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Evaluation of Anti-Oxidants Potential of Turmeric, Vitamins C and E in Doxorubicin-Induced Oxidative Stress in Wistar Rats

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Abstract

Doxorubicin (DOX) is a well-known anticancer drug in treatment of various cancers such as leukemias, lymphomas, soft-tissue sarcomas and solid tumors. Doxorubicin produces its toxicity due to generation of reactive oxygen species (ROS), production of superoxide anion and hydroxyl radical. This study investigated the anti-oxidant potentials of turmeric root extract in Doxorubicin induced toxicity in the presence of vitamins C and E in Wistar rats. In this study, 54 adult Wistar rats were divided into 9 groups of six animals each. Group 1 animals served as control (normal saline), group 2 animals served as negative control, and received Doxorubicin (DOX), group 3 animals were given DOX and turmeric, group 4 animals received DOX and vitamin C, group 5 animals received DOX and vitamin E, group 6 animals received DOX, vitamins C and turmeric, group 7 animals received DOX, vitamin E and turmeric, while group 8 animals received DOX, vitamin C and vitamin E and finally, group 9 animals receive DOX, 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 oxidative stress analysis. 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. All these are reversed on administration of turmeric root extract, vitamins C and E individually and collectively.

Keywords: Anti-oxidants, turmeric, vitamins C and E, Doxorubicin, Oxidative stress

Introduction

The anthracycline antibiotic adriamycin, or doxorubicin (DOX), is a well-known anticancer drug in treatment of various tumors. Doxorubicin was firstly used clinically in cancer therapy in the late 1960s. It is considered as one of the most potent antitumor anthracyclines. DOX could be administered alone or with other chemotherapeutic agents in the treatment protocols of many types of cancers such as leukemias, lymphomas, soft-tissue sarcomas and solid tumors. Doxorubicin's among many adverse effects is male infertility (Zanetti et al., 2007). Although a number of possible toxic mechanisms have been recognized following exposure to DOX, 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 addition, DOX metabolism produces superoxide anion and hydroxyl radical which lead to toxic manifestation in the cellular membrane of the normal cells. Also, it has been reported that this toxicity is mediated through cardiac tissues inflammation (Abushouk 2017). Between the importance of DOX in cancer treatment and the increase of in incidence of its toxicity, it has become increasingly important to find pharmacological remedies with protective effects against these adverse effects.

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Turmeric is a golden spice derived from the rhizome of the *Curcuma longa* plant, which belongs to the Zingiberaceae family (Gupta et al., 2013). Since ancient times, turmeric has been used as the principal ingredient of dishes originating from Bangladesh and India for its color, flavor, and taste. It is also used in social and religious ceremonies in Ayurvedic and folk medicines against various ailments, including gastric, hepatic, gynecological, and infectious diseases (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. Calebin-A, vanillic acid, vanillin, quercetin, and other phenolic compounds have also previously been identified from turmeric (Miean and Mohamed 2001 and Gupta et al., 2013). Curcumin possesses anti-inflammatory, immunomodulatory, and antiatherogenic activities and is a potent inhibitor of various reactive oxygen-generating enzymes (Ara´ujo et al., 2001 and Chainani-Wu 2003). It has been used in indigenous herbal medicine for the treatment of inflammatory and liver disorders. Antioxidative properties of curcumin are well documented. Curcumin is a potent scavenger of reactive oxygen species including superoxide anion radicals and hydroxyl radicals. It has also been reported to inhibit erythrocyte lipid peroxidation (Borra et al., 2013). Curcumin administration attenuated the arsenic, gentamicin, and acetaminophen-induced oxidative stress in rats (El-Demerdash et al., 2009 and Cekmen et al., 2009). Curcumin also prevented free radical formation-induced myocardial ischemia and paraquat induced lung injury in rats (Manikandan et al., 2004). Additionally, curcumin protected against diazinon-induced toxicity in blood, liver, and erythrocyte of male Wistar rats (Messarah et al., 2013). 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).

Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA, or oxidative stress is defined as a "state harmful to the body, which arises when oxidative reactions exceed antioxidant reactions because the balance between them has been lost." However, oxidative stress also has a useful role in physiologic adaptation and in the regulation of intracellular signal transduction. Therefore, a more useful definition of oxidative stress may be a "state where oxidative forces exceed the antioxidant systems due to loss of the balance between them" (Toshikazu and Yuji 2002). Oxidative stress develops from an imbalance between free radical productions, often increased through dysfunctional mitochondria, and reduced antioxidant defences (Mohora et al., 2007 and Small et al., 2012). The anti-oxidant enzymes and parameters usually evaluated during oxidative stress study includes among others; Superoxide dismutases (SOD), Reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GP_X) and Malondialdehyde (MDA) etc. Superoxide dismutases play a central role in the metabolism of reactive oxygen species. They greatly accelerate the dismutation of superoxide radicals. The two major forms in humans are copper/zinc and manganese superoxide dismutase. All glutathione peroxidises reduce hydrogen peroxide and alkyl hydroperoxides. The catalase activity is usually determined by its catalytic function (Chance 1947). In fact, it dismutates hydrogen peroxide in water and oxygen. Glutathione is a tripeptide (L-g-glutamyl-L-cysteinylglycine) that serves several essential functions within the cell. The role of intracellular glutathione in the detoxification of xenobiotics and reactive oxygen species has been well established. Furthermore, it is a coenzyme for various enzymes such as glutathione peroxidase, which plays an essential protective role against oxidative stress. The protective mechanism, results in an increased formation of intracellular glutathione disulphide, (oxidized form) (DeLeve and Kaplowitz 1001).

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Methods

Animals

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

Sample Collection

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

Extraction Method

The root of the plant was left to dry at room temperature between 32 – 35^{o} C after collection and cleaning until they attained a constant weight. The extraction method that was used was adopted from Hanan et al, (2013) which is the cold maceration extraction protocol, with minute adjustments. The powdered turmeric root bark of about 50g was soaked in 70% ethanol of about 1000ml in a 2 litre flask and mixed forcefully at 1hr intermission, for 12 hrs and allowed to settle over-night (35^{o}C) to allow for adequate extraction. Subsequently, the concoction was filtered by means of a filter paper with pore size of 0.45milli-pore. The concentration of the extract was increase using rotary evaporation process at 40^{o}C and 200 rpm. The final semi-solid extract was obtained by drying the content of the rotary evaporator over a steam bath at 40^{o}C. The resultant extract obtained 23% yield, was kept safe at room temperature in desiccators, until it was needed for the study.

Experimental Design

54 adult Wistar rats were divided into nine groups of six animals each. Group 1 animals served as control (normal saline 0.2ml), group 2 animals served as negative control, and received Doxorubicin (DOX), group 3 animals were given DOX and turmeric, group 4 animals received DOX and vitamin C, group 5 animals received DOX and vitamin E, group 6 animals received DOX, vitamins C and turmeric, group 7 animals received DOX, vitamin E and turmeric, while group 8 animals received DOX, vitamin C and vitamin E and finally, group 9 animals receive DOX, vitamin C, vitamin E and turmeric. The animals were administered the following doses of the drugs and extract; vitamin C was given at a dose of 90mg/70kg/day, Vitamin E was give at a dose of 22.4 IU /70kg/day, DOX was administered at a dose of 10-20mg/m^{2} once a week, while turmeric was administered at a dose of 500mg/kg/day. The sequence of administration of these drugs as describe above continued for a period of 28 days, but the animals were sacrificed under diethyl ether anesthesia, on day 14 and day 28th. Blood samples were collected from each animal from the various groups for oxidative stress 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

Measurement of serum antioxidants activities and oxidative stress

Plasma levels of total antioxidant status (TAS) were determined using DPPH Method (1, 1 diphenyl 2, picryl hydrazyl) (Bondet et al., 1997), Lipid peroxidation was determined by measuring the thiobarbituric acid

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reactive substances (TBARS) produced during lipid peroxidation (Ohkawa ET AL., 1979), GSH was measured by the method of Beutler et al. (1963), Plasma activities of superoxide dismutase (SOD) were determined using the method of Misra and Fridovich, (Misra Fridovich 1972) and measured at 480nm. Glutathione peroxidase (GPX) was assayed by the method proposed by Reddy et al. (1995). In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts H_2O_2 to H_2O and O_2. The coloured product formed was measured colorimetrically at 430nm. Catalase (CAT) was determined using direct colorimetric method of Sinha (1971). The method is based on the fact that dichromate is reduced to chromic acetate when heated in the presence of H_2O_2. The chromic acetate produced was measured colorimetrically at 570nm.

Statistical Analysis

The results are presented as Mean ± Standard error of mean. Differences between means were assessed using Analysis of variance (ANOVA) and post test using LSD multiple comparison test (Mead, & Curnow, 1982).

Results

The result of Superoxide dismutase (SOD) is presented in figure 1. There was a significant reduction ($p \leq 0.05$) of SOD level caused by DOX after 14 and 28 days of its administration. These were reversed towards normal on the 28th day by the turmeric root extract, as well as the combinations of turmeric, Vitamin C and Vitamin E. Also turmeric and the combination of the two vitamins reversed the reduction towards normal on day 14. A similar effect was observed for the combination of Vitamins C and E. Figure 2 shows the result of the effect of turmeric root extract, Vitamins C and E on Malondialdehyde (MDA) in Doxorubicin induce-toxicity. It was observed that administration of DOX for 14 and 28 days caused a significant increase (≤ 0.05) in the level of MDA. These were reversed towards normal by turmeric on the 28th day. Similar results were observed with the combination of turmeric and vitamins C and E. This reversal was more pronounced with the combination of turmeric, vitamin C and vitamin E on both the 14th and 28th days. Administration of DOX for 14 and 28 days each caused a significant reduction (≤ 0.05) in the serum level of Glutathione peroxidase (GPx) as presented in figure 3. These reductions were raised back towards normal by the turmeric root extract on days 14 and 28, but were full reversed to normal level on day 28 by turmeric, vitamin C and vitamin E combination. The individual combination of turmeric and the vitamins raised back the GPx concentration towards normal but were not as effective as the combination of the three. Doxorubicin administration to the animals caused a significant reduction (≤ 0.05) in the serum level of Catalase (CAT), but administration of the turmeric root extract as well as vitamins C and E antagonised this effect, although the antagonistic effect was more noticeable with the turmeric, and vitamins C and E combination on the both the 14th and 28th day. These findings are presented in figure 4. Figure 5 presents the result of the effect of turmeric root extract, Vitamins C and E on Glutathione (GSH) in Doxorubicin induce-toxicity. It was observed that administration of DOX to the animals caused a significant decrease (≤ 0.05) in the serum level of Glutathione (GSH). On the contrary, administration of the turmeric root extract to the animals resulted in a reversal of action, leading to an increase in GSH values. This increase was even more noticeable when turmeric was combined with vitamins C and E. The highest effect on the GSH was on day 28, with the group of turmeric and the two vitamins C and E combiation. The serum total antioxidant status (TAS), was significantly reduced on administration of DOX to the animals. On the other hand, administration of turmeric root extract and its combination with the individual vitamins caused a reversal of this decreasing trend towards normal levels. It was revealed from the results that the effect of turmeric in combination with the two vitamins was more potent on the 28th day. These findings are presented in figure 6. The result also revealed a time-dependent pattern, showing more effect as the time increased; this was observed in all the oxidative stress parameters studied.

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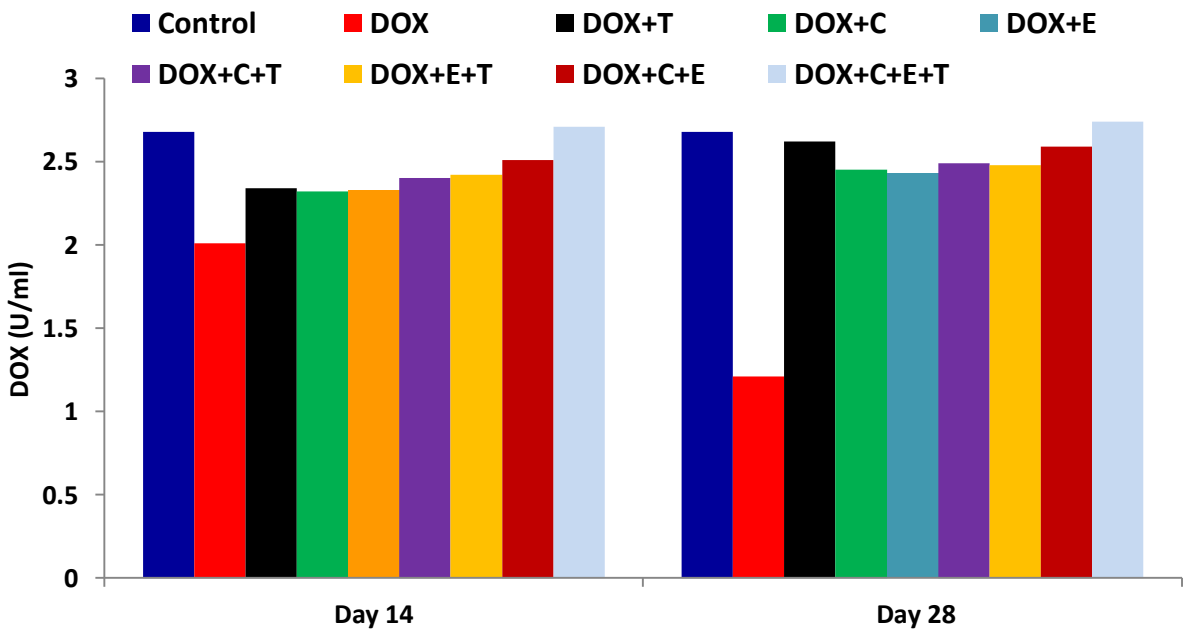

Figure 1: Effect of turmeric root extract, Vitamins C and E on Superoxide dismutase (SOD) in Doxorubicin induce-toxicity

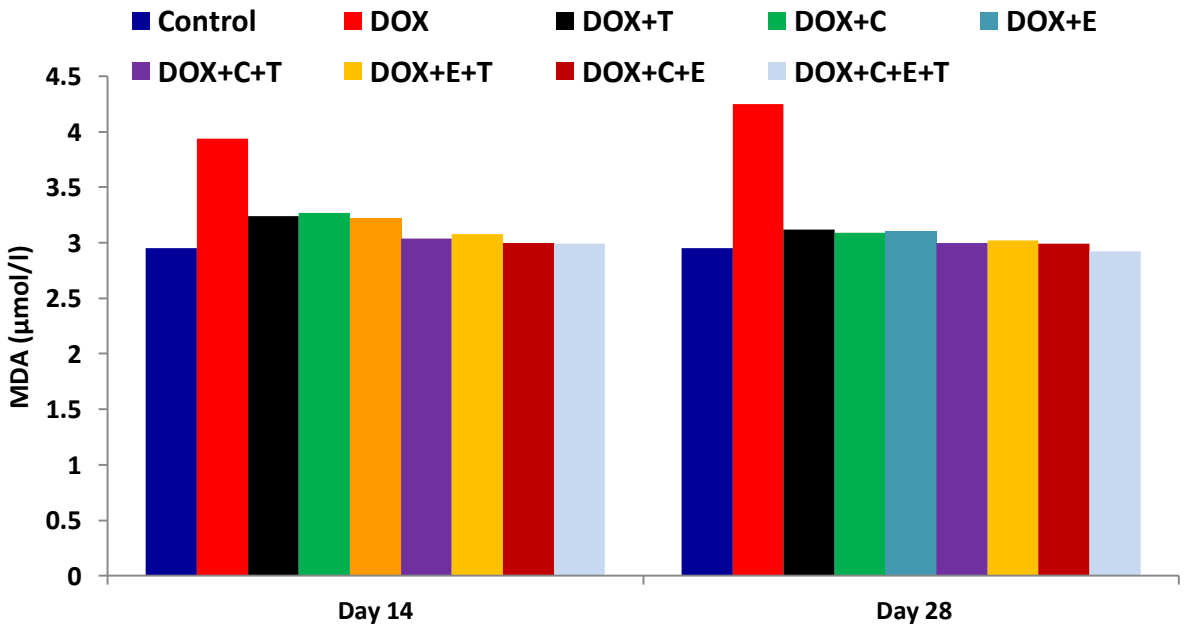

Figure 2: Effect of turmeric root extract, Vitamins C and E on Malondialdehyde (MDA) in Doxorubicin induce-toxicity

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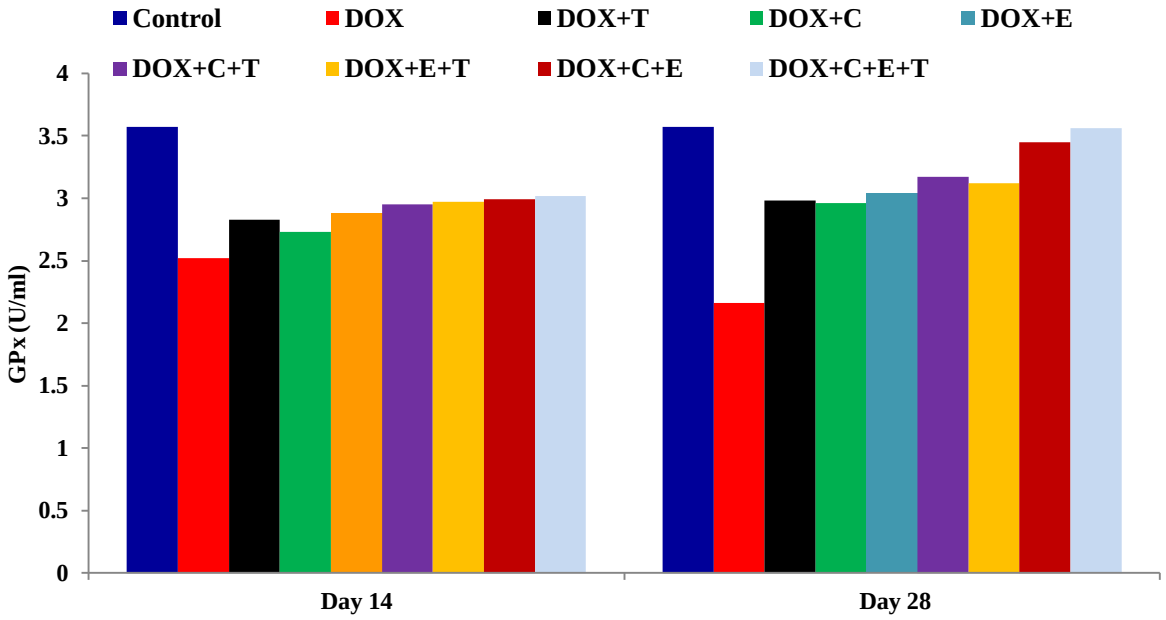

Figure 3: Effect of turmeric root extract, Vitamins C and E on Glutathione peroxidase (GPx) in Doxorubicin induce-toxicity

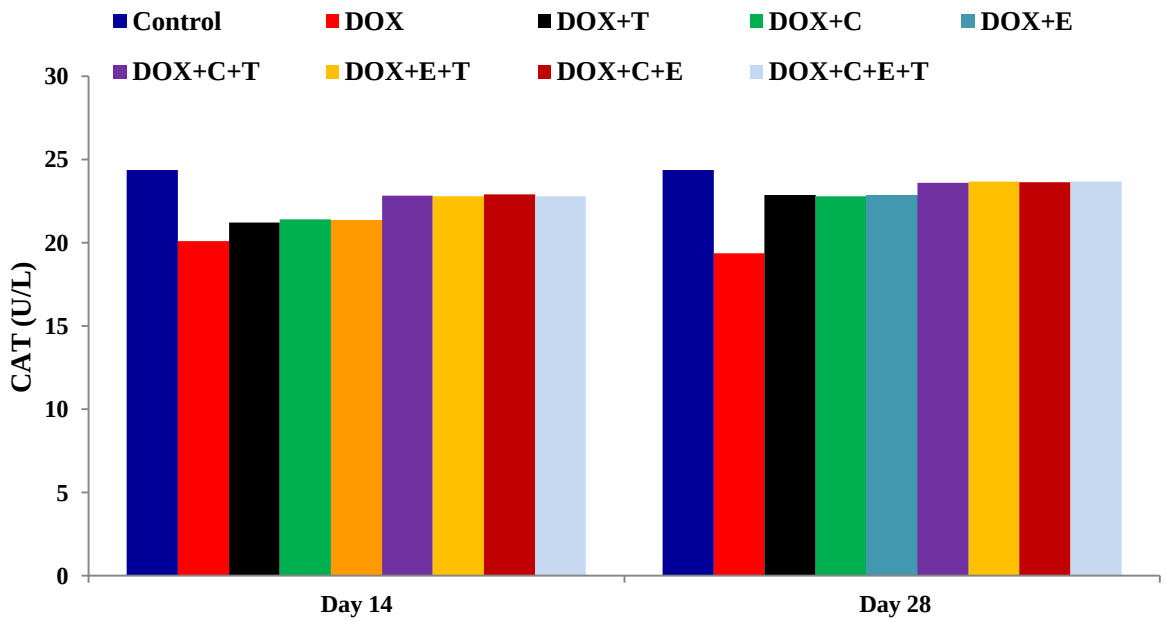

Figure 4: Effect of turmeric root extract, Vitamins C and E on Catalase (CAT) in Doxorubicin induce-toxicity

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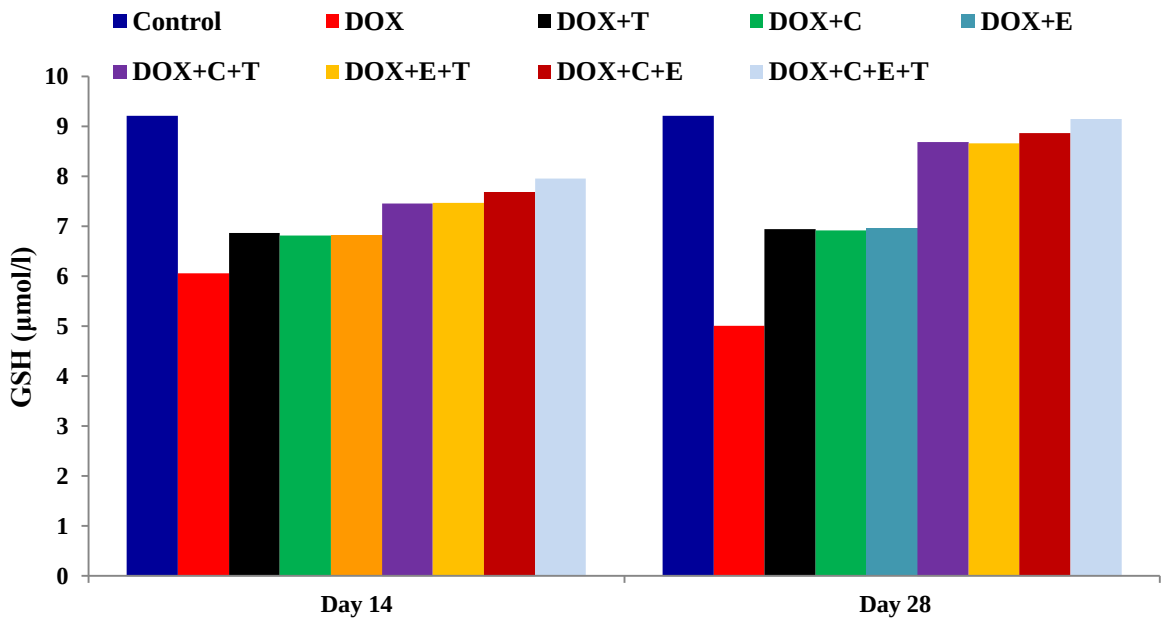

Figure 5: Effect of Tturmeric root extract, Vitamins C and E on Glutathione (GSH) in Doxorubicin induce-toxicity

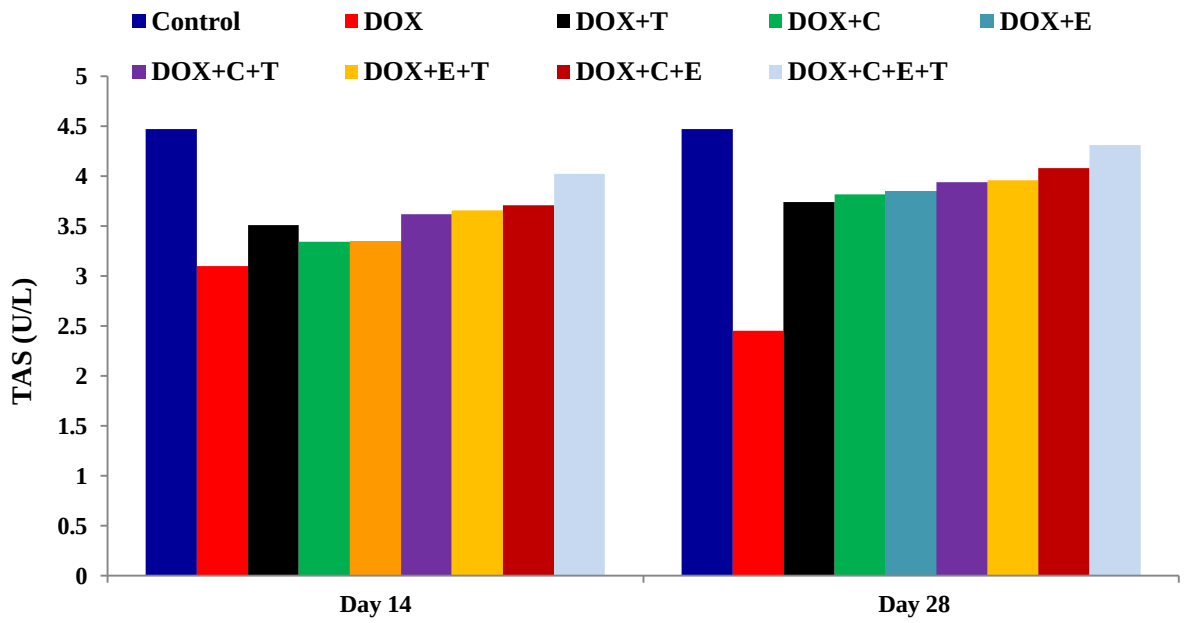

Figure 6: Effect of turmeric root extract, Vitamins C and E on Total antioxidant status (TAS) in Doxorubicin induce-toxicity

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Discussion

It is now a common knowledge that there is an increase prevalence of cancers and tumours among the general population all over the world, thus there is a need to explore new and more effective ways of treatments modalities, with less adverse effects. For this very essence, this present study was carried out to investigate the effect of turmeric on doxorubicin induced toxicity in the presence of vitamins C and E, since doxorubicin is common and widely used in the treatment of various cancers, not without serious deleterious effects, of which several authors have attributed these to be related with an induction of increase oxidative stress (Patil and Balaraman 2009; Gamal 2012 and Abdel-Wahab et al., 2003) etc. In this study, it was revealed that administration of DOX, affected all the oxidative stress biomarkers studied. These markers include Superoxide dismutases (SOD), reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GP_X) and Malondialdehyde (MDA) and serum total antioxidant status (TAS). Administration of DOX caused a significant reduction in the serum concentration of SOD, GPx, CAT, GSH and TAS as well as an increase in the serum level of MDA, which by implication is a decrease in their activities. It was further observed that turmeric, vitamin C, vitamin E and combinations all countered the effect of DOX on these biomarkers.

Oxidative stress results when there is increased production of free radicals or decreased activity of counter-actors, antioxidants or both in a combination (Suchitra et al., 2013). Thus by implication, DOX may have caused an increased production of free radicals, which is in agreement with the findings of Patil and Balaraman (2009) and Gamal 2012. Antioxidant which can either be enzymatic (SOD, CAT, GPx) or non-enzymatic (GSH, TAS, Vitamin C, A etc) protects against the effect (s) of free radicals in order to maintain homeostatic balance of reactive oxygen species (Edem et al., 2012; Osadolor et al., 2014, and Olaniyan and Babatunde 2016).

SOD plays a major role as first line of the antioxidant defense system by catalyzing the dismutation of superoxide radical to form hydrogen peroxide (an oxidant) and molecular oxygen (Edem et al., 2012 and Ho et al., 1998). Thus by implication DOX may have caused over production of superoxide radicals, which over whelmed the intrinsic serum level of SOD thereby causing a reduction in its activity. This might have been responsible for the reduction in the serum level of SOD, following DOX treatment. The reversal of these deleterious effects of DOX by turmeric, vitamins C and E as well as their combination, implies free radical scavenging properties of these substances, thus confirming their anti-oxidant capabilities as has been reported by other scholars (El-Demerdash et al., 2009 and Cekmen et al., 2009).

Catalase (CAT) catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) (Chelikani et al., 2004).

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The reduction in the serum levels of CAT following DOX treatment, which by implication is a reduction in its activity, may not be unconnected with over production of ROS, which agrees with the findings of other researchers as stated above, i.e. DOX by implication may have caused over production of ROS including H_2O_2, which suppress the level of CAT in the serum, thus reducing their activities. As stated above, the administration of turmeric, vitamins C and E as well as their combination, caused an increase of CAT retuning it towards normal level. This is probably because, the extract and the drugs both individually and collectively scavenges, and eradicated the free radicals (ROS including H_2O_2), thus reducing the pressure exacted on the biological anti-oxidant (CAT), eventually returning its concentration back to normal.

GPx is a selenium dependent enzyme with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Igharo et al., 2016). Significant reduction in SOD, CAT and GPx activity might be an indication of accumulation of H_2O_2 that required to mump up these reactive species. Thus by implication DOX caused over production of H_2O_2 as well as lipid peroxidation which lead to the reduction of these anti-oxidants levels in the serum, which agrees with the findings of Abdel-Wahab et al., (2003), who reported that oxygen radical-induced damage of lipids in membrane is the key factor for DOX-induced toxicity or a possible increased formation of free radicals that could lead to oxidative damage (Surajudeen et al., 2014 and Ho et al., 1998) as a result of overwhelming antioxidant activities of all these enzymes. With the observed effects of turmeric, vitamins C and E as well as their combination, it is obvious that they all individually and collectively possess free radical scavenging properties, which allows their actions to reverse these toxic effects caused by DOX administration.

Malondialdehyde (MDA) is secondary products of lipid peroxidation, thus its concentration is increased during oxidative stress and decreases in the presence of anti-oxidants. The increasing concentration of MDA is an evidence of tissue damage caused by increased free radicals. The adverse effects of DOX results mainly from its essential tendency to produce free radicals and block antioxidant enzymes in various tissues (Yeh et al., 2009). The current study and other similar studies confirmed that lipid peroxidation, a downstream chain reaction started by free radicals, was triggered by DOX as revealed by the increased level of lipoperoxidation product (MDA) (Asmis et al., 2006). Also, DOX voluntarily auto-oxidizes in the presence of oxygen, producing superoxide and consequently other ROS, which can stimulate lipid peroxidation (Howell and Shalet 2001). These effects were all reversed by turmeric, vitamins C and E as well as their combination, confirming their anti-oxidant potentials.

Reduced glutathione (GSH) reduces the oxidized form of the enzyme glutathione peroxidase, (GSSG) which in turn reduces hydrogen peroxide (H_2O_2) dangerous reactive species within the cell (Igharo et al., 2016). Thus the

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significant reduction in the levels of GSH as observed in our result of DOX administration, may be majorly due to over production of H_2O_2 thus consummately engaging the activities of the enzyme glutathione peroxidise, to reduce cellular toxicity, which in turn causes a reduction in the levels of GSH in its attempt to reduce the oxides of the enzyme glutathione peroxidase. This is a valid inference following the reprts of other researchers on the activities of DOX (Patil and Balaraman 2009 and Gamal 2012). Significant reduction in plasma levels of reduced glutathione (GSH) and TAS are thus a result of overwhelming antioxidant effects to reduce free radicals generated. Thus the ability of turmeric, vitamins C and E and their combination to reverse the adverse effects observed with DOX on GSH and TAS, is by implication a demonstration of anti-oxidant potentials.

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. All these are reversed on administration of turmeric root extract, vitamins C and E individually and collectively.

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