oxidative stress in renal tissues is characterized by elevated malondialdehyde (a marker of lipid peroxidation) and lowered reduced glutathione levels [10, 11 and 12], as well as lowered activities of catalase [13] glutathione peroxidase and superoxide dismutase. [14, 12]. In addition to oxidative damage, doxorubicin toxicity also induces inflammatory changes in kidney tissues [12]. Doxorubicin-induced nephrotoxicity causes increased capillary porosity and glomerular shrinking [12]. It is characterized by increased plasma levels of creatinine, urea [13, 11] and uric acid, [11] and increased plasma lactate dehydrogenase activity [11], as well as reduced renal Ca²⁺-ATPase, Mg²⁺-ATPase and Na⁺, K⁺-ATPase activities [15, 16].

Turmeric is a golden spice derived from the rhizome of the *Curcuma longa* plant, which belongs to the Zingiberaceae family [17]. Among the phytochemical constituents found in turmeric, curcumin is the one mostly reported to possess antioxidant properties. Curcumin possesses anti-inflammatory, immunomodulatory, and antiatherogenic activities and is a potent inhibitor of various reactive oxygen-generating enzymes [18, 19]. 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 [20]. Curcumin administration attenuated the arsenic, gentamicin, and acetaminophen-induced oxidative stress in rats [21, 22]. Curcumin also prevented free radical formation-induced myocardial ischemia and paraquat induced lung injury in rats [23]. Furthermore, curcumin protected against diazinon-induced toxicity in blood, liver, and erythrocyte of male Wistar rats [24]. Curcumin is a potent anti-oxidant and free radical scavenger [25]. It inhibits lipid peroxidation [26] and also inhibits Nitric Oxide Synthase (NOS) over-expression [27, 28]. Also, Isirima and Christian [29], had reported the anti-oxidant potentials of turmeric. In their study, they found that turmeric demonstrated anti-oxidant properties by reversing the significant reduction in the serum concentration of SOD, GPx, CAT, GSH and TAS as well as the increase serum level of MDA, caused by doxorubicin.

2. Methods

2.1. 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 [30].

2.2. Kits

The sodium and potassium kits were products of Atlas Medical, Cowley Rd, Cambridge, UK; while the chloride, magnesium, creatinine and urea kits were products of Agappe Diagnostics Switzerland, GmbH.

2.3. 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.

2.4. Extraction Method

The root of the plant was left to dry at room temperature between 32 – 35° C after collection and cleaning until they attained a constant weight. The extraction method that was used was adopted from Hanan et al, [31] 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°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°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°C. The resultant extract obtained 23% yield, was kept safe at room temperature in desiccators, until it was needed for the study.

2.5. Experimental Design

Fifty four 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 given at a dose of 22.4 IU /70kg/day, DOX was administered at a dose of 10-20mg/m² once a week, while turmeric was administered at a dose of 500mg/kg/day. The sequence of administration of these drugs as describe above continued for a period of 28 days, but the animals were sacrificed under diethyl ether anesthesia, on day 14 and day 28th. Blood samples were collected from each animal from the various groups for renal function test analysis. The animals were grouped as shown below;

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

2.6. Determination of Serum Urea

Serum urea levels were determined using urease modified Barthelot reaction [32] by a ready-made auto analyzer kit for this purpose, which can be measured of serum. The measured levels of serum urea were expressed in mg/dl.

2.7. Determination of Serum Creatinine

Serum creatinine concentrations were determined according to Jaffe reaction [33] using a ready-made auto analyzer kit for this purpose, which can be measured of serum. The measured levels of serum creatinine were expressed in mg/dl.

2.8. Ion Assay

The calcium, chloride, cholesterol, magnesium, potassium, sodium and triglyceride contents of the homogenates were assayed according to the kits manufacturers' instructions.

2.9. Statistical analysis

Mean values \pm S. E. M. were calculated for each parameter. For the determination of significant differences, Means were compared using the one-way Analysis of variance (ANOVA) test and the significance between the study groups were tested by employing the Post Hoc, multiple comparison test with Dunnett. P values <0.05 were considered as a level of statistical significance.

3. Results

Figure 1 presents the effect of turmeric on serum urea (mmol/l) in doxorubicin-induced toxicity in Wistar rats after 14 days of simultaneous drug treatment, showing that doxorubicin (Dox) caused a significant increase ($p \leq 0.05$) in the serum urea (13.58 ± 1.31) when compared to the control (7.45 ± 1.23). This increase was reversed towards normal by turmeric alone (9.26 ± 1.17) or in combination with vitamins C and E (7.94 ± 1.81). These observations were similar to those in figure 2 with serum urea values of (13.98 ± 1.57), (7.45 ± 1.23) and (8.86 ± 1.64) for doxorubicin, control and turmeric respectively, after 28 days of concomitant drug treatment. Figure 3 presents the effect of turmeric on creatinine level (mmol/l) in doxorubicin-induced toxicity in Wistar rats after 14 days of concomitant drug treatment, showing that doxorubicin (Dox) caused a significant increase ($p \leq 0.05$) in the serum creatinine level (129.74 ± 3.18) when compared to the control (89.56 ± 2.14). This increase was reversed towards normal by turmeric alone (103.56 ± 2.12) or in combination with vitamins C and E (92.11 ± 2.13). These observations were similar to those in figure 4 with serum creatinine level of (135.74 ± 3.26), (89.56 ± 2.14) and (101.28 ± 2.24) for doxorubicin, control and turmeric respectively, after 28 days of simultaneous drug treatment.

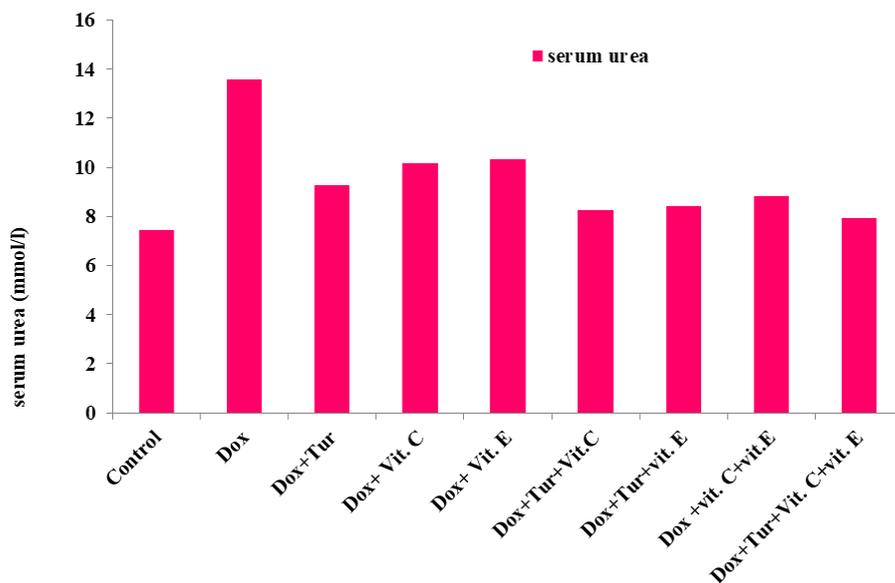


Figure 1 Effect of turmeric on serum urea (mmol/l) in doxorubicin-induced toxicity in wistar Rats after 14 days of drug treatment

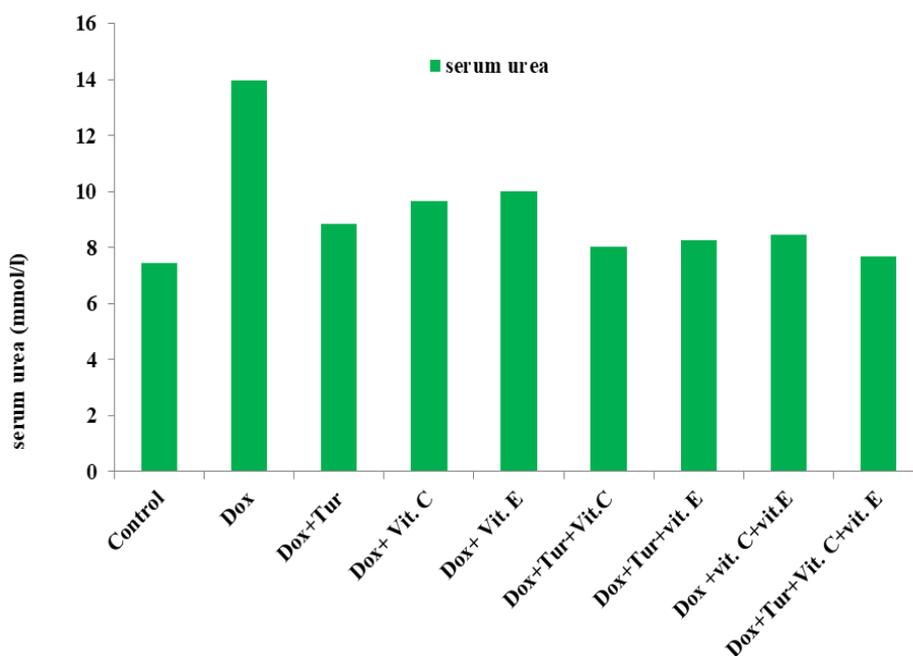


Figure 2 Effect of turmeric on serum urea (I/I) in doxorubicin-induced toxicity in wistarRatsafter 28 days of drug treatment.

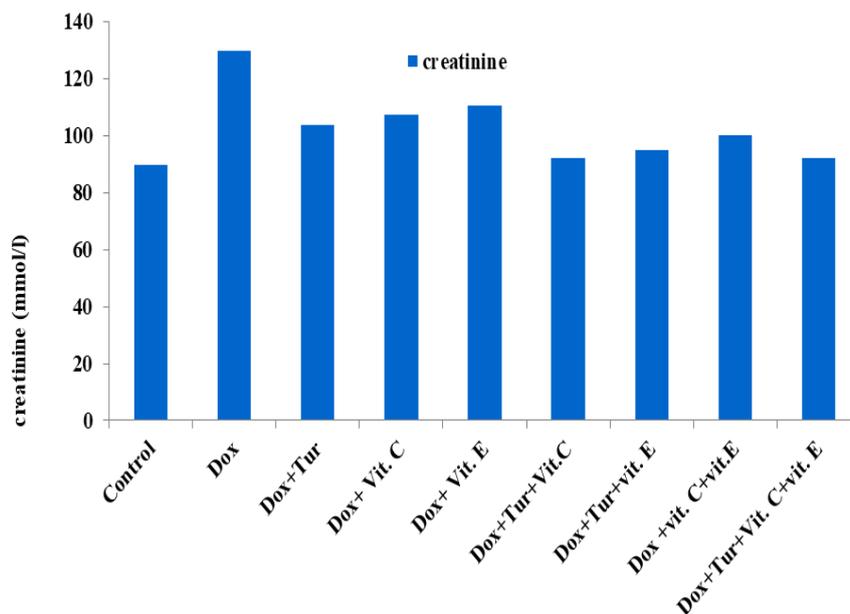


Figure 3 Effect of turmeric on creatinine (mmol/l) in doxorubicin-induced toxicity in Wistar Rats 14 days of drug treatment.

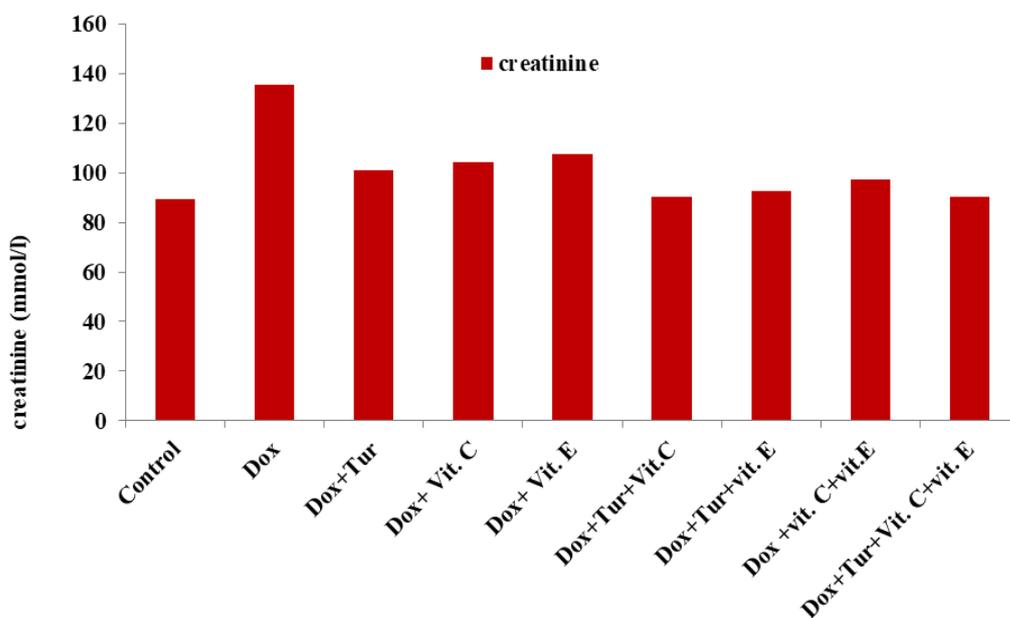


Figure 4 Effect of turmeric on creatinine (mmol/l) in doxorubicin-induced toxicity in Wistar Rats 28 days of drug treatment.

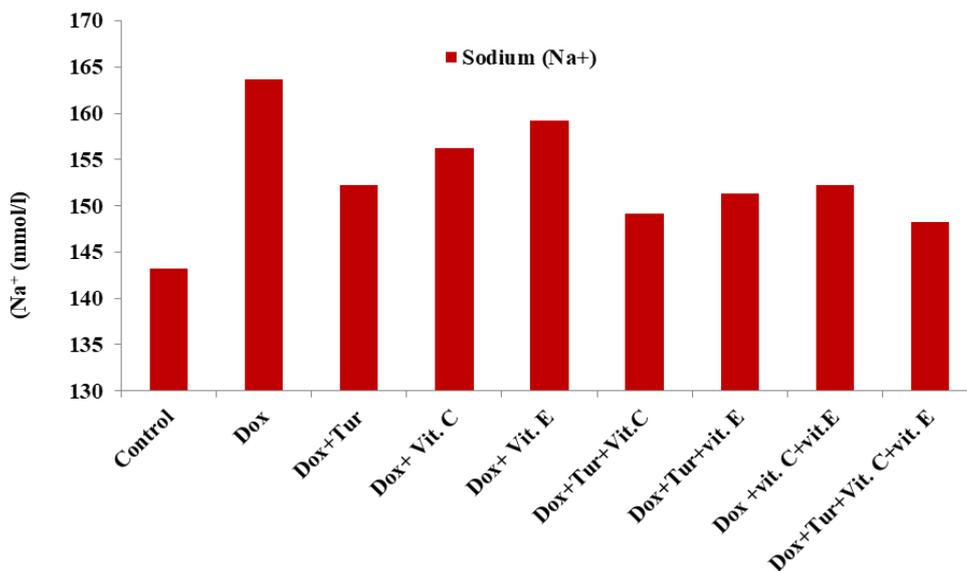


Figure 5 Effect of turmeric on sodium $[(Na^+)(mmol/l)]$ in doxorubicin-induced toxicity in Wistar Rats 14 days of drug treatment.

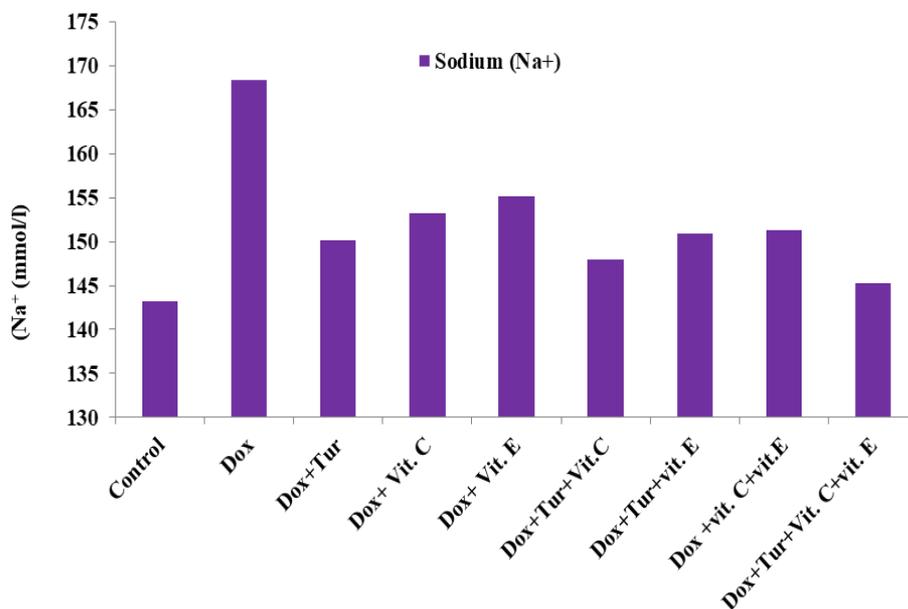


Figure 6 Effect of turmeric on sodium $[(Na^+)(mmol/l)]$ in doxorubicin-induced toxicity in Wistar Rats 28 days of drug treatment.

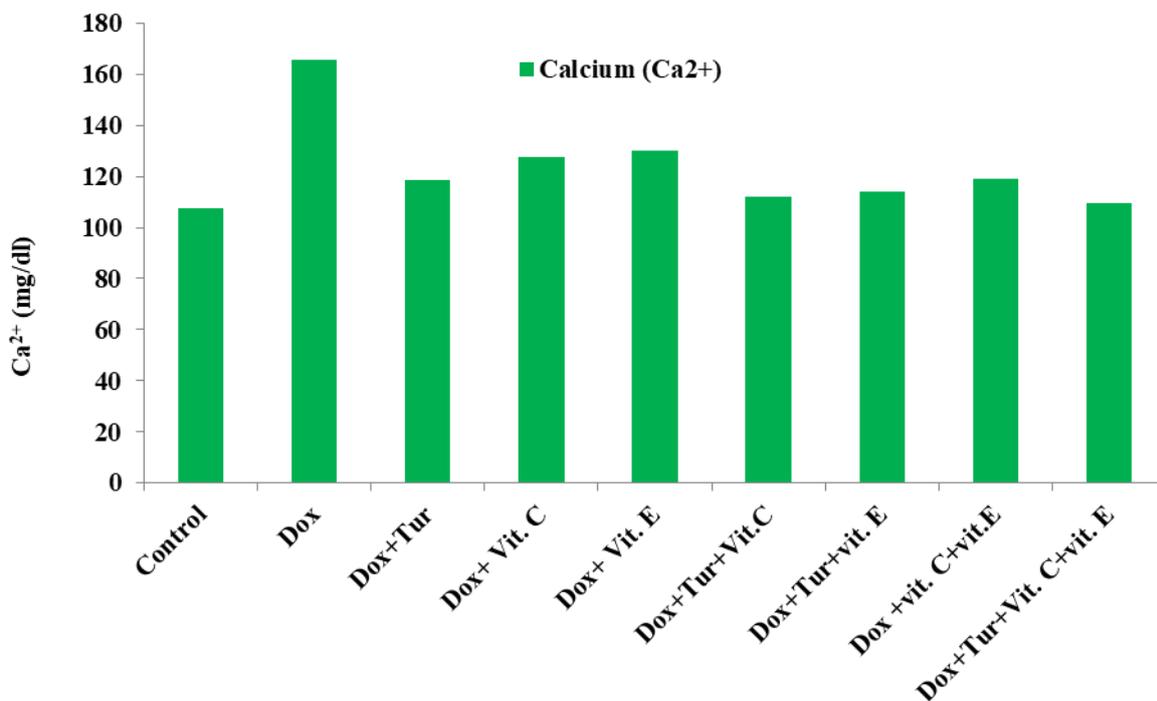


Figure 7 Effect of turmeric on calcium [(Ca²⁺)(mg/dl)] in doxorubicin-induced toxicity in Wistar Rats 14 days of drug treatment.

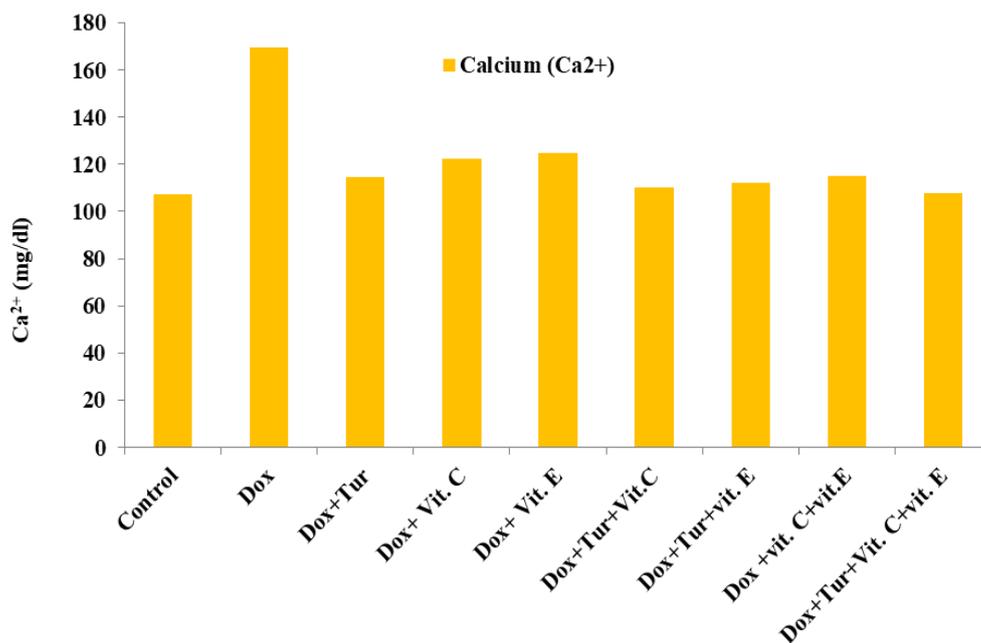


Figure 8 Effect of turmeric on calcium [(Ca²⁺)(mg/dl)] in doxorubicin-induced toxicity in Wistar Rats 28 days of drug treatment.

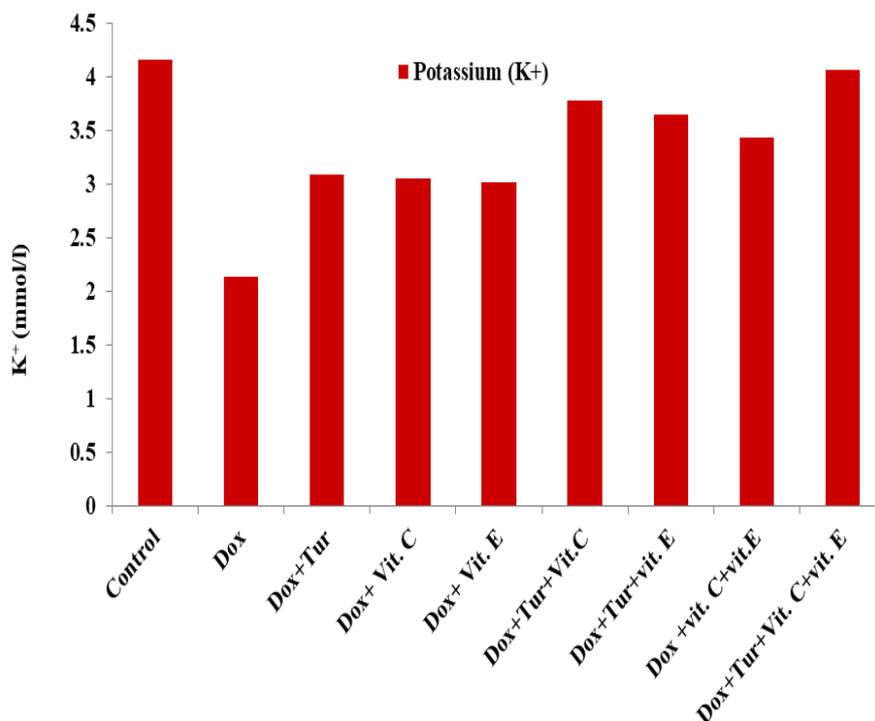


Figure 9 Effect of turmeric on potassium [(K⁺)(mmol/l)] in doxorubicin-induced toxicity in Wistar Rats 14 days of drug treatment.

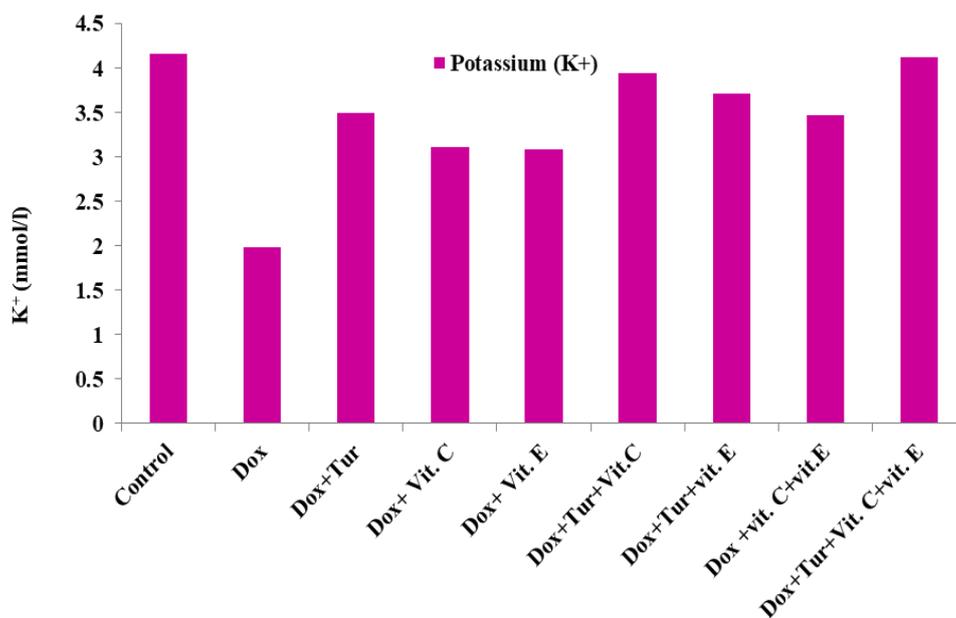


Figure 10 Effect of turmeric on potassium [(K⁺)(mmol/l)] in doxorubicin-induced toxicity in Wistar Rats 28 days of drug treatment.

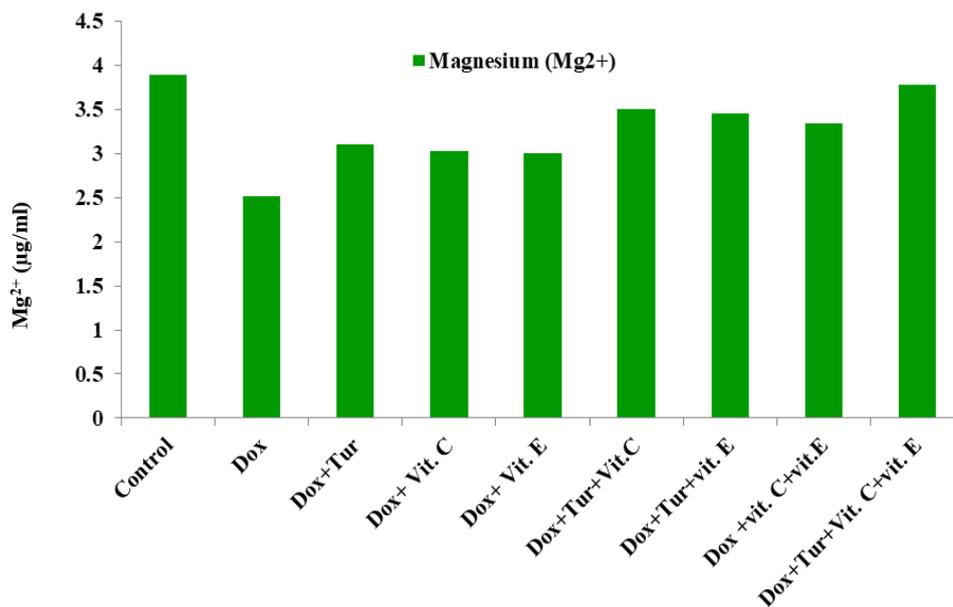


Figure 11 Effect of turmeric on Magnesium [(Mg²⁺)(ug/ml)] in doxorubicin-induced toxicity in Wistar Rats 14 days of drug treatment.

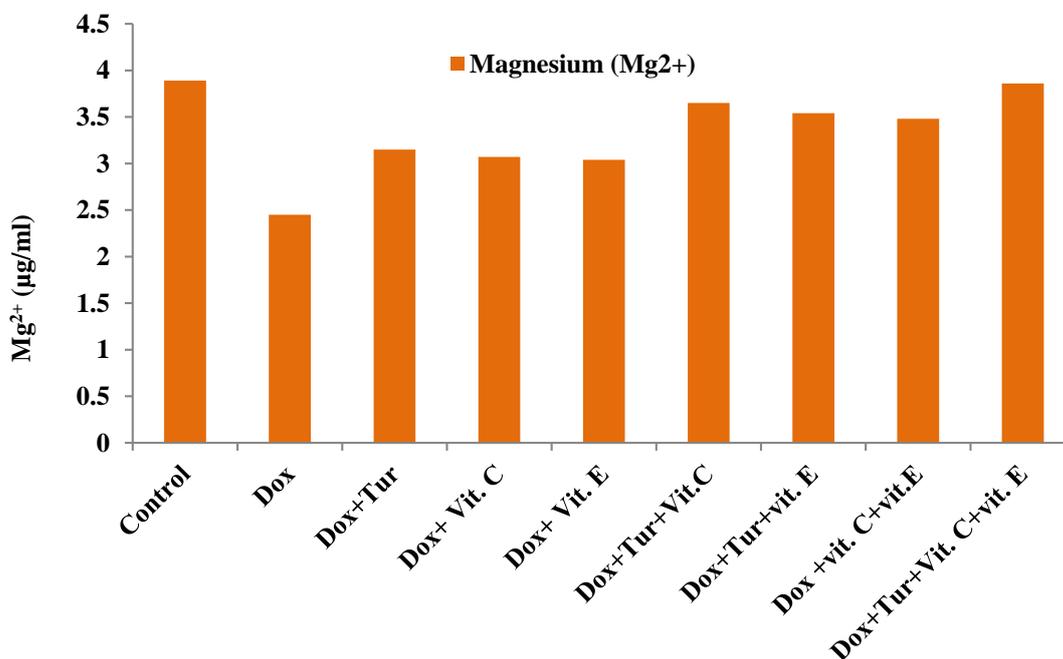


Figure 12 Effect of turmeric on Magnesium [(Mg²⁺) (µg/ml)] doxorubicin-induced toxicity in Wistar Rats 28 days of drug treatment

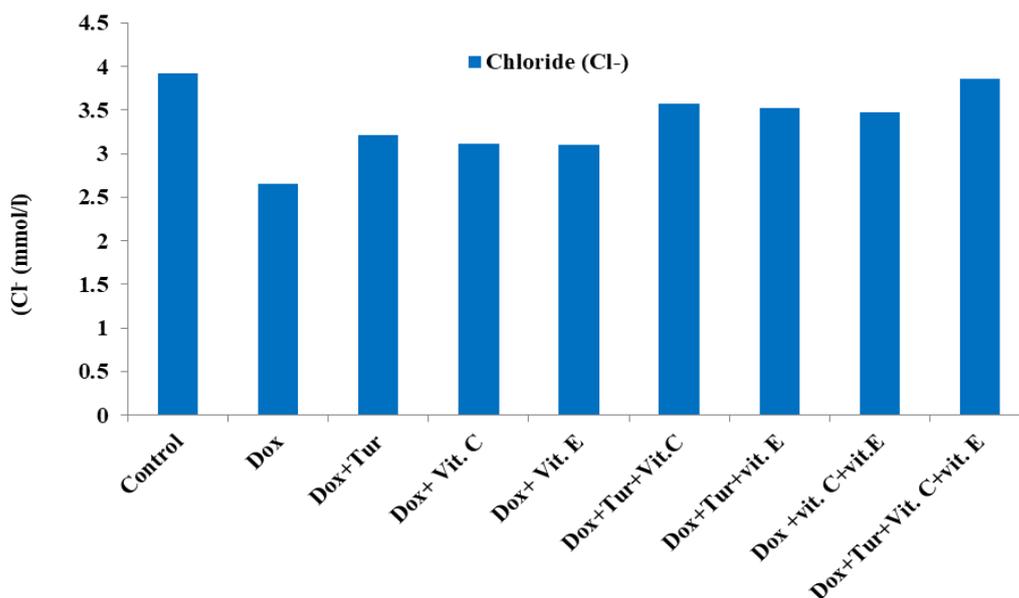


Figure 13 Effect of turmeric on Chloride [(Cl⁻)(mmol/l)] in doxorubicin-induced toxicity in Wistar Rats 14 days of drug treatment.

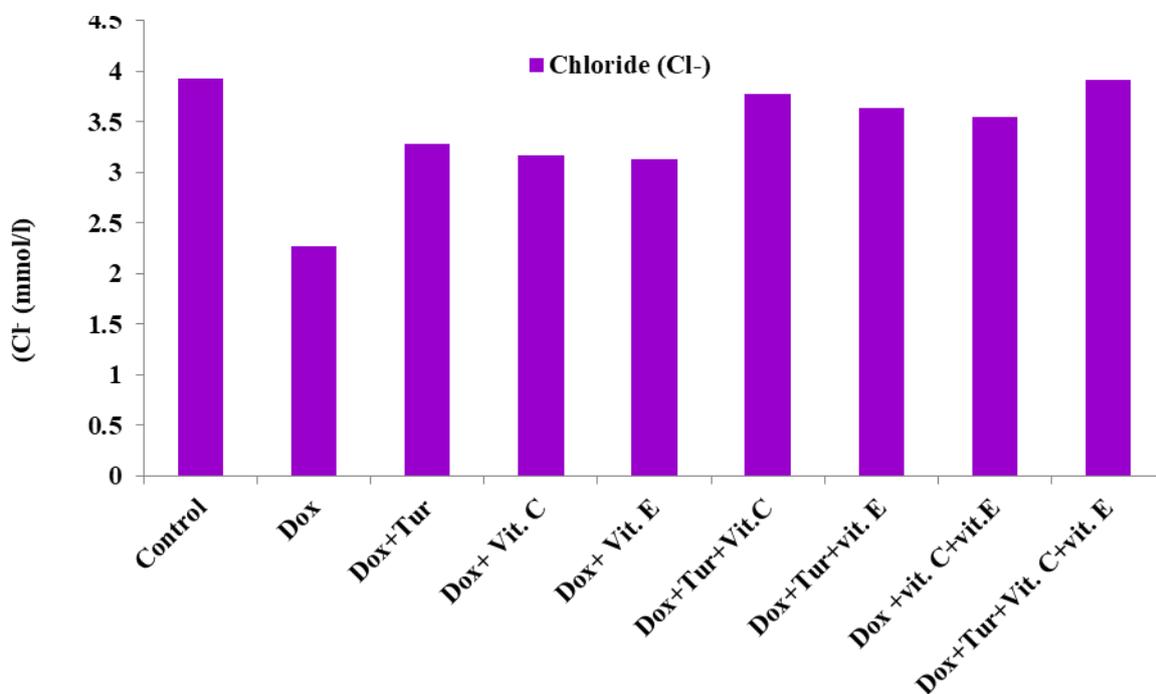


Figure 14 Effect of turmeric on Chloride [(Cl⁻)(mmol/l)] in doxorubicin-induced toxicity in Wistar Rats 28 days of drug treatment.

Figure 5 presents the effect of turmeric on sodium [(Na⁺) (mmol/l)] in doxorubicin-induced toxicity in Wistar rats after 14 days of simultaneous drug treatment, showing that doxorubicin (Dox) caused a significant increase ($p \leq 0.05$) in the serum level of sodium ion (163.61 ± 2.49) when compared to the control (143.20 ± 2.65). This increase was reversed towards normal by turmeric alone (152.20 ± 2.14) or in combination with vitamins C and E (148.24 ± 1.67). These observations were similar to those in figure 6 with sodium ion levels of (168.34 ± 1.58), (143.20 ± 2.65) and (150.20 ± 2.17) for doxorubicin, control and turmeric respectively, after 28 days of simultaneous drug treatment. Figure 7 presents the effect of turmeric on calcium [(Ca²⁺) (mg/dl)] in doxorubicin-induced toxicity in Wistar rats after 14 days of simultaneous drug treatment, showing that doxorubicin (Dox) caused a significant increase ($p \leq 0.05$) in the serum

level of calcium ion (165.92 ± 2.18) when compared to the control (107.41 ± 2.61). This increase was reversed towards normal by turmeric alone (118.38 ± 2.52) or in combination with vitamins C and E (109.33 ± 2.13). These observations were similar to those in figure 8 with serum calcium levels of (169.34 ± 2.35), (107.41 ± 2.61) and (114.57 ± 2.41) for doxorubicin, control and turmeric respectively, after 28 days of concomitant drug treatment. Figure 9 presents the effect of turmeric on potassium [(K⁺) (mmol/l)] in doxorubicin-induced toxicity in Wistar rats after 14 days of concomitant drug treatment, showing that doxorubicin (Dox) caused a significant decrease ($p \leq 0.05$) in the serum level of potassium ion (2.14 ± 0.57) when compared to the control (4.16 ± 0.81). This decrease was reversed towards normal by turmeric alone (3.09 ± 0.24) or in combination with vitamins C and E (4.07 ± 0.52). These observations were similar to those in figure 10 with serum potassium levels of (1.98 ± 0.42), (4.16 ± 0.81) and (3.49 ± 0.17) for doxorubicin, control and turmeric respectively, after 28 days of simultaneous drug treatment. Figure 11 presents the effect of turmeric on magnesium [(Mg²⁺) (µg/ml)] in doxorubicin-induced toxicity in Wistar rats after 14 days of concomitant drug treatment, showing that doxorubicin (Dox) caused a significant decrease ($p \leq 0.05$) in the serum level of magnesium (2.51 ± 0.18) when compared to the control (3.89 ± 0.26). This decrease was reversed towards normal by turmeric alone (3.11 ± 0.14) or in combination with vitamins C and E (3.78 ± 0.31). These observations were similar to those in figure 12 with serum magnesium levels of (2.45 ± 0.13), (3.89 ± 0.26) and (3.15 ± 0.41) for doxorubicin, control and turmeric respectively, after 28 days of simultaneous drug treatment. Figure 13 presents the effect of turmeric on chloride [(Cl⁻) (mmol/l)] in doxorubicin-induced toxicity in Wistar rats after 14 days of simultaneous drug treatment, showing that doxorubicin (Dox) caused a significant decrease ($p \leq 0.05$) in the serum chloride levels (2.65 ± 0.44) when compared to the control (3.92 ± 0.56). This decrease was reversed towards normal by turmeric alone (3.22 ± 0.15) or in combination with vitamins C and E (3.86 ± 0.13). These observations were similar to those in figure 14 with serum chloride levels of (2.27 ± 0.82), (3.92 ± 0.56) and (3.28 ± 0.12) for doxorubicin, control and turmeric, after 28 days of concomitant drug treatment.

4. Discussion

Administration of doxorubicin to Wistar rats for 14 and 28 days caused a significant increase in the serum levels of urea, creatinine, sodium (Na⁺), calcium (Ca²⁺), and a decrease serum level of Chloride (Cl⁻), magnesium (Mg²⁺) and potassium (K⁺). Plasma biomarkers such as creatinine and urea concentrations are usually monitored to evaluate glomerular function, because of their inverse relationship with the latter [34, 35, 36, ad 37], because, the biochemical and physiological alterations of kidney tissue could affect the extracellular space due to the intense relationship between the cell environment and the extracellular space [8]. The alteration in these kidney function parameters is in line with other studies. For instance Adamis et al., [7] found that doxorubicin toxicity disrupted kidney function which had a profound effect on total body metabolism. Also, it was reported that doxorubicin toxicity is characterized by increased plasma levels of creatinine, urea [13, 11], and uric acid, [11]. Manal et al., [9] equally reported nephrotoxicity that caused significant increases in serum markers of kidney damage, creatinine and urea in mice, while Ayla et al., [5] added that doxorubicin induced nephrotoxicity is associated with progressive glomerular damage associated with atrophic tubular lesion. In the same vein, Anil et al., [38], report a significant increase in sodium (Na⁺), calcium (Ca²⁺), and a decrease serum level of chloride (Cl⁻) and potassium (K⁺) by doxorubicin in dogs. Doxorubicin toxicity also induces inflammatory changes in kidney tissues [12] and doxorubicin-induced nephrotoxicity causes increased capillary porousness and glomerular shrinking [12]. Thus the elevated concentrations of renal calcium and sodium, as well as the lowered chloride, magnesium and potassium, induced by doxorubicin in this study, is reflective of compromised membranes of the renal tissues. The mechanism of doxorubicin nephrotoxicity was reported to be correlated with its cyto-toxicity, manifested by the destruction of cell membranes and other cellular components as a result of DNA intercalation [6]. This cyto-toxicity is assumed to be facilitated by oxidative stress induced by excess reactive oxygen species (ROS). ROS activity alters specific intracellular structures such as proteins, lipids and nuclear DNA [1]. Doxorubicin-induced oxidative stress in renal tissues is characterized by elevated malondialdehyde (a marker of lipid peroxidation) and lowered reduced glutathione levels [10, 11, 12], as well as lowered activities of catalase, [13] glutathione peroxidase and superoxide dismutase [14, 12]. However, a concomitant administration of turmeric alone or with vitamins C and or E along with doxorubicin prevented these doxorubicin-induced abnormal electrolyte imbalances. Therefore, the reduction in the plasma creatinine and urea levels, produced by the extracts, is suggestive of their capacity to protect the nephrons from doxorubicin-induced damage, [12] and thus preserve the functional capacity of the glomerular filtration apparatus. The subsequent improvement in ion transport then led to improved electrolyte balance, especially, offsetting doxorubicin-induced calcium overload. This may be the mechanism of nephroprotective activities of the extracts. The potential to prevent the doxorubicin-induced abnormal electrolyte imbalances, by the extract may be related with its anti-oxidant potential, owing to the role of increased oxidative stress in kidney damage. This is posited because the phytochemical in turmeric with the highest concentration (curcumin), has been reported to possess anti-inflammatory, immunomodulatory, and antiatherogenic activities as well as a potential to inhibit various reactive

oxygen-generating enzymes [18, 19]. It has also been reported to have a potent anti-oxidant and free radical scavenger capacity [25] and to inhibit lipid peroxidation [26]. Also in one study, it was reported to attenuate the arsenic, gentamicin, and acetaminophen-induced oxidative stress in rats [21, 22]. The evidence is even more illuminating with the report of Isirima and Christian [29], on the ability of turmeric extract to prevent the significant reduction in the serum concentration of SOD, GPx, CAT, GSH and TAS as well as the increase serum level of MDA, caused by doxorubicin. Thus turmeric presents a promising prospect as resources for prevention or management of doxorubicin-induced renal toxicity. This could be exploited in cancer chemotherapy after due clinical trials.

5. Conclusion

Doxorubicin caused a significant increase in the serum levels of urea, creatinine, sodium (Na⁺), calcium (Ca²⁺), and a decrease serum level of chloride (Cl⁻), magnesium (Mg²⁺) and potassium (K⁺) in Wistar rats. These abnormal electrolyte imbalances were prevented by administration of turmeric alone or in combination with vitamins C and or E along with doxorubicin concomitantly.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest in connection with this paper.

Statement of ethical approval

This study was duly approved by the ethical committee of the School of Science Laboratory Technology, University of Port Harcourt.

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