Journal of Complementary and Alternative Medical Research
2(3): 1-27, 2017; Article no.JOCAMR.30604

SCIENCEDOMAIN *international*
www.sciencedomain.org

Attenuation of Doxorubicin-induced Oxidative Stress and Organ Damage in Experimental Rats by *Theobroma cacao* Stem Bark

A. M. Kosoko[1*], O. J. Olurinde[1] and O. E. Oyinloye[2]

[1]Department of Biochemistry, College of Biosciences (COLBIOS), Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria.
[2]Department of Pharmacology, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AMK, OJO and OOE designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AMK managed the experimental process. Author OJO identified the species of plant and managed the literature searches, analyses of the study, performed the biochemical analyses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2017/30604
Editor(s):
(1) Ahmed Moussa, Pharmacognosy and Api-Phytotherapy Research Laboratory, Mostaganem University, Algeria.
Reviewers:
(1) Edet Okon Akpanyung, University of Uyo, Uyo, Nigeria.
(2) Weiting Wang, Centre for Pharmacodynamics Research, Tianjin Institute of Pharmaceutical Research, Tianjin, China.
(3) Salisu Maiwada Abubakar, Bayero University Kano, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/18245

Original Research Article

Received 21st November 2016
Accepted 15th December 2016
Published 17th March 2017

ABSTRACT

Eighty rats, randomly selected, were divided into three (3) treatment groups: pre-, co- and post-treatment; consisting of 6 sub-groups each (5 rats per sub-group); baseline, normal saline (2 ml), α-lipoic acid (20 mg/kg body weight), and 200 mg/kg, 400 mg/kg, or 800 mg/kg body weight *Theobroma cacao* stem bark aqueous extract (TCAE). Animals in the pre-treatment group were intoxicated with a single dose (20 mg/kg) of doxorubicin (DOX) intraperitoneally followed by 7 days oral administration of normal saline, α-lipoic acid or TCAE; co-treatment group were co-administered 2.86 mg/kg body weight of DOX with either normal saline, α-lipoic acid or TCAE orally for 7 days while post-treatment group were administered normal saline, α-lipoic acid or TCAE orally for 7 days and on the 8th day, intoxicated with a single dose of DOX (20 mg/kg intraperitoneally).

**Corresponding author: E-mail: kosokoam@funaab.edu.ng, kosoko_am2002@yahoo.com;*

Animals were sacrificed (pre- and post-treatment groups on the ninth day while the co-treatment group on the 8th day), blood samples collected by retro-orbital plexus, kidneys and spleen samples harvested for biochemical and histopathological investigations. A significant elevation in plasma urea and creatinine concentrations with perturbation in plasma electrolyte (K^+, Na^+, Cl^-, HCO_3^-) concentrations were observed resulting from DOX intoxication. DOX caused a significant increase in activities of splenic ACP, LDH, γ-GT and ALP activities. A significant increase in renal and splenic concentrations of H_2O_2 generated, MDA and PC; XO, MPX and NOX activities while the concentrations of GSH, AsA and α-TOC; and the activities of SOD, CAT, GST and GPX were significantly reduced following DOX intoxication. Treatment with TCAE significantly attenuated renal and splenic tissue damage and pro-oxidant markers precipitated by DOX intoxication while restoring normalcy to tissue antioxidant markers. *Theobroma cacao* stem bark aqueous extract presents a potential candidate in the prevention of renal and splenic injury and dysfunction related to doxorubicin intoxication.

Keywords: Chemoprevention; Theobroma cacao; doxorubicin; renal toxicity; spleen toxicity; oxidative stress.

1. INTRODUCTION

Since the introduction of doxorubicin (DOX) for the treatment of cancer in 1969, this compound has demonstrated high antitumor efficacy. DOX's cytotoxic effect on malignant cells, as well as its toxic effects on various organs is thought to be related to its DNA intercalation and cell membrane lipid binding activities. It has been suggested, that DOX-induced apoptosis may be an integral component of the cellular mechanism of action responsible for its therapeutic effects, toxicities, or both [1]. In spite of its high antitumor efficacy, DOX's use in chemotherapy has been largely limited due to its cardiac, renal, pulmonary, testicular, spleen and hematological toxicities [2,3]. DOX chemotherapy employs chemical agents to discontinue the growth and annihilate cancer cells even at remote sites from the source of primary tumor. However, it does not discriminate between a cancer and normal cell and eradicates not only fast-growing cancer cells but also other rapidly growing cells in the body [4]. An escalating amount of fact suggest that a simultaneous application of chemotherapy and chemo-preventive agents with antioxidant action may augment the efficacy of chemotherapeutics [5,6]. Although the mechanism underlying the severe cytotoxicity of DOX is not fully clear, reactive oxygen species (ROS) are assumed to be a key factor in the toxicity of DOX and events controlling this oxidative injury are extensively appreciated [7]. The administration of DOX leads to production of hydroxyl radicals, hydrogen peroxide and superoxide anions. NADPH-cytochrome P_{450} converts DOX to a semiquinone free radical which then leads to the generation of superoxide anion and hydroxyl radicals causing membrane lipid peroxidation (LPO) [8]. α-Lipoic acid (ALA) is a unique and potent antioxidant. It can deliver antioxidant activity in both fat and water-soluble mediums, and it is capable of having an antioxidant effect in both its oxidized (LA) and reduced (DHLA [dihydrolipoic acid]) forms [9]. This effectively allows LA to deliver its antioxidant effect to any cell or tissue type, as well as to any subcellular compartment, in the body [10]. It appears to be particularly effective in recharging enzymes in the mitochondria, the "energy centers" of the cells while vitamin C and reduced glutathione are absolutely essential to good health, LA can be considered a master antioxidant orchestrator, facilitating the optimal interactions among the other antioxidants. DHLA directly recharges vitamin C and indirectly recharges vitamin E. LA also increases intracellular reduced glutathione levels and coenzyme Q_{10} levels [11]. The drugs of today's modern society are products of research and development, whose raw materials are naturally occurring materials obtained from plants; either in the roots, stems, leaves, fruits and seeds [12,13]. More research focus had been shifted to medicinal plants because of the presence of more effective compounds in them and their historical use, and the fact that many people over the world rely on them for the treatment of infectious and non-infectious diseases [14]. These plant chemicals were/are isolated from a wide array of plants and even those plants already known are being discovered of having new and interesting physiological properties, *Theobroma cacao* (simply known as cacao) as an example [15]. The studies on *Theobroma cacao*, their related products and its various parts have become an area of interest owing to their health-promoting properties which were

attributed to their phenolic compounds, mainly flavonoids and phenolic acids [16]. *Theobroma cacao* and its parts had been considered from long time, as a food-rich polyphenols. In *Theobroma cacao* and chocolate, the monomeric modules of the main flavonoids are flavan-3-ols, epicatechin and catechin, and polymers of these are proanthocynidins, which are called procyanidins [17]. Cocoa and its compounds have drawn recently a lot of attention because of its contributory role as a chemopreventive agent. In several studies, the highly anti-oxidative effect of *Theobroma cacao* has been demonstrated, comparing with other products, a special characteristic related to its high content of procyanidins which in turn prevent the oxidation of cholesterol-LDL [18,19,20,21]. In experimental trails, cocoa phenolics have presented several beneficial effects against platelet aggregation [22], high blood pressure [23], atherosclerosis [25], hyperglycemia and hyperchoelstrolemia [25,26], inflammation [27], hepatocarcinogenesis [25], DNA damage and clastogenic effect [28]. Flavonoids are naturally occurring substances that acquire diverse pharmacological properties and curative applications, this is endorsed to the phenolic structures which have antioxidant efficiency and restrain free radical-mediated processes. Flavonoids are comprised of several classes, including flavonols, flavones, flavanols and flavans [4]. The spleen is an organ found in virtually all vertebrates. Similar in structure to a large lymph node, it acts primarily as a blood filter. The spleen plays important roles in regard to red blood cells and the immune system. It removes old red blood cells and holds a reserve of blood, which can be valuable in case of hemorrhagic shock, and also recycles iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is removed in the liver [29]. The spleen synthesizes antibodies in its white pulp and removes antibody-coated bacteria and antibody-coated blood cells by way of blood and lymph node circulation [30]. The kidneys are bean-shaped organs that serve several essential regulatory roles in vertebrates. Their main function is the balance of electrolytes in the blood, along with homeostasis of pH. They also remove excess organic molecules from the blood, and it is by this action that their best-known function is performed: the removal of waste products of metabolism. Kidneys are essential to the urinary system and also serve homeostatic functions such as regulation of electrolytes, maintainace of acid-base balance and regulation of blood pressure, as natural filter of the blood and remove water-soluble wastes which are diverted into the bladder. It is also involved in the excretion of nitrogenous wastes such as urea and ammonium during the production of urine and for the reabsorption of water, glucose and amino acids, production of hormones (calcitriol and erythropoietin) and the enzyme renin [31]. Most research on the medicinal properties of *Theobroma cacao* focuses on the plant's seed and pod with only a few established studies on the stem bark. In this study we evaluated the potential spleenonephro-protective activities of *Theobroma cacao* stem bark aqueous extract against DOX induced organ toxicity and oxidative stress [24].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagent

Sodium hydroxide, Sodium chloride, doxorubicin, α-Lipoic acid, formalin, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, urea test kits (RANDOX), creatinine (RANDOX), chloride test kits (TECO), bicarbonate (TECO), potassium test kit (Micropoint), sodium test kit (Micropoint), diethylether, ethanol, xylene, paraffin wax, haemotoxylin, eosin and distilled water. All reagents and chemicals used were of analytical grade.

2.2 Preparation of Extract

Freshly peeled stem barks of *Theobroma cacao* tree were collected in a village farm at Ekiti, Ekiti state southwest Nigeria. The plant part were identified and authenticated at the Department of Botany, University of Ibadan, Nigeria. The fresh stem bark of *Theobroma cacao* were allowed to air-dry to a constant weight at room temperature in a well-ventilated room for a period of four weeks. Conventional extraction process described by Koul [32] was adopted.

2.3 Animals

Eighty (80) Inbred male Wistar rats, weighing between 100 and 210 g were purchased from the Animal House of the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were kept in well-ventilated cages in the departmental animal house at room

temperature (28–30°C) and under controlled light cycles (12h light:12h dark) for two weeks acclimatization before the commencement of the experiment. They were maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water ad libitum. Rats handling and treatments conform to guidelines of the National Institute of Health (NIH publication 85–23, 1985) for laboratory animal care and use. The study was approved by the College of Biosciences, Federal University of Agriculture Abeokuta Animal Ethics Committee.

2.4 Experimental Design

Animals were randomly selected, after acclimatization, and distributed into four (4) groups, viz; baseline, pre-treatment, co-treatment and post-treatment groups, with each group except the baseline group further sub-divided into five different sub-groups of five rats per sub-group as follows: normal saline, α-lipoic acid, 200TCAE, 400TCAE or 800TCAE groups.

2.4.1 Pre-treatment group

This group comprises of 25 rats divided into five sub-groups of five rats each. All the rats were administered single dose of 20 mg/kg body weight DOX intraperitoneally on the first day. After 24 hours, oral treatment with either normal saline (negative control), 20 mg/kg body weight α-lipoic acid (positive control), 200 mg/kg body weight TCAE, 400 mg/kg body weight TCAE or 800 mg/kg body weight TCAE respectively in each group was conducted for seven days. The rats were fasted overnight and sacrificed 24 hours after the last treatment.

2.4.2 Co-treatment group

This group comprises of 25 rats divided into five sub-groups of five rats each. A dose of 2.86 mg/kg body weight doxorubicin was co-administered intraperitoneally with either normal saline (negative control), 20 mg/kg body weight α-lipoic acid (positive control), 200 mg/kg body weight TCAE, 400 mg/kg body weight TCAE or 800 mg/kg body weight TCAE respectively in each group for seven days orally. The rats were fasted overnight and sacrificed 24 hours after the last administration.

2.4.3 Post-treatment group

This group comprises of 25 rats divided into five sub-groups of five rats each. The rats were first treated with normal saline (negative control), 20 mg/kg body weight α-lipoic acid (positive control), 200 mg/kg body weight TCAE, 400 mg/kg body weight TCAE or 800 mg/kg body weight TCAE orally respectively in each group for seven days. Single dose of 20 mg/kg body weight DOX was administered intraperitoneally on the eight day, the rats fasted overnight and sacrificed 24 hours after the last intoxication.

2.4.4 Baseline group

This group comprises of five rats administered normal saline orally per day for seven days, fasted overnight and sacrificed 24 hours after the last administration.

2.4.5 Preparation of tissues

Rats were fasted overnight and sacrificed 24h after the last treatment. Spleen and kidneys were quickly removed and washed in ice-cold 1.15% KCl solution to remove blood stain, dried and weighed. Part of these tissues were fixed in 10% formalin solution and used for histopathology. The remaining tissues were homogenized separately in 4 volumes of 50mM phosphate buffer, pH 7.4 and centrifuged at 10,000 × g for 15 min to obtain post-mitochondrial fraction (PMF). Procedures were carried out at temperature of 4°C.

2.4.6 Preparation of plasma

Blood samples were collected in lithium heparin anticoagulant tubes by retro-orbital venous plexus. Plasma was prepared by centrifugation at 3000 × g for 15 min in a Beckman bench centrifuge. The clear supernatant was used for the estimation of biochemical indices of renal dysfunction.

2.5 Determination of Total Peroxy Radical Trapping Activity

The peroxyl radical scavenging activity was determined by thiocyanate method using α-tocopherol as standard as described by Yildirim et al. [33].

2.6 Determination of Nitric Oxide Radical Scavenging Activity

Nitric oxide scavenging activity can be estimated by the use of Griess Illosvoy reaction [34]. The compound sodium nitroprusside is known to

decompose in aqueous solution at physiological pH 7.2 producing $NO^{\cdot}$. Scavengers of nitric oxide compete with oxygen leading to reducing production of nitrite ions.

2.7 Determination of Total Flavonoid Content

The determination of the flavonoid content was carried out a described by Zhishen et al. [35]. Catechin was used as positive reference standard.

2.8 Biochemical Assays

Plasma bicarbonate ion (HCO_3^-) determination was done using back titration method described by Tietz et al. [36]. The colometeric determination of chloride (Cl^-) in the plasma was done according to the method described by Tiezt et al. [36]. Plasma sodium (Na^+) and potassium (K^+) ions were determined by methods described by Tietz et al. [36]. Plasma urea and creatinine levels were determined by the method of Talke and Schubert [37] and Jaffe [38] respectively. Splenic alkaline phosphatase (ALP) activity was determined according to the method described by Tietz et al. [36] and as modified by Wright et al. [39] using Randox kits, gamma-glutamyl transferase (γ-GT) activity was monitored according to the method described by Szasz [40], acid phosphatase (ACP) activity was determined according to the method described by Tietz et al. [36] while lactate dehydrogenase (LDH) activity was determined according to the method described by Bower [41]. Splenic and renal hydrogen peroxide (H_2O_2) concentration was quantified based on Wolf's method [42], protein carbonyl (PC) concentration was carried out by following method described by Levine et al. [43], malondialdehyde (MDA) concentration was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation. This was measured using method of Moore and Roberts [44], myeloperoxidase (MPX) activity was determined using method of Klebanoff et al. [45], NADPH oxidase (NOX) activity was measured by the method of Reusch and Burger [46], xanthine oxidase (XO) activity was determined according to the method of Bergmeyer et al. [47], glutathione-S-transferase (GST) activity was determined according to Habig et al. [48], enzymatic assay of glutathione peroxidase (GPX) activity was determined following the method described by Wendel [49], catalase (CAT) activity was determined according to the method of Sinha et al. [50], the activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich [51], the method of Beutler et al. [52] was followed for the determination of reduced glutathione (GSH) concentration, ascorbic acid (AsA) concentration was quantified according to the method of Nino and Shah [53] and concentration of α–tocopherol (α-toc) was carried out following the procedure of Kayden et al. [54].

2.9 Histopathological Examination of Spleen and Renal Sections

The tissues were excised and immediately fixed in 10% buffered formalin at the end of the experiment. The tissue specimens were embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Four micrometer (4 µm) thick serial histological sections were obtained from the paraffin blocks and stained with hematoxylin and eosin. The sections were examined under light microscope by a histopathologist (who was ignorant of the treatment groups) to evaluate pathological changes and photomicrographs were taken [55].

2.10 Statistical Analysis of Data

Values were expressed as mean ± standard deviation of five animals per group. Data were analysed using one-way ANOVA followed by the post-hoc Duncan multiple range test using SPSS (V20.0). Values were considered statistically significant at $p<0.05$.

3. RESULTS

3.1 Total Flavonoid Content, NITRIC Oxide Scavenging Activity, Total Peroxyl Radical Trapping Activity and Effects of *Theobroma cacao* on Relative Organ Weights of DOX-exposed Rats

Table 1 revealed that DOX intoxication caused a significant decrease and insignificant increase in kidney and spleen weights of experimental animals respectively relative to baseline ($p<0.05$). Pre-, co- and post-treatment of experimental animals with 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *T. cacao* caused a significant increase in spleen weight of experimental animals. A significant dose dependent increase in spleen and kidney weights were observed with the administration of TCAE in co- and post-treatment, while an insignificant

increase in spleen and kidney weights were observed in rats pre-treated with different doses of TCAE ($p < 0.05$). *Theobroma cacao* stem bark aqueous extract showed presence of significant dose dependent amount of flavonoid relative to the standard, catechin with a significant dose dependent scavenging ability for nitric oxide radicals relative to ascorbic acid and a significant dose dependent ability to trap peroxy radicals ($p < 0.05$) (Figs. 1, 2 and 3 respectively). However, the activities of the standards were significantly higher the TCAE.

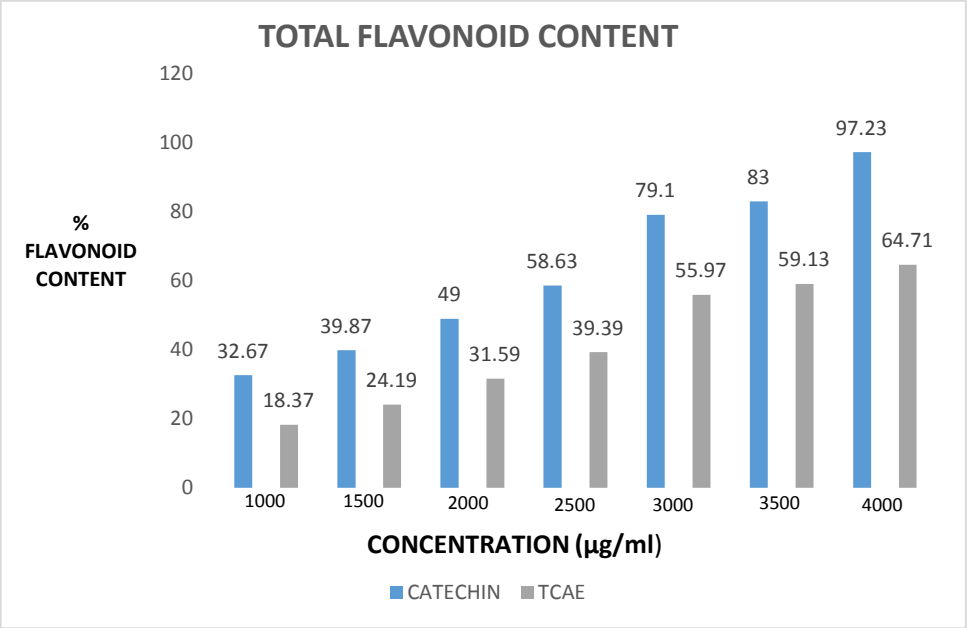


Fig. 1. Total flavonoid content of TCAE

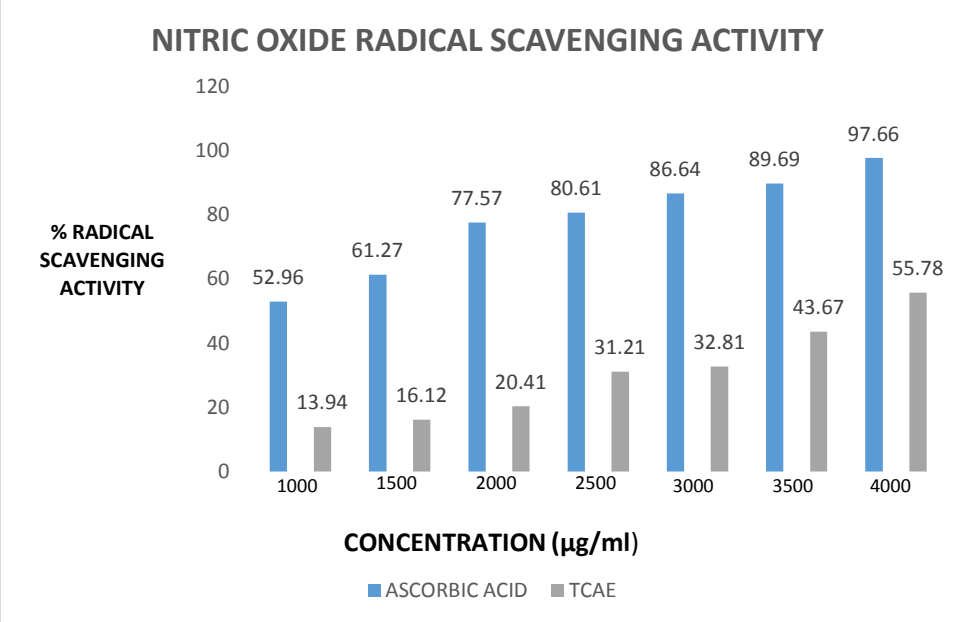


Fig. 2. Nitric oxide radical scavenging activity of TCAE
Values are means of percentages of inhibition of three replicates
TCAE – Theobroma cacao aqueous extract

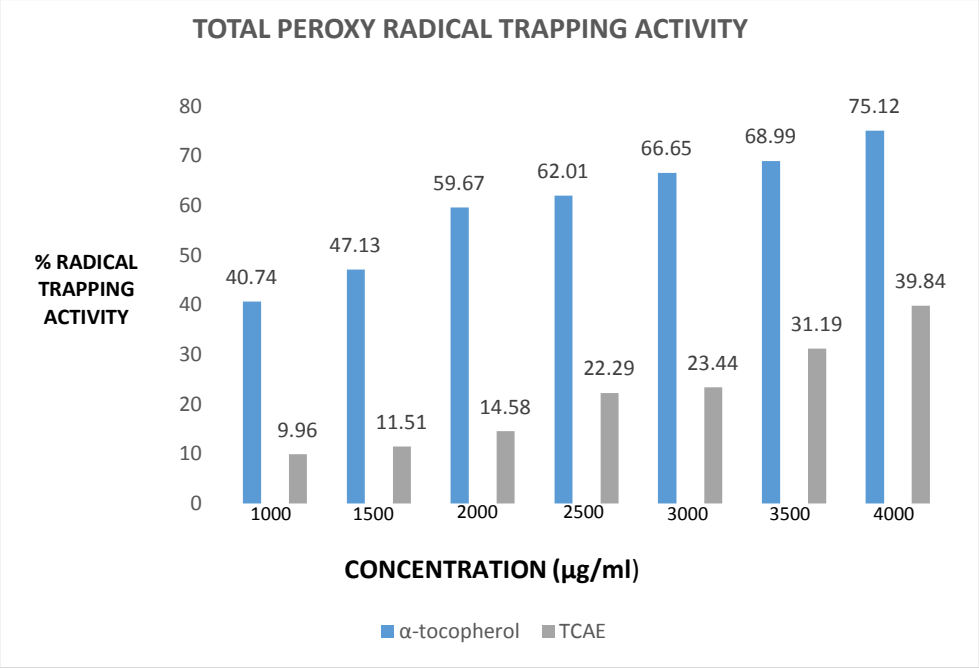


Fig. 3. Total peroxy radical trapping activity of TCAE
Values are means of percentages of inhibition of three replicates
TCAE – Theobroma cacao aqueous extract

3.2 Effects of *Theobroma cacao* on Plasma Electrolytes (Na^+, K^+, Cl^- and HCO_3^- Ions), Urea and Creatinine Concentration in DOX-exposed Rats

Doxorubicin intoxication caused a significant increase in plasma creatinine and urea concentrations of experimental rats compared with the baseline group ($p < 0.05$). Significant dose-dependent reduction in creatinine and urea concentrations were observed in *Theobroma cacao* stem bark aqueous extract treated groups relative to DOX-intoxicated group ($p < 0.05$) (Table 2).

It was observed from Table 3 that doxorubicin intoxication caused a significant increase in plasma potassium ion concentration in experimental rats compared to baseline group ($p < 0.05$). Significant decrease was observed in the three modes of treatment with TCAE except for pre-treatment with 400 mg/kg body weight which decreases insignificantly relative to DOX-intoxicated group. Also, a significant decrease in plasma sodium, chloride and bicarbonate ions concentration were observed in DOX-intoxicated rats compared with baseline group were observed ($p < 0.05$). Pre-, co- and post-treatment modes with 200 mg/kg, 400 mg/kg or 800 mg/kg body weight TCAE groups caused a significant increase in electrolyte concentrations relative to DOX-intoxicated group ($p < 0.05$).

3.3 Effects of *Theobroma cacao* on Splenic Alkaline Phosphatase, Acid Phosphatase, Lactate Dehydrogenase and γ-Glutamyl Transferase Activities in DOX-Exposed Rats

From Tables 4, 5, 6 and 7, doxorubicin intoxication induced a significant increase in splenic ALP, ACP, LDH and γ-GT activities respectively in experimental rats relative to the baseline group. Pre-, co- or post-treatment with *Theobroma cacao* stem bark aqueous extract caused a significant dose-dependent reduction in the splenic toxicity marker enzyme activities across the three modes of treatments compared with DOX-intoxicated groups ($p<0.05$).

Table 1. Relative organ weights (g) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	Spleen				Kidneys			
	Pre-treatment	**CO-treatment**	**Post-treatment**	**Baseline**	**Pre-treatment**	**Co-treatment**	**Post-treatment**	**Baseline**
Normal saline	0.130± 0.052$^{\alpha}$	0.162±0.014$^{\alpha\gamma}$	0.408±0.032$^{\alpha\gamma}$	----------	0.49 ± 0.02	0.50 ± 0.03	0.49 ± 0.02	---------
α-lipoic acid	0.132±0.040$^{\alpha}$	0.26±0.064$^{\alpha\beta}$	0.479±0.061$^{\alpha\beta}$	----------	0.43 ± 0.01	0.50 ± 0.04	0.47 ± 0.05	---------
200TCAE	0.136±0.06$^{\alpha}$	0.284±0.046$^{\alpha\beta}$	0.515±0.067$^{\alpha\beta}$	----------	0.40 ± 0.05 $^{\beta}$	0.47 ± 0.02	0.55 ± 0.15 $^{\alpha\gamma}$	---------
400TCAE	0.332±0.014$^{\alpha\beta\gamma}$	0.411±0.017$^{\alpha\beta\gamma}$	0.616±0.052$^{\beta\gamma}$	----------	0.48 ± 0.02	0.45 ± 0.02	0.53 ± 0.06 $^{\alpha}$	---------
800TCAE	0.340±0.064$^{\alpha\beta\gamma}$	0.444±0.068$^{\alpha\beta\gamma}$	0.67±1.80$^{\beta\gamma}$	----------	0.44 ± 0.01	0.52 ± 0.05 $^{\alpha}$	0.51 ± 0.05 $^{\alpha}$	---------
Baseline	----------	----------	----------	**0.628±0.12**	----------	----------	----------	**0.43±0.03**

Values are expressed as mean±standard deviation (n=5). Significant at p<0.05
α= significant difference compared with baseline.
β= significant difference compared with normal saline
γ= significant difference compared with α – lipoic acid

Table 2. Plasma creatinine and urea concentrations in DOX-induced renal toxicity and the ameliorative role of TCAE

Parameters	**Treatment mode**	**NS**	**ALA**	**200 TCAE**	**400 TCAE**	**800 TCAE**	**Baseline**
Creatinine	Pre	2.186±0.005$^{\alpha\gamma}$	1.594±0.162$^{\beta}$	1.292± 0.256$^{\beta}$	1.178±0.013$^{\beta\gamma}$	2.38± 0.100$^{\beta}$	
(mg/dl)	Co	1.966±0.102$^{\alpha\gamma}$	0.512±0.008$^{\alpha\beta}$	0.820±0.134$^{\alpha\beta}$	0.730±0.193$^{\alpha\beta}$	0.760±0.104$^{\alpha\beta}$	1.496± 0.356
	Post	1.718±0.203$^{\gamma}$	1.138±0.340$^{\beta}$	0.702±0.372$^{\alpha\beta\gamma}$	1.278±0.809$^{\beta}$	0.826±0.065$^{\alpha\beta}$	
Urea (mg/dl)	Pre	80.35±1.23 $^{\alpha\gamma}$	65.24±0.52 $^{\alpha\beta}$	61.61±1.17 $^{\alpha\beta\gamma}$	59.67±1.04 $^{\alpha\beta\gamma}$	58.74±1.01$^{\alpha\beta\gamma}$	
	Co	76.23±0.39$^{\alpha\gamma}$	66.14±0.51 $^{\alpha\beta}$	64.53 ± 0.88 $^{\alpha\beta}$	62.83±1.05$^{\alpha\beta\gamma}$	60.42±3.91$^{\alpha\beta\gamma}$	51.47± 0.67
	Post	71.17±0.59$^{\alpha\gamma}$	60.51±0.98 $^{\alpha\beta}$	59.64 ± 0.88 $^{\alpha\beta}$	55.52±1.15 $^{\alpha\beta\gamma}$	53.66±3.49 $^{\alpha\beta\gamma}$	

Table 3. Plasma electrolyte concentrations in DOX-induced renal toxicity and the modulatory role of TCAE

Parameters	Treatment mode	NS	ALA	200 TCAE	400 TCAE	800 TCAE	Baseline
Chloride	Pre	280.74±0.39$^{\alpha\gamma}$	378.64±7.16$^{\alpha\beta}$	359.80±14.29$^{\alpha\beta\gamma}$	307.26 ±1.50$^{\alpha\beta\gamma}$	306.66 ±2.75$^{\alpha\beta\gamma}$	324.76 ±3.14
(mEq/L)	Co	314.64 ±8.65	321.60 ±3.82	316.70 ±6.80	330.82 ±5.33$^{\beta}$	372.72±9.18$^{\alpha\beta\gamma}$	
	Post	310.08±2.04$^{\alpha\gamma}$	348.32 ±3.03$^{\alpha\beta}$	348.12 ±5.26$^{\alpha\beta}$	360.42±16.90$^{\alpha\beta\gamma}$	363.44±19.71$^{\alpha\beta\gamma}$	
Bicarbonate	Pre	59.22 ± 0.27$^{\alpha\gamma}$	85.58 ± 9.99$^{\alpha\beta}$	137.98 ± 4.60$^{\alpha\beta\gamma}$	136.72 ±6.20$^{\alpha\beta\gamma}$	110.58±14.50$^{\alpha\beta\gamma}$	97.00 ± 3.73
(mmol/L)	Co	80.54 ± 1.89$^{\alpha}$	82.00 ± 7.52$^{\alpha}$	110.34 ±3.59$^{\alpha\beta\gamma}$	83.10 ± 8.54$^{\alpha}$	77.48 ± 4.09$^{\alpha}$	
	Post	73.54 ± 4.63$^{\alpha\gamma}$	95.68 ± 3.73$^{\beta}$	91.92 ± 1.09$^{\beta}$	90.44 ± 4.25$^{\beta}$	94.72 ± 1.71$^{\beta}$	
Potassium	Pre	16.82 ± 0.13$^{\alpha\gamma}$	16.04 ± 0.43$^{\alpha}$	14.96 ± 0.16$^{\alpha\beta}$	15.50 ± 1.79$^{\alpha}$	15.32 ± 2.45$^{\alpha\beta}$	5.09 ± 0.57
(mmol/L)	Co	16.00 ± 0.83$^{\alpha\gamma}$	12.12 ± 3.35$^{\alpha\beta}$	6.62 ± 0.28$^{\alpha\beta\gamma}$	7.76 ± 1.36$^{\alpha\beta\gamma}$	8.12 ± 1.27$^{\alpha\beta\gamma}$	
	Post	14.12 ± 1.08$^{\alpha\gamma}$	11.72 ± 1.08$^{\alpha\beta}$	7.80 ± 0.88$^{\alpha\beta\gamma}$	9.72 ± 0.59$^{\alpha\beta\gamma}$	7.38 ± 0.29$^{\alpha\beta\gamma}$	
Sodium	Pre	335.20 ±5.26$^{\alpha\gamma}$	476.80±36.69$^{\alpha\beta}$	463.20 ±12.04$^{\alpha\beta}$	437.20±22.65$^{\alpha\beta\gamma}$	472.50 ±27.55$^{\alpha\beta}$	505.16±12.62
(mmol/L)	Co	351.22±29.01$^{\alpha\gamma}$	399.28±06.99$^{\alpha\beta}$	437.00±11.94$^{\alpha\beta\gamma}$	401.20 ±14.90$^{\alpha\beta}$	416.00 ±28.97$^{\alpha\beta}$	
	Post	343.88 ±24.11$^{\alpha}$	450.00±15.90$^{\alpha\beta}$	457.44±16.95$^{\alpha\beta}$	468.84 ±14.91$^{\alpha\beta}$	474.04±17.29$^{\alpha\beta}$	

Values are expressed as mean±Standard deviation (n=5). Means bearing different superscripts (α, β and γ) are significant at p<0.05 compared with Baseline, Normal saline, and α – lipoic acid respectively. (NS denotes normal saline, ALA denotes α-lipoic acid, 200TCAE, 400TCAE and 800TCAE denotes 200mg, 400mg and 800mg/kg body weight of Theobroma cacao aqueous extract respectively)

Table 4. Alkaline phosphatase (ALP) activity (IU/L) in DOX-induced spleenotoxicity and protective role of *Theobroma cacao* stem bark aqueous extract (TCAE)

	Pre-treatment	Co-treatment	Post-treatment	Baseline
Normal saline	2.34± 0.239$^{\alpha\gamma}$	1.88±0.209$^{\alpha\gamma}$	1.64±3.840$^{\alpha\gamma}$	_____
α–Lipoic- acid	1.50±0.167$^{\alpha\beta}$	1.21±0.122$^{\alpha\beta}$	1.05±0.097$^{\alpha\beta}$	_____
200 TCAE	1.29±0.074$^{\alpha\beta\gamma}$	1.09±0.075$^{\alpha\beta\gamma}$	0.984±0.84$^{\alpha\beta\gamma}$	_____
400TCAE	1.14±0.050$^{\alpha\beta\gamma}$	1.10±0.02$^{\alpha\beta\gamma}$	0.601±0.23$^{\alpha\beta\gamma}$	_____
800TCAE	1.05±0.087$^{\alpha\beta\gamma}$	1.01±0.111$^{\alpha\beta\gamma}$	0.48±0.244$^{\alpha\beta\gamma}$	____
Baseline	_____	_____	_____	**0.161±1.0**

Table 5. Acid phosphatase (ACP) activity (IU/L) in DOX-induced spleenotoxicity and the protective properties of *Theobroma cacao* stem bark aqueous extract (TCAE)

Groups / Treatments	Pre-treatment	Co-treatment	Post-treatment	Baseline
Normal saline	2.24±0.239$^{\alpha\gamma}$	1.88±0.209$^{\alpha\gamma}$	1.64±0.384$^{\alpha\gamma}$	_____
α–Lipoic- acid	1.50±0.167$^{\alpha\beta}$	1.21±0.122$^{\alpha\beta}$	1.05±0.097$^{\alpha\beta}$	_____
200 TCAE	1.29±0.074$^{\alpha\beta\gamma}$	1.09±0.072$^{\alpha\beta\gamma}$	0.98±0.209$^{\alpha\beta\gamma}$	_____
400TCAE	1.14±0.50$^{\alpha\beta\gamma}$	1.08±0.021$^{\alpha\beta\gamma}$	0.88±0.20$^{\alpha\beta\gamma}$	_____
800TCAE	1.05 6±0.206$^{\alpha\beta\gamma}$	1.03±0.021$^{\alpha\beta\gamma}$	0.676±0.243$^{\alpha\beta\gamma}$	____
Baseline	_____	_____	_____	**0.133±0.35**

Values are expressed as mean ± standard deviation (n=5). Significant at p< 0.05. α= significant difference compared with baseline group. β= significant difference compared with normal saline group. γ = significant difference compared with α-lipoic –acid. 200 TCAE = 200mg/kg body weight of Theobroma cacao stem bark aqueous extract. 400 TCAE = 400mg/kg body weight of Theobroma cacao stem bark aqueous extract. 800 TCAE = 800mg/kg body weight of Theobroma cacao stem bark aqueous extract

Table 6. Lactate dehydrogenase (LDH) activity (IU/L) in doxorubicin-induced spleenotoxicity and the protective properties of *Theobroma cacao* stem bark aqueous extract (TCAE)

Groups / Treatments	Pre-treatment	Co-treatment	Post-treatment	Baseline
Normal Saline	7.29±0.202$^{\alpha\gamma}$	7.00±0.234$^{\alpha\gamma}$	6.51±0.183$^{\alpha\gamma}$	_____
α–Lipoic- acid	6.65±0.106$^{\alpha\beta}$	5.82±0.264$^{\alpha\beta}$	5.47±0.181$^{\alpha\beta}$	_____
200 TCAE	6.01±0.16$^{\alpha\beta}$	5.41±0.139$^{\alpha\beta}$	4.81±0.177$^{\alpha\beta\gamma}$	_____
400TCAE	5.46±0.126$^{\alpha\beta\gamma}$	4.90±0.120$^{\alpha\beta\gamma}$	4.15±0.175$^{\alpha\beta\gamma}$	_____
800TCAE	4.97±0.104$^{\alpha\beta\gamma}$	4.48±0.088$^{\alpha\beta\gamma}$	3.544±0.65$^{\alpha\beta\gamma}$	____
Baseline	_____	_____	_____	**2.81±0.123**

3.4 Effects of *Theobroma cacao* on Antioxidant Parameters, and Histology of Tissues of DOX-exposed Rats

From Table 8, doxorubicin intoxication caused a significant elevation in renal and splenic hydrogen peroxide, malondialdehyde and protein carbonyl concentrations of experimental rats compared with baseline group (p<0.05). Pre-, co- or post-treatment with either 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *Theobroma cacao* stem bark aqueous extract caused a significant dose dependent reduction in hydrogen peroxide, malondialdehyde and protein carbonyl concentrations in the renal and splenic tissues of experimental rats relative to DOX-exposed group (p<0.05). Table 9 revealed significant elevation in renal and splenic NADPH oxidase, xanthine oxidase and myeloperoxidase activities of experimental rats following DOX intoxication compared with baseline group (p<0.05). A dose-dependent reduction in these enzyme's activities were observed following treatment with either 200, 400 or 800 mg/kg body weight in the three (3) modes of treatment with *Theobroma cacao*

stem back aqueous extract relative to the DOX-intoxicated group ($p<0.05$). The result in Table 10 also indicated that doxorubicin administration caused a significant decrease in catalase and superoxide dismutase activities in renal and splenic tissues of experimental rats compared with baseline group ($p<0.05$). There were significant increase in these enzymes' activities in renal and splenic tissues following co- and post-treatment with 200mg/kg, 400mg/kg or 800mg/kg body weight *T. cacao* compared with DOX-intoxicated group ($p<0.05$). The increase in enzymes' activities in renal and splenic tissues following pre-treatment with 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *T. cacao* were insignificant compared with DOX-intoxicated group ($p<0.05$). The result in Table 11 revealed that doxorubicin intoxication caused a significant reduction in reduced glutathione concentration, glutathione peroxidase and glutathione S-transferase activities in renal and splenic tissues of experimental rats compared with baseline group. A significant dose dependent elevations in reduced glutathione concentration and the activities of the enzymes were observed following co- and post-treatment with 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *T. cacao* groups. The observed elevation in reduced glutathione concentration and the activities of the enzymes following pre-treatment with 200 mg/kg, 400 mg/kg and 800 mg/kg body weight *T. cacao* groups were insignificant relative to the rats in DOX-intoxicated group. The result in Table 12 reported a significant decrease in renal and splenic α-tocopherol and ascorbic acid concentrations among DOX-intoxicated rats relative to the baseline group ($p<0.05$). A significant increase in renal α-tocopherol and ascorbic acid concentrations were observed following co- and post-treatment with 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *T. cacao* groups while the increase in pre-treatment with 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *T. cacao* groups were insignificant relative to DOX-intoxicated group ($p<0.05$). A dose dependent increase in splenic ascorbic acid and α-tocopherol concentrations were also observed following pre-, co- or post-treatment with 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *T. cacao* groups compared relative to DOX-intoxicated group.

Figs. 4 to 9 reveals that DOX-intoxication caused severe degeneration and necrosis of renal tubular and glomerular epithelial cells; and a severe generalized congestion of the splenic tissues. The severe to moderate degeneration and necrosis of renal tubular and glomerular epithelial cells observed with pre-, co- and post-treatment with graded doses of *T. cacao* might be due to the insufficiency of the 7 days treatment to completely reverse the pathological lesions associated with DOX intoxication. On the other hand, seven day pre-, co- and post-treatment with graded doses of *T. cacao* preserved the morphology and restored the architectural layout of the splenic epithelial tissue and completely reversed the congestion of the splenic tissue resulting from DOX intoxication.

Table 7. Gamma-glutamyl-transferase (γ-GT) activity (IU/L) in doxorubicin-induced spleenotoxicity and protective properties of *Theobroma cacao* stem bark aqueos extract (TCAE)

Groups / Treatments	Pre-treatment	Co-treatment	Post-treatment	Baseline
Normal Saline	$9.43\pm0.133^{\alpha\gamma}$	$7.94\pm0.245^{\alpha\gamma}$	$7.88\pm0.152^{\alpha\gamma}$	_____
α–Lipoic- acid	$4.36\pm0.287^{\alpha\beta}$	$4.15\pm0.453^{\alpha\beta}$	$3.2\pm0.124^{\alpha\beta}$	_____
200 TCAE	$3.98\pm0.957^{\alpha\beta\gamma}$	$4.32\pm0.456^{\alpha\beta}$	$3.2\pm0.996^{\alpha\beta}$	_____
400TCAE	$3.09\pm0.243^{\alpha\beta\gamma}$	$3.08\pm0.147^{\alpha\beta\gamma}$	$2.71\pm0.117^{\alpha\beta\gamma}$	_____
800TCAE	$3.38\pm0.253^{\alpha\beta\gamma}$	$3.28\pm0.157^{\alpha\beta\gamma}$	$3.38\pm0.253^{\alpha\beta\gamma}$	_____
Baseline	_____	_____	_____	**1.16±0.23**

Values are expressed as mean ± standard deviation (n=5). Significant at $p<0.05$. α= significant difference compared with baseline group. β= significant difference compared with normal saline group. γ = significant difference compared with α-lipoic acid. 200 TCAE = 200mg/kg body weight of Theobroma cacao stem bark aqueous extract. 400 TCAE = 400mg/kg body weight of Theobroma cacao stem bark aqueous extract. 800 TCAE = 800mg/kg body weight of Theobroma cacao stem bark aqueous extract

Table 8. Effects of *Theobroma cacao* on hydrogen peroxide, protein carbonyl and malondialdehyde concentrations in renal and splenic tissues of DOX-exposed rats

Treatment	Kidneys			Spleen		
	Hydrogen peroxide (µmol/mg protein)	Protein carbonyl (nmol/mg protein)	Malondialdehyde (units/mg protein)	Hydrogen peroxide (µmol/mg protein)	Protein carbonyl (nmol/mg protein)	Malondialdehyde (units/mg protein)
PRE-NS	7.887±0.565$^{\alpha\gamma}$	14.866±0.475$^{\alpha\gamma}$	13.632±0.394$^{\alpha\gamma}$	10.078±0.078$^{\alpha\gamma}$	18.57±0.34$^{\alpha\gamma}$	17.08±0.34$^{\alpha\gamma}$
PRE-ALA	2.369±0.260$^{\alpha\beta}$	4.336±0.475$^{\alpha\beta}$	3.986 ± 0.436$^{\alpha\beta}$	2.82±0.406$^{\alpha\beta}$	5.65±1.406$^{\alpha\beta}$	5.10±1.406$^{\alpha\beta}$
PRE-200 mgTCAE	1.441±0.056$^{\alpha\beta\gamma}$	2.640±0.102$^{\alpha\beta\gamma}$	2.415 ± 0.957$^{\alpha\beta\gamma}$	1.84±0.71$^{\alpha\beta\gamma}$	3.34±0.471$^{\alpha\beta\gamma}$	3.104±0.471$^{\alpha\beta\gamma}$
PRE-400mgTCAE	0.945±0.031$^{\alpha\beta\gamma}$	1.730±0.057$^{\alpha\beta\gamma}$	1.609 ± 0.052$^{\alpha\beta\gamma}$	1.23±0.32$^{\alpha\beta\gamma}$	2.8135±0.687$^{\alpha\beta\gamma}$	2.035±0.687$^{\alpha\beta\gamma}$
PRE-800mgTCAE	0.575±0.012$^{\alpha\beta\gamma}$	1.052±0.021$^{\alpha\beta\gamma}$	0.967 ± 0.019$^{\alpha\beta\gamma}$	0.74±0.104$^{\alpha\beta\gamma}$	1.33±0.243$^{\alpha\beta\gamma}$	1.22±0.243$^{\alpha\beta\gamma}$
Co-NS	5.393±0.192$^{\alpha\gamma}$	9.870±0.351$^{\alpha\gamma}$	9.071 ± 0.323$^{\alpha\gamma}$	6.99±0.234$^{\alpha\gamma}$	12.72±0.611$^{\alpha\gamma}$	11.72±0.411$^{\alpha\gamma}$
Co-ALA	1.604±0.134$^{\alpha\beta}$	3.025±0.245$^{\alpha\beta}$	3.698 ± 0.225$^{\alpha\gamma}$	2.11±0.264$^{\alpha\beta}$	3.36±0.355$^{\alpha\beta}$	3.34±0.355$^{\alpha\beta}$
Co-200 mgTCAE	1.009±0.093$^{\alpha\beta\gamma}$	1.849±0.170$^{\alpha\beta\gamma}$	1.642 ± 0.156$^{\alpha\beta\gamma}$	0.972±1.39$^{\alpha\beta\gamma\sigma}$	2.29±0.63$^{\alpha\beta\gamma}$	2.02±1.63$^{\alpha\beta\gamma}$
Co-400 mgTCAE	0.645±0.030$^{\alpha\beta\gamma}$	1.201±0.064$^{\alpha\beta\gamma}$	1.119 ± 0.065$^{\alpha\beta\gamma}$	0.81±0.120$^{\alpha\beta\gamma\sigma}$	1.55±0.123$^{\alpha\beta\gamma}$	1.41±0.756$^{\alpha\beta\gamma}$
Co-800 mgTCAE	0.388±0.015$^{\alpha\beta\gamma}$	0.710±0.028$^{\alpha\beta\gamma}$	0.652 ± 0.026$^{\alpha\beta\gamma}$	0.504± 0.88$^{\alpha\beta\gamma}$	0.996±0.21$^{\alpha\beta\gamma}$	0.848± 0.568$^{\alpha\beta\gamma}$
POST-NS	3.867±0.126$^{\alpha\gamma}$	7.078±0.233$^{\alpha\gamma}$	6.506± 0.215$^{\alpha\gamma}$	4.95±0.173$^{\alpha\gamma}$	9.02±0.49$^{\alpha\gamma}$	8.32±0.723$^{\alpha\gamma}$
POST-ALA	1.315±0.373$^{\alpha\beta}$	2.041±0.204$^{\alpha\beta}$	1.876 ± 0.189$^{\alpha\beta}$	1.42±0.142$^{\alpha\beta\delta}$	2.61±0.240$^{\alpha\beta}$	2.40±0.240$^{\alpha\beta}$
POST-200 mgTCAE	0.695±0.031$^{\alpha\beta\gamma}$	1.272±0.056$^{\alpha\beta\gamma}$	1.169 ±0.051$^{\alpha\beta\gamma}$	0.88±0.392$^{\alpha\beta\gamma}$	1.62±0.54$^{\alpha\beta\gamma}$	1.48±0.54$^{\alpha\beta\gamma}$
POST-400 mgTCAE	0.464±0.024$^{\alpha\beta\gamma}$	0.050±0.043$^{\alpha\beta\gamma}$	0.781±0.040$^{\alpha\beta\gamma}$	0.594±0.675$^{\alpha\beta\gamma}$	1.08±0.475$^{\alpha\beta\gamma}$	1.12±0.675$^{\alpha\beta\gamma}$
POST-800 mgTCAE	0.281 ±0.006$^{\beta\gamma}$	0.515±0.011$^{\alpha\beta\gamma}$	0.473 ± 0.010$^{\alpha\beta\gamma}$	0.357±1.008$^{\alpha\beta\gamma}$	0.65±0.128$^{\alpha\beta\gamma}$	0.672±1.08$^{\alpha\beta\gamma}$
Baseline	**0.126±0.004$^{\beta\gamma}$**	**0.233 ± 0.006$^{\beta\gamma}$**	**0.213 ± 0.006$^{\beta\gamma}$**	**0.161±0.1**	**0.29±0.23**	**0.27±0.11**

Values are expressed as mean±standard deviation (n=5). Significance at p<0.05. PRE = pre-treatment, CO = co-treatment, POST = post-treatment. NS = DOX-intoxicated normal saline treated group (negative control), ALA = DOX-intoxicated α-lipoic acid treated group (positive control), 200mgTCAE = DOX-intoxicated 200mg/kg body weight T. cacao treated group, 400mgTCAE = DOX-intoxicated 400mg/kg body weight T. cacao treated group, 800mgTCAE = DOX-intoxicated 800mg/kg body weight T. cacao treated group

Table 9. Effects of *Theobroma cacao* on NADPH oxidase, xanthine oxidase and myeloperoxidase activities in renal and splenic tissues of DOX-exposed rats

Treatment	Kidneys			Spleen		
	NADPH oxidase (units/mg protein)	Xanthine oxidase (units/mg protein)	Myeloperoxidase (units/mg protein)	NADPH oxidase (units/mg protein)	Xanthine Oxidase (units/mg protein)	Myeloperoxidase (units/mg protein)
PRE-NS	24.105±0.247$^{\alpha\gamma}$	25.903±0.241$^{\alpha\gamma}$	25.469±0.817$^{\alpha\gamma}$	31.49±0.334$^{\alpha\gamma}$	33.49±0.634$^{\alpha\gamma}$	33.34±0.123$^{\alpha\gamma}$
PRE-ALA	7.253 ± 0.794$^{\alpha\beta}$	7.558 ±0.687$^{\alpha\beta}$	7.874 ± 0.862$^{\alpha\beta}$	11.02±0.636$^{\alpha\beta}$	9.67±1.436$^{\alpha\beta}$	9.65±0.336$^{\alpha\beta}$
PRE-200 mgTCAE	4.396 ± 0.174$^{\alpha\beta\gamma}$	4.691 ±0.209$^{\alpha\beta\gamma}$	4.771 ± 0.189$^{\alpha\beta\gamma}$	5.676±0.241$^{\alpha\beta\gamma}$	6.02±0.571$^{\alpha\beta\gamma}$	6.45±0.281$^{\alpha\beta\gamma}$
PRE-400 mgTCAE	2.929 ± 0.095$^{\alpha\beta\gamma}$	3.219 ±0.153$^{\alpha\beta\gamma}$	3.141 ± 0.104$^{\alpha\beta\gamma}$	3.53±0.287$^{\alpha\beta\gamma}$	4.05±0.287$^{\alpha\beta\gamma}$	3.82±0.287$^{\alpha\beta\gamma}$
PRE-800 mgTCAE	1.760 ± 0.035$^{\alpha\beta\gamma}$	1.907 ±0.062$^{\alpha\beta\gamma}$	1.911 ± 0.038$^{\alpha\beta\gamma}$	2.47±0.343$^{\alpha\beta\gamma}$	2.42±0.343$^{\alpha\beta\gamma}$	2.42±0.443$^{\alpha\beta\gamma}$
Co-NS	16.509±0.508$^{\alpha\gamma}$	17.842±0.576$^{\alpha\gamma}$	18.155±0.638$^{\alpha\gamma}$	20.56±0.341$^{\alpha\gamma}$	22.44±0.411$^{\alpha\gamma}$	22.165±0.341$^{\alpha\gamma}$
Co-ALA	5.060 ± 0.410$^{\alpha\beta}$	5.462 ± 0.361$^{\alpha\beta}$	5.493 ± 0.445$^{\alpha\beta}$	7.06±0.355$^{\alpha\beta}$	6.82±0.155$^{\alpha\beta}$	7.14±0.571$^{\alpha\beta}$
Co-200 mgTCAE	3.092 ±0.283$^{\alpha\beta\gamma}$	3.211 ±0.256$^{\alpha\beta\gamma}$	3.244 ± 0.307$^{\alpha\beta\gamma}$	3.76±0.563$^{\alpha\beta\gamma}$	4.4±0.863$^{\alpha\beta\gamma}$	3.976±0.363$^{\alpha\beta\gamma}$
Co-400 mgTCAE	1.974 ± 0.090$^{\alpha\beta\gamma}$	2.120 ±0.097$^{\alpha\beta\gamma}$	2.128 ± 0.109$^{\alpha\beta\gamma}$	2.48±0.105$^{\alpha\beta\gamma}$	2.75±0.205$^{\alpha\beta\gamma}$	2.68±0.205$^{\alpha\beta\gamma}$
Co-800 mgTCAE	1.187± 0.040$^{\alpha\beta\gamma}$	1.300 ±0.052$^{\alpha\beta\gamma}$	1.309 ± 0.047$^{\alpha\beta\gamma}$	1.55±0.84$^{\alpha\beta\gamma}$	1.67±0.184$^{\alpha\beta\gamma}$	1.57±0.84$^{\alpha\beta\gamma}$
POST-NS	11.839±0.391$^{\alpha\gamma}$	12.713±0.420$^{\alpha\gamma}$	12.852±0.425$^{\alpha\gamma}$	15.15±0.504$^{\alpha\gamma}$	16.27±0.274$^{\alpha\gamma}$	16.15±0.234$^{\alpha\gamma}$
POST-ALA	3.414 ± 0.341$^{\alpha\beta}$	3.666 ± 0.367$^{\alpha\beta}$	3.706 ± 0.371$^{\alpha\beta}$	4.371±0.124$^{\alpha\beta}$	4.46±0.124$^{\alpha\beta}$	4.74±0.124$^{\alpha\beta}$
POST-200mgTCAE	2.128 ± 0.094$^{\alpha\beta\gamma}$	2.285 ±0.101$^{\alpha\beta\gamma}$	2.310 ± 0.102$^{\alpha\beta\gamma}$	2.72±0.388$^{\alpha\beta\gamma}$	2.92±0.95$^{\alpha\beta\gamma}$	2.95±0.488$^{\alpha\beta\gamma}$
POST-400mgTCAE	1.421 ± 0.072$^{\alpha\beta\gamma}$	1.527 ±0.077$^{\alpha\beta\gamma}$	1.543 ±0.078$^{\alpha\beta\gamma}$	1.819±0.92$^{\alpha\beta\gamma}$	1.95±0.475$^{\alpha\beta\gamma}$	1.98±0.92$^{\alpha\beta\gamma}$
POST-800mgTCAE	0.861 ± 0.019$^{\alpha\beta\gamma}$	0.925 ±0.020$^{\alpha\beta\gamma}$	0.936 ± 0.020$^{\alpha\beta\gamma}$	1.10±0.83$^{\alpha\beta\gamma}$	1.18±0.125$^{\alpha\beta\gamma}$	1.32±0.83$^{\alpha\beta\gamma}$
Baseline	**0.387 ± 0.011$^{\beta\gamma}$**	**0.415 ±0.012$^{\beta\gamma}$**	**0.420 ± 0.013 $^{\beta\gamma}$**	**0.55±0.062**	**0.531±0.131**	**0.52±0.132**

Values are expressed as mean±standard deviation (n=5). Significance at p<0.05. PRE = pre-treatment, CO = co-treatment, POST = post-treatment. NS = DOX-intoxicated normal saline treated group (negative control), ALA = DOX-intoxicated α-lipoic acid treated group (positive control), 200mgTCAE = DOX-intoxicated 200mg/kg body weight T. cacao treated group, 400mgTCAE = DOX-intoxicated 400mg/kg body weight T. cacao treated group, 800mgTCAE = DOX-intoxicated 800mg/kg body weight T. cacao treated group

Table 10. Effects of *Theobroma cacao* on catalase and superoxide dismutase activities in renal and splenic tissues of DOX-exposed rats

Treatment	Kidneys		Spleen	
	Catalase (µmol/mg protein)	Superoxide dismutase (units/mg protein)	Catalase (µmol/mg protein)	Superoxide dismutase (units/mg protein)
PRE-NS	$6.250 \pm 0.113^{\alpha}$	$6.263 \pm 1.269^{\alpha}$	$2.66 \pm 0.181^{\alpha\gamma}$	$8.63 \pm 1.18^{\alpha\gamma}$
PRE-ALA	$10.483 \pm 0.616^{\alpha}$	$10.982 \pm 0.646^{\alpha}$	$13.18 \pm 0.08^{\alpha\beta}$	$14.18 \pm 0.58^{\alpha\beta}$
PRE-200 mgTCAE	$5.722 \pm 0.374^{\alpha}$	$5.995 \pm 0.392^{\alpha}$	$6.69 \pm 0.433^{\alpha\beta\gamma}$	$7.80 \pm 0.933^{\alpha\beta\gamma}$
PRE-400 mgTCAE	$7.886 \pm 0.229^{\alpha}$	$8.261 \pm 0.240^{\alpha}$	$10.06 \pm 0.519^{\alpha\beta\gamma}$	$10.39 \pm 0.519^{\alpha\beta\gamma}$
PRE-800 mgTCAE	$8.880 \pm 0.084^{\alpha}$	$9.302 \pm 0.088^{\alpha}$	$11.36 \pm 0.1463^{\alpha\beta\gamma}$	$11.79 \pm 0.63^{\alpha\beta\gamma}$
Co-NS	$11.958 \pm 0.760^{\alpha\gamma}$	$12.817 \pm 0.795^{\alpha\gamma}$	$15.29 \pm 0.341^{\alpha\gamma}$	$16.22 \pm 0.441^{\alpha\gamma}$
Co-ALA	$18.275 \pm 3.164^{\alpha\beta}$	$19.145 \pm 3.314^{\alpha\beta}$	$23.49 \pm 0.61^{\alpha\beta}$	$21.81 \pm 0.361^{\alpha\beta}$
Co-200 mgTCAE	$23.758 \pm 4.113^{\alpha\beta}$	$24.889 \pm 4.309^{\alpha\beta}$	$32.08 \pm 0.263^{\alpha\beta\gamma}$	$33.08 \pm 0.553^{\alpha\beta\gamma}$
Co-400 mgTCAE	$31.484 \pm 2.505^{\alpha\beta\gamma}$	$32.983 \pm 2.624^{\alpha\beta\gamma}$	$40.4 \pm 0.328^{\alpha\beta\gamma}$	$42.4 \pm 0.428^{\alpha\beta\gamma}$
Co-800 mgTCAE	$44.963 \pm 6.056^{\beta\gamma}$	$44.686 \pm 6.816^{\alpha\beta\gamma}$	$54.48 \pm 0.85^{\alpha\beta\gamma}$	$60.40 \pm 0.155^{\alpha\beta\gamma}$
POST-NS	$22.751 \pm 1.172^{\alpha\gamma}$	$23.834 \pm 1.228^{\beta\gamma}$	$29.23 \pm 0.153^{\alpha\gamma}$	$30.23 \pm 0.353^{\alpha\gamma}$
POST-ALA	$58.750 \pm 4.493^{\alpha\beta}$	$61.546 \pm 4.706^{\alpha\beta}$	$75.200 \pm 0.424^{\alpha\beta}$	$78.77 \pm 0.424^{\alpha\beta}$
POST-200 mgTCAE	$76.370 \pm 5.840^{\alpha\beta\gamma}$	$80.010 \pm 6.118^{\alpha\beta\gamma}$	$97.70 \pm 0.123^{\alpha\beta\gamma}$	$102.42 \pm 0.133^{\alpha\beta\gamma}$
POST-400 mgTCAE	$99.875 \pm 7.637^{\alpha\beta\gamma}$	$104.62 \pm 8.000^{\alpha\beta\gamma}$	$127 \pm 0.99^{\alpha\beta\gamma}$	$133.92 \pm 0.829^{\alpha\beta\gamma}$
POST-800 mgTCAE	$124.26 \pm 10.081^{\alpha\beta\gamma}$	$133.39 \pm 11.836^{\alpha\beta\gamma}$	$159.23 \pm 0.83^{\alpha\beta\gamma}$	$165.54 \pm 1.53^{\alpha\beta\gamma}$
Baseline	**$49.503 \pm 1.144^{\beta\gamma}$**	**$51.859 \pm 1.198^{\beta\gamma}$**	**63.3±0.142**	**66.36±0.342**

Values are expressed as mean ± standard deviation (n=5). Significance at p<0.05. PRE = pre-treatment, CO = co-treatment, POST = post-treatment. NS = DOX-intoxicated normal saline treated group (negative control), ALA = DOX-intoxicated α-lipoic acid treated group (positive control), 200mgTCAE = DOX-intoxicated 200mg/kg body weight T. cacao treated group, 400mgTCAE = DOX-intoxicated 400mg/kg body weight T. cacao treated group, 800mgTCAE = DOX-intoxicated 800mg/kg body weight T. cacao treated group

Table 11. Effects of *Theobroma cacao* on reduced glutathione concentration, glutathione S-transferase and glutathione peroxidase activities in renal and splenic tissues of DOX-exposed rats

Treatment	Kidneys			Spleen		
	Reduced glutathione (µg/ml)	Glutathione-S-Transferase (µmol/min/mg protein)	Glutathione peroxidase (GSH consumed/mg protein)	Reduced glutathione (µg/ml)	Glutathione-S-Transferase (µmol/min/mg protein)	Glutathione peroxidase (GSH consumed/mg protein)
PRE-NS	1.38 ± 0.05 $^{\alpha}$	66.59 ± 0.07 $^{\alpha}$	6.72 ± 0.12 $^{\alpha}$	1.71± 0.352$^{\alpha\gamma}$	8.66±0.418$^{\alpha\gamma}$	8.29± 0.452$^{\alpha\gamma}$
PRE-ALA	2.28 ± 0.13 $^{\alpha}$	11.41 ± 0.67 $^{\alpha}$	11.58 ± 0.68 $^{\alpha}$	2.91±0.719 $^{\alpha\beta}$	14.62±0.758$^{\alpha\beta}$	12.10±0.59$^{\alpha\beta}$
PRE-200 mgTCAE	1.22 ± 0.10 $^{\alpha}$	6.11 ± 0.50 $^{\alpha}$	6.20 ± 0.51 $^{\alpha}$	1.5±0.441$^{\alpha\beta\gamma}$	7.85±0.433$^{\alpha\beta\gamma}$	8.34±0.341$^{\alpha\beta\gamma}$
PRE-400 mgTCAE	1.70 ± 0.05 $^{\alpha}$	8.58 ± 0.25 $^{\alpha}$	8.62 ± 0.25 $^{\alpha}$	2.19±0.62$^{\alpha\beta\gamma}$	11.30± 0.319$^{\alpha\beta\gamma}$	11.67±0.426$^{\alpha\beta\gamma}$
PRE-800 mgTCAE	1.93 ± 0.02 $^{\alpha}$	9.66 ± 0.09 $^{\alpha}$	9.80 ± 0.09 $^{\alpha}$	2.46±0.204$^{\alpha\beta\gamma}$	11.2±0.63$^{\alpha\beta\gamma}$	13.22±0.104$^{\alpha\beta\gamma}$
Co-NS	2.66 ± 0.16 $^{\alpha\gamma}$	13.31 ±0.83$^{\alpha\gamma}$	13.21± 0.84$^{\alpha\gamma}$	3.32±0.234$^{\alpha\gamma}$	16.09±0.191$^{\alpha\gamma}$	17.00±0.434$^{\alpha\gamma}$
Co-ALA	4.22 ± 0.69 $^{\alpha\beta}$	19.88 ±3.44$^{\alpha\beta}$	20.18± 3.49$^{\alpha\beta}$	5.08±0.264$^{\alpha\beta}$	27.90±0.56$^{\alpha\beta}$	27.37±0.564$^{\alpha\beta}$
Co-200 mgTCAE	5.16 ± 0.89 $^{\alpha\beta}$	27.48 ±4.48$^{\alpha\beta\gamma}$	26.24± 4.54$^{\alpha\beta\gamma}$	6.6±1.146$^{\alpha\beta\gamma}$	34.63±0.453$^{\alpha\beta\gamma}$	35.11±0.46$^{\alpha\beta\gamma}$
Co-400 mgTCAE	6.84 ± 0.54 $^{\alpha\beta\gamma}$	34.47 ±2.82$^{\alpha\beta\gamma}$	35.08± 2.92$^{\alpha\beta\gamma}$	8.75±0.230$^{\alpha\beta\gamma}$	43.84±0.428$^{\alpha\beta\gamma}$	43.98±0.230$^{\alpha\beta\gamma}$
Co-800 mgTCAE	9.77 ± 1.31 $^{\alpha\beta\gamma}$	48.92 ±6.59$^{\beta\gamma}$	49.65± 6.69$^{\alpha\beta\gamma}$	12.03±1.68$^{\alpha\beta\gamma}$	61.40±0.255$^{\alpha\beta\gamma}$	63.07±0.88$^{\alpha\beta\gamma}$
POST-NS	4.94 ± 0.25$^{\alpha\gamma}$	24.75 ± 1.28$^{\alpha\gamma}$	25.13± 1.29$^{\alpha\gamma}$	6.32±0.32$^{\alpha\gamma}$	31.16±0.633$^{\alpha\gamma}$	32.16±0.283$^{\alpha\gamma}$
POST-ALA	12.77 ± 0.98$^{\alpha\beta}$	63.92 ± 4.89$^{\alpha\beta}$	64.88± 4.96$^{\alpha\beta}$	16.34±1.181$^{\alpha\beta}$	81.81±0.324$^{\alpha\beta}$	83.04±1.18$^{\alpha\beta}$
POST-200 mgTCAE	16.60 ± 1.26$^{\alpha\beta\gamma}$	83.10 ± 6.35$^{\alpha\beta\gamma}$	84.34 ± 6.45$^{\alpha\beta\gamma}$	21.24±1.67$^{\alpha\beta\gamma}$	106.04±0.733$^{\alpha\beta\gamma}$	107.06±1.17$^{\alpha\beta\gamma}$
POST-400 mgTCAE	21.71 ± 1.66$^{\alpha\beta\gamma}$	108.66± 8.31$^{\alpha\beta\gamma}$	110.29± 8.43$^{\alpha\beta\gamma}$	27.78±1.12$^{\alpha\beta\gamma}$	139.92±0.329$^{\alpha\beta\gamma}$	141.03±1.175$^{\alpha\beta\gamma}$
POST-800 mgTCAE	27.01 ± 2.19$^{\alpha\beta\gamma}$	135.20±10.97$^{\alpha\beta\gamma}$	137.22±11.13$^{\alpha\beta\gamma}$	34.55±2.80$^{\alpha\beta\gamma}$	173.05±1.53$^{\alpha\beta\gamma}$	175.23±1.44$^{\alpha\beta\gamma\delta}$
Baseline	**29.11 ± 0.67 $^{\beta\gamma}$**	**53.86 ± 1.24 $^{\beta\gamma}$**	**54.67 ± 1.26 $^{\beta\gamma}$**	**37.62±0.322**	**68.93±0.34**	**69.97±0.53**

Values are expressed as mean±standard deviation (n=5). Significance at p<0.05. PRE = pre-treatment, CO = co-treatment, POST = post-treatment. NS = DOX-intoxicated normal saline treated group (negative control), ALA = DOX-intoxicated α-lipoic acid treated group (positive control), 200mgTCAE = DOX-intoxicated 200mg/kg body weight T. cacao treated group, 400mgTCAE = DOX-intoxicated 400mg/kg body weight T. cacao treated group, 800mgTCAE = DOX-intoxicated 800mg/kg body weight T. cacao treated group

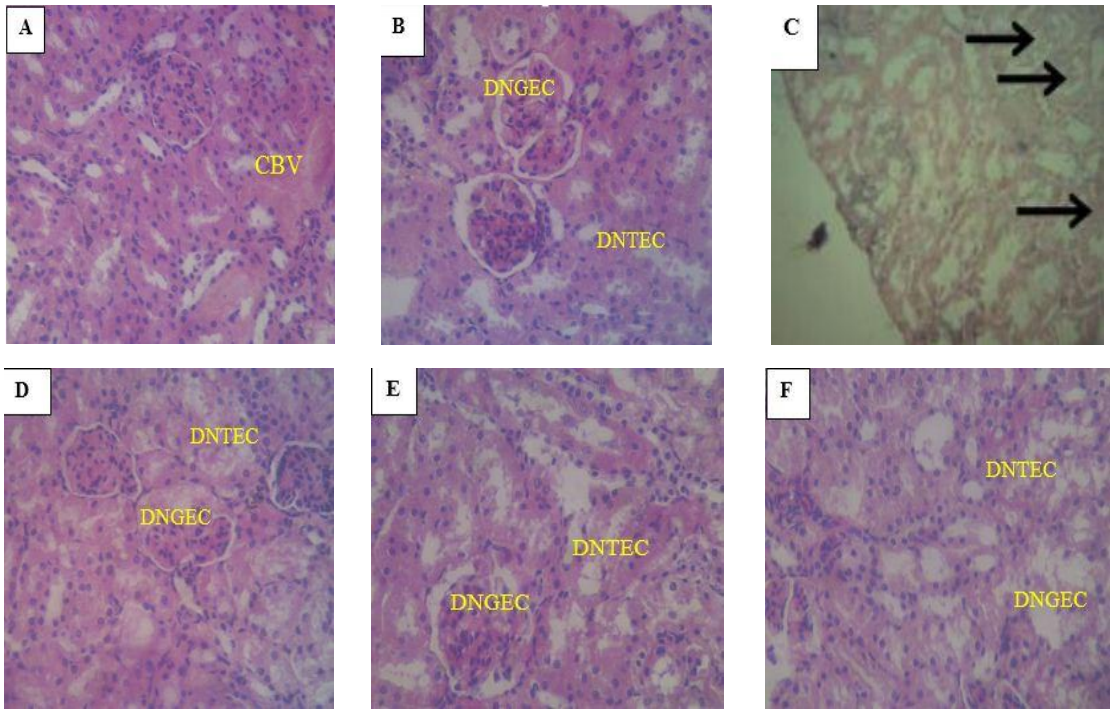


Fig. 4. Histology of kidney tissue sections. Photomicrograph showing effects of *Theobroma cacao* in rats pre-intoxicated with doxorubicin (x400; H&E)

Plates A: Showing mild Congestion of blood vessel (CBV)

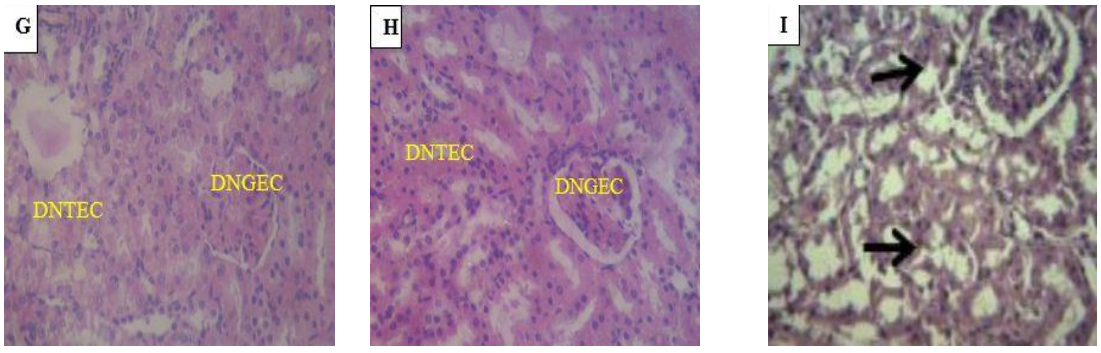
Plates B: Showing severe degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)

Plates C: Showing severe degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)

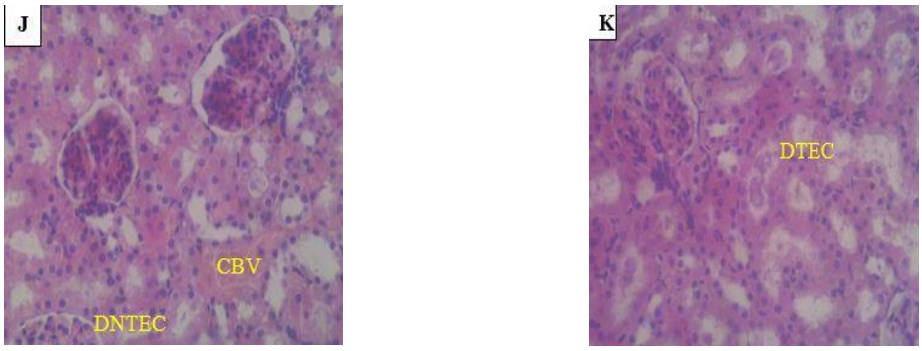
Plates D: Showing severe degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)

Plates E: Showing severe degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)

Plates F: Showing severe degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)



Co-NS **Co-ALA** **Co-200TCAE**



Co-400TCAE **Co-800TCAE**

Fig. 5. Histology of kidney tissue sections. Photomicrograph showing effects of *Theobroma cacao* in rats co-intoxicated with doxorubicin (x400; H&E)
Plates G: showing severe degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)
Plates H: showing moderate degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)
Plates I: Showing moderate degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)
Plates J: showing moderate congestion of blood vessels (CBV), degeneration and necrosis of tubular epithelial cells (DNTEC)
Plates K: Showing moderate degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)

Table 12. Effects of *Theobroma cacao* on α-tocopherol and ascorbic acid concentrations in renal and splenic tissues of DOX-exposed rats

Treatment	Kidneys		Spleen	
	α-Tocopherol (μmol/L)	Ascorbic acid (μmol/L)	α-Tocopherol (μmol/L)	Ascorbic acid (μmol/L)
PRE-NS	$5.64 \pm 0.08^{\alpha}$	$6.01 \pm 0.03^{\alpha}$	$7.22 \pm 0.122^{\alpha\gamma}$	$7.71 \pm 0.152^{\alpha\gamma}$
PRE-ALA	$9.48 \pm 0.56^{\alpha}$	$10.25 \pm 0.60^{\alpha}$	$12.70 \pm 0.164^{\alpha\beta}$	$12.96 \pm 0.640^{\alpha\beta}$
PRE-200mgTCAE	$5.07 \pm 0.41^{\alpha}$	$5.60 \pm 0.37^{\alpha}$	$6.42 \pm 0.668^{\alpha\beta\gamma}$	$7.12 \pm 0.464^{\alpha\gamma}$
PRE-400mgTCAE	$7.05 \pm 0.21^{\alpha}$	$7.71 \pm 0.22^{\alpha}$	$8.86 \pm 0.62^{\alpha\beta\gamma}$	$8.26 \pm 0.62^{\alpha\beta\gamma}$
PRE-800mgTCAE	$8.03 \pm 0.08^{\alpha}$	$8.68 \pm 0.08^{\alpha}$	$10.19 \pm 0.164^{\alpha\beta\gamma}$	$11.19 \pm 0.164^{\alpha\beta\gamma}$
Co-NS	$10.81 \pm 0.69^{\alpha\gamma}$	$11.70 \pm 0.74^{\alpha\gamma}$	$13.0 \pm 0.014^{\alpha\gamma}$	$15.40 \pm 1.014^{\alpha\gamma}$
Co-ALA	$16.52 \pm 2.86^{\alpha\beta}$	$17.87 \pm 3.09^{\alpha\beta}$	$21.30 \pm 3.72^{\alpha\beta}$	$24.34 \pm 3.264^{\alpha\beta}$
Co-200mgTCAE	$21.48 \pm 3.71^{\alpha\beta}$	$23.23 \pm 4.02^{\alpha\beta\gamma}$	$27.82 \pm 4.146^{\alpha\beta\gamma}$	$31.144 \pm 0.146^{\alpha\beta\gamma}$
Co-400mgTCAE	$28.72 \pm 2.40^{\alpha\beta\gamma}$	$30.79 \pm 2.45^{\alpha\beta\gamma}$	$36.81 \pm 3.317^{\alpha\beta\gamma}$	$39.52 \pm 0.317^{\alpha\beta\gamma}$
Co-800mgTCAE	$38.57 \pm 5.88^{\alpha\beta\gamma}$	$43.97 \pm 5.93^{\beta\gamma}$	$52.02 \pm 7.738^{\alpha\beta\gamma}$	$55.45 \pm 0.768^{\alpha\beta\gamma\delta\varepsilon}$
POST-NS	$20.57 \pm 1.06^{\alpha\gamma}$	$22.25 \pm 1.15^{\alpha\gamma}$	$26.33 \pm 1.35^{\alpha\gamma}$	$28.46 \pm 1.32^{\alpha\gamma}$
POST-ALA	$53.11 \pm 4.06^{\alpha\beta}$	$57.46 \pm 4.39^{\alpha\beta}$	$67.99 \pm 5.56^{\alpha\beta}$	$73.54 \pm 0.561^{\alpha\beta}$
POST-200mgTCAE	$69.05 \pm 5.28^{\alpha\beta\gamma}$	$74.70 \pm 5.71^{\alpha\beta\gamma}$	$88.39 \pm 6.767^{\alpha\beta\gamma}$	$95.61 \pm 0.767^{\alpha\beta\gamma}$
POST-400mgTCAE	$90.30 \pm 6.91^{\alpha\beta\gamma}$	$97.68 \pm 7.47^{\alpha\beta\gamma}$	$115.58 \pm 8.52^{\alpha\beta\gamma}$	$125.05 \pm 1.52^{\alpha\beta\gamma}$
POST-800mgTCAE	$113.35 \pm 9.11^{\alpha\beta\gamma}$	$121.53 \pm 9.86^{\alpha\beta\gamma}$	$122.75 \pm 1.80^{\alpha\beta\gamma}$	$155 \pm 1.80^{\alpha\beta\gamma\delta\varepsilon}$
Baseline	**$44.75 \pm 1.03^{\beta\gamma}$**	**$48.42 \pm 1.19^{\beta\gamma}$**	**57.97±0.32**	**61.97±0.322**

Values are expressed as mean±standard deviation (n=5). Significance at p<0.05. PRE = pre-treatment, CO = co-treatment, POST = post-treatment. NS = DOX-intoxicated normal saline treated group (negative control), ALA = DOX-intoxicated α-lipoic acid treated group (positive control), 200mgTCAE = DOX-intoxicated 200mg/kg body weight T. cacao treated group, 400mgTCAE = DOX-intoxicated 400mg/kg body weight T. cacao treated group, 800mgTCAE = DOX-intoxicated 800mg/kg body weight T. cacao treated group

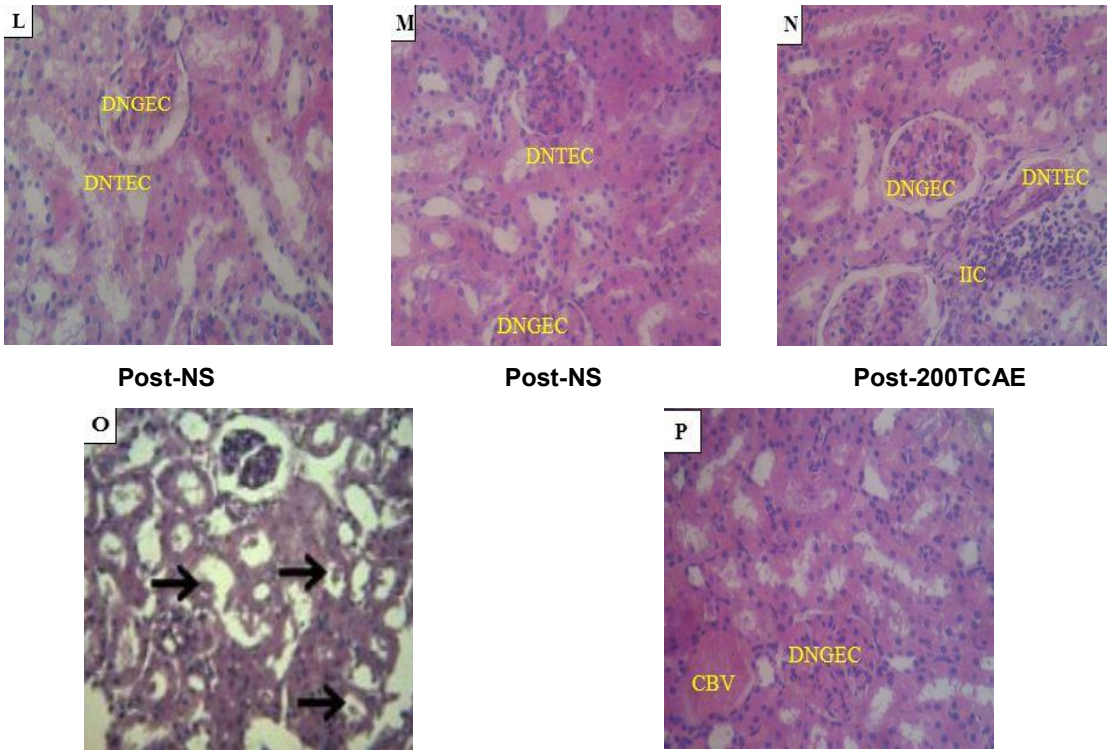
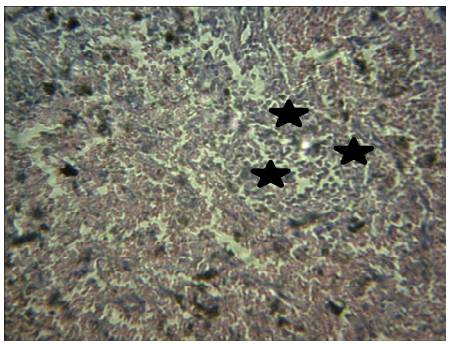


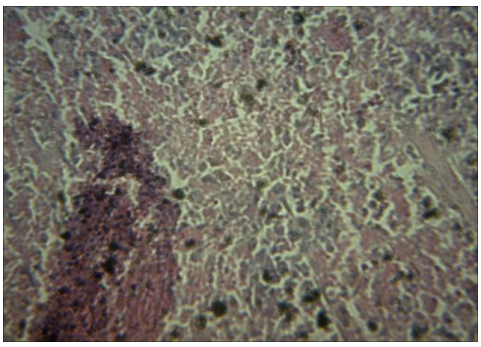
Fig. 6. Histology of kidney tissue sections. Photomicrograph showing effects of *Theobroma cacao* in rats post-intoxicated with doxorubicin (x400; H&E)
Plates L: Showing severe degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)
Plates M: Showing moderate degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)
Plates N: Showing moderate degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC) and infiltration by inflammatory cells (IIC)
Plates O: Showing moderate congestion of blood vessels (CBV), degeneration and necrosis of tubular epithelial cells (DNTEC)
Plates P: Showing moderate degeneration and necrosis of tubular (DNTEC) and glomerular epithelial cells (DNGEC)

A. DOX pre-intoxicated normal saline treated



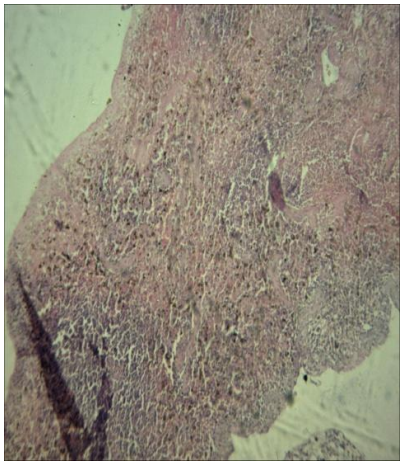
The section of spleen showing a severe generalized congestion of the spleen

B. DOX pre-intoxicated 20 mg/kg α-lipoic acid treated



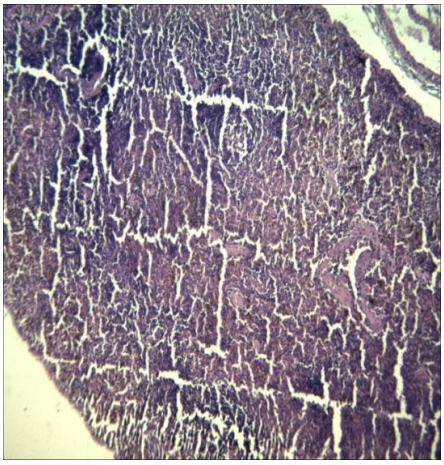
The section of spleen showing no visible lesions seen

C. DOX pre-intoxicated 200 mg/kg *T. cacao* treated



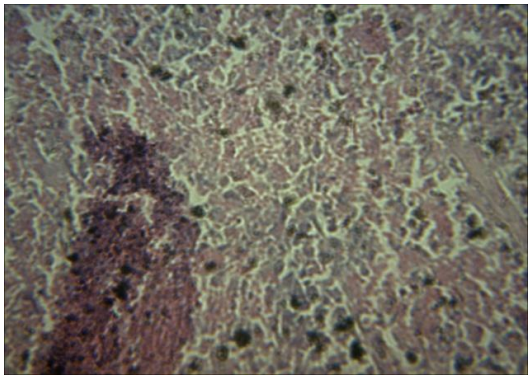
The section of spleen showing no visible lesions seen

D. DOX pre-intoxicated 400 mg/kg *T. cacao* treated



The section of spleen showing no visible lesions seen

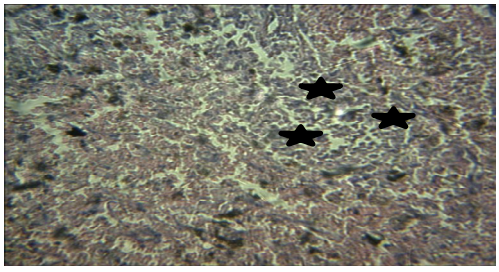
E. DOX pre-intoxicated 800 mg/kg *T. cacao* treated



The section of spleen showing no visible lesions seen

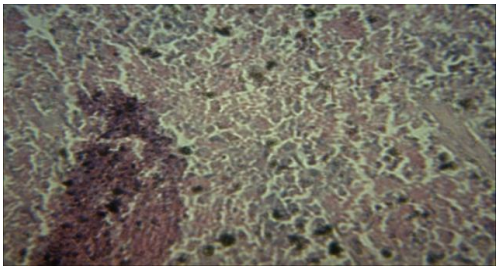
Fig. 7. Histology of spleen tissue sections. Photomicrograph showing effects of *Theobroma cacao* in rats pre-intoxicated with doxorubicin (x400; H&E)

A. DOX-intoxicated normal saline co-treated



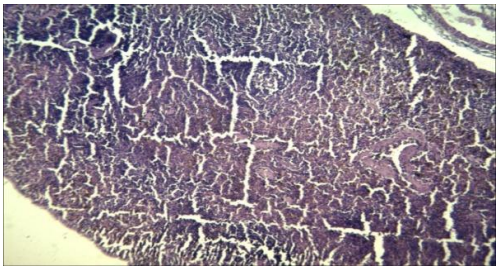
The section of spleen showing a severe generalised congestion of the spleen

B. DOX-intoxicated alpha lipoic acid co-treated



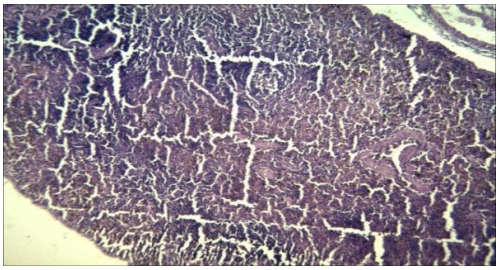
The section of spleen showing no visible lesions seen

C. DOX-intoxicated 200 mg/kg *T. cacao* co-treated



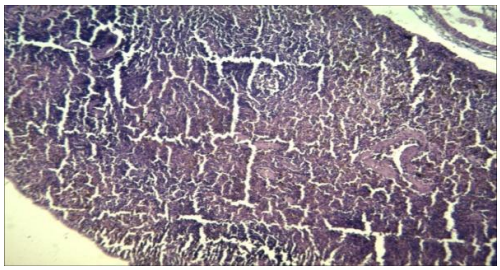
The section of spleen showing no visible lesions seen

D. DOX-intoxicated 400 mg/kg *T. cacao* co-treated



The section of spleen showing no visible lesions seen

E. DOX-intoxicated 800 mg/kg *T. cacao* co-treated



The section of spleen showing no visible lesions seen

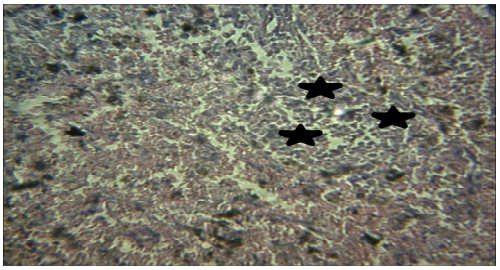
Fig. 8. Histology of spleen tissue sections. Photomicrograph showing effects of *Theobroma cacao* in rats co-intoxicated with doxorubicin (x400; H&E)

4. DISCUSSION

With the possession of unpaired electrons, free radicals are usually unstable and highly reactive. Peroxyl radical is a key step in lipid peroxidation and is an important cause of cell membrane destruction and thus tissue damage [56]. It has also been suggested that oxidative modification of low-density lipoprotein (LDL) is the main cause of atherosclerosis, which is highly related to peroxyl radical formation [57]. Antioxidants can scavenge the free radicals and inhibit lipid peroxidation. Antioxidants are added to a variety of foods to prevent or deter free radical-induced lipid oxidation of food [58]. Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer. In LDL oxidation, the amount of TBARS, the breakdown product of LDL during lipid peroxidation, can be used as an index of lipid peroxidation [59]. Our results showed that *T. cacao* stem bark aqueous extract showed significant dose-dependent peroxyl radical scavenging potential but not as much as the standard, α-tocopherol (Fig. 3). As phenolic antioxidants are suggested to act as inhibitors of LDL oxidation by means of free radical scavenging, so it is expected that the presence of these compounds in these extracts might be responsible for inhibiting the LDL oxidation by donating the hydrogen atom [60]. The main interest of the recent research suggests that LDL oxidation may play an important role in the pathogenesis of atherosclerotic complications, including coronary heart disease (CHD). The radical-mediated oxidative chain reaction is a possible mechanism involved in LDL oxidation. LDL oxidation is believed to be a complex and multistep process involving both lipid and protein fractions through different mechanisms [61]. Antioxidants, including vitamins C and E, flavonoids, and other plant phenolics, have been shown to suppress LDL oxidation and delay the development of heart diseases [62]. Nitric oxide is a free radical generated by endothelial cells, macrophages, neurons etc., and it is involved in the regulation of various physiological process [63]. Excess concentration of NO is associated with several diseases [64,65]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radicals [31,66]. In the present study, the

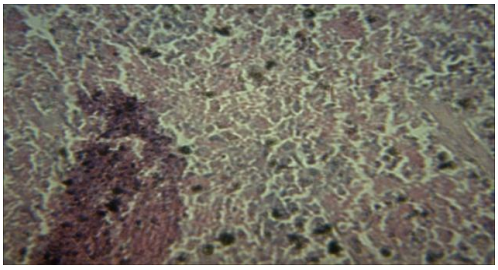
T. cacao showed a significant dose-dependent activity in scavenging nitric oxide and in competing with oxygen to react with nitric oxide (Fig. 2). The standard, ascorbic acid exhibited the highest activity in scavenging nitric oxide. Phenolic and polyphenolic compounds are ubiquitous in plants. They are a major class of phytochemicals with interesting functional properties that are of potential benefit to human health. Numerous studies have linked the antioxidant properties of *T. cacao* extracts to their flavonoid and phenolic compounds [67,68,69]. The higher value of the phenolic and flavonoid contents in TCAE may be due to the polar nature of these components. Our observations corroborate the findings of other studies which have shown that TCAE have good antioxidant properties (Fig. 1), which may be linked to its high polyphenolic content [70,71,72].

A. DOX post-intoxicated normal saline treated



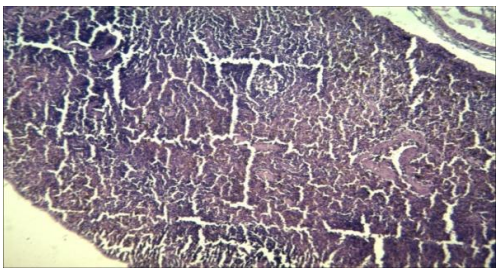
The section of spleen showing a severe generalised congestion of the spleen

B. DOX post-intoxicated α-lipoic acid treated



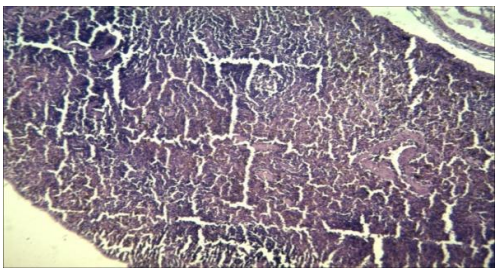
The section of spleen showing no visible lesions seen

C. DOX post-intoxicated 200 mg/kg *T. cacao* treated



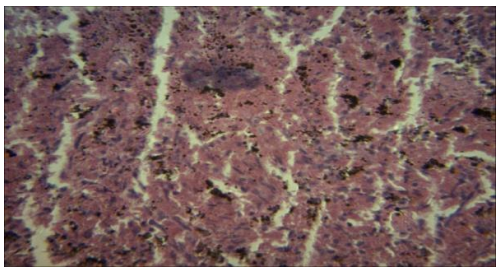
The section of spleen showing no visible lesions seen

D. DOX post-intoxicated 400 mg/kg *T. cacao* treated



The section of spleen showing no visible lesions seen

E. DOX post-intoxicated 800 mg/kg *T. cacao* treated



The section of spleen showing no visible lesions seen

Fig. 9. Histology of spleen tissue sections. Photomicrograph showing effects of *Theobroma cacao* in rats post-intoxicated with doxorubicin (x400; H&E)

Doxorubicin (DOX), a quinone-containing anthracycline antibiotic, is an important agent against a wide spectrum of human neoplasms. However, its toxicity limits usage in cancer chemotherapy [73]. It has been shown that free radicals are involved in doxorubicin-induced toxicities [74]. It has been reported that doxorubicin caused severe damage in some organs like liver, heart, spleen and kidneys [75]. The chemical structure of doxorubicin causes the generation of free radicals and the induction of oxidative stress that correlates with cellular injury [76]. Doxorubicin causes an imbalance between free oxygen radicals (ROS) and antioxidants. The disturbance in oxidant–antioxidant systems results in tissue injury that is demonstrated with lipid peroxidation and protein oxidation in tissue [77].

In the present study, our results elucidated a very highly significant increase in the level of urea and creatinine levels in the serum of DOX-intoxicated rats when compared to normal rats. The elevated serum levels of urea and creatinine indicate reduced ability of the kidney to eliminate toxic metabolic substances. This is in accordance with Injac et al. [78] who investigated that DOX-induced nephrotoxicity causes increased capillary permeability and glomerular atrophy. Moreover, these results are in agreement with previously published reports of El-Moselhy and El-Sheikh [79] who demonstrated that DOX caused deterioration in renal function as it significantly increased blood urea nitrogen (BUN), creatinine, compared to control, with distortion in normal renal histology. Also the elevated levels of these parameters seen in the current study run in parallel with those of Ayla et al. [2], and Abou Seif [80] who showed that DOX caused a marked rise in serum urea, creatinine and potassium ion, splenic lactate dehydrogenase (LDH), acid phosphatase (ACP), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) activities with concurrent depression in chloride, bicarbonate and sodium levels. Concomitant to these biochemical changes in kidney were observed in DOX-administered animals. The previous deleterious biochemical alterations of the present study were associated with a marked elevation of kidney and spleen hydrogen peroxide (H_2O_2)generated, lipid peroxidation (malondialdehyde; MDA), protein carbonylation (PC), myeloperoxidase (MPX), NADPH oxidase (NOX), xanthine oxidase (XO) and a significant decrease of non-enzymatic antioxidant (reduced glutathione (GSH), α-tocopherol (α-toc) and ascorbic acid (AsA)) content and enzymatic antioxidants (catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione-S-transferase (GST)) enzyme activities. These results are in agreement with many other authors [81,82,83] who stated that one of the most prevailing hypothesis of kidney and spleen damage from DOX administration is the ability of the drug to produce reactive oxygen species (ROS) and suppress antioxidant defense mechanism. They also revealed that the increased lipid peroxidation play a critical role in kidney and spleen injury. Yeh et al. [84] reported that rats administrated with DOX showed increase in the level of lipid peroxidation (MDA) and depressed antioxidant enzymes activities (SOD, GPX and GSH) and elevate apoptotic index. Mohan et al. [85] reported that DOX might cause excessive consumption, reduced production or chemical deactivation of these enzymes. The putative role of oxidative stress in the induction of DOX nephrosis and splenotoxicity may be supported by the protective effect of the activation of superoxide dismutase on the development of DOX nephropathy and splenopathy [86]. DOX-induced organ toxicity is well documented [87, 85, 78]. DOX induced a severe nephrotic syndrome with massive albuminuria, proteinuria, hyperlipidemia, hypo-albuminemia and hypoproteinemia; and splenopathy with depression in the activities of LDH, GGT and ALP. These changes were associated with a marked decrease in the antioxidant defense of the kidney and spleen as manifested by the significant increase in lipid peroxides, GSH depletion and a significant decrease in CAT activity. The changes reflect many functional alterations such as a drop in glomerular filtration rate, glomerular capillary damage and tubulotoxicity; thus approved that DOX induced pathogenesis of nephropathy through involvement of free radicals [88].

The treatment of doxorubicin-administered animals with *Theobroma cacao* stem bark aqueous extract (TCAE) successfully improved the elevated splenic LDH, ACP, ALP and GGT activities; serum creatinine, urea and potassium; and depressed chloride, bicarbonate and sodium concentrations. A markedly decrease in splenic LDH, ACP, ALP and γ-GT activities, serum levels of potassium ion, urea and creatinine with elevation in chloride, bicarbonate and sodium concentrations as compares with DOX-intoxicated rats had been recorded in the present study. The results of the present study are in agreement with previously published authors

Gokcimen et al. [75] and Khalifa et al. [89] who concluded that high flavonoids content of *Theobroma cacao* may inhibit general toxicity, renal and splenic damaging effects of DOX. Restoration in the levels of H_2O_2, MDA and PC; MPX, NOX and XO after administration of TCAE could be related to its ability to scavenge reactive oxygen species, thus preventing further damage to membrane lipids and cellular proteins. Our results are in line with previous studies by Kalender et al. [82] who have shown that *Theobroma cacao* leaves extract exhibits excellent antioxidant property. In many studies, *Theobroma cacao* leaves extract neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect [90]. Kalender et al. [82] concluded that *Theobroma cacao* leaves extract treatment has protective effect against DOX induced hepatotoxicity, nephrotoxicity and splenotoxicity. The previous results of current study are in agreement with previously published reports [91,92,93] where concluded that the flavonoids in *T. cacao* may inhibit the generation of highly reactive species from H_2O_2 by chelating transition metal ions, resulting in chemoprevention of cancer. Biological activity of flavonoids in vivo in most cases seems to be associated with its antioxidant activity, chelation of Fe (III), and suppression of hydroxyl radical formation in DOX organ toxicity. Flavonoids are an antioxidant acting as a potent inhibitor of iron-catalyzed radical formation by DOX; by chelating free iron and blocking its coordination sites [91]. Moreover, Sakac et al. [94] observed the inhibition of hydroxyl radical generation over a wide range of DOX/iron ratio (1: 5.5–1: 22) and found that one flavonoid molecule could bind up to 6 DOX molecule. From the histopathology results, it was observed that there were some histological lesions in all the treatment groups despite the seven days treatment. This can be adduced to the insufficency of the treatment days to completely restored normalcy and totally eliminate the pathological lesions observed due to DOX intoxication. There might be a need to increase the number of days of treatment to say 2 or 3 weeks. The improvement of kidney and spleen integrity and function may be mediated via the antioxidant activity of TCAE. This is confirmed by the current study which revealed a significant decrease in activities of MPX, NOX and XO; which are potent free radical generators, lipid peroxidation and protein carbonylation and increase in concentrations of α-tocopherol and ascorbic acid; CAT, SOD, GPX and GST activities and GSH levels.

5. CONCLUSION

The treatment with *Theobroma cacao* stem bark aqueous extract potentially attenuated the deleterious effects of DOX-induced toxicity in kidney and spleen. This improvement effect in organs injury may be mediated via enhancement of the antioxidant defense system.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Injac R, Strukelj B. Recent advances in protection against doxorubicin-induced toxicity. Cancer Research Treatment. 2008;7:497-516.
2. Ayla S, Seckin I, Tanriverdi G, Cengiz M, Eser M, Soner BC, Oktem G. Doxorubicin induced nephrotoxicity: Protective effect of nicotinamide. Int. J. Cell Biology. 2011; 9. Article ID 390238
3. Mohan I, Kumar K, Naidu M, Khan M, Sundaram C. Protective effect of cordipro against doxorubicin-induced cardiotoxicity in mice. Phytomedicine. 2006;13(4):222–229.
4. Rashid Summya, Nemat Ali, Sana Nafees, Shiekh Tanveer Ahmad, Wani Arjumand, Syed Kazim Hasan, Sarwat Sultana. Alleviation of doxorubicin-induced nephrotoxicity and hepatotoxicity by chrysin in Wistar rats. Toxicol Mech Methods. 2013;23(5):337–345.
5. Aydin B, Unsal M, Sekeroglu ZA, Gu¨lbahar Y. The antioxidant and antigenotoxic effects of pycnogenol® on rats treated with cisplatin. Biol Trace Elem Res. 2011;142:638–650.
6. Lee CK, Son SH, Park KK, et al. Licochalcone a inhibits the growth of colon carcinoma and attenuates cisplatin-induced toxicity without a loss of chemotherapeutic efficacy in mice. Basic Clin Pharmacol Toxicol. 2008;103:48–54.
7. Reddy PN, Reddy PS, Rao MR. Studies on the effect of doxorubicin on MDA, NO2, NO3, Se-GSH peroxidase and SOD levels

in albino rat tissues. Afr J Biotechnol. 2007;6:2303–2309.
8. Oz E, Ilhan MN. Effects of melatonin in reducing the toxic effects of doxorubicin. Mol Cell Biochem. 2006;286:11-15.
9. Goraca A, Huk-Kolega H, Piechota A, et al. Lipoic acid—biological activity and therapeutic potential. Pharmacological Reports. 2011;63:849-858.
PMID: 22001972
10. Rochette L, Ghibu S, Richard C, Zeller M, Cottin Y, Vergely C. Direct and indirect antioxidant properties of α-lipoic acid and therapeutic potential. Molecular Nutrition & Food Research. 2013;57(1):114-125.
11. Kleinkauf-Rocha J, Bobermin L, Machado M, et al. Lipoic acid increases glutamate uptake,glutamine synthetase activity and glutathione content in C6 astrocyte cell line. International Journal of Developmental Neuroscience. 2013;31: 165-170.
12. Odugbemi T, Fabeku P, Akinsulire T. Outlines and pictures of medicinal plants from Nigeria. University of Lagos Press. 2006;87.
13. Burkill HM. Useful plants of West Tropical Africa. 2nd.Ed. Royal Botanic Gardens New York. 1994;1:252-253.
14. Martinez MJ, Betancourt J, ALonzo-Gonzal ez N, Jauregai A. Screening of some cuban medicinal plants for antimicrobial activity. Ethnopharmacol. 1996;52:171-174.
15. Wood GAR, Lass RA. Shade and nutrition. Cocoa, Forth Edition. 1985;166-194.
16. Kelm MA, Johnson JC, Robbins RJ, Hammerstone JF, Schmitz HH. High-performance liquid chromatography separation and purification of cacao (*Theobroma cacao* L.). 2006;23:156–161.
17. Ranneh Y, Ali F, Esa NM. The protective effect of cocoa (*Theobroma cacao L.*) in colon cancer. J Nutr Food Sci. 2013;3: 193.
18. Lee KW, Kim YJ, Lee HJ, Lee CY. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem. 2003;51: 7292-7295.
19. Gu L, House SE, Wu X, Ou B, Prior RL. Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. J Agric Food Chem. 2006;54:4057-4061.
20. Vinson JA, Proch J, Bose P, Muchler S, Taffera P, et al. Chocolate is a powerful *ex vivo* and *in vivo* antioxidant, an antiatherosclerotic agent in an animal model, and a significant contributor to antioxidants in the European and American diets. J Agric Food Chem. 2006;54:8071-8076.
21. Weisburger JH. Chemopreventive effects of cocoa polyphenols on chronic diseases. Exp Biol Med (Maywood). 2001;226:891-897.
22. Steinberg FM, Bearden MM, Keen CL. Cocoa and chocolate flavonoids: Implications for cardiovascular health. J Am Diet Assoc. 2003;103:215-223.
23. Buijsse B, Feskens EJ, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: The Zutphen elderly study. Arch Intern Med. 2006;166: 411-417.
24. Kurosawa T, Itoh F, Nozaki A, Nakano Y, Katsuda S, et al. Suppressive effect of cocoa powder on atherosclerosis in Kurosawa and Kusanagi-hyper-cholesterolemic rabbits. J Atheroscler Thromb. 2005;12:20-28.
25. Amin I, Koh BK, Asmah R. Effect of cacao liquor extract on tumor marker enzymes during chemical hepatocarcinogenesis in rats. J Med Food. 2004;7:7-12.
26. Ruzaidi A, Abbe Maleyki MJ, Amin I, Nawalyah AG, Muhajir H. Protective effect of polyphenol-rich extract prepared from Malaysian cocoa (*Theobroma cacao*) on glucose levels and lipid profiles in streptozotocin-induced diabetic rats. J Sci Food Agric. 2005;88:1442-1447.
27. Selmi C, Mao TK, Keen CL, Schmitz HH, Eric Gershwin M. The anti-inflammatory properties of cocoa flavanols. J Cardiovasc Pharmacol. 2006;47:S163-S171.
28. Yamagishi M, Osakabe N, Natsume M, Adachi T, Takizawa T, et al. Anticlastogenic activity of cacao: inhibitory effect of cacao liquor polyphenols against mitomycin C-induced DNA damage. Food Chem Toxicol. 2001;39:1279-1283.
29. Mebius RE, Kraal G. Structure and function of the spleen. Nature reviews. Immunology. 2005;5(8):606–616.
DOI: 10.1038/nri1669
PMID 16056254
30. Jia T, Pamer EG. Immunology. dispensable but not irrelevant. Science. 2009;325(5940):549–550.
DOI: 10.1126/science.1178329
PMC 2917045
PMID 19644100

31. Cotran RS, Kumar V, Collins T. Robbin's pathological basis of disease. 6th ed (Thomson Press (I) Ltd, Noida, India). 1999;1.
32. Koul A, Ghara AR, Gangar SC. Chemomodulatory effects *of Azadirachta indica* on the hepatic status of skin tumor bearing mice. J. Int. Sci. 2006;20(3):169-177.
33. Yildirim A, Oktay M, Bilaoglu V. The antioxidant activity of the leaves of *Cydonia vulgaris*. Turk J Med Sci. 2012;31: 23-27.
34. Garrat DC. The quantitative analysis of drugs. Chapman and Hall Ltd., Japan. 1964;3:456-458.
35. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64:555-559.
36. Tietz NW, Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry and molecular diagnostics. 1953. 4th ed. St. Louis, Mo. Elsevier Saunders; c2006.
37. Talke HSGE, Schubert GE. Enzymatic urea determination in the blood and serum in the Warburg optical test. Klinische Wochenschrift. 1965;43:174.
38. Jaffe M. Hoppe-Seylers Z. physiol. Chem. 1886;10:391.
39. Wright HU, Gawehn K, Grassl M. Methods of enzymatic analysis. 2nd edn., Academic Press Inc., New York.1997;1:521-522.
40. Szasz G. A kinetic photometric method for serum gamma glutamyl transpeptidase. Clin. Chem. 1969;15:124-136.
41. Bowers Jr GN. Lactic dehydrogenase. Standard Methods of Clinical Chemistry. 1963;4:163.
42. Wolf Z, Akim AM, Taufiq-Yap YH, Abdul Hamid R, Kasran R. *In Vitro* antioxidant and antiproliferative activities of methanolic plant part extracts of *Theobrom cacao.* Molecules. 1994;19:18317-18331.
43. Levine R, Garland D, Oliver CN. Determination of carbonyl content of oxidatively modified proteins. Methods Enzymol. 1990;186:464-478.
44. Moore K, Roberts LJ. Measurement of lipid peroxidation. Free Radical Research. 1998;28(6):659-671.
45. Klebanoff SJ. Myeloperoxidase: Friend and foe. Journal of Leukocyte Biology. 2005; 77(5):598-625.
46. Reusch VM, Burger MM. Journal of Biological Chemistry. 1974;249:5337-5345.
47. Bergmeyer HU, Gawehn K, Grassl M. Methods of enzymatic analysis. 2nd edn., Academic Press Inc., New York.1974;1: 521-522.
48. Habig TD, Bergmeyer HU, Gawehn K, Grassl M. Method of enzymatic analysis. 2nd edn., Academic Press Inc., New York. 1974;1:521-522.
49. Wendel A. Glutathione peroxidase. Enzymatic basis of detoxication. 1980;1: 333-353.
50. Sinha JD, Bergmeyer HU, Gawehn K, Grassl M. Methods of enzymatic analysis. 2nd edn., Academic Press Inc., New York. 1971;(1):521-522.
51. Misra A, Fridovich M. Serum superoxide dismutase is associated with vascular structure and function in hypertensive and diabetic patients. Oxidative Medicine and Cellular Longevity. 1972;23:184-193.
52. Beutler E, Duren O, Kelly BM. Improved method for the determination of blood glutathione. J. Lab & Clin. Med. 1963; 61(5):882-888.
53. Nino HV, Shah W. Vitamins. In: Tietz NW. Editor. Fundamentals of Clinical Chemistry. 2nd edition. WB Saunders, Philadelphia. 1986;547-550.
54. Kayden HJ, Chow CK, Bjornson LK. Spectrophotometric method for determination of tocopherol in red blood cells. Journal of Lipid Research. 1973; 14(5):533-540.
55. Krause EH, Lee WJ, Lee SA, Kim EH, Cho EH, Jeong E, Kim DW, Kim MS, Park JY, Park KG, Lee HJ, Lee IK, Lim S, Jang HC, Lee KH, Lee KU. Effects of alpha-lipoic acid on body weight in obese subjects. Am J Med. 2001;124:85.e1–85.e8
56. Halliwell B. Antioxidant characterization. Biochem. Pharm. 1995;49:1341–1348.
57. Brown MS, Goldstein J. Lipoprotein metabolism in the macrophage. Ann. Rev. Biochem. 1983;52:223–261.
58. Angelo AJ. Lipid oxidation in foods. Crit Rev Food Sci Nutr. 1996;36:175-224.
59. Ernster L. Lipid peroxidation in biological membranes: Mechanisms and implications. In Yagi K (Ed.), Active oxygens, lipid peroxides and antioxidants. Tokyo, Japan: Japan Scientific Societies Press. 1993;1-38
60. Frankel EN, Meyer AS. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J. Sci. Food Agric. 2000;80:1925–1941.

61. Marnett LJ. Oxyradicals and DNA damage. Carcino. 2000;21:361-370.
62. Steer P, Millgard J, Sarabi DM, Basu S, Vessby B, Kahan T. Cardiac and vascular structure and function are related to lipid peroxidation and metabolism. Lipid. 2012;37:231–236.
63. Lata H, Ahuja GK. Role of free radicals in health and disease. Indian J Physio Allied Sci. 2012;57:124.
64. Ialenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacol. 1993;110:701.
65. Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990's. Nature. 1993;362:801.
66. Sainani GS, Manika JS, Sainani RG. Oxidative stress: A key factor in pathogenesis of chronic diseases. Med Uptake. 1997;1:1.
67. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem. 1999;47(10):3954-3962.
68. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J Food Composition and Analysis. 2007;20:337-345.
69. Rice-Evans CA, Miller NJ, Bolwell PJ, Bramley PM, Pridham JM. The relative activities of plant-derived polyphenolic flavonoid. Free Radical Res. 2012;22:375-383.
70. Hii CL, Law CL, Suzannah S, Misnawi, Cloke M. Polyphenols in Cocoa (*Theobroma cacao* L.). As. J. Food Ag-Ind. 2013;2(04):702-722.
71. Locatelli M, Travaglia F, Giovannelli L, Coïsson JD, Bordiga, M, Pattarino F, Arlorio M. Clovamide and phenolics from cocoa beans (*Theobroma cacao* L.) inhibit lipid peroxidation in liposomal systems. Food Research International. 2013;50:129-134.
72. Othman A, Ismail A, Ghani NA, Adenan I. Antioxidant capacity and phenolic content of cocoa beans. Food Chemistry. 2007; 100:1523-1530.
73. Singal PK, Deally CM, Weinberg LE. Subcellular effects of adriamycin in the heart: A concise review. J. Mol. Cell. Cardiol. 1987;99:817–828.
74. Yagmurca M, Hasan E, Mustafa I, Songurd M, Ucare, Ersin F. Caffeic acid phenethyl ester as a protective agent against doxorubicin nephrotoxicity in rats. Clinica Chimica Acta. 2004;348(1-2): 27-34.
75. Gokcimen A, Cim A, Tola HT, Bayram D, Kocak A, Ozgüner F, Ayata A. Protective effect of N-acetylcysteine,caffeic acid and vitamin E on doxorubicin hepatotoxicity. Human & Experimental Toxicology. 2007; 26:519–525.
76. Saad SY, Najjar TA, Al-Rikabi AC. The preventive role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. Pharmacol. Res. 2001;43:211–218.
77. Karaman A, Fadillioglu E, Turkmen E, Tas E, Yilmaz Z. Protective effects of leflunomide against ischemia reperfusion injury of the rat liver. Pediatr. Surg. Int. 2006;22:428–434.
78. Injac R, Boskovic M, Perse M, Koprivec-Furlan E, Cerar A, Djordjevic A, Strukelj B. Acute doxorubicin nephrotoxicity in rats with malignant neoplasm can be successfully treated with fullerenol C60 (OH) 24 via suppression of oxidative stress. Pharmacol. Rep. 2008;60:742–749.
79. El-Moselhy MA, El-Sheikh AAK. Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity. Biomed. Pharmacother. 2014;68:101-110.
80. Abou Seif HS. Protective effects of rutin and hesperidin against doxorubicin-induced nephrotoxicity. Beni-Suef University Journal of Applied Sciences. 2012;1(2):1–18.
81. Abd El-Aziz MA, Othman AI, Amer M, ElMissiry MA. Potential protective role of angiotensin-converting enzyme inhibitors captopril and enalapril against adriamycin-induced acute cardiac and hepatic toxicity in rats. J. Appl. Toxicol. 2001;21:469–473.
82. Kalender Y, Yel M, Kalender S. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of vitamin E and catechin. Toxicology. 2005; 209(1):39-45.
83. Yagmurcaa M, Orhan B, Hakan M, Onder S, Ahmet N, Ozcan K, Ahmet S. Protective effects of erdosteine on doxorubicininduced hepatotoxicity in rats. Archives of Medical Research. 2007; 38(4):380-385.
84. Yeh Y, Liu T, Wang L, Lee H, Ting C, Lee W. A standardized extract of *Ginkgo biloba* suppresses doxorubicin-induced oxidative stress and P53-mediated mitochondrial

apoptosis in rats' testes. British Journal of Pharmacology. 2009;156(1):48–61.

85. Mohan M, Kamble S, Gadhi P, Kasture S. Protective effect of *Solanum torvum* on doxorubicin-induced nephrotoxicity in rats. Food Chem Toxicol. 2010;4:436–440.
86. Okasora T, Takikawa T, Utsunomiya Y, Senoh I, Hayashibara H, Shiraki K, Shimizu F. Suppressive effect of superoxide dismutase on adriamycin nephropathy. Nephron. 1992;60:199–203.
87. Yilmaz S, Atessahin A, Sahna E, Karahan I, Ozer S. Protective effect of lycopene on adriamycin-induced nephrotoxicity and nephrotoxicity. Toxicology. 2006;218:164–171.
88. Badary OA, Abdel-naim AB, Abdel-wahab MH, Hamada FMA. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. Toxicology. 2000;143:219–226.
89. Khalifa Fares K, Khalil Fatma A, Barakat Heba A, Hassan Marwa M. Protective role of wheat germ and grape seed oils in chlorpyrifosinduced oxidative stress, biochemical and histological alterations in liver of rats. Australian Journal of Basic and Applied Sciences. 2011;5(10):54–66.
90. Quiles JL, Huertas JR, Battino M, Mataix J, Ramirez-Tortosa MC. Antioxidant nutrients and adriamycin toxicity. Toxicology. 2002; 180:79–95.
91. Zajdel A, Wilczok A, Węglarz L, Dzierżewicz Z. Phytic acid inhibits lipid peroxidation *in vitro*. BioMed. Research International; 2013.
DOI: 10.1155/2013/147307
92. Verghese M, Rao DR, Chawan CB, Walker LT, Shackelford L. Anticarcinogenic effect of phytic acid (IP6): Apoptosis as a possible mechanism of action. 2006;39: 1093–1098.
93. Midorikawa K, Murata M, Oikawa S, Hiraku Y, Kawanishi S. Protective effect of phytic acid on oxidative DNA damage with reference to cancer chemoprevention. Biochemical and Biophysical Research Communications. 2001;288(3):552-557.
94. Sakac M, Canadanovic-Brunet J, Misan A, Tumbas V, Medi D. Antioxidant activity of phytic acid in lipid model system. Food Technology and Biotechnology. 2010; 48(4):524–529.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18245