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DOXORUBICIN INDUCED NEURO- AND CARDIOTOXICITIES IN EXPERIMENTAL RATS: PROTECTION AGAINST OXIDATIVE DAMAGE BY *THEOBROMA CACAO* STEM BARK

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ABSTRACT

80 rats, randomly selected, were divided into 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), 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *Theobroma cacao* stem bark aqueous extract (TCAE). All rats except for baseline group were intoxicated with 20mg/kg body weight doxorubicin (DOX) intraperitoneally. The animals in pre- or post-treatment group received a single dose of DOX (20 mg/kg body weight) intraperitoneally 24h before or after 7 days' oral administration with TCAE respectively while those in 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. Animals were sacrificed (pre- and post- treatment groups were sacrificed on the ninth day while the co-treatment group sacrificed on the 8th day). Brain and heart tissue samples were harvested for enzyme markers of toxicity, oxidative stress and histopathological examinations. DOX intoxication caused significant decrease in activities of LDH and ACP, and increase in γGT and ALP activities in brain tissues while causing a significant increase in LDH, ACP, γGT activities and decrease in ALP activity in the cardiac tissues. DOX intoxication caused a significant increase in concentrations of H_2O_2 generated, MDA and PC, XO, MPx and NOX activities with concomitant decrease in CAT, SOD, GPx and GST activities, and in concentrations of GSH, AsA and α-Toc in brain and cardiac tissues. Pre-, co- and post-treatment with TCAE at either 200 mg/kg, 400 mg/kg or 800 mg/kg body weight significantly reversed the oxidative damage to the organs induced by DOX-intoxication. The result affirmed that *T. cacao* stem bark aqueous extract protected against DOX induced oxidative damage in brain and cardiac tissues of experimental rats.

KEYWORDS: Chemoprevention; Theobroma cacao; Doxorubicin; Oxidative stress; Neurotoxicity; Cardiotoxicity.

1.0 INTRODUCTION

Doxorubicin (DOX) obtained from soil actinomycetes *Streptococcus peucetius* is a powerful drug used for the treatment of solid tumors such as those arising in the breast, bile ducts, endometrial tissue, esophagus and liver, osteosarcomas, soft-tissue sarcomas and non-Hodgkin's lymphoma (Tikoo *et al.*, 2011). DOX is known as a powerful anthracycline antibiotic widely used to treat many human cancers, but significant cardiotoxicity and brain damage (Kuznetsova *et al.*, 2011), hepatotoxicity (Patela *et al.*, 2010), nephrotoxicity (Mohana *et al.*, 2010) and testicular toxicity (Trivedi *et al.*, 2011) limits its clinical application. A number of studies were conducted for antioxidants screening from the natural medicine aiming to minimize oxidative injury by DOX. Several natural antioxidants have been shown to alleviate the DOX-induced cell damage without compromising its anti-tumor efficacy in the animal studies (Xin *et al.*, 2011). Over the past few years, the antioxidant and health-promoting properties of cocoa (***Theobroma cacao***) and cocoa-related products have been thoroughly investigated. Polyphenols, widely distributed in plant foods, are the main antioxidant-active fraction of cocoa and within polyphenols, flavanols and procyanidins have been identified as the active antioxidant agents of cocoa and dark plain chocolate (Martínez *et al.*, 2012). More than 200 studies have reported that various parts of the cocoa plant, e.g., cocoa beans (prepared as chocolate), the bark, flower, pulp, and leaf, and cocoa butter have been used for medicinal purposes. The phenolic compounds in cocoa contain bioactive compounds that have potential health benefits for chronic diseases such as inflammation, cardiovascular illness, neurodegenerative disorders and cancer (Schinella *et al.*, 2010)**. α-Lipoic acid (ALA)** also known as thioctic acid (TA) and 1,2 dithiolane −3-

pentanoic acid, is a naturally occurring substance, that is essential for the function of different enzymes of oxidative metabolism. It is believed that ALA or its reduced form, dihydrolipoic acid (DHLA) have many biochemical functions acting as biological antioxidants, as metal chelators, reducing the oxidized forms of other antioxidant agents such as vitamin C and E and glutathione (GSH) and modulating the signaling transduction of several pathways, like insulin and nuclear factor kappa B (NF-kB) (Golbidi *et al*., 2011). **Brain** is the main organ of the human nervous system. It is located in the head, protected by the skull. It has the same general structure as the brains of other mammals, but with a more developed cerebral cortex. Despite being protected by the thick bones of the skull, suspended in cerebrospinal fluid, and isolated from the bloodstream by the blood–brain barrier, the human brain is susceptible to damage and disease (Canadian Cancer Society, 2015). **Heart** is a muscular organ in humans and other animals, which pumps blood through the blood vessels of the circulatory system and also assists in the removal of metabolic wastes. The heart is located in the middle compartment of the mediastinum in the chest. The heart pumps blood through both circulatory systems. In addition, the blood carries nutrients from the liver and gastrointestinal tract to various organs of the body, while transporting waste to the liver and kidneys (Michelakis *et al*, 2014). The aim of the study is to investigate the protective potential of *Theobroma cacao* stem bark aqueous extract against DOX-induced oxidative damage in the brain and heart in experimental rats.

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagent

Sodium hydroxide, sodium chloride, doxorubicin, α-lipoic acid, formalin, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were purchased from Sigma Chemical Co., Saint Louis, MO, USA. Diethylether, ethanol, xylene, paraffin wax, haemotoxylin and eosin were purchased from Sigma Chemical Co., (St Louis, Mo USA). All other chemicals were supplied by Zayo Company, Jos, Nigeria, which is an accredited supplier of Sigma and BDH chemicals in Nigeria. All reagents and chemicals used were of analytical grade (greater than or equal to 99.7%).

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 was identified and authenticated at the Department of Botany, University of Ibadan, Nigeria. The fresh stem bark of *Theobroma cacao* was 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 (2006) was adopted.

2.3 Animals

Eighty (80) Inbred male Wistar rats, weighing between 100 and 210g 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.

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 24h, 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 24h after the last treatment.

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 24h after the last administration.

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 24h after the last intoxication.

4. Baseline group

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

2.5 Preparation of tissues

Rats were fasted overnight and sacrificed 24h after the last treatment. Brain and heart tissue samples 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.

2.6. Hydrogen peroxide scavenging assay

Plant extract (4 mL) prepared in distilled water at various concentration was mixed with 0.6 mL of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using following equation (Oktay et al., 2003).

$$S\% = [\,(A_{control} - A_{sample})/A_{control}\,] \times 100$$

2.7 Cupric ion reducing capacity assay (CUPRAC)

1 mL 10 mM cupric chloride, 1 mL 7.5 mM neocuproine and 1 mL 1 M ammonium acetate buffer of pH 7 solutions were added to test tubes containing 2 mL of distilled water. The plant extract in different concentration were added to each test tube separately. These mixtures were incubated for half an hour at room temperature and measured against blank at 450 nm. Ascorbic acid was used as positive reference standard (Apak et al., 2014).

2.8 Metal ion chelating activity

The plant extract in different concentration were added to each test tube separately (150 µL), 0.25 mM $FeCl_2$ solution (50 µL) was added. After 5 min, the reaction was initiated by adding 1.0 mM ferrozine solution (100 µL). Absorbance at 545 nm was recorded after 10 min of incubation at room temperature. A reaction mixture containing methanol (150 µL) instead of substance solution served as a control. Ascorbic acid was used as the chelating standard. Chelating activity was calculated using A_{cont} (absorbance of the negative control, e.g., blank solution without test compound) and A_{sample} (absorbance of the substance solution). Chelating activity was expressed as EC_{50}, the concentration that chelates 50% of Fe^{2+} ions (Chew et al., 2009).

2.9 Superoxide radical scavenging activity

The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (NBT) to a purple formazan. The 1 mL reaction mixture contained phosphate buffer (20 mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various extract concentrations (0–20 µg/mL) of sample solution. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated. All tests were performed six times. Ascorbic acid was used as positive control (Liu et al., 1997).

2.10. Peroxynitrite scavenging assay

An acidic solution (0.6 M HCl) of 5 mL H_2O_2 (0.7 M) was mixed with 5 mL 0.6 M KNO_2 on an ice bath for 1 s and 5 mL of ice-cold 1.2 M NaOH was added. Excess H_2O_2 was removed by treatment with granular MnO_2 prewashed with 1.2 M NaOH and the reaction mixture was left overnight at -20°C. Peroxynitrite solution was collected from the top of the frozen mixture and the concentration was measured spectrophotometrically at 302 nm (ε = 1670 $M^{-1}cm^{-1}$). An Evans Blue bleaching assay was used to measure peroxynitrite scavenging activity. The reaction mixture contained 50 mM phosphate buffer (pH 7.4), 0.1 mM DTPA, 90 mM NaCl, 5 mM KCl, 12.5 µM Evans Blue, various doses of plant extract (1000–4000 µg/ml) and 1 mM peroxynitrite in a final volume of 1 ml. After incubation at 25°C for 30 min the absorbance was measured at 611 nm. The percentage scavenging of $ONOO^-$ was calculated by comparing the results of the test and blank samples. All tests were performed six times. Gallic acid was used as the reference compound (Beckman and Beckman, 1990).

2.11 Total antioxidant capacity

The plant extract in different concentration ranging from 1000–4000 µg/ml were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard (Jayaprakasha et al., 2002).

2.12 Reducing power ability

The extract (0.75 mL) at various concentrations was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride

($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power (Meir et al., 1995). Ascorbic acid was used as positive reference standard.

2.13 Biochemical assays

Brain and cardiac alkaline phosphatase (ALP) activity was determined according to the method described by Bassey et al., (1946) and as modified by Wright et al., (1972) using Randox kits, gamma-glutamyl transferase (γ-GT) activity was monitored according to the method described by Szasz (1969), acid phosphatase (ACP) activity was determined according to the method described by Brandt et al. (1980) while lactate dehydrogenase (LDH) activity was determined according to the method described by Bower (1963). Tissues hydrogen peroxide (H_2O_2) concentration was quantified based on Wolff's method (1991), protein carbonyl (PC) concentration was carried out by following method described by Levine *et al*., (1994), 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 (1998), myeloperoxidase (MPX) activity was determined using method of Klebanoff et al., (2005), NADPH oxidase (NOX) activity was measured by the method of Reusch and Burger (1974), xanthine oxidase (XO) activity was determined according to the method of Bergmeyer et al., (1974), glutathione-S-transferase (GST) activity was determined according to Habig *et al*, (1974), enzymatic assay of glutathione peroxidase (GPX) activity was determined following the method described by Rotruck et al (1973), catalase (CAT) activity was determined according to the method of Sinha et al., (1971), the activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich (1972), the method of Beutler *et al.,* (1963) was followed for the determination of reduced glutathione (GSH) concentration, ascorbic acid (AsA) concentration was quantified according to the method of Omaye et al (1979) and concentration of α–tocopherol (α-toc) was carried out following the procedure of Kayden et al., (1973).

2.14 Histopathological examination of brain and heart 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 (4µm) 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 (Krause, 2001).

2.15 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 Hydrogen peroxide scavenging activity, Cupric ion reducing capacity activity, Metal ion chelating activity, Superoxide radical scavenging activity, Peroxynitrite scavenging activity, Total antioxidant capacity, Reducing power ability and effects of *Theobroma cacao* on relative organ weights of DOX-exposed rats

Table 3.1.1 revealed that DOX intoxication caused a significant decrease in brain and heart weights of experimental rats relative to baseline ($p<0.05$). Pre-, co- or post-treatment of experimental animals with 200 mg/kg body weight *T. cacao* caused a further decrease in brain weight of experimental rats. Pre-, co- or post-treatment of experimental animals with 400 mg/kg or 800 mg/kg body weight *T. cacao* caused an insignificant change in brain weight of experimental rats. Pre-, co- or post-treatment with 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *T. cacao* caused a significant increase in heart weight of experimental rats ($p<0.05$). *Theobroma cacao* stem bark aqueous extract (TCAE) showed significant dose dependent increase in hydrogen peroxide scavenging activity, cupric ion reducing activity, metal ion chelating activity, superoxide radical scavenging activity, peroxynitrite scavenging activity, total antioxidant capacity and reducing power ability ($p < 0.05$) (figures 3.1.1, 3.1.2, 3.1.3, 3.1.4, 3.1.5, 3.1.6, 3.1.7 and 3.1.8 respectively). However, the activities of the standards were significantly higher in the assays than TCAE.

Table 3.1.1: Relative organ weights (g) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				HEART			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	$1.65 \pm 0.04^{\alpha\gamma}$	$1.59 \pm 0.06^{\alpha}$	$1.65 \pm 0.11^{\alpha\gamma}$	----------	$3.672 \pm 0.703^{\alpha\gamma}$	$4.788 \pm 0.435^{\alpha}$	$4.651 \pm 0.754^{\alpha\beta\gamma}$	---------
α-LIPOIC ACID	$1.23 \pm 0.21^{\beta}$	1.60 ± 0.04	$1.41 \pm 0.90^{\beta}$	----------	$3.347 \pm 0.433^{\beta}$	4.930 ± 0.537	$5.122 \pm 0.206^{\alpha\beta}$	---------
200TCAE	$1.40 \pm 0.33^{\beta\gamma}$	$1.48 \pm 0.16^{\beta\gamma}$	$1.40 \pm 0.26^{\beta}$	----------	$4.452 \pm 0.066^{\alpha\beta\gamma}$	$4.221 \pm 0.223^{\alpha\beta\gamma}$	$5.756 \pm 0.712^{\alpha\beta\gamma}$	---------
400TCAE	$1.71 \pm 0.13^{\gamma}$	$1.55 \pm 0.11^{\gamma}$	$1.15 \pm 0.31^{\alpha\beta\gamma}$	----------	$4.965 \pm 0.623^{\alpha\beta\gamma}$	$5.643 \pm 0.365^{\alpha\beta\gamma}$	$5.438 \pm 0.212^{\alpha\beta\gamma}$	---------
800TCAE	$1.68 \pm 0.01^{\gamma}$	1.60 ± 0.28	1.62 ± 0.38	----------	$4.753 \pm 0.002^{\alpha\beta\gamma}$	$5.556 \pm 0.702^{\alpha\beta\gamma}$	$5.231 \pm 0.501^{\alpha\beta}$	---------
BASELINE	----------	----------	----------	1.73 ± 0.26	----------	----------	----------	7.63 ± 0.459

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

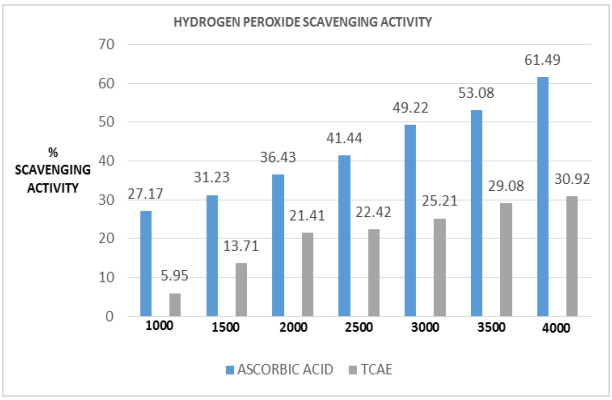


Figure 3.1.1: Hydrogen peroxide scavenging activity of TCAE

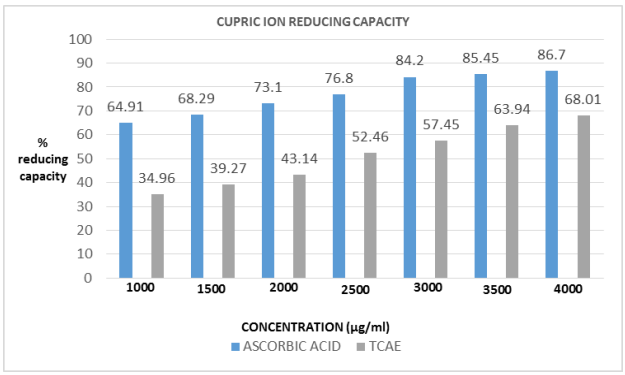


Figure 3.1.2: Cupric ion reducing capacity activity of TCAE

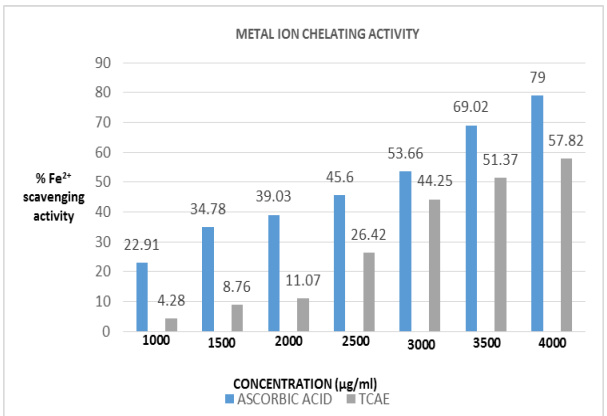


Figure 3.1.3: Metal ion chelating activity of TCAE

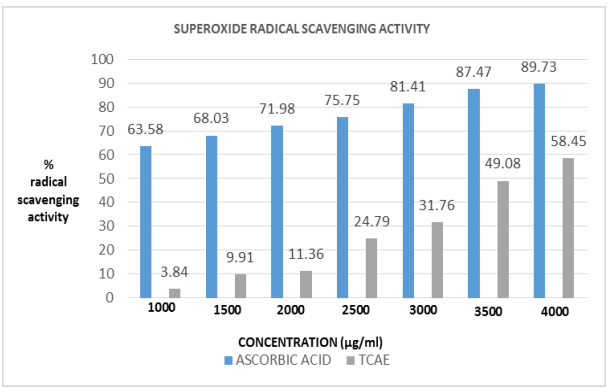


Figure 3.1.4: Superoxide radical scavenging activity of TCAE

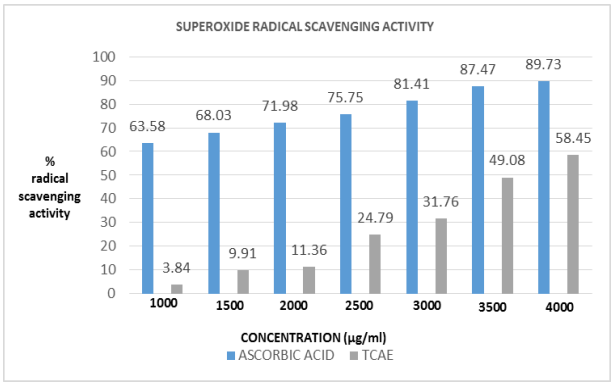


Figure 3.1.5: Superoxide radical scavenging activity of TCAE

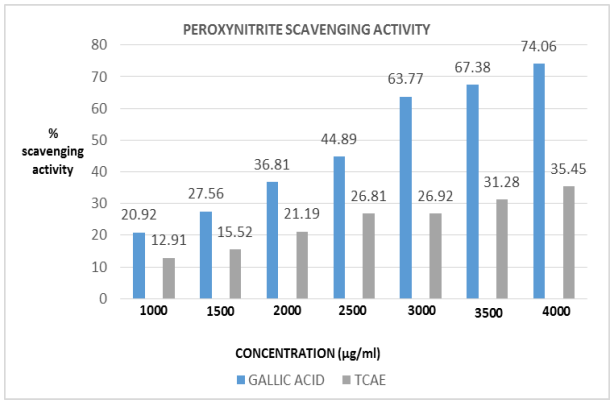


Figure 3.1.6: Peroxynitrite scavenging activity of TCAE

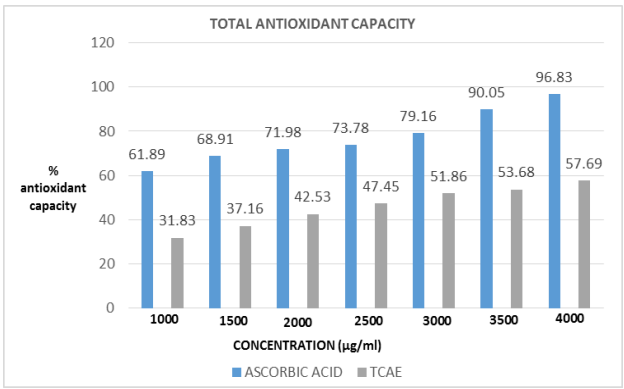


Figure 3.1.7: Total antioxidant capacity of TCAE

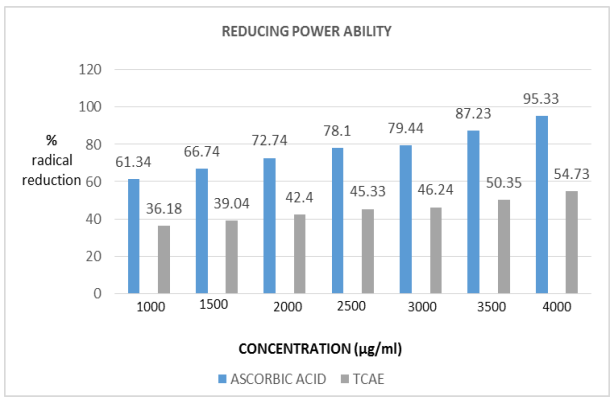


Figure 3.1.8: Reducing power ability of TCAE

3.2: Effects of *Theobroma cacao* on brain and cardiac alkaline phosphatase, acid phosphatase, lactate dehydrogenase and γ-glutamyl transferase activities in DOX-exposed rats

From tables 3.2.1, 3.2.2, 3.2.3 and 3.2.4, doxorubicin intoxication induced a significant changes and perturbation in brain and cardiac 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 apparent dose-dependent resolution to normalcy by the intoxication (comparable to baseline group) in the activities of brain and cardiac toxicity marker enzymes across the three modes of treatments compared with DOX-intoxicated groups ($p<0.05$).

Table 3.2.1: Alkaline phosphatase (ALP) activity (IU/L) in DOX-induced toxicity and protective role of *Theobroma cacao* stem bark aqueous extract (TCAE)

	BRAIN				HEART			
	PRE-TREATMENT	CO-TREATMENT	POST-TREATMENT	BASELINE	PRE-TREATMENT	CO-TREATMENT	POST-TREATMENT	BASELINE
NORMAL SALINE	21.66 ±1.16$^{\alpha\gamma}$	21.59 ±0.92$^{\alpha\gamma}$	21.31 ±1.57$^{\alpha}$	----------	0.944 ± 0.016$^{\alpha\gamma}$	3.927 ± 0.079$^{\alpha\gamma}$	10.15 ±0.312$^{\alpha\beta\gamma}$	---------
α-LIPOIC ACID	4.53 ± 0.33$^{\alpha\beta}$	4.63 ± 0.27$^{\alpha\beta}$	10.73 ±1.14$^{\beta}$	----------	6.614 ± 0.311$^{\alpha\beta}$	8.990 ± 0.926$^{\alpha\beta}$	16.01 ±0.374$^{\alpha\beta\gamma}$	---------
200TCAE	3.86 ± 0.49$^{\alpha\beta}$	4.28 ± 0.43$^{\alpha\beta}$	11.32 ±2.75$^{\beta\gamma}$	----------	22.64 ±0.122$^{\alpha\beta\gamma}$	28.94 ±0.341$^{\alpha\beta\gamma}$	31.44 ±1.113$^{\alpha\beta\gamma}$	---------
400TCAE	5.19 ± 0.35$^{\alpha\beta}$	5.55 ± 0.54$^{\alpha\beta}$	11.10 ±1.15$^{\beta}$	----------	29.84 ±0.178$^{\alpha\beta\gamma}$	34.98 ±0.091$^{\alpha\beta\gamma}$	36.97 ±0.377$^{\alpha\beta\gamma}$	---------
800TCAE	4.99 ± 0.52$^{\alpha\beta}$	5.27 ± 0.29$^{\alpha\beta}$	10.75 ±0.95$^{\beta}$	----------	32.99 ±0.056$^{\alpha\beta\gamma}$	37.97 ±0.734$^{\alpha\beta\gamma}$	39.47 ±0.060$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	10.53 ±1.16	----------	----------	----------	44.53±1.327

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. 200TCAE = 200mg/kg body weight of *Theobroma cacao* stem bark aqueous extract. 400TCAE = 400mg/kg body weight of *Theobroma cacao* stem bark aqueous extract. 800TCAE = 800mg/kg body weight of *Theobroma cacao* stem bark aqueous extract.

Table 3.2.2: Acid phosphatase (ACP) activity (IU/L) in DOX-induced toxicity and the protective properties of *Theobroma cacao* stem bark aqueous extract (TCAE)

	BRAIN				HEART			
	PRE-TREATMENT	CO-TREATMENT	POST-TREATMENT	BASELINE	PRE-TREATMENT	CO-TREATMENT	POST-TREATMENT	BASELINE
NORMAL SALINE	2.06 ± 0.05$^{\alpha\gamma}$	2.90 ± 0.78$^{\alpha}$	2.36 ± 0.02$^{\alpha\gamma}$	----------	31.60 ± 0.647$^{\alpha\gamma}$	21.14 ± 0.955$^{\alpha\gamma}$	28.18 ± 0.458$^{\alpha\gamma}$	---------
α-LIPOIC ACID	3.10 ± 0.48$^{\beta}$	2.75 ± 0.28$^{\alpha}$	2.90 ± 0.56$^{\alpha\beta}$	----------	16.84 ± 0.164$^{\alpha\beta}$	12.24 ± 0.534$^{\alpha\beta}$	19.66 ± 0.374$^{\alpha\beta}$	---------
200TCAE	2.36 ± 0.16$^{\alpha\beta\gamma}$	2.96 ± 0.11$^{\alpha}$	2.24 ± 0.16$^{\alpha\gamma}$	----------	11.74 ± 0.562$^{\alpha\beta}$	8.23 ± 1.037$^{\alpha\beta\gamma}$	8.435 ±0.898$^{\alpha\beta\gamma}$	---------
400TCAE	2.55 ± 0.14$^{\alpha\beta\gamma}$	2.48 ± 0.31$^{\alpha\beta\gamma}$	2.78 ± 0.23$^{\alpha\beta}$	----------	11.54 ± 0.362$^{\alpha\beta}$	5.423 ±0.435$^{\alpha\beta\gamma}$	8.937 ±0.638$^{\alpha\beta\gamma}$	---------
800TCAE	2.54 ± 0.22$^{\beta\gamma}$	2.08 ± 0.16$^{\beta\gamma}$	2.54 ± 0.31	----------	7.34 ± 0.586$^{\alpha\beta\gamma}$	4.778 ±0.857$^{\alpha\beta\gamma}$	7.348 ±0.467$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	3.44 ± 0.18	----------	----------	----------	2.632±0.453

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. 200TCAE = 200mg/kg body weight of *Theobroma cacao* stem bark aqueous extract. 400TCAE = 400mg/kg body weight of *Theobroma cacao* stem bark aqueous extract. 800TCAE = 800mg/kg body weight of *Theobroma cacao* stem bark aqueous extract.

Table 3.2.3: Lactate dehydrogenase (LDH) activity (IU/L) in doxorubicin-induced toxicity and the protective properties of *Theobroma cacao* stem bark aqueous extract (TCAE)

	BRAIN				HEART			
	PRE-TREATMENT	CO-TREATMENT	POST-TREATMENT	BASELINE	PRE-TREATMENT	CO-TREATMENT	POST-TREATMENT	BASELINE
NORMAL SALINE	1.62 ± 0.01$^{\alpha\gamma}$	1.63 ± 0.04$^{\alpha\gamma}$	1.62 ± 0.02$^{\alpha\gamma}$	----------	180.23 ±1.142$^{\alpha\gamma}$	141.32±1.423$^{\alpha\gamma}$	136.02±1.273$^{\alpha\gamma}$	---------

α-LIPOIC ACID	3.32 ± 0.07$^{\beta}$	3.35 ± 0.04$^{\beta}$	3.16 ± 0.56$^{\beta}$	----------	153.32±0.347$^{\alpha\beta}$	121.22±1.082$^{\alpha\beta}$	116.54±1.838$^{\alpha\beta\gamma}$	---------
200TCAE	3.21 ± 0.01$^{\beta}$	3.28 ± 0.16$^{\beta}$	3.59 ± 0.16$^{\alpha\beta}$	----------	94.11 ±0.380$^{\alpha\beta\gamma}$	79.56 ±0.802$^{\alpha\beta\gamma}$	80.39 ±3.320$^{\alpha\beta\gamma}$	---------
400TCAE	3.19 ± 0.04$^{\beta}$	3.43 ± 0.11$^{\beta}$	3.41 ± 0.23$^{\beta}$	----------	79.05 ±2.913$^{\alpha\beta\gamma}$	76.11 ±0.432$^{\alpha\beta\gamma}$	78.01 ±0.717$^{\alpha\beta\gamma}$	---------
800TCAE	3.62 ± 0.14$^{\alpha\beta\gamma}$	3.51 ± 0.28$^{\beta}$	3.41 ± 0.31$^{\alpha\beta\gamma}$	----------	72.01 ±1.005$^{\alpha\beta\gamma}$	69.84 ±0.738$^{\alpha\beta\gamma}$	68.48 ±1.021$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	3.29 ± 0.01	----------	----------	----------	63.01±0.893

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. 200TCAE = 200mg/kg body weight of *Theobroma cacao* stem bark aqueous extract. 400TCAE = 400mg/kg body weight of *Theobroma cacao* stem bark aqueous extract. 800TCAE = 800mg/kg body weight of *Theobroma cacao* stem bark aqueous extract.

Table 3.2.4: Gamma-glutamyl-transferase (γ-GT) activity (IU/L) in doxorubicin-induced toxicity and protective properties of *Theobroma cacao* stem bark aqueos extract (TCAE)

	BRAIN				**HEART**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	5.65 ± 0.27$^{\alpha\gamma}$	5.80 ± 0.30$^{\alpha\gamma}$	5.66 ± 0.25$^{\alpha\gamma}$	----------	83.12 ±0.672$^{\alpha\beta\gamma}$	79.71 ±0.058$^{\alpha\beta\gamma}$	81.93 ± 0.393$^{\alpha\gamma}$	---------
α-LIPOIC ACID	2.83 ± 0.61$^{\alpha\beta}$	2.69 ± 0.44$^{\beta}$	2.86 ± 0.49$^{\beta}$	----------	68.32±0.987$^{\alpha\beta}$	65.77 ±0.982$^{\alpha\beta}$	63.34 ± 0.943$^{\alpha\beta}$	---------
200TCAE	3.38 ± 0.28$^{\beta\gamma}$	2.47 ± 0.23$^{\alpha\beta}$	2.30 ± 0.40$^{\alpha\beta\gamma}$	----------	57.94 ±0.091$^{\alpha\beta\gamma}$	56.12 ±0.899$^{\alpha\beta\gamma}$	58.53 ±0.602$^{\alpha\beta\gamma}$	---------
400TCAE	3.44 ± 0.17$^{\beta\gamma}$	3.55 ± 0.21$^{\alpha\beta\gamma}$	3.00 ± 0.11$^{\beta}$	----------	56.24 ±0.982$^{\alpha\beta\gamma}$	55.41 ±0.623$^{\alpha\beta\gamma}$	56.84 ±0.731$^{\alpha\beta\gamma}$	---------
800TCAE	3.37 ± 0.08$^{\alpha\beta\gamma}$	2.35 ± 0.25$^{\alpha\beta}$	2.99 ± 0.48$^{\beta}$	----------	53.73 ±0.589$^{\alpha\beta\gamma}$	52.53 ±0.216$^{\alpha\beta\gamma}$	49.84 ±0.644$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	3.05 ± 0.44	----------	----------	----------	45.22±0.881

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

3.3: Effects of *Theobroma cacao* on antioxidant parameters, and histology of tissues of DOX-exposed rats

From tables 3.3.1, 3.3.2 and 3.3.3, doxorubicin intoxication caused a significant elevation in hydrogen peroxide, malondialdehyde and protein carbonyl concentrations in the heart and cardiac tissues of the experimental rats compared with baseline group ($p<0.05$). Pre-, co- or post-treatment with either 200mg/kg, 400mg/kg or 800mg/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 tissues of experimental rats relative to DOX-exposed group ($p<0.05$). Tables 3.3.4, 3.3.5 and 3.3.6 revealed a significant elevation in brain and cardiac myeloperoxidase, NADPH oxidase and xanthine oxidase activities of experimental rats following DOX intoxication compared with baseline group ($p<0.05$). A dose-dependent reduction in these enzymes' activities were observed following treatment with either 200, 400 or 800mg/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 tables 3.3.7 and 3.3.8 also indicated that doxorubicin administration caused a significant decrease in catalase and superoxide dismutase activities in studied tissues of experimental rats compared with baseline group ($p<0.05$). There was significant increase in these enzymes' activities following pre-, 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 result in tables 3.3.9, 3.3.10 and 3.3.11 revealed that doxorubicin intoxication caused a significant reduction in the activities of glutathione peroxidase and glutathione S-transferase with concomitant decline in reduced glutathione concentration in the tissues of experimental rats compared with baseline group. A significant dose dependent elevation in the studied glutathione metabolism markers were observed following pre-, co- and post-treatment with 200mg/kg, 400mg/kg or 800mg/kg body weight *T. cacao* groups. The result in tables 3.3.12 and 3.3.13 reported a significant decrease in brain and cardiac α-tocopherol and ascorbic acid concentrations among DOX-intoxicated rats relative to the baseline group ($p<0.05$). A significant increase in brain and cardiac α-tocopherol and ascorbic acid concentrations were observed following pre-, co- and post-treatment with 200mg/kg, 400mg/kg or 800mg/kg body weight *T. cacao* relative to DOX-intoxicated group ($p<0.05$).

Table 3.3.1: Hydrogen peroxide (H_2O_2) concentration (µmol/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				HEART			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	11.79 ± 0.29$^{\alpha}$	7.89 ± 0.61$^{\alpha}$	5.63 ± 0.34$^{\alpha}$	--------	9.715 ± 0.441$^{\alpha\gamma}$	6.432 ± 0.293$^{\alpha\gamma}$	4.584± 0.151$^{\alpha\beta\gamma}$	--------
α-LIPOIC ACID	3.42 ± 0.39$^{\alpha\beta}$	2.34 ± 0.17$^{\alpha\beta}$	1.62 ± 0.29$^{\alpha\beta}$	--------	2.793 ± 0.310$^{\alpha\beta}$	1.934 ± 0.205$^{\alpha\beta}$	1.321± 0.132$^{\alpha\beta\gamma}$	--------
200TCAE	2.09 ± 0.09$^{\alpha\beta}$	1.44 ± 0.24$^{\alpha\beta}$	1.01 ± 0.78$^{\alpha\beta}$	--------	1.707± 0.076$^{\alpha\beta\gamma}$	1.117± 0.141$^{\alpha\beta\gamma}$	0.823± 0.036$^{\alpha\beta\gamma}$	--------
400TCAE	1.29 ± 0.24$^{\alpha\beta}$	0.98 ± 0.09$^{\alpha\beta}$	0.41 ± 0.03$^{\alpha\beta}$	--------	1.123± 0.047$^{\alpha\beta\gamma}$	0.767± 0.039$^{\alpha\beta\gamma}$	0.551± 0.027$^{\alpha\beta\gamma}$	--------
800TCAE	0.84 ± 0.04$^{\alpha\beta}$	0.56 ± 0.07$^{\alpha\beta}$	0.41 ± 0.02$^{\beta}$	--------	0.679±0.017$^{\alpha\beta\gamma}$	0.464± 0.017$^{\alpha\beta\gamma}$	0.333± 0.007$^{\alpha\beta\gamma}$	--------
BASELINE	--------	--------	--------	**0.18 ± 0.01**	--------	--------	--------	**0.15 ± 0.01**

Table 3.3.2: Malondialdehyde (MDA) concentration (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				HEART			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	19.32 ± 0.29$^{\alpha\beta\gamma}$	13.28 ± 0.61$^{\alpha\gamma}$	9.49 ± 0.34$^{\alpha\gamma}$	--------	16.22 ± 0.572$^{\alpha\gamma}$	10.82 ± 0.494$^{\alpha\gamma}$	7.712 ± 0.252$^{\alpha\gamma}$	--------
α-LIPOIC ACID	5.77 ± 0.69$^{\alpha\beta}$	4.11 ± 0.37$^{\alpha\beta}$	2.97 ± 0.29$^{\alpha\beta}$	--------	4.69 ± 0.521$^{\alpha\beta}$	3.24 ± 0.344$^{\alpha\beta\gamma}$	2.223 ± 0.222$^{\alpha\beta}$	--------
200TCAE	3.54 ± 0.17$^{\alpha\beta\gamma}$	2.42 ± 0.24$^{\beta}$	1.01 ± 0.78$^{\beta}$	--------	2.87 ± 0.128$^{\alpha\beta\gamma}$	1.982 ±0.237$^{\alpha\beta\gamma}$	1.385 ±0.061$^{\alpha\beta\gamma}$	--------
400TCAE	2.29 ± 0.09$^{\beta\gamma}$	1.58 ± 0.09$^{\beta\gamma}$	1.14 ± 0.03$^{\beta}$	--------	1.892 ±0.081$^{\alpha\beta\gamma}$	1.294 ±0.432$^{\alpha\beta\gamma}$	0.925 ±0.046$^{\alpha\beta\gamma}$	--------
800TCAE	1.19 ± 0.04$^{\alpha\beta}$	0.96 ± 0.07$^{\beta\gamma}$	0.68 ± 0.02$^{\beta}$	--------	1.142 ±0.021$^{\alpha\beta\gamma}$	0.789 ±0.029$^{\alpha\beta\gamma}$	0.561 ±0.012$^{\alpha\beta\gamma}$	--------
BASELINE	--------	--------	--------	**0.31 ± 0.01**	--------	--------	--------	**0.252±0.007**

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 3.3.3: Protein carbonyl (PC) concentration (nmol/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				HEART			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	PRE-TREATMENT	CO-TREATMENT	POST-TREATMENT	**BASELINE**
NORMAL SALINE	21.22 ± 0.14$^{\alpha\gamma}$	14.45 ± 1.66$^{\alpha\gamma}$	10.25 ± 0.34$^{\alpha\gamma}$	--------	17.56 ± 0.507$^{\alpha\gamma}$	11.77 ± 0.537$^{\alpha\gamma}$	8.388 ± 0.277$^{\alpha\gamma}$	--------
α-LIPOIC ACID	6.27 ± 0.69$^{\alpha\beta}$	4.35 ± 0.33$^{\alpha\beta}$	2.97 ± 0.34$^{\alpha\beta}$	--------	5.111 ± 0.567$^{\alpha\beta}$	3.532 ± 0.375$^{\alpha\beta}$	2.419 ± 0.242$^{\alpha\beta}$	--------
200TCAE	3.84 ± 0.17$^{\alpha\beta\gamma}$	2.64 ± 0.32$^{\alpha\beta\gamma}$	2.97 ± 0.29$^{\alpha\beta}$	--------	3.124±0.139$^{\alpha\beta\gamma}$	2.154±0.258$^{\alpha\beta\gamma}$	1.507±0.067$^{\alpha\beta\gamma}$	--------
400TCAE	2.51 ± 0.09$^{\alpha\beta\gamma}$	1.73 ± 0.09$^{\alpha\beta\gamma}$	2.24 ± 0.11$^{\alpha\beta\gamma}$	--------	2.062±0.087$^{\alpha\beta\gamma}$	1.404±0.072$^{\alpha\beta\gamma}$	0.877±0.044$^{\alpha\beta\gamma}$	--------

800TCAE	1.55 ± 0.04 αβγ	1.04 ± 0.07 αβγ	0.76 ± 0.02 βγ	--------	1.242±0.031 αβγ	0.849±0.032 αβγ	0.531±0.011 αβγ	--------
BASELINE	--------	--------	--------	**0.31 ± 0.01**	--------	--------	--------	**0.274±0.008**

Table 3.3.4: Myeloperoxidase (MPX) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				**HEART**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	35.24 ± 0.22 αγ	24.17 ± 1.19 αγ	17.22 ± 0.64 αγ	--------	30.54 ± 0.992 αγ	21.37 ± 0.976 αγ	15.23 ± 0.503 αγ	--------
α-LIPOIC ACID	10.49 ± 1.17 αβ	7.27 ± 0.83 αβ	4.97 ± 0.49 αβ	--------	8.084 ± 1.032 αβ	6.414 ± 0.681 αβ	4.391 ± 0.432 αβ	--------
200TCAE	6.42 ± 0.29 αβγ	4.42 ± 0.58 αβγ	3.09 ± 0.15 αβγ	--------	5.673 ± 0.254 αβγ	3.915 ± 0.472 αβγ	2.737 ± 0.121 αβγ	--------
400TCAE	4.02 ± 0.07 αβγ	2.88 ± 0.18 αβγ	2.07 ± 0.11 αβγ	--------	3.742 ± 0.158 αβγ	2.552 ± 0.131 αβγ	1.829 ± 0.092 αβγ	--------
800TCAE	2.77 ± 0.07 αβγ	1.74 ± 0.07 αβγ	1.27 ± 0.06 βγ	--------	1.965 ± 0.057 αβγ	1.542 ± 0.059 αβγ	1.108 ± 0.023 αβγ	--------
BASELINE	--------	--------	--------	**0.61 ± 0.08**	--------	--------	--------	**0.497±0.014**

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 3.3.5: NADPH oxidase (NOX) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				**HEART**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	38.59 ± 0.44 αγ	26.27 ± 1.19 α	18.69 ± 0.64 αγ	--------	28.31 ± 0.677 αγ	19.69 ± 0.899 αγ	14.03 ± 0.463 αγ	--------
α-LIPOIC ACID	11.39 ± 1.27 αβ	7.87 ± 0.83 αβ	5.37 ± 0.49 αβ	--------	8.56 ± 0.949 αβ	5.908 ± 0.627 αβ	4.046 ± 0.405 αβ	--------
200TCAE	6.96 ± 0.31 αβγ	4.82 ± 0.58 αβγ	3.36 ± 0.15 αβγ	--------	5.23 ± 0.234 αβγ	3.626±0.432 αβγ	2.523±0.111 αβγ	--------
400TCAE	4.59 ± 0.19 αβγ	3.13 ± 0.18 αβγ	2.24 ± 0.11 αβγ	--------	3.45 ± 0.147 αβγ	2.342±0.121 αβγ	1.684±0.085 αβγ	--------
800TCAE	2.55 ± 0.17 αβγ	1.89 ± 0.07 αβγ	1.36 ± 0.06 βγ	--------	2.07 ± 0.053 αβγ	1.423±0.047 αβγ	1.028±0.222 αβγ	--------
BASELINE	--------	--------	--------	**0.61 ± 0.08**	--------	--------	--------	**0.458±0.013**

Table 3.3.6 Xanthine oxidase (XO) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				**HEART**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	37.88 ± 0.53 αγ	24.17 ± 1.19 αγ	18.69 ± 0.64 αγ	--------	31.09±0.219 αγ	21.37 ± 0.976 αγ	15.23 ± 0.503 αγ	--------
α-LIPOIC ACID	11.25 ± 1.26 αβ	7.79 ± 0.83 αβ	5.39 ± 0.53 αβ	--------	9.284 ± 1.031 αβ	6.416 ± 0.681 αβ	4.392 ± 0.439 αβ	--------

200TCAE	6.89 ± 0.67 αβγ	4.75 ± 0.58 αβγ	3.36 ± 0.15 αβγ	--------	5.674±0.254 αβγ	3.912±0.470 αβγ	2.731±0.121 αβγ	--------
400TCAE	4.02 ± 0.27 αβγ	2.88 ± 0.18 αβγ	2.24 ± 0.11 αβγ	--------	3.745±0.158 αβγ	2.551±0.131 αβγ	1.829±0.092 αβγ	--------
800TCAE	2.55 ± 0.07 αβγ	1.87 ± 0.07 αβγ	1.37 ± 0.06 βγ	--------	2.256±0.057 αβγ	1.542±0.059 αβγ	1.108±0.024 αβγ	--------
BASELINE	--------	--------	--------	**0.31 ± 0.06**	--------	--------	--------	**0.498±0.014**

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 3.3.7: Catalase (CAT) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				**HEART**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	8.88 ± 0.13 αγ	17.46 ± 1.54 α	18.69 ± 1.84 αγ	--------	7.114 ± 1.352 αγ	14.33 ± 1.162 αγ	69.62 ± 5.322 αγ	--------
α-LIPOIC ACID	15.39 ± 0.89 α	27.42 ± 6.26 α	85.45 ± 6.53 αβ	--------	12.42 ± 0.269 αβ	22.34 ± 4.842 αβ	90.57 ± 6.926 αβ	--------
200TCAE	8.18 ± 0.67 αγ	35.65 ± 7.75 αβγ	151.11±8.48 αβγ	--------	6.426±0.655 αβγ	29.04±6.292 αβγ	118.30±9.051 αβγ	--------
400TCAE	11.46 ± 0.43 αγ	45.62 ± 4.18 αβ	151.81±0.11 αβγ	--------	9.296±0.351 αβγ	37.17±3.402 αβγ	147.20±11.94 αβγ	--------
800TCAE	13.89 ± 0.17 αγ	64.08 ± 9.57 βγ	151.81±0.06 αβγ	--------	10.50±0.129 αβγ	52.21±7.802 αβγ	110.30±0.023 αβγ	--------
BASELINE	--------	--------	--------	**72.00 ± 1.66**	--------	--------	--------	**58.67±1.351**

Table 3.3.8: Superoxide dismutase (SOD) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				**HEART**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	9.17 ± 0.28 αγ	18.45 ± 0.54 α	34.35 ± 1.84 αγ	--------	7.52 ± 0.183 αγ	15.01 ± 1.212 αγ	28.24 ± 1.458 αγ	--------
α-LIPOIC ACID	15.99 ± 0.99 α	28.87 ± 6.26 α	89.56 ± 6.53 αβ	--------	13.01 ± 0.765 αβ	23.40 ± 5.07 αβ	65.98 ± 5.57 αβ	--------
200TCAE	15.94 ± 0.74 αγ	37.38 ± 8.58 α	111.21±8.88 αβγ	--------	6.981±0.569 αβγ	30.43±6.594 αβγ	94.82 ± 7.24 αβγ	--------
400TCAE	11.92 ± 0.49 αγ	47.79 ± 4.85	151.52±11.64 αβγ	--------	9.731±0.368 αβγ	38.93±3.572 αβγ	124.00±9.487 αβγ	--------
800TCAE	13.36 ± 0.22 αγ	67.13 ± 10.57 β	181.85±56.91 αβγ	--------	11.00±0.135 αβγ	54.69±8.185 αβγ	155.10±14.28 αβγ	--------
BASELINE	--------	--------	--------	**75.43 ± 1.73**	--------	--------	--------	**61.46±1.423**

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 3.3.9: Glutathione peroxidase (GSH consumed/ mg protein) activity in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				HEART			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	9.77 ± 0.01$^{\alpha}$	19.46 ± 1.54$^{\alpha}$	36.35 ± 1.84$^{\alpha\gamma}$	--------	10.79 ± 0.013$^{\alpha\gamma}$	15.83 ± 1.282$^{\alpha\gamma}$	29.77 ± 1.532$^{\alpha\gamma}$	--------
α-LIPOIC ACID	16.79 ± 0.99$^{\alpha\beta}$	30.27 ± 6.26$^{\alpha}$	94.27 ± 7.22$^{\alpha\beta}$	--------	15.41 ± 3.654$^{\alpha\beta}$	24.67 ± 5.346$^{\alpha\beta}$	76.89 ± 5.882$^{\alpha\beta}$	--------
200TCAE	9.04 ± 0.76$^{\alpha}$	39.38 ± 6.58$^{\alpha}$	151.00 ± 7.68$^{\alpha\beta\gamma}$	--------	7.364±0.600$^{\alpha\beta\gamma}$	25.29 ± 6.953$^{\alpha\beta}$	99.96±7.648$^{\alpha\beta\gamma}$	--------
400TCAE	12.62 ± 0.49$^{\alpha\beta}$	50.38 ± 4.65$^{\alpha\beta\gamma}$	153.42±10.00$^{\alpha\beta\gamma}$	--------	10.26±0.388$^{\alpha\gamma}$	41.04±3.762$^{\alpha\beta\gamma}$	130.72±9.992$^{\alpha\beta\gamma}$	--------
800TCAE	16.66 ± 0.22$^{\alpha\beta}$	70.76 ± 10.57$^{\beta\gamma}$	192.45±49.91$^{\alpha\beta\gamma}$	--------	11.60±0.142$^{\alpha\gamma}$	57.65±8.864$^{\alpha\beta\gamma}$	162.60±13.19$^{\alpha\beta\gamma}$	--------
BASELINE	--------	--------	--------	**70.43 ± 1.63**	--------	--------	--------	**64.79±1.493**

Table 3.3.10: Glutathione S-transferase (µmol/min/mg protein) activity in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				HEART			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	9.77 ± 0.08$^{\alpha\gamma}$	19.46 ± 1.54$^{\alpha}$	36.30 ± 1.84$^{\alpha\gamma}$	--------	7.905 ± 0.701$^{\alpha\gamma}$	15.59 ± 1.263$^{\alpha\gamma}$	29.33 ± 1.571$^{\alpha\gamma}$	--------
α-LIPOIC ACID	16.79 ± 0.99$^{\alpha}$	29.87 ± 6.56$^{\alpha}$	92.36 ± 7.22$^{\alpha\beta}$	--------	13.51 ± 0.794$^{\alpha\beta}$	24.31 ± 5.267$^{\alpha\beta}$	75.75 ± 5.792$^{\alpha\beta}$	--------
200TCAE	9.04 ± 0.76$^{\alpha\gamma}$	39.38 ± 6.58$^{\alpha}$	121.21±9.28$^{\alpha\beta\gamma}$	--------	7.253±0.594$^{\alpha\beta\gamma}$	31.60±6.846$^{\alpha\beta\gamma}$	98.18±7.512$^{\alpha\beta\gamma}$	--------
400TCAE	12.62 ± 0.49$^{\alpha\gamma}$	38.79 ± 6.85$^{\alpha}$	151.42±11.04$^{\alpha\beta\gamma}$	--------	10.11±0.387$^{\alpha\beta\gamma}$	40.44±3.703$^{\alpha\beta\gamma}$	128.79±9.842$^{\alpha\beta\gamma}$	--------
800TCAE	14.06 ± 0.22$^{\alpha\gamma}$	67.13 ± 10.57$^{\beta}$	181.85±56.91$^{\alpha\beta\gamma}$	--------	11.43±0.140$^{\alpha\beta\gamma}$	56.80±8.492$^{\alpha\beta\gamma}$	160.00±13.02$^{\alpha\beta\gamma}$	--------
BASELINE	--------	--------	--------	**78.34 ± 1.83**	--------	--------	--------	**63.83±1.475**

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 3.3.11: Reduced glutathione (µg/ml) concentration in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				HEART			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	1.86 ± 0.12$^{\alpha}$	3.82 ± 3.31$^{\alpha}$	7.19 ± 0.37$^{\alpha}$	--------	1.588 ± 0.007$^{\alpha\gamma}$	3.115 ± 0.252$^{\alpha\gamma}$	8.863 ± 0.301$^{\alpha\gamma}$	--------
α-LIPOIC ACID	3.31 ± 0.19$^{\alpha}$	5.96 ± 1.29$^{\alpha}$	18.57 ± 1.42$^{\alpha\beta}$	--------	2.703 ± 3.651$^{\alpha\beta}$	4.852 ± 1.052$^{\alpha\beta}$	15.13 ± 1.152$^{\alpha\beta}$	--------
200TCAE	1.78 ± 0.15$^{\alpha}$	7.75 ± 1.68$^{\alpha}$	24.17 ± 1.85$^{\alpha\beta}$	--------	1.443 ± 0.600$^{\alpha}$	6.315±1.364$^{\alpha\beta\gamma}$	19.67±1.502$^{\alpha\beta\gamma}$	--------

400TCAE	2.48 ± 0.09$^{\alpha}$	9.91 ± 0.91$^{\alpha}$	31.57 ± 2.41$^{\alpha\beta}$	--------	2.023±0.388$^{\alpha\beta\gamma}$	8.072±2.903$^{\alpha\beta\gamma}$	25.72±1.964$^{\alpha\beta\gamma}$	--------
800TCAE	2.82 ± 0.34$^{\alpha}$	13.93 ± 2.08$^{\beta}$	39.28 ± 3.19$^{\alpha\beta}$	--------	2.287 ± 0.142$^{\alpha\beta}$	11.34±1.692$^{\alpha\beta\gamma}$	32.00±2.515$^{\alpha\beta\gamma}$	--------
BASELINE	--------	--------	--------	**42.35 ± 0.97**	--------	--------	--------	**34.50±0.797**

Table 3.3.12: α-tocopherol concentration (µmol/L) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				**HEART**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	8.77 ± 0.01$^{\alpha}$	11.66 ± 0.14$^{\alpha}$	29.32 ± 1.54$^{\alpha}$	--------	6.61 ± 0.014$^{\alpha\gamma}$	12.96 ± 1.052$^{\alpha\gamma}$	24.38 ± 1.255$^{\alpha\gamma}$	--------
α-LIPOIC ACID	13.79 ± 0.81$^{\alpha}$	11.66 ± 5.81$^{\alpha}$	77.26 ± 5.91$^{\alpha\beta}$	--------	11.23 ± 0.661$^{\alpha\beta}$	20.20 ± 4.373$^{\alpha\beta}$	62.95 ± 4.812$^{\alpha\beta}$	--------
200TCAE	7.39 ± 0.60$^{\alpha}$	32.32 ± 7.56$^{\alpha\beta}$	101.00 ± 7.70$^{\alpha\beta}$	--------	6.029 ± 0.491$^{\alpha\gamma}$	26.26±5.679$^{\alpha\beta\gamma}$	81.84±6.252$^{\alpha\beta\gamma}$	--------
400TCAE	10.32 ± 0.39$^{\alpha}$	41.25 ± 1.03$^{\alpha\beta}$	131.42 ± 1.00$^{\alpha\beta}$	--------	8.406±0.317$^{\alpha\beta\gamma}$	33.60±3.082$^{\alpha\beta\gamma}$	107.00±8.182$^{\alpha\beta\gamma}$	--------
800TCAE	11.66 ± 0.41$^{\alpha}$	57.94 ± 9.37$^{\alpha\beta}$	162.50±49.90$^{\alpha\beta}$	--------	9.499±0.116$^{\alpha\beta\gamma}$	47.20±7.058$^{\alpha\beta\gamma}$	47.20 ±7.058$^{\alpha\beta\gamma}$	--------
BASELINE	--------	--------	--------	**70.42 ± 1.63**	--------	--------	--------	**53.04±1.226**

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 = 200 mg/kg body weight of *Theobroma cacao* stem bark aqueous extract. 400 TCAE = 400 mg/kg body weight of *Theobroma cacao* stem bark aqueous extract. 800 TCAE = 800 mg/kg body weight of *Theobroma cacao* stem bark aqueous extract.

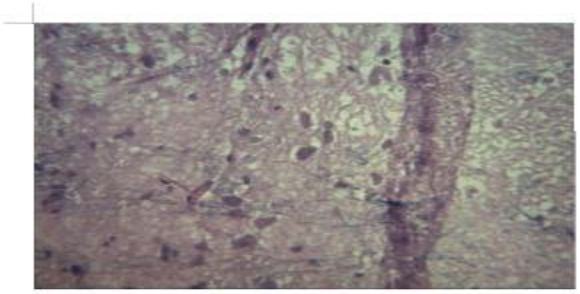
Table 3.3.13: Ascorbic acid concentration (µmol/L) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	BRAIN				**HEART**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
NORMAL SALINE	8.49 ± 0.39$^{\alpha}$	17.54 ± 1.14$^{\alpha}$	32.67 ± 1.84$^{\alpha}$	--------	7.03 ± 0.155$^{\alpha\gamma}$	14.02 ± 1.136$^{\alpha\gamma}$	26.37 ± 1.352$^{\alpha\gamma}$	--------
α-LIPOIC ACID	14.91 ± 0.88$^{\alpha}$	26.82 ± 5.81$^{\alpha}$	83.53 ± 6.39$^{\alpha\beta}$	--------	12.15 ± 0.714$^{\alpha\beta}$	21.85 ± 4.735$^{\alpha\beta}$	68.10 ± 5.308$^{\alpha\beta}$	--------
200TCAE	8.00 ± 0.65$^{\alpha}$	34.87 ± 7.56$^{\alpha\beta}$	101.09 ± 8.30$^{\alpha\beta}$	--------	6.525 ± 0.531$^{\alpha\gamma}$	28. 41±6.152$^{\alpha\beta\gamma}$	88.53±6.765$^{\alpha\beta\gamma}$	--------
400TCAE	11.16 ± 0.42$^{\alpha}$	46.62 ± 1.03$^{\alpha\beta}$	141.40±10.90$^{\alpha\beta}$	--------	9.098±0.343$^{\alpha\beta\gamma}$	36.35±3.333$^{\alpha\beta\gamma}$	115.70±8.853$^{\alpha\beta\gamma}$	--------
800TCAE	12.61 ± 0.16$^{\alpha}$	62.67 ± 9.37$^{\alpha\beta}$	171.90±14.30$^{\alpha\beta}$	--------	10.27±0.126$^{\alpha\beta\gamma}$	51.06±7.365$^{\alpha\beta\gamma}$	144.00±43.47$^{\alpha\beta\gamma}$	--------
BASELINE	--------	--------	--------	**70.42 ± 1.63**	--------	--------	--------	**57.38±1.324**

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.

Figure 3.2.1: HISTOPATHOLOGY OF THE BRAIN SECTIONS

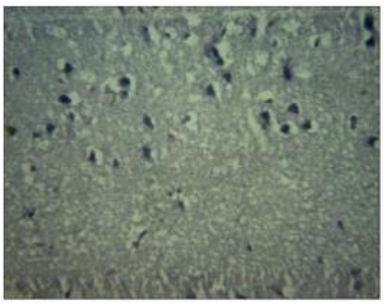
Baseline



There is no visible lesions.

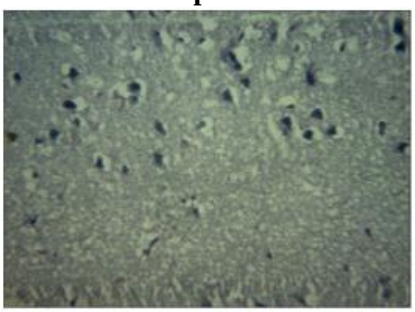
PRE-TREATMENT MODE

Normal saline



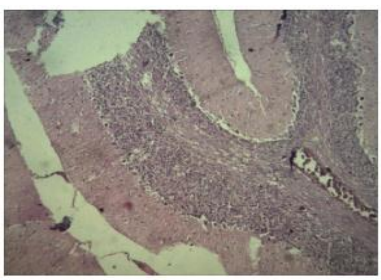
There is a diffuse moderate spongiosis of the cerebral cortex

α-Lipoic acid



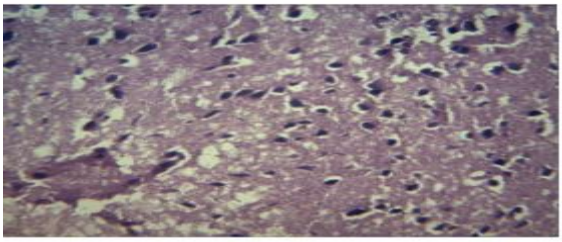
There is a diffuse moderate spongiosis of the cerebral cortex

200TCAE



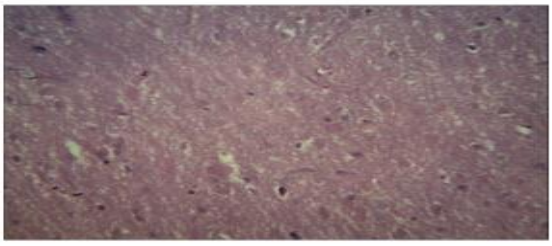
Some vessels of the cerebellum are severely congested

400TCAE



There is a mild diffuse spongiosis

800TCAE



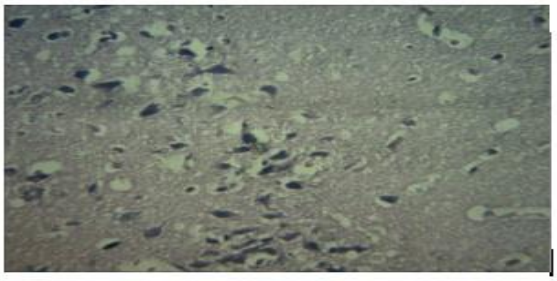
There is no visible lesion seen

CO-TREATMENT MODE

Normal saline

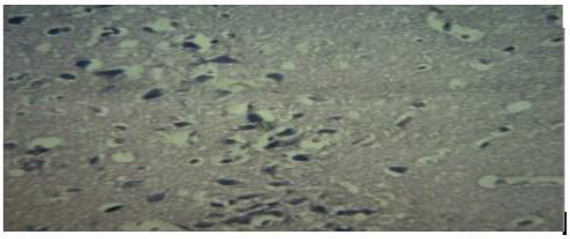
There is no visible lesion shown

α-Lipoic acid



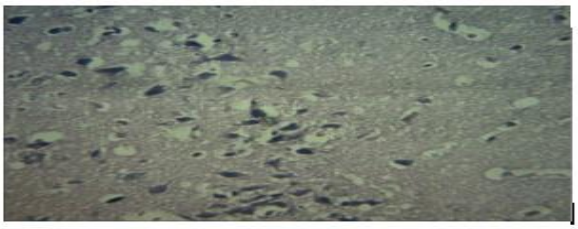
There is no visible lesion seen.

200TCAE



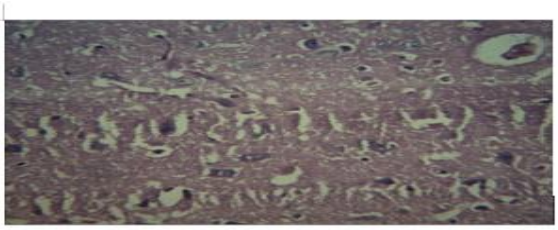
There is no visible lesion seen.

400TCAE



There is no visible lesion seen.

800TCAE



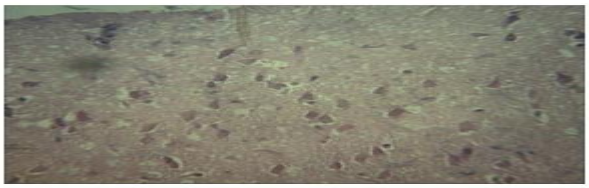
There is no visible lesion seen.

POST-TREATMENT MODE

Normal saline

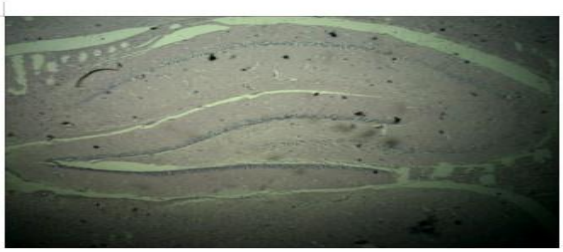
There is no visible lesion seen.

α-Lipoic acid



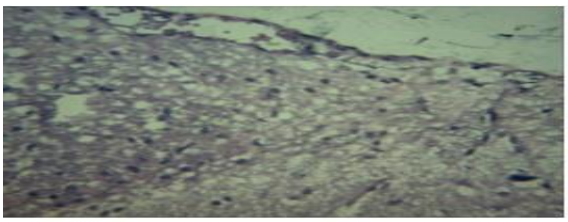
There is no visible lesion shown

200TCAE



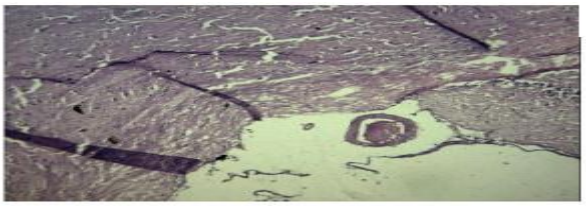
There is no visible lesion seen.

400TCAE



There are sub meningeal spongiosis

800TCAE

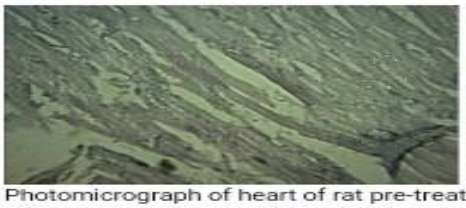


There is no visible lesion shown.

Figure 3.2.2: HISTOPATHOLOGY OF THE HEART SECTIONS

Photomicrograph of heart of rat in baseline. Group. No visible lesions seen.

Pre-treatment group



Photomicrograph of heart of rat pre-treated with normal saline. No visible lesions seen

.

Photomicrograph of heart of rat pre-treated with ALA. No visible lesions seen

Photomicrograph of heart of rat pre-treated with 200mg/kg TCAE No visible lesions seen.

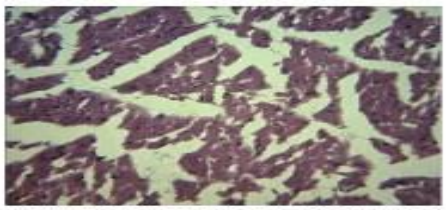
Photomicrograph of heart of rat pre-treated with 400mg/kg TCAE. No visible lesions seen

Photomicrograph of heart of rat pre-treated with 800mg/kg TCAE No visible lesions seen

Co-treament group

Photomicrograph of heart of rat co-treated with normal saline. No visible lesions seen.

.



Photomicrograph of heart of rat co-treated with ALA. No visible lesions seen

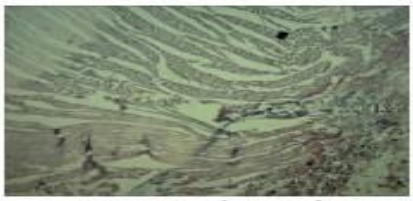
Photomicrograph of heart of rat co-treated with 200mg/kg TCAE. No visible lesions seen

Photomicrograph of heart of rat co-treated with 400mg/kg TCAE. No visible lesions seen.

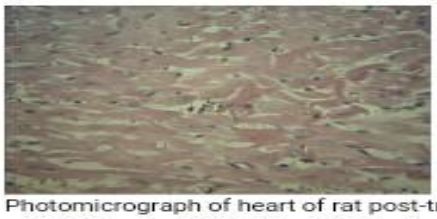
Photomicrograph of heart of rat co-treated with 800mg/kg TCAE No visible lesions seen

Post-treament group

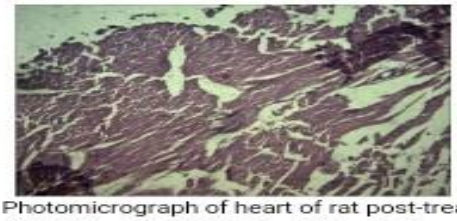
Photomicrograph of heart of rat post-treated with normal saline. No visible lesions seen.



Photomicrograph of heart of rat post-treated with ALA. No visible lesions seen



Photomicrograph of heart of rat post-treated with 200mg/kg TCAE. No visible lesions seen



Photomicrograph of heart of rat post-treated with 400mg/kg TCAE. No visible lesions seen.

4.0 DISCUSSION

Doxorubicin (DOX) is a widely used chemotherapeutic agent in the treatment of tumors with a major side effect on the brain and most especially cardiac toxicity. High concentration of DOX leads to a high redox reactivity in these tissues. DOX-increased ROS generation resulted in the oxidation of lipids, proteins, and signaling molecules (Kuznetsov *et al*., 2011). The principal mechanism of DOX is chelating DNA, inhibiting topoisomerase II and producing free radicals to kill cancer cells (Chen *et al*., 2013). α-Lipoic acid (ALA) and its reduced form DHLA, are considered as powerful natural antioxidant agents with a scavenging capacity for many reactive oxygen species (ROS). ALA/DHLA have some important advantages over other antioxidant agents such as vitamin E and C, because they have amphiphilic properties that confer their antioxidant actions in the membrane as well as in the cytosol. ALA/DHLA can also regenerate other antioxidant substances such as vitamin C, vitamin E and the ratio of reduced/oxidized glutathione (GSH/GSSG) (Newsholme *et al*., 2012). The present result showed that treating animal with α-lipoic acid improved and reversed the biochemical changes induced in the heart and brain tissues by DOX intoxication. This result correlates with the findings of Li *et al* (2013) where it was reported that α-lipoic acid ameliorates oxidative stress by increasing aldehyde dehydrogenase-2 activity in the heart and brain and also caused a significant fall in the lipid peroxide concentration.

The phenolic compounds in *T. cacao* stem bark contain bioactive compounds that have potential health benefits for chronic diseases such as inflammation, cardiovascular illness, neurodegenerative disorders, and cancer (Schinella *et al*., 2010)**.** This present study revelaed the protective potential of *Theobroma cacao* stem back aqueous extract (TCAE) on brain and cardiac enzymes and oxidative damages caused by doxorubicin induced toxicity. These findings also correlate with Zainal et al (2014) where it was stated that consumption of cocoa *T. cacao* stem bark and which have high antioxidant activity, could be beneficial in decreasing the damage from genotoxic and epigenetic carcinogens, and inhibiting the complex processes leading to cancer. *T. cacao* stem bark because of its polyphenolic compounds has become an important potential chemopreventive and therapeutic natural agent. Cocoa flavonoids influenced several important biological activities *in vitro* and *in vivo* by their free radical scavenging ability or through the regulation of signal transduction pathways to stimulate apoptosis, inhibit inflammation, cellular proliferation, apoptosis, angiogenesis and metastasis (Zainal *et al*., 2014).

LDH catalyzes the conversion of pyruvate to lactate and back, as it converts NADH to NAD^+. LDH is found extensively in body tissues, such as blood cells and heart muscle (Cruz *et al*., 2012). The result of the present study revealed that DOX intoxication caused a significant reduction and increase in LDH activity in brain and heart tissues respectively while pre-, co-, and post-treatment with TCAE caused a significant dose-dependent reversal in LDH activity compared with DOX-intoxicated untreated rats. This was supported by

the work of Koti *et al* (2013) where a significant modulation in LDH activity was observed during tissue damage. ACPs have had considerable impact as tools of clinical investigation and intervention. One particular example is tartrate resistant acid phosphatase, which is detected in the serum in raised amounts (Sarosiek *et al*., 2015). The result of the present study revealed that DOX intoxication caused a significant decrease and increase in ACP activity in brain and heart tissues respectively while pre-, co-, and post-treatment with TCAE caused a significant dose-dependent reversal in ACP activity compared with DOX-intoxicated untreated rats. This experimental result correlate with the work of Koti *et al* (2013) where it was stated that a modulation in the activities of cardiac and brain enzymes (LDH, GGT and ACP) was observed as a result of cardiac and brain damage caused by DOX. ALP activity on endothelial cell is responsible, in part, for the conversion of adenosine nucleotides to adenosine, a potent vasodilator and anti-inflammatory mediator that can protect tissues from the ischemic damage that results from injury (Swamy *et al*., 2012). The result of the present study revealed that DOX intoxication caused a significant increase and reduction in ALP activity in brain and heart tissues respectively while pre-, co-, and post-treatment with TCAE caused a significant dose-dependent reversal in ALP activity compared with DOX-intoxicated untreated rats. This was supported by the work of Abramowitz *et al*., 2010 where modulation in ALP activity in heart and brain were reported as an indicator of cardiac and brain damage. Gamma-glutamyl transferase (GGT) is a cell-surface protein contributing to the extracellular catabolism of glutathione (GSH). The enzyme is produced in many tissues. High levels of GGT have been associated in populations with increased risk of atherosclerotic cardiovascular disease (CVD) and brain damage (Kaya *et al*., 2014). In the present study, we revealed that DOX intoxication caused a significant increase in GGT activity in cardiac and brain tissues while pre-, co-, and post-treatment with TCAE caused a significant dose-dependent reduction in GGT activity in these tissues. This was supported by the work of Ragavendran *et al* (2012) where similar elevations in cardiac and brain enzymes activities in rats following challenge with a single cumulative dose of DOX was reported. According to Vijay *et al* (2011), GGT was reported as an important marker of tissue injury especially during clinical follow-up of DOX therapy.

DOX administration induced oxidative stress on these tissues as manifested by the alterations observed in both enzymatic and non-enzymatic cardiac antioxidant defense systems. From the present study, it was clear that DOX intoxication significantly increased concentrations of hydrogen peroxide generated, malondialdehyde (MDA) and protein carbonyl (PC). This is in agreement with the findings of Brett *et al* (2015) where it was reported that cells exposed to increasing concentration of DOX had an increase in concentrations of hydrogen peroxide generated, malondialdehyde (MDA) and protein carbonyl (PC) due to metabolic reductive activation of DOX to a semiquinone. Pre-, co-, and post-treatment with TCAE caused a significant dose-dependent reduction in H_2O_2, MDA and PC concentrations. This also agrees with the work of Zainal *et al* (2014) where it was reported that cocoa flavonoids influenced several important biological activities *in vitro* and *in vivo* by their free radical scavenging ability. In this study, DOX intoxication caused a significant increase in the activities of enzymes implicated in free radical generation: myeloperoxidase (MPX), NADPH oxidase (NOX) and xanthine oxidase (XO). This agrees with the work of Daniel *et al* (2008) where exposure of rats to DOX led to an increase in the activities of MPX, NOX and XO due to ability of DOX to bioactivate mitomycin C to generate oxygen radicals. Pre-, co-, and post-treatment with TCAE caused a significant dose-dependent reduction in these enzymes activities. This correlate with the work of Crozier *et al* (2012) as a result of its ability to inhibit the complex processes leading to cancer. Administration of TCAE reversed the DOX induced oxidative damage and significantly increased the antioxidant enzymes (catalase, superoxide dismutase, glutathione S-transferase and glutathione peroxidase). This is in agreement with the findings of Saratchandran and Cherupally (2012) where it was stated that reduction in these enzymic antioxidant activities is associated with a marked increase in cardiac and brain lipid peroxidation as manifested by increased MDA level. This study also revealed that DOX intoxication significantly decreased the concentration of non-enzymatic antioxidant (reduced glutathione, ascorbic acid and α-tocopherol). This is in agreement with Kuznetsov *et al*, (*2011*) where it was reported that high concentration of DOX leads to a high redox reactivity in the heart and brain. Pre-, co-, and post-treatment with TCAE significantly reversed the decrease observed in the concentrations of these non-enzymic markers caused by DOX intoxication. This finding correlates with the work of Golbidi *et al*, (2011), where both cocoa and ALA was reported to act as biological antioxidants, as metal chelators, reducing the oxidized forms of other antioxidant agents such as vitamins C and E and reduced glutathione. The tissue histology showed no visible lesions. This is in agreement with Blanco *et al* (2012) who stated that the neurotoxicity and cardiotoxicity of DOX remains difficult to predict and is often not detected until years after the completion of chemotherapy.

5.0 CONCLUSION

Thus, the result of the present study affirmed that *T. cacao* stem bark aqueous extract protected against DOX induced oxidative damage in the brain and heart of experimental rats.

6.0 REFERENCES

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