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Effects of turmeric on Liver function Parameters in Doxorubicin-induced oxidative stress in Wistar rats

Isirima, Joshua Charles* and Okoroafor, Dorcas Okayo

1. Isirima, Joshua Charles; Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Choba-Port Harcourt, 500272, Nigeria Email Address joshuasrm964@gmail.com, Phone number: 08076080566

2. Okoroafor, Dorcas Okayo; Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Choba-Port Harcourt, 500272, Nigeria

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ABSTRACT: Doxorubicin (DOX) is a well-known anticancer drug in treatment of various cancers including leukemias, lymphomas, soft-tissue sarcomas and solid tumors. Doxorubicin produces its toxicity in the liver by causing hepatic injury leading to an increase in permeability of the hepatocyte membrane and a subsequent leakage of transaminases into the blood thereby causing elevation of these in the circulation. This study investigated the effect of turmeric on liver function parameters 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 (DOX), group 3 animals were given DOX and turmeric, group 4 animals received DOX and vitamin C, group 5 animals received DOX and vitamin E, group 6 animals received DOX, vitamins C and turmeric, group 7 animals received DOX, vitamin E and turmeric, while group 8 animals received DOX, vitamin C and vitamin E and finally, group 9 animals receive DOX, vitamin C, vitamin E and turmeric. The experiment lasted for 28 days and blood samples were collected from each animal from the various groups for liver function assay. Doxorubicin caused a significant increase in the serum levels of aspartate aminotransferase, alanine aminotransferase, alanine aminotransferase, total and conjugate bilirubins and a significant decrease in the serum levels of albumin and total protein. These changes were prevented by turmeric alone or in combined concomitant administration with vitamins C and or E and doxorubicin.

Keywords: Turmeric, Liver function Parameters, Doxorubicin, oxidative stress, Wistar rats

I. INTRODUCTION

Doxorubicin is a product of Streptomyces peucetius var. Caesius. It is a prototype agent of anthracycline antibiotics (Blum and Carter 1974). It is one of the most effective antineoplastic drugs, frequently used against ovarian, breast, testicular, lung, thyroid 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 discouragements in 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). Doxorubicin has also been reported to cause hepatotoxic effects related to histo-morphological changes and liver function parameters during the cause of cancer chemotherapy, as well as in several animal studies. In a study to investigate the protective role of Phyllantus niruri extract in doxorubicin-induced myocardial toxicity in rats, it found that the level of enzymes like Alanine Amino Transferase (ALT) and Aspartate Aminotransferase (AST) increases; indicating Dox induced toxicity to liver and kidney (Thippeswamy et al., 2011). Also in another study by Anil et al., (2019), to investigate the effect of Doxorubicin on haematological and blood biochemical profile of health dogs, it was found that there was a significant decrease in the total plasma proteins, albumin and globulin, whereas there was a non-significant increase in ALT and AST whereas a significant increase in ALP (Anil 2019). Studies have attributed an increase in liver enzyme activity to be related to hepatic injury as indicated by Mohd et al. (2013), who stated that an increased liver enzyme activity is the basis, or which liver disease is often first suspected, as they reported that an injury to the hepatic cells causes a distortion of metabolic function. They also

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remarked that it is the liver test that reveals the integrity of the hepatocyte membrane, necrosis of hepatocytes or biliary epithelia, cholestasis or induction phenomenon. Another study reported that in the presence of liver disease or injury, the hepatocyte membrane becomes more permeable and some of the enzymes leak out into the blood circulation, thus resulting in moderate elevations of transaminases in the blood (Nwachukwu, et al., 2015). Studies attempt to attribute the toxicity of DOX in the liver to be related an increase in oxidative stress. The main pathogenic mechanism appears to involve the generation of reactive oxygen species (ROS) (Patil and Balaraman 2009 and Gamal 2012). Some experimental studies have shown that ROS are important agents for tissue damage (Malekirad et al., 2011). It has been demonstrated that oxygen radical-induced damage of lipids in membrane is the key factor for DOX-induced toxicity (Abdel-Wahab et al., 2003). In the liver, oxidative stress caused by increased ROS production can result from two different ways, the most common occurring when the semiquinone form of DOX reacts with O^2 producing O^{2*-} and H_2O_2, and an alternative way occur through NADPH oxidases, the principal extra-mitochondrial producers of ROS in hepatocytes (Kassner et al., 2008). NADPH-oxidases are present in small levels but their levels increase in response to extracellular stimulus, such as DOX treatment (Kassner et al., 2008). The literature shows that DOX-induced ROS production leads to an increase in lipid peroxidation and a reduction in the activities of SOD, CAT and glutathione peroxidase (GPx), as well as DNA damage, and a decrease in GSH levels, which confirm DOX hepatotoxicity (Odom et al., 1992; Ortiz et al., 2008 and Kalendera et al., 2005). Between the importance of DOX in cancer treatment and the increase in incidence of its toxicity, it has become increasingly essential to find pharmacological remedies with protective effects against DOX adverse effects,

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

II. METHODS

Animals

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

Sample collection

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

Extraction Method

The root of the plant was left to dry at room temperature between 32 – 35^o C after collection and cleaning until they attained a

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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. Blood samples were collected from each animal from the various groups for liver 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

Liver Function Test Analysis

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) determinations were carried out using Randox automated method and the methods of Reitman and Frankel (1957) and Schmidt and Schmidt (1963). On the other hand, alkaline phosphatase (ALP) determination was carried outusing Randox automated method and the methods of Klein and Babson (1960) and Babson, et al. (1966). In a similar manner, total protein (TP) determination was carried out using Randox automated method and the method of Tietz (1995), while albumin (ALB) and bilirubin (BIL) determinations were carried out using Randox automated method and the methods of Grant (1987) and Doumas, et al. (1971)

Statistical analysis

Mean values ± S. E. M. were calculated for each parameter. For the determination of significant differences, Means were compared using the oneway 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.

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III. RESULTS

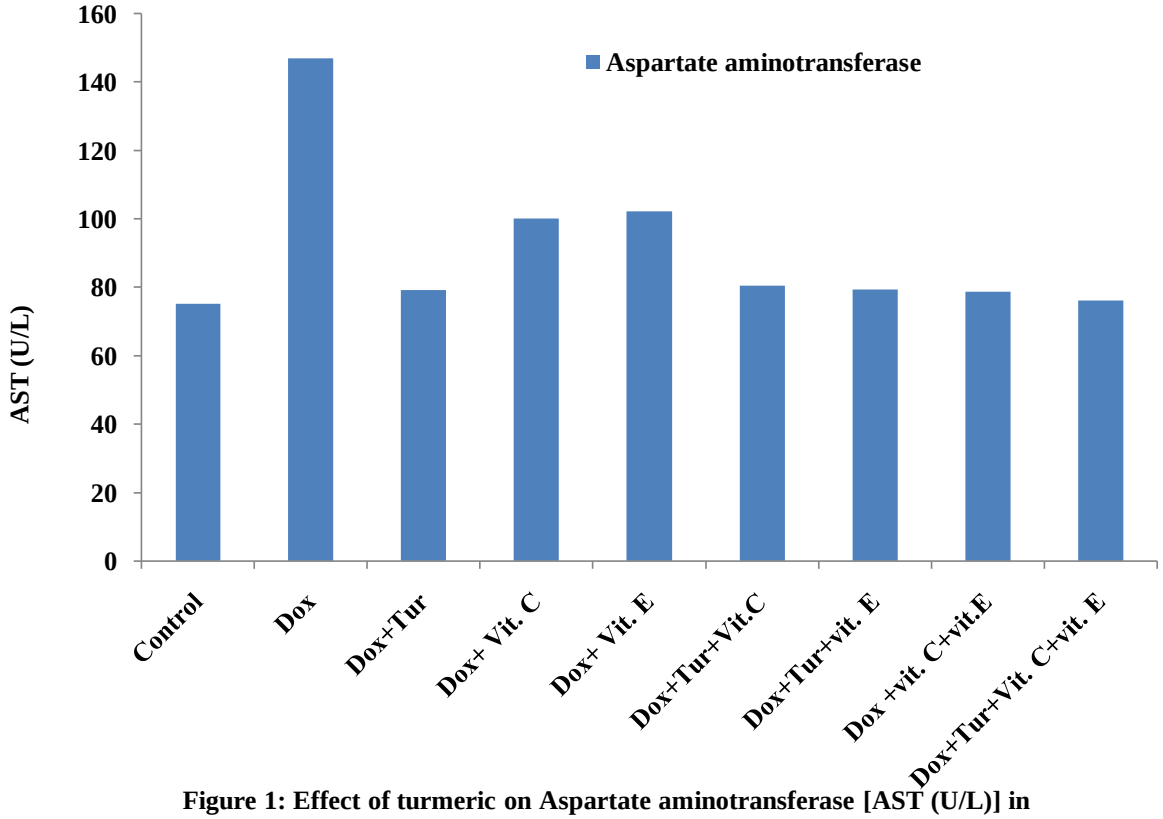

Figure 1: Effect of turmeric on Aspartate aminotransferase [AST (U/L)] in doxorubicin-induced toxicity in Wistar Rats after 14 days of administration

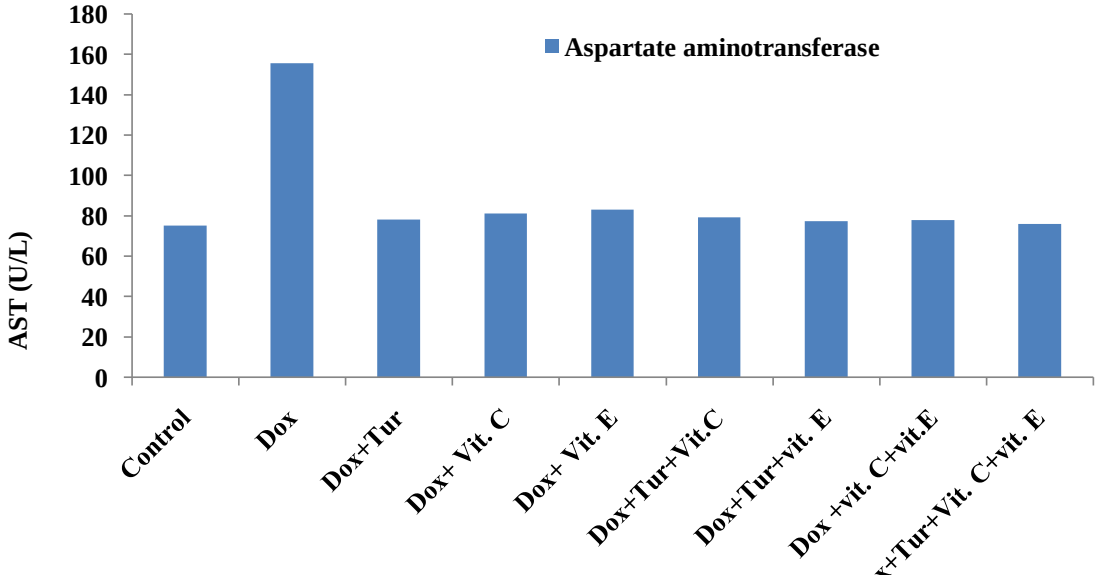

Figure 2: Effect of turmeric on Aspartate aminotransferase [AST (U/L)] in doxorubicin-induced toxicity in Wistar Rats after 28 days of administration

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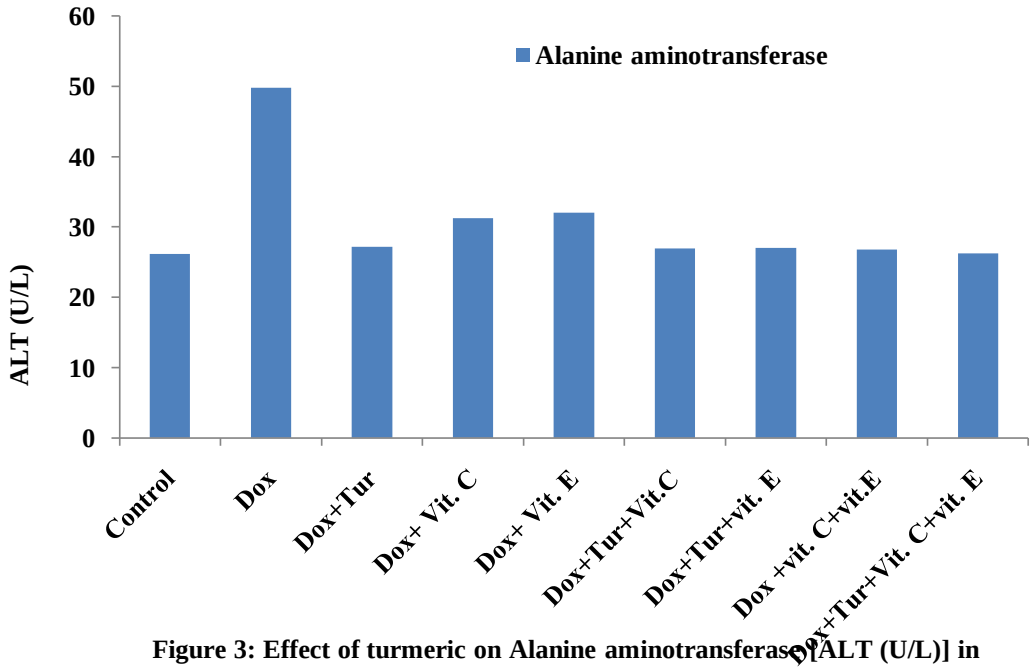

Figure 3: Effect of turmeric on Alanine aminotransferase [ALT (U/L)] in doxorubicin-induced toxicity in Wistar Rats after 14 days of administration

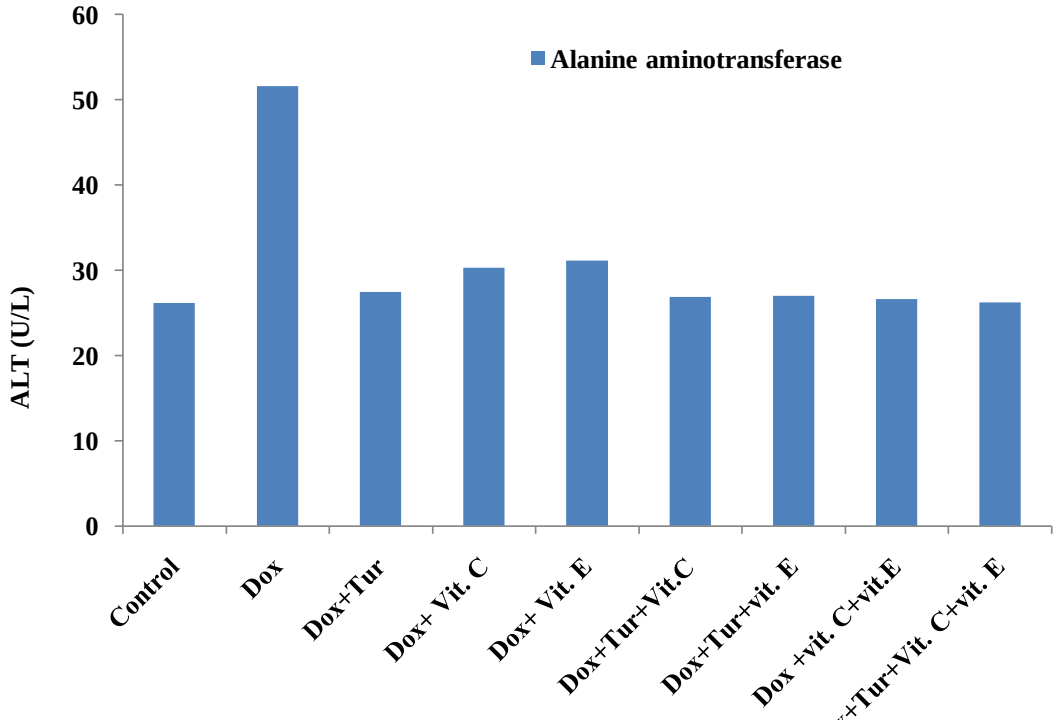

Figure 4: Effect of turmeric on Alanine aminotransferase [ALT (U/L)] in doxorubicin-induced toxicity in Wistar Rats after 28 days of administration

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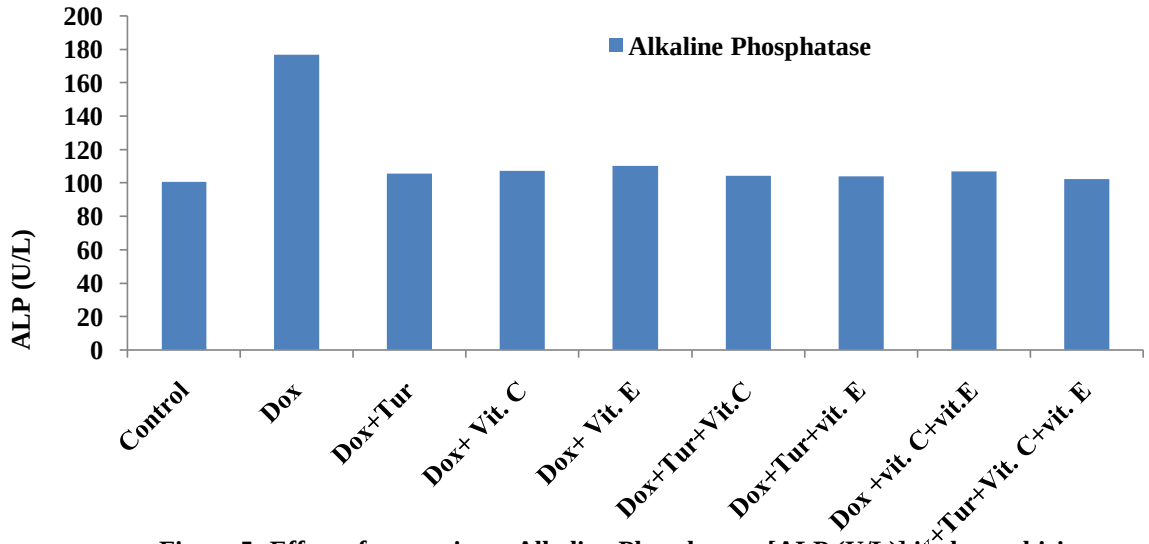

Figure 5: Effect of turmeric on Alkaline Phosphatase [ALP (U/L)] in doxorubicin-induced toxicity in Wistar Rats after 14 days of administration

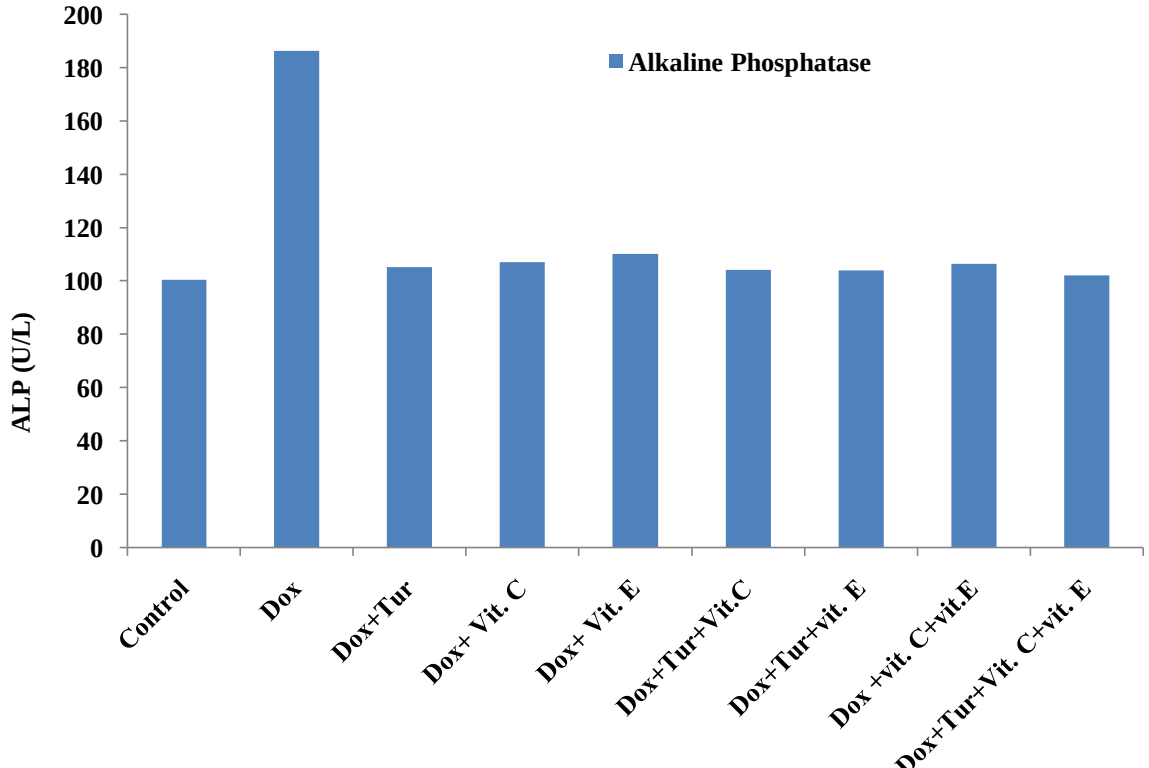

Figure 6: Effect of turmeric on Alkaline Phosphatase [ALP (U/L)] in doxorubicin-induced toxicity in Wistar Rats after 28 days of administration

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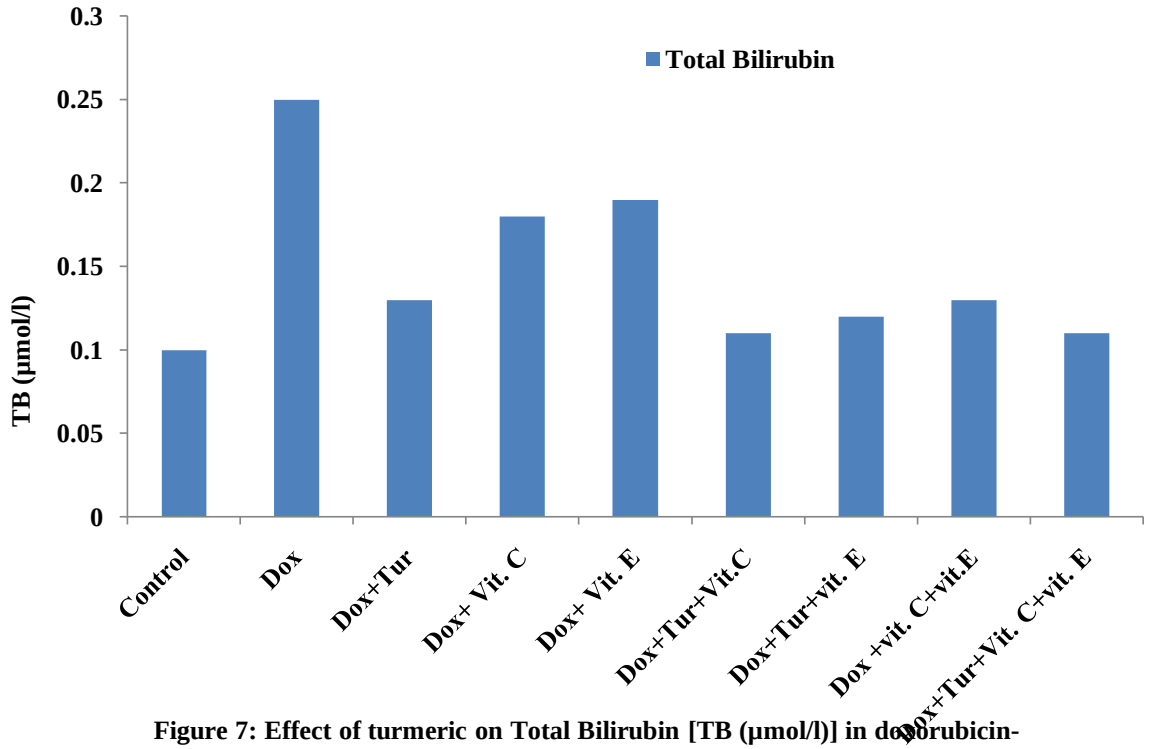

Figure 7: Effect of turmeric on Total Bilirubin [TB (µmol/l)] in doxorubicin-induced toxicity in Wistar Rats after 14 days of administration

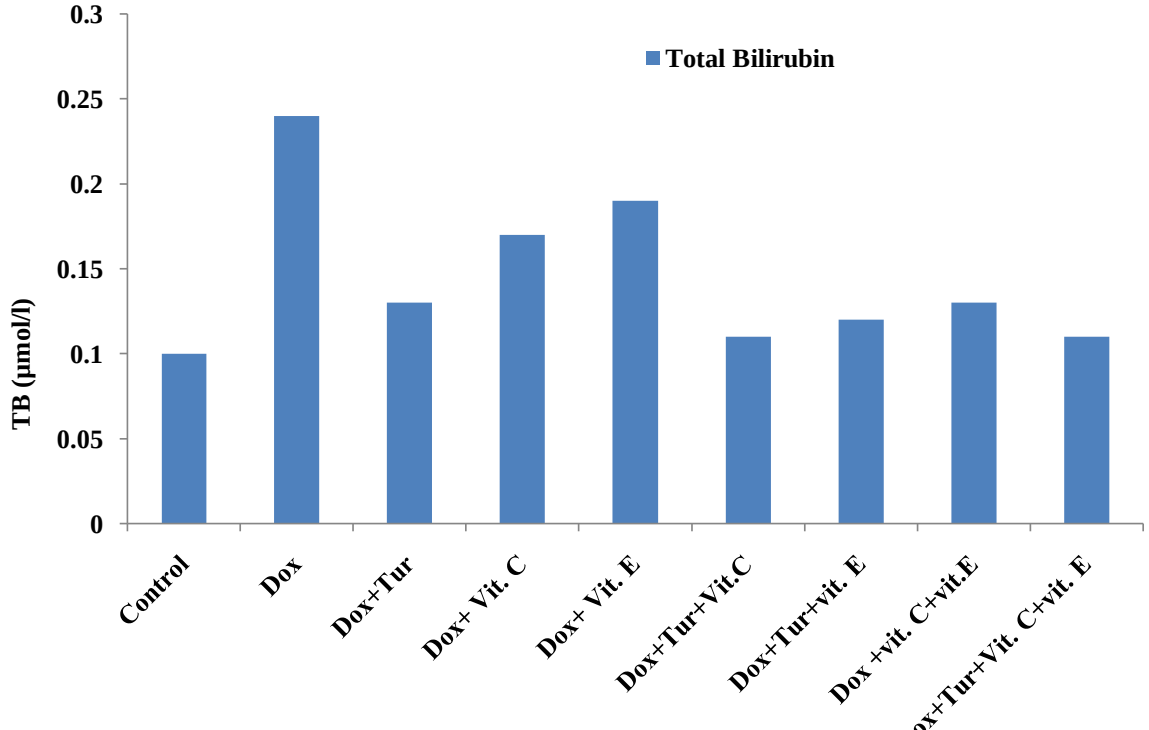

Figure 8: Effect of turmeric on Total Bilirubin [TB (µmol/l)] in doxorubicin-induced toxicity in Wistar Rats after 28 days of administration

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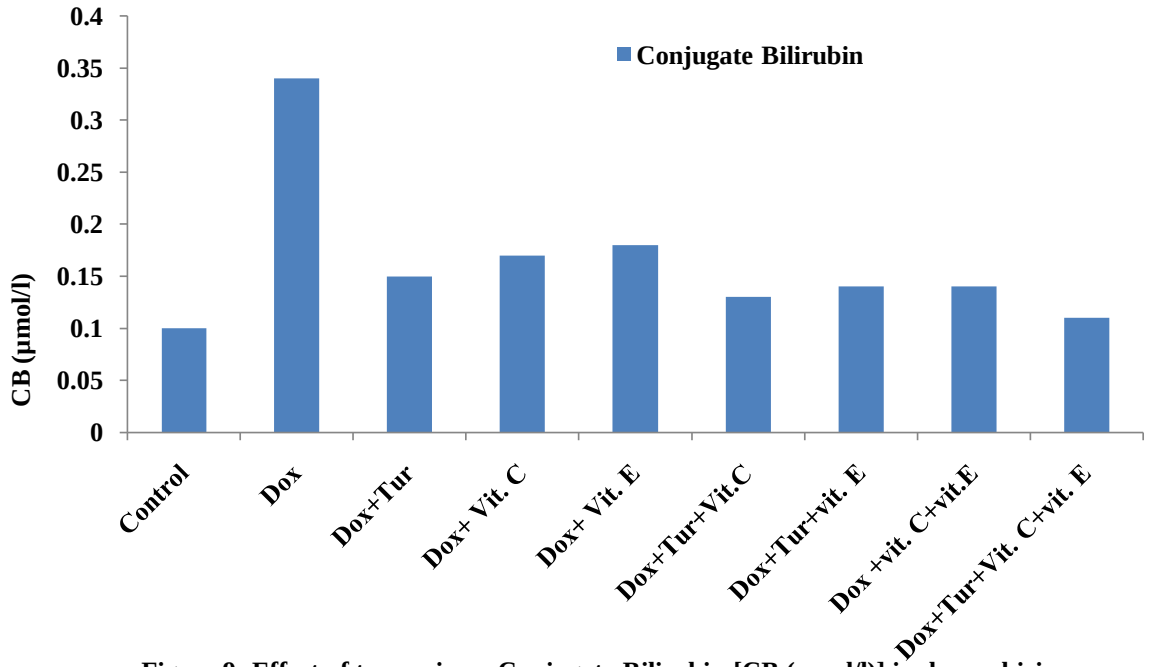

Figure 9: Effect of turmeric on Conjugate Bilirubin [CB (µmol/l)] in doxorubicin-induced toxicity in Wistar Rats 14 days of administration

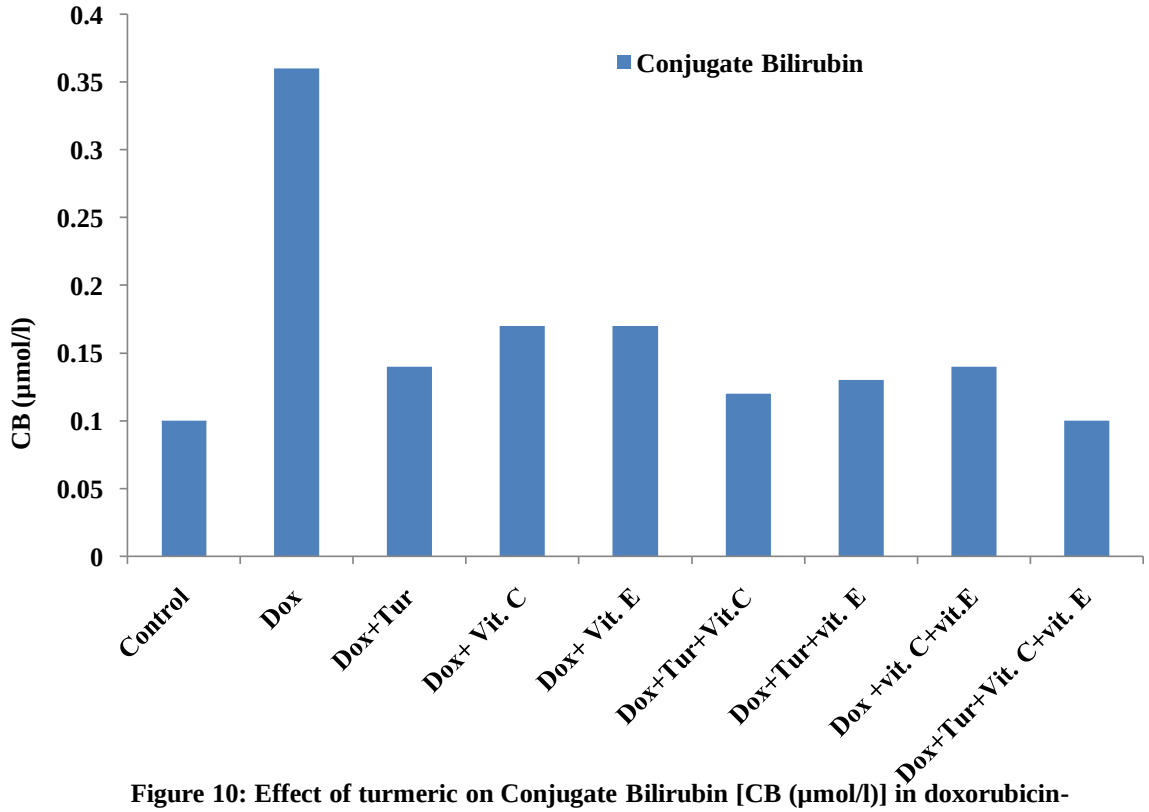

Figure 10: Effect of turmeric on Conjugate Bilirubin [CB (µmol/l)] in doxorubicin-induced toxicity in Wistar Rats 28 days of administration

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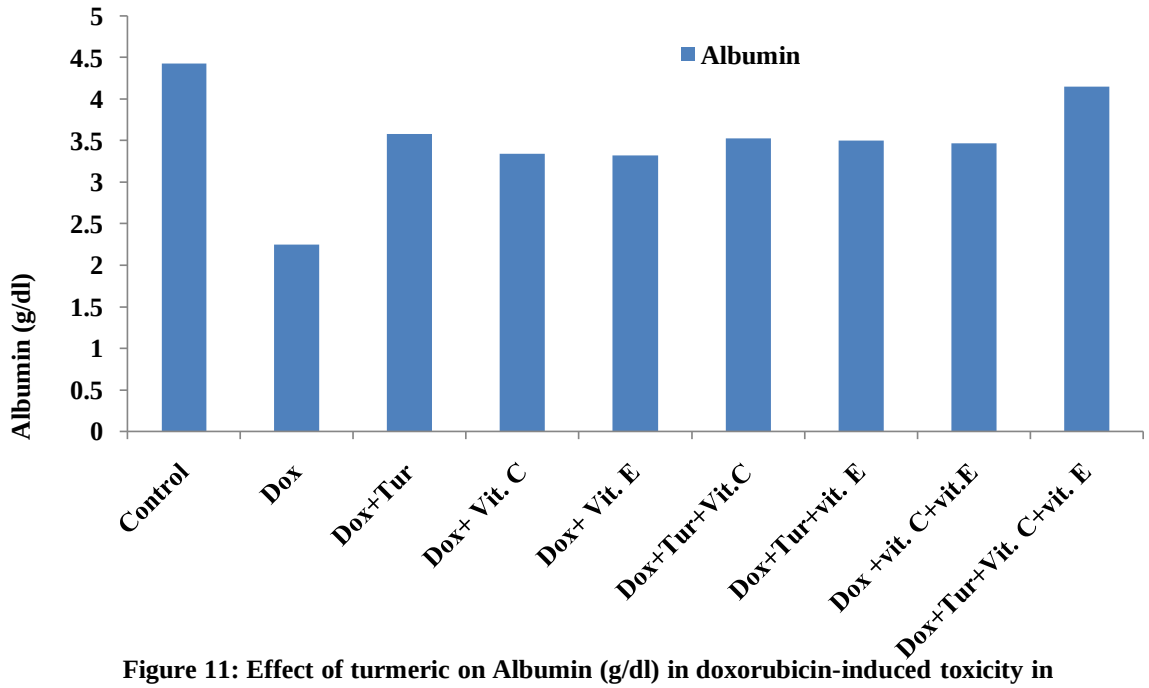

Figure 11: Effect of turmeric on Albumin (g/dl) in doxorubicin-induced toxicity in Wistar Rats 14 days of administration

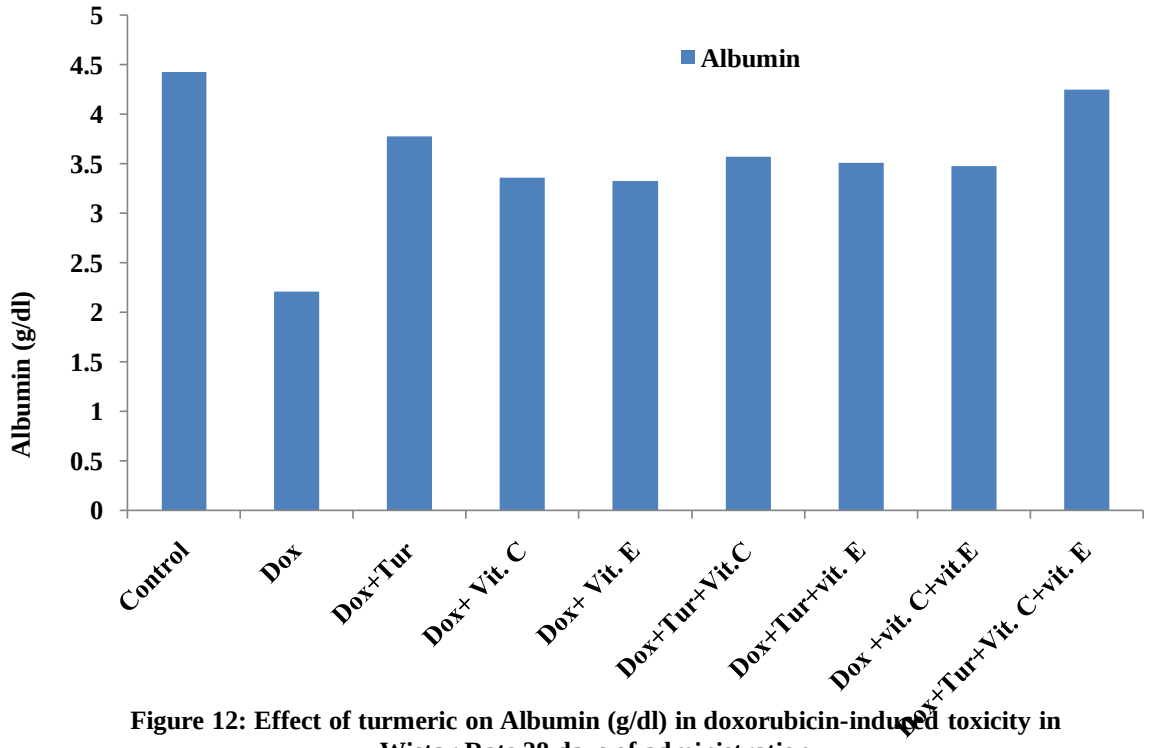

Figure 12: Effect of turmeric on Albumin (g/dl) in doxorubicin-induced toxicity in Wistar Rats 28 days of administration

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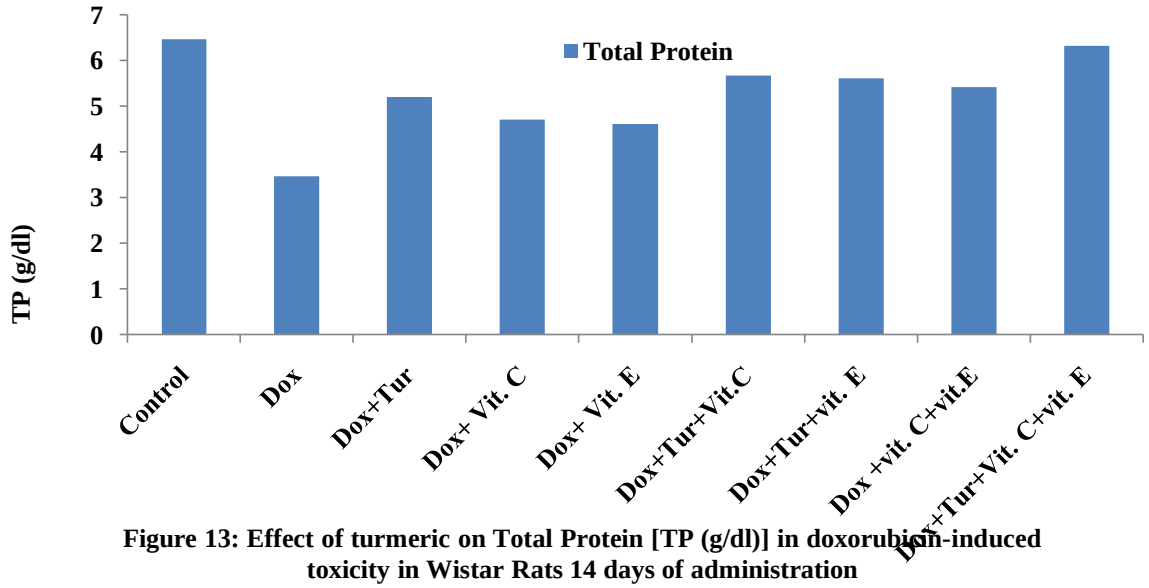

Figure 13: Effect of turmeric on Total Protein [TP (g/dl)] in doxorubicin-induced toxicity in Wistar Rats 14 days of administration

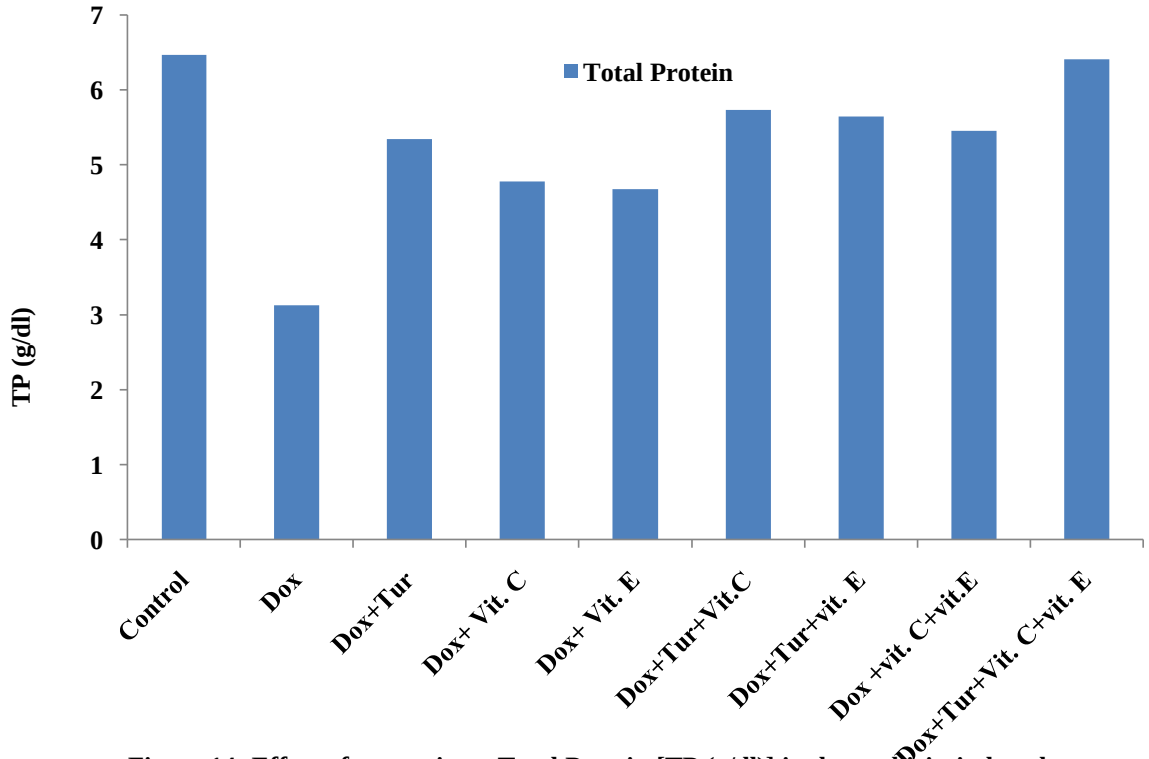

Figure 14: Effect of turmeric on Total Protein [TP (g/dl)] in doxorubicin-induced toxicity in Wistar Rats 28 days of administration

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Figure 1 presents the effect of turmeric on aspartate aminotransferase [AST (U/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 aspartate aminotransferase (147.04±3.62) when compared to the control (75.20±3.06). This increase was reversed towards normal by turmeric alone (79.24±2.12) or in combination with vitamins C (80.41±2.16) or/and E (79.38±1.04)/(76.11±0.88) respectively. These observations were similar to those in figure 2 with aspartate aminotransferase values of (155.67±7.14), (75.20±3.06) and (78.31±1.18) for doxorubicin, control and vitamin C respectively, after 28 days of concomitant drug treatment. Figure 3 presents the effect of turmeric on alanine aminotransferase [ALT (U/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 level of alanine aminotransferase (49.82±1.01) when compared to the control (26.16±0.57). This increase was reversed towards normal by turmeric alone (27.18±1.11) or in combination with vitamins C (26.98±1.17) or/and E (26.76±1.36)/(26.22±1.19) respectively. These observations were similar to those in figure 4 with alanine aminotransferase values of (51.61±1.34), (26.16±0.57) and (27.48±1.01) for doxorubicin, control and vitamin C respectively, after 28 days of simultaneous drug treatment. Figure 5 presents the effect of turmeric on alkaline phosphatase [ALP (U/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 alkaline phosphatase (177.01±11.13) when compared to the control (100.56±8.32). This increase was reversed towards normal by turmeric alone (105.46±6.26) or in combination with vitamins C (104.24±5.31) or/and E (104.12±5.28)/(102.27±4.32) respectively. These observations were similar to those in figure 6 with alkaline phosphatase values of (186.34±12.72), (100.56±8.32) and (105.22±7.41) for doxorubicin, control and vitamin C respectively, after 28 days of simultaneous drug treatment. Figure 7 presents the effect of turmeric on total bilirubin [TB (µmol/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 total bilirubin (0.25±0.14) when compared to the control (0.10±0.06). This increase was reversed towards normal by turmeric alone (0.13±0.11) or in combination with vitamins C (0.11±0.05) or/and E (0.12±0.03)/(0.11±0.02) respectively. These observations were similar to those in figure 8 with total bilirubin values of (0.26±0.19), (0.10±0.06) and (0.13±0.12) for doxorubicin, control and vitamin C respectively, after 28 days of concomitant drug treatment. Figure 9 presents the effect of turmeric on conjugate bilirubin [CB (µmol/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 level of conjugate bilirubin (0.34±0.18) when compared to the control (0.10±0.03). This increase was reversed towards normal by turmeric alone (0.15±0.14) or in combination with vitamins C (0.13±0.17) or/and E (0.14±0.03)/(0.11±0.02) respectively. These observations were similar to those in figure 10 with conjugate bilirubin values of (0.36±0.17), (0.10±0.03) and (0.14±0.29) for doxorubicin, control and vitamin C respectively, after 28 days of simultaneous drug treatment. Figure 11 presents the effect of turmeric on albumin (g/dl) 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 albumin (2.25±0.23) when compared to the control (4.43±0.56). This decrease was reversed towards normal by turmeric alone (3.58±0.21) or in combination with vitamins C (3.53±0.17) or/and E (3.50±0.24)/(4.15±0.14) respectively. These observations were similar to those in figure 12 with albumin values of (2.21±0.12), (4.43±0.56) and (3.78±0.25) for doxorubicin, control and vitamin C respectively, after 28 days of simultaneous drug treatment. Figure 13 presents the effect of turmeric on total protein [TP (g/dl)] 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 level of total protein (3.47±0.35) when compared to the control (6.47±0.72). This decrease was reversed towards normal by turmeric alone (5.21±0.17) or in combination with vitamins C (5.68±0.29) or/and E (5.62±0.14)/(6.32±0.41) respectively. These observations were similar to those in figure 14 with total protein values of (3.13±0.28), (6.47±0.72) and (5.35±0.24) for doxorubicin, control and vitamin C respectively, after 28 days of concomitant drug treatment.

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IV. DISCUSSION

Administration of doxorubicin to Wistar rats for 14 and 28 days caused a significant increase in the serum levels of aspartate aminotransferase, alanine aminotransferase, alanine aminotransferase, total and conjugate bilirubins and a significant decrease in the serum levels of albumin and total protein. These findings are in agreement with the observations in other studies (Thippeswamy et al., 2011 and Anil et al., 2019). On the contrary, a simultaneous administration of turmeric alone or with vitamins C and or E and doxorubicin prevented these abnormal changes in the liver function parameters. Studies have attributed an increase in liver enzyme activity to correlate with hepatic disease or injury (Mohd et al. (2013). It has been revealed that, in the presence of liver disease or injury, the hepatocyte membrane becomes more permeable and some of the enzymes leak out into the blood circulation, thus resulting in moderate elevations of transaminases in the blood (Nwachukwu, et al., 2015). Attempts have been made to attribute the toxicity of doxorubicin in the liver to be associated with an increase in oxidative stress (generation of reactive oxygen species (ROS) (Patil and Balaraman 2009 and Gamal 2012). Studies have shown that ROS are important agents for tissue damage (Malekirad et al., 2011). It has been demonstrated that oxygen radical-induced damage of lipids in membrane is the key factor for doxorubicin-induced toxicity (Abdel-Wahab et al., 2003). Research has shows that doxorubicin-induced ROS production leads to an increase in lipid peroxidation and a reduction in the activities of SOD, CAT and glutathione peroxidase (GPx), as well as cause DNA damage, and a decrease in GSH levels, which confirm doxorubicin hepatotoxicity (Odom et al., 1992; Ortiz et al., 2008 and Kalendera et al., 2005). The observations on turmerics' hepatoprotective potentials against doxorubicin induced liver toxicity in this study, is in agreement with several other research findings. It was reported that turmeric contains curcuminoids which consist of three major components including curcumin (77 %), demethoxycurcumin (17 %) and bisdemethoxycurcumin (3 %) (Aggarwal et al.,2003). On the other hand, curcumin with the highest content as reported above has been reported to possess anti-oxidant potentials. Curcumin possesses anti-inflammatory, immunomodulatory, and antiatherogenic activities and is a potent inhibitor of various reactive oxygen-generating enzymes, a potent anti-oxidant and scavenger of reactive oxygen species including superoxide anion radicals and hydroxyl radicals (Ara´ujo et al., 2001; Fujisawa et al., 2004 and Chainani-Wu 2003). Curcumin was also reported to inhibit erythrocyte lipid peroxidation (Borra et al., 2013). Curcumin found to protect against diazinon-induced toxicity in blood, liver, and erythrocyte of male Wistar rats (Messarah et al., 2013). It inhibits lipid peroxidation (Sreejayan-Rao 1994) and also inhibits Nitric Oxide Synthase (NOS) over-expression (Spinas 1999 and Pan et al., 2000). Also, Isirima and Christian (2021), had reported the anti-oxidant potentials of turmeric. In their study, they found that turmeric demonstrated anti-oxidant properties 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. Thus turmeric presents a promising compliment for doxorubicin combine therapy which could be exploited in cancer chemotherapy after due clinical trials.

V. CONCLUSION

Doxorubicin caused a significant increase in the serum levels of aspartate aminotransferase, alanine aminotransferase, alanine aminotransferase, total and conjugate bilirubins and a significant decrease in the serum levels of albumin and total protein in Wistar rats. These changes were prevented by turmeric alone or in combined concomitant administration with vitamins C and or E and doxorubicin.

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