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THEOBROMA CACAO STEM BARK AMELIORATES DOXORUBICIN-INDUCED OXIDATIVE STRESS, TESTICULAR AND LUNGS DAMAGE IN EXPERIMENTAL RATS

Kosoko A. M.*, Olurinde O. J. and Akinloye O. A.

Department of Biochemistry, College of Biosciences (COLBIOS), Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria.

***Corresponding Author: Kosoko A. M.**

Department of Biochemistry, College of Biosciences (COLBIOS), Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria.

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ABSTRACT

Doxorubicin **(DOX),** a chemotherapy drug, is used to treat different types of cancer but its clinical use has been limited by dose-dependent multi-organ toxicities especially to the lungs, testes, heart and liver. Eighty rats, randomly selected, were divided into three (3) treatment groups: pre-, co- and post-treatment groups; consisting of 6 sub-groups each (5 rats per sub-group); baseline, normal saline (2ml), α- lipoic acid (20mg/kg body weight), and 200mg/kg, 400mg/kg or 800mg/kg body weight *Theobroma cacao* stem bark aqueous extract (TCAE) sub-groups. All animals except those in baseline sub-group were intoxicated with 20mg/kg body weight DOX intraperitoneally. Animals in the pre-treatment group were administered a single dose of DOX followed by 7 days oral administration of normal saline, α- lipoic acid or graded doses of TCAE; co-treatment group were co-administered 2.86 mg/kg body weight 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. Animals were sacrificed (pre- and post- treatment group were sacrificed on the 9th day while the co-treatment group sacrificed on the 8th day), blood samples collected by retro-orbital plexus, testes and lungs harvested for biochemical assays and histopathological investigations. Data were analyzed using SPSS 20.0 statistical tool, significance of difference calculated using one-way analysis of variance (ANOVA) and Duncan multiple range test. DOX intoxication caused a significant increase in testicular and lungs acid phosphatase, lactate dehydrogenase, γ-glutamyl transferase activities with a concomitant decrease in alkaline phosphatase activity. DOX intoxication also caused a significant increase in the concentrations of hydrogen peroxide, malondialdehyde and protein carbonyl; activities of myeloperoxidase, NADPH oxidase and xanthine oxidase while significantly reducing the concentrations of reduced glutathione, ascorbic acid and α-tocopherol; and activities of catalase, superoxide dismutase, glutathione-S-transferase and glutathione peroxidase. Treatment with TCAE significantly ameliorated markers of testicular and lungs damage and pro-oxidant markers while elevating the organs antioxidant status. TCAE possess a potential ameliorative property against DOX induced testicular and lungs damage and oxidative stress.

KEYWORDS: Chemoprevention; Theobroma cacao; Doxorubicin; Testicular toxicity; Lungs toxicity; Oxidative stress.

1.0 INTRODUCTION

Doxorubicin (DOX), an anthracycline, derived from *Streptomyces peucetius var. Caesius* in the 1970's, is a chemotherapeutic agent which has been used effectively in treating acute leukaemia and malignant lymphoma and solid tumours (Sridwi, 2011). Doxorubicin has a broad spectrum of potent activity against many different types of cancers, including a variety of solid tumours such as breast, lung, gastric, ovarian, thyroid, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, sarcoma and pediatric cancers (Tikoo *et al*., 2011; Sridwi, 2011). Doxorubicin exhibits profound toxicity to the reproductive system, adversely affecting male fertility (Thiagarajan, 2011). The evaluation of the damage caused by doxorubicin can contribute to further studies against harmful side effects of doxorubicin because such effects are similar between man and rodents in many aspects (Sridwi, 2011). *Theobroma cacao is* a small 4-8m (13-36ft) tall evergreen tree, native to the deep tropical regions of central and south America. It belongs to the genus *Theobroma*, classified under the subfamily *Sterculioidea* of the mallow family *Malvaceae*. Cocoa trees are found wild in the rain forest of the western hemisphere from 18°N to 15°S, which is from Mexico to the southern edge of the Amazon forests. Cocoa and its compounds have drawn recently a lot of attention

because of its contributory role as a chemopreventive agent (Yazan et al., 2013). It is widely used in the manufacture of chocolate. 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). Most research on the medicinal properties of *Theobroma cacao* are centered on the pod and seed with only a few un established works on the stem bark. α-Lipoic acid (ALA), thiotic acid (TA), is a natural compound chemically named 1, 2-dithiolane-3-pentanoic acid ($C_8H_{14}O_2S_2$) is essential for the function of different enzymes of oxidative metabolism (Golbidi *et al*., 2011). ALA is commonly found in dietary components such as vegetables (spinach, broccoli, tomato) and meats, mainly viscera and also in many dietary supplements. ALA has shown to improve endothelial dysfunction (Wray *et al*., 2012) and to reduce oxidative stress post exercise training (McNeilly *et al*., 2011). It also protects against the development of atherosclerosis and inhibits the progression of an already established atherosclerosis plaque (Ying *et al.*, 2011). In humans, ALA is synthesized by the liver and other tissues (also found naturally in our diets), and functions as a cofactor within pyruvate dehydrogenase complex and α keto-glutarate dehydrogenase complex (Saeid *et al*., 2011). ALA and its active reduced counterpart, dihydrolipoic acid (DHLA), have been shown to combat oxidative stress by scavenging reactive oxygen species (ROS), regeneration of exogenous and endogenous antioxidants such as vitamins C and E, and GSH (Saeid *et al*., 2011). Testicles (testes) are oval shaped organs of the male reproductive system that lie in the scrotum, secured at either end by a structure called the spermatic cord. The testes are responsible for making testosterone, the primary male sex hormone, and for generating sperm (Murdakai *et al*., 2011). The pathogenesis of testicular injury is initiated by the participation of toxicants or by their bioconversion to potentially toxic metabolites. These metabolites can be electrophilic chemicals or free radical, which either elicits an immune response or directly affect the biochemistry of the cells by interacting with cellular molecules leading to protein dysfunction, lipid peroxidation, DNA damage, oxidative stress and depletion of natural antioxidants (Kamboj and Kalia, 2013)**.** The lungs are a pair of spongy air-filled organs located on either side of the chest (thorax), consisting of elastic sacs with branching passages into which air is drawn, in order to allow passage of oxygen into the blood and removal of carbon dioxide from the blood. The trachea (windpipe) conducts inhaled air into the lungs through its tubular branches, called bronchi, which then divides into smaller branches (bronchioles). The bronchioles eventually end in clusters of microscopic air sacs called alveoli in which oxygen from the air is absorbed into the blood (Wikimania, 2015). Carbondioxide, a waste product of metabolism, travels from the blood to the alveoli, where it can be exhaled. The lungs are covered by a thin tissue layer called the pleura.

The aim of this study is to determine the ameliorative potential of *T. cacao* stem bark aqueous extract (TCAE) on doxorubicin (DOX) induced testicular and lungs damage and oxidative stress in experimental rats.

2.0 MATERIALS AND METHODS

2.1 PREPARATION OF THE AQUEOUS 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 (2006) was adopted.

2.2 CHEMICALS AND REAGENTS

Doxorubicin (20mg adriamycin hydrochloride), Pharmacia Italia S.P.A. Italy was used in this study. All other chemicals used were of analytical grade.

2.3 IN VITRO ANTIOXIDANT ASSAY OF THEOBROMA CACAO STEM BARK AQUEOUS EXTRACT (TCAE)

2.3.1 Total phenolic content

The total phenolic content was determined by using the Folin-Ciocalteu reagent according to the method of Singleton et al., (1999)

2.3.2 Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by the ability of the extract to scavenge the hydroxyl radicals generated by Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction) (Halliwell et al., 1981)

2.3.3 ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

The spectrophotometric assay of $ABTS^+$ radical scavenging activity was determined according to the method of Re et al., (1999) with some modification.

2.3.4 DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging activity

The free radical scavenging activity was measured by a modified DPPH assay (Blois, 1958).

2.4 ANIMALS AND TREATMENTS

The experimental wistar rats (*Rattus novergicus)* weighing 140 – 150g were obtained from the Animal Unit of the University of Ibadan, Nigeria. Eighty (80) healthy wistar rats were kept in a well aerated wire-meshed up and down wooden cage under normal atmospheric conditions with 12hours light/dark cycle. They were allowed access to water and commercial grower feeds *ad-libitum* from Animal Care Nigeria

limited. The rats were allowed to acclimatize for eight weeks before the study commenced. The experimental rats were divided into four viz: pre-treatment, co-treatment, post-treatment and baseline group, with each group except the baseline group further sub-divided into five different sub-groups of five rats per sub-group as follows:

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 20mg/kg body weight DOX intraperitoneally on the first day. After 24hours, oral treatment of normal saline (negative control), 20mg/kg body weight α-lipoic acid (positive control), 200mg/kg body weight TCAE, 400mg/kg body weight TCAE or 800mg/kg body weight TCAE respectively in each group was conducted for seven days. The rats were fasted overnight and sacrificed 24hours 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 2.86mg/kg body weight doxorubicin was co-administered intraperitoneally with normal saline (negative control), 20mg/kg body weight α-lipoic acid (positive control), 200mg/kg body weight TCAE, 400mg/kg body weight TCAE or 800mg/kg body weight TCAE respectively in each group for seven days orally. The rats were fasted overnight and sacrificed 24hours 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), 20mg/kg body weight α-lipoic acid (positive control), 200mg/kg body weight TCAE, 400mg/kg body weight TCAE or 800mg/kg body weight TCAE orally respectively in each group for seven days. Single dose of 20mg/kg body weight DOX was administered intraperitoneally on the eight day, the rats fasted overnight and sacrificed 24 hours 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 24 hours after the last administration.

2.5 SAMPLE COLLECTION

The animals were anaesthetized using diethylether and dissected. Blood samples were collected via retro-orbital plexus and transferred into lithium heparin bottles for enzyme analyses. Testicles and lungs were harvested and transferred into a pre-cooled normal saline, rinsed and mopped with filter paper. A section of the tissues were excised and transferred into 10% formalin for histopathological investigation while known weight of the tissue samples were homogenized in 4 volumes of the homogenizing potassium phosphate buffer pH 7.4 using a Teflon homogenizer. The resulting homogenate was centrifuged at 5000rpm at 4^0C for 10minutes to obtain the post-mitochondrial fraction (PMF). The supernatant was collected and stored for biochemical assays.

2.6 BIOCHEMICAL ANALYSES

Testicular and lungs 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 Li et al. (1873) while lactate dehydrogenase (LDH) activity was determined according to the method described by Bower (1963). Testicular and lungs hydrogen peroxide concentration was quantified based on Wolff's method (1991). Tissue protein carbonyl concentration was carried out by following method described by Levine *et al*., (1994). Malondialdehyde 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 activity was determined using method of Klebanoff et al., 2005. NADPH oxidase activity was measured by the method of Reusch and Burger (1974). Xanthine oxidase activity was determined according to the method of Bergmeyer et al., (1974). Glutathione-S-transferase activity was determined according to Habig *et al*, (1974). Enzymatic assay of glutathione peroxidase activity was determined following the method described by Rotruck et al., (1973). Catalase activity was determined according to the method of Sinha A. (1971). The activity of superoxide dismutase 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 concentration was quantified according to the method of Nino and Shah (1986). Concentration of α–tocopherol was carried out following the procedure of Kayden et al., (1973).

2.7 HISTOPATHOLOGICAL EXAMINATION OF LUNGS AND TESTICULAR 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. Five micrometer thick serial histological sections were obtained from the paraffin blocks and stained with hematoxylin and eosin. The sections were examined under light microscope to evaluate pathological changes and photomicrographs were taken (Krause, 2001).

2.8 STATISTICAL ANALYSIS OF DATA

The data obtained from various tests were analyzed using statistical package for social sciences (SPSS) V20.0 and the results presented as mean ± standard deviation.

Group means were compared by one-way analysis of variance (ANOVA) followed by post hoc test/ test of significance using Duncan multiple test at $p < 0.05$.

3.0 RESULTS

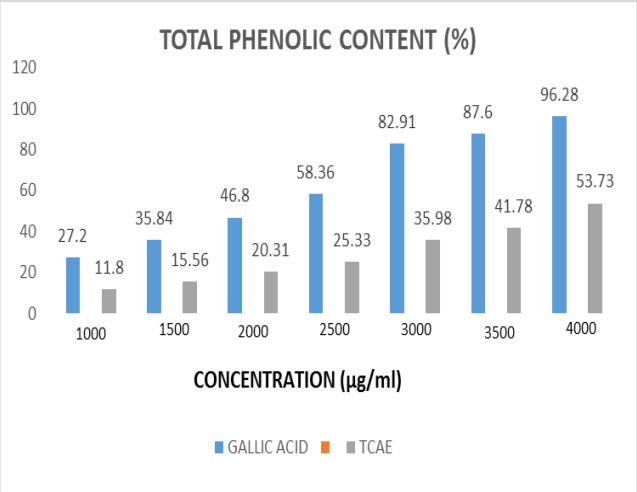


Figure 3.1: Total phenolic content of TCAE

Values are means of percentages of inhibition of three replicates
TCAE – Theobroma cacao aqueous extract.

The standard, gallic acid, exhibited highest phenolic content relative to Theobroma cacao stem bark. The phenolic content of Theobroma cacao stem bark extract increased in a concentration dependent manner.

Hydroxyl radical scavenging assay shows the ability of the extracts and standard ascorbic acid to inhibit hydroxyl radical-mediated deoxyribose degradation in a Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture.

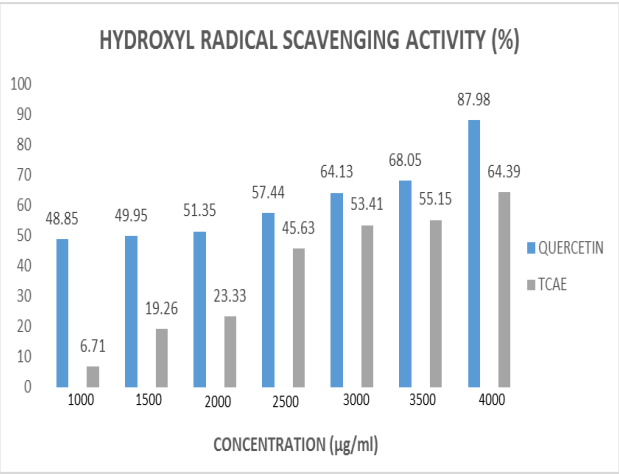


Figure 3.2: Hydroxyl radical scavenging activity

Values are means of percentages of inhibition of three replicates
TCAE – Theobroma cacao aqueous extract

Theobroma cacao and the standard showed a significant scavenging ability for hydroxyl radicals.

A concentration-dependent scavenging activity of DPPH radical was shown by Theobroma cacao stem bark aqueous extract and Annona muricata methanol leaves extract.

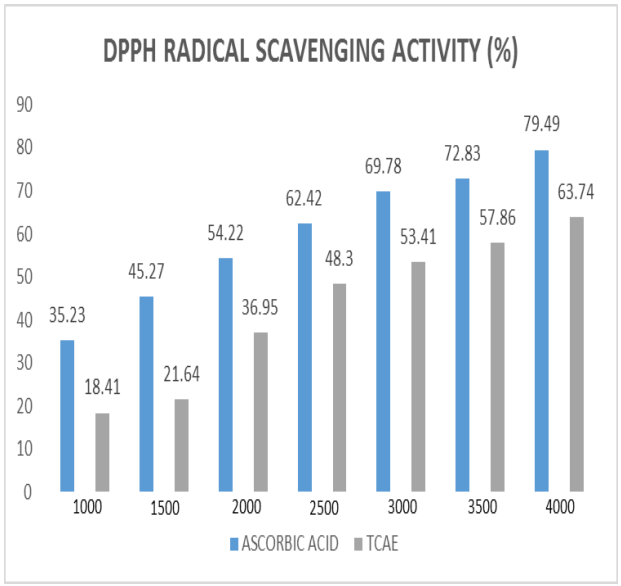


Figure 3.3: DPPH radical scavenging ability

Values are means of percentages of inhibition of three replicates
TCAE – Theobroma cacao aqueous extract

Theobroma cacao and the standard shows significant ability to scavenge DPPH radicals.

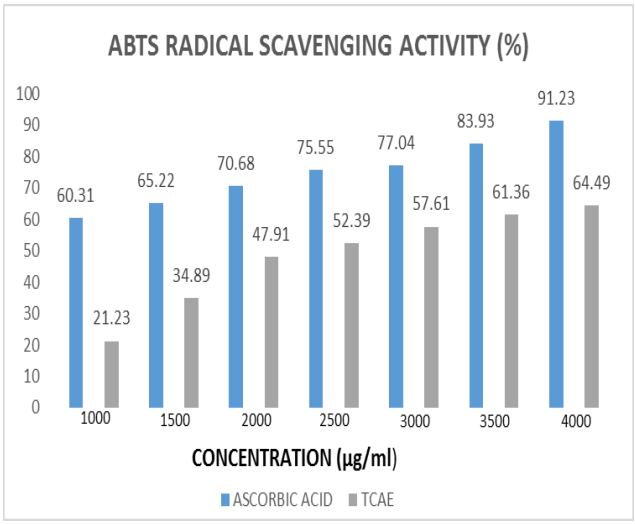


Figure 3.4: ABTS radical scavenging activity

Values are means of percentages of inhibition of three replicates
TCAE – Theobroma cacao aqueous extract

Theobroma cacao and the standard showed a significant ability to scavenge ABTS radicals.

Table 3.1: Effect of DOX intoxication on sperm morphometrics and the ameliorative role of TCAE.

	PRE-TREATMENT					CO-TREATMENT					POST-TREATMENT					BASELINE
PARAMETER	**NS**	**ALA**	**200 TCAE**	**400 TCAE**	**800 TCAE**	**NS**	**ALA**	**200 TCAE**	**400 TCAE**	**800 TCAE**	**NS**	**ALA**	**200 TCAE**	**400 TCAE**	**800 TCAE**	
Active	**20**	**30**	**30**	**30**	**35**	**25**	**60**	**30**	**70**	**50**	**35**	**50**	**60**	**50**	**55**	75
Sluggish	**20**	**30**	**30**	**30**	**15**	**20**	**30**	**60**	**25**	**25**	**40**	**30**	**25**	**25**	**40**	25
Death	**60**	**40**	**45**	**30**	**50**	**10**	**10**	**10**	**10**	**25**	**30**	**15**	**10**	**25**	**10**	Nil
Viscosity	+++	++	+++	+++	+++	++++	+++	+++	++++	+++	++	++	+++	++++	++++	++++
Tailless head	**40**	**20**	**25**	**15**	**15**	**Nil**	**Nil**	**20**	**10**	**20**	**Nil**	**15**	**10**	**Nil**	**10**	5
Headless tail	**10**	**16**	**15**	**15**	**15**	**10**	**10**	**Nil**	**10**	**Nil**	**15**	**15**	**10**	**7**	**Nil**	5
Rudimentary head	**30**	**36**	**35**	**10**	**40**	**15**	**40**	**20**	**Nil**	**15**	**15**	**10**	**20**	**15**	**15**	10
Bent tail	**10**	**20**	**18**	**25**	**10**	**35**	**20**	**25**	**30**	**Nil**	**Nil**	**15**	**15**	**10**	**40**	Nil
Curve mouth pieces	**40**	**40**	**35**	**50**	**30**	**30**	**25**	**30**	**40**	**70**	**40**	**35**	**45**	**30**	**35**	58
Coiled tail	**30**	**10**	____	**10**	**10**	**10**	**5**	**5**	**10**	**7**	**30**	**Nil**	**5**	**10**	**10**	Nil
% of abnormal	**30**	**32**	**45**	**50**	**50**	**10**	**5**	**45**	**20**	**7**	**30**	**30**	**15**	**10**	**20**	10
Pus cell	**8-10**	**5-7**	**4-7**	**6-8**	**5-7**	**9-11**	**10-12**	**6-7**	**8-10**	**10-12**	**10-12**	**10-12**	**10-12**	**7-10**	**8-10**	8-10
RBC	**2-3**	**1-2**	**1-2**	**4-6**	**1-2**	**1-2**	**2-3**	**4-6**	**5-7**	**3-6**	**5-6**	**5-6**	**5-6**	**3-5**	**Nil**	4-6
Yeast cell	++	+	**Nil**	+	**Nil**	**Few**	+	++	**Few**	+	+	+	+	**Nil**	**Nil**	Nil
Bacteria cells	++	+	**Nil**	+	+	+	++	++	++	++	++	++	+	++	+	+
Sperm count	**59.9**	**62.9**	**61.4**	**60.5**	**82**	**307.5**	**340.6**	**395**	**314.5**	**366.6**	**326.7**	**371.3**	**350.6**	**367**	**326.8**	425

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.

The table above revealed that a significant alteration was observed in the sperm morphometrics following DOX intoxication. Normalcy was restored on the sperm morphometrics with pre-, co- and post- TCAE treatment in a dose dependent manner.

Table 3.2: Organ weights (g) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	$0.695\pm0.007^{\alpha}$	$1.450\pm0.070^{\alpha\gamma}$	$1.657\pm0.099^{\alpha}$	----------	$0.15\pm0.07^{\alpha\gamma}$	$0.23\pm0.03^{\alpha\gamma}$	$0.49\pm0.01^{\alpha\gamma}$	---------
α-lipoic acid	$0.730\pm0.252^{\alpha}$	$1.699\pm0.014^{\alpha\beta}$	$1.679\pm0.007^{\alpha}$	----------	$1.11\pm0.03^{\beta}$	$0.74\pm0.01^{\alpha\beta}$	$1.23\pm0.02^{\alpha\beta}$	---------
200TCAE	$1.340\pm0.254^{\alpha\beta\gamma}$	$1.525\pm0.021^{\alpha\gamma}$	$1.680\pm0.124^{\alpha}$	----------	$0.83\pm0.02^{\alpha\beta\gamma}$	$0.82\pm0.04^{\alpha\beta\gamma}$	$1.03\pm0.01^{\alpha\beta}$	---------
400TCAE	$1.840\pm0.226^{\beta\gamma}$	$1.690\pm0.014^{\alpha\beta}$	$1.793\pm0.015^{\beta\gamma}$	----------	$0.99\pm0.02^{\alpha\beta\gamma}$	$0.83\pm0.01^{\alpha\beta\gamma}$	$1.14\pm0.04^{\alpha\beta\gamma}$	---------
800TCAE	$1.750\pm0.071^{\beta\gamma}$	$1.795\pm0.007^{\beta}$	$1.585\pm0.077^{\alpha\gamma}$	----------	$0.77\pm0.02^{\alpha\beta\gamma}$	$1.02\pm0.01^{\alpha\beta\gamma}$	$1.29\pm0.02^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	1.872±0.064	----------	----------	----------	1.02±0.01

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

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.

The table above revealed that DOX intoxication caused a significant decrease in the testicular weight of experimental animals. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in the testicular weight of experimental animals.

Doxorubicin intoxication caused a significant decrease in lung weight compared to baseline. A significant dose dependent increase in lung weight was observed with the administration of TCAE in co and post-treatment, while an insignificant increase in the lung weight was observed in rats pre-treated with different concentrations of TCAE.

Table 3.3: Acid phosphatase (ACP) activity (IU/L) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	$16.35\pm1.060^{\alpha\gamma}$	$14.68\pm0.417^{\alpha\gamma}$	$11.39\pm0.007^{\alpha\gamma}$	----------	$18.03\pm0.75^{\alpha\gamma}$	$2.70\pm0.41^{\alpha\gamma}$	$9.05\pm0.29^{\alpha\gamma}$	---------
α-lipoic acid	$13.35\pm0.636^{\alpha\beta}$	$10.90\pm0.084^{\alpha\beta}$	$7.270\pm0.367^{\alpha\beta}$	----------	$5.52\pm0.61^{\alpha\beta}$	$3.84\pm0.29^{\alpha\beta}$	$2.61\pm0.26^{\alpha\beta}$	---------
200TCAE	$9.900\pm0.424^{\alpha\beta\gamma}$	$7.535\pm0.077^{\alpha\beta\gamma}$	$5.865\pm0.091^{\alpha\beta\gamma}$	----------	$3.37\pm0.15^{\alpha\beta\gamma}$	$2.35\pm0.20^{\alpha\beta\gamma}$	$1.63\pm0.07^{\alpha\beta\gamma}$	---------
400TCAE	$8.720\pm0.127^{\alpha\beta\gamma}$	$6.435\pm0.063^{\alpha\beta\gamma}$	$4.920\pm0.098^{\alpha\beta\gamma}$	----------	$2.25\pm0.79^{\alpha\beta\gamma}$	$1.52\pm0.07^{\alpha\beta\gamma}$	$1.09\pm0.05^{\alpha\beta\gamma}$	---------
800TCAE	$8.165\pm0.205^{\alpha\beta\gamma}$	$4.985\pm0.035^{\alpha\beta\gamma}$	$4.650\pm0.134^{\alpha\beta\gamma}$	----------	$1.34\pm0.24^{\alpha\beta\gamma}$	$0.92\pm0.03^{\alpha\beta\gamma}$	$0.66\pm0.01^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	3.800±0.424	----------	----------	----------	0.29±0.01

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

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.

The table above revealed that DOX intoxication caused a significant increase in testicular ACP activity of experimental animals. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant decrease in testicular ACP activity of experimental animals. Doxorubicin intoxication induced a significant increase in ACP activity in the lungs for the three modes of treatment. A dose dependent decrease in ACP activity was observed with the administration of TCAE compared with other groups.

Table 3.4: Alkaline phosphatase (ALP) activity (IU/L) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	$5.850\pm0.070^{\alpha\gamma}$	$5.595\pm0.417^{\alpha\gamma}$	$7.815\pm0.232^{\alpha\gamma}$	----------	$1.63\pm0.27^{\alpha\gamma}$	$4.18\pm0.89^{\alpha\gamma}$	$8.36\pm0.63^{\gamma}$	---------

α-lipoic acid	7.780±0.311$^{\alpha\beta}$	8.440±0.452$^{\alpha\beta}$	10.50±0.987$^{\alpha\beta}$	----------	9.96±0.45$^{\alpha\beta}$	12.40±0.40$^{\alpha\beta}$	9.14±0.96$^{\alpha\beta}$	---------
200TCAE	10.40±0.572$^{\alpha\beta\gamma}$	10.60±0.558$^{\alpha\beta\gamma}$	13.94±0.077$^{\alpha\beta\gamma}$	----------	5.08±0.65$^{\alpha\beta\gamma}$	19.96±0.05$^{\alpha\beta\gamma}$	11.50±0.85$^{\alpha\beta\gamma}$	---------
400TCAE	11.36±0.077$^{\alpha\beta\gamma}$	12.45±0.091$^{\alpha\beta\gamma}$	14.72±0.321$^{\alpha\beta\gamma}$	----------	3.42±0.24$^{\alpha\beta\gamma}$	3.36±0.96$^{\alpha\beta\gamma}$	7.66±0.45$^{\alpha\beta\gamma}$	---------
800TCAE	11.93±0.056$^{\alpha\beta\gamma}$	13.64±0.339$^{\alpha\beta\gamma}$	15.82±0.219$^{\alpha\beta\gamma}$	----------	2.56±0.16$^{\alpha\beta\gamma}$	3.01±0.02$^{\alpha\beta\gamma}$	6.74±0.04$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	16.53±2.42	----------	----------	----------	8.62±2.48

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

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.

The table above revealed that DOX intoxication caused a significant decrease in ALP activity in the testes. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in the ALP activity in the testes of experimental animals. Doxorubicin intoxication induced a significant decrease in ALP activity in the lungs for the three modes of treatment. An increase in ALP activity was observed with the administration of 200mg/kg bodyweight TCAE across the three modes of treatment but the administration of 400 and 800mg/kg bodyweight TCAE caused a decrease in ALP activity.

Table 3.5: γ-Glutamyl Transferase (γ-GT) activity (IU/L) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				**LUNGS**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	43.12±1.294$^{\alpha\gamma}$	43.71±0.058$^{\alpha\gamma}$	36.93±0.098$^{\alpha\gamma}$	----------	3.35±0.40$^{\alpha\gamma}$	4.09±0.38$^{\alpha\gamma}$	3.40±0.89$^{\alpha\gamma}$	---------
α-lipoic acid	38.32±0.459$^{\alpha\beta}$	37.78±0.466$^{\alpha\beta}$	33.66±0.325$^{\alpha\beta}$	----------	2.06±0.38$^{\beta}$	0.93±0.10$^{\alpha\beta}$	1.86±0.18$^{\alpha\beta}$	---------
200TCAE	36.82±0.091$^{\alpha\beta\gamma}$	35.76±1.271$^{\alpha\beta\gamma}$	30.56±0.622$^{\alpha\beta\gamma}$	----------	2.43±0.46$^{\beta}$	2.36±0.20$^{\beta\gamma}$	0.73±0.04$^{\alpha\beta\gamma}$	---------
400TCAE	35.89±0.982$^{\alpha\beta\gamma}$	35.43±0.321$^{\alpha\beta\gamma}$	28.92±0.630$^{\alpha\beta\gamma}$	----------	1.29±0.12$^{\alpha\beta\gamma}$	2.27±0.37$^{\beta\gamma}$	1.48±0.43$^{\alpha\beta}$	---------
800TCAE	35.16±0.219$^{\alpha\beta\gamma}$	34.11±0.956$^{\alpha\beta\gamma}$	26.15±0.692$^{\alpha\beta\gamma}$	----------	3.52±0.27$^{\alpha\gamma}$	0.78±0.05$^{\alpha\beta\gamma}$	2.80±0.08$^{\beta\gamma}$	---------
BASELINE	----------	----------	----------	23.22±0.551	----------	----------	----------	2.52±0.97

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

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.

The table above revealed that DOX intoxication caused a significant elevation in γ-GT activity in the testes. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant reduction in the γ-GT activity in the testes of experimental animals. Doxorubicin intoxication induced a significant increase in γ-GT activity in the lungs for the three modes of treatment. A significant dose dependent decrease in γ-GT activity was observed with the

administration of TCAE in co-treatment group. In pre-treatment group, administration of 200 and 400mg/kg TCAE caused a significant decrease in γ-GT activity while 800mg/kg TCAE caused an insignificant increase in γ-GT activity. In post-treatment, administration of TCAE caused a significant decrease in γ-GT activity in the lung tissue in a dose-dependent manner.

Table 3.6: Lactate dehydrogenase (LDH) activity (IU/L) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	50.53±0.601$^{\alpha\gamma}$	46.21±0.242$^{\alpha\gamma}$	39.38±0.537$^{\alpha\gamma}$	----------	2.09±0.24$^{\alpha}$	1.54±0.39$^{\alpha\gamma}$	1.64±0.02$^{\alpha\gamma}$	---------
α-lipoic acid	43.61±0.777$^{\alpha\beta}$	40.95±0.760$^{\alpha\beta}$	36.42±0.339$^{\alpha\beta}$	----------	1.97±0.73$^{\alpha}$	3.02±0.35$^{\beta}$	2.30±0.89$^{\alpha\beta}$	---------
200TCAE	37.33±0.169$^{\alpha\beta\gamma}$	35.96±0.390$^{\alpha\beta\gamma}$	35.05±0.235$^{\alpha\beta\gamma}$	----------	2.20±0.49$^{\alpha}$	2.26±0.49$^{\alpha\beta\gamma}$	3.24±0.19$^{\beta\gamma}$	---------
400TCAE	35.79±0.261$^{\alpha\beta\gamma}$	35.11±1.510$^{\alpha\beta\gamma}$	33.85±0.063$^{\alpha\beta\gamma}$	----------	2.27±0.85$^{\alpha}$	2.31±0.88$^{\alpha\beta\gamma}$	3.42±0.29$^{\beta\gamma}$	---------
800TCAE	35.08±0.837$^{\alpha\beta\gamma}$	33.93±0.639$^{\alpha\beta\gamma}$	32.28±0.544$^{\alpha\beta\gamma}$	----------	2.62±0.89$^{\alpha\gamma}$	3.06±0.57$^{\beta\gamma}$	4.06±0.52$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	27.05±2.69	----------	----------	----------	3.21±0.41

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

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.

The table above revealed that DOX intoxication caused a significant elevation in LDH activity in the testes. Pre-, co and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant reduction in the LDH activity in the testes the of experimental animals. Doxorubicin intoxication induced a significant decrease in LDH activity in the lungs for the three modes of treatment. A significant dose dependent increase in LDH activity was observed with the administration of TCAE in co and post-treatment groups. There was no significant difference in LDH activity in rats pre-treated with TCAE (200mg/kg, 400mg/kg or 800mg/kg) compared with normal saline group.

Table 3.7: Hydrogen peroxide (H_2O_2) concentration (µmol/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	7.665±0.100$^{\alpha\gamma}$	5.599±0.100$^{\alpha\gamma}$	3.992±0.131$^{\alpha\gamma}$	----------	11.28±0.35$^{\alpha\gamma}$	7.55±0.35$^{\alpha\gamma}$	5.38±0.17$^{\alpha\gamma}$	---------
α-lipoic acid	2.341±0.270$^{\alpha\beta}$	1.534±0.183$^{\alpha\beta}$	1.151±0.115$^{\alpha\beta}$	----------	3.28±0.36$^{\alpha\beta}$	2.27±0.24$^{\alpha\beta}$	1.55±0.16$^{\alpha\beta}$	---------
200TCAE	1.477±0.614$^{\alpha\beta\gamma}$	1.030±0.180$^{\alpha\beta\gamma}$	0.717±0.031$^{\alpha\beta\gamma}$	----------	2.01±0.61$^{\alpha\beta\gamma}$	1.38±0.17$^{\alpha\beta\gamma}$	0.97±0.04$^{\alpha\beta\gamma}$	---------
400TCAE	1.026±0.085$^{\alpha\beta\gamma}$	0.659±0.035$^{\alpha\beta\gamma}$	0.479±0.024$^{\alpha\beta\gamma}$	----------	1.32±0.06$^{\alpha\beta\gamma}$	0.90±0.05$^{\alpha\beta\gamma}$	0.65±0.03$^{\alpha\beta\gamma}$	---------
800TCAE	0.588±0.019$^{\alpha\beta\gamma}$	0.386±0.041$^{\alpha\beta\gamma}$	0.290±0.006$^{\alpha\beta\gamma}$	----------	0.79±0.02$^{\alpha\beta\gamma}$	0.54±0.02$^{\alpha\beta\gamma}$	0.39±0.01$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	0.130±0.039	----------	----------	----------	0.13±0.04

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

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.

The table above revealed that DOX intoxication caused a significant increase in the generation of reactive oxygen species (H_2O_2 inclusive) in the testes. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant reduction in H_2O_2 generated in the testes of experimental animals. Doxorubicin intoxication caused a significant increase in H_2O_2 generation in the lungs across the three modes of treatment. A dose dependent decrease in H_2O_2 concentration was observed with the administration of TCAE compared with other groups across the three modes of treatment

Table 3.8: Malondialdehyde (MDA) concentration (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	13.72±0.169 $^{\alpha\gamma}$	9.464±0.242 $^{\alpha\gamma}$	6.715±0.221 $^{\alpha\gamma}$	----------	11.28±0.35$^{\alpha\gamma}$	7.55±0.35$^{\alpha\gamma}$	5.38±0.17$^{\alpha\gamma}$	---------
α-lipoic acid	4.015±0.345 $^{\alpha\beta}$	2.831±0.157$^{\alpha\beta}$	1.936±0.193 $^{\alpha\beta}$	----------	3.28±0.36$^{\alpha\beta}$	2.27±0.24$^{\alpha\beta}$	1.55±0.16$^{\alpha\beta}$	---------
200TCAE	2.501±0.097 $^{\alpha\beta\gamma}$	1.615±0.148 $^{\alpha\beta\gamma}$	1.207±0.053 $^{\alpha\beta\gamma}$	----------	2.01±0.61$^{\alpha\beta\gamma}$	1.38±0.17$^{\alpha\beta\gamma}$	0.97±0.04$^{\alpha\beta\gamma}$	---------
400TCAE	1.590±0.074 $^{\alpha\beta\gamma}$	1.113±0.055 $^{\alpha\beta\gamma}$	0.806±0.040 $^{\alpha\beta\gamma}$	----------	1.32±0.06$^{\alpha\beta\gamma}$	0.90±0.05$^{\alpha\beta\gamma}$	0.65±0.03$^{\alpha\beta\gamma}$	---------
800TCAE	1.018±0.053 $^{\alpha\beta\gamma}$	0.685±0.026 $^{\alpha\beta\gamma}$	0.488±0.010 $^{\alpha\beta\gamma}$	----------	0.79±0.02$^{\alpha\beta\gamma}$	0.54±0.02$^{\alpha\beta\gamma}$	0.39±0.01$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	0.219±0.006	----------	----------	----------	0.13±0.04

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

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.

The table above revealed that DOX intoxication caused a significant increase in MDA concentration in the testes. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant reduction in MDA concentration in the testes of experimental animals. Doxorubicin intoxication caused a significant increase in MDA generation in the lungs across the three modes of treatment. A dose dependent reduction in MDA concentration was observed with the administration of TCAE compared with other groups across the three modes of treatment.

Table 3.9: Protein carbonyl (PC) concentration (nmol/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	14.40±0.821 $^{\alpha\gamma}$	10.25±0.468 $^{\alpha\gamma}$	7.306±0.241 $^{\alpha\gamma}$	----------	20.21±0.01$^{\alpha\gamma}$	13.82±0.61$^{\alpha\gamma}$	9.85±0.325$^{\alpha\gamma}$	---------
α-lipoic acid	4.453±0.496 $^{\alpha\beta}$	3.076±0.326 $^{\alpha\beta}$	2.107±0.210 $^{\alpha\beta}$	----------	6.00±0.66$^{\alpha\beta}$	4.15±0.44$^{\alpha\beta}$	2.84±0.246$^{\alpha\beta}$	---------
200TCAE	2.721±0.121 $^{\alpha\beta\gamma}$	1.876±0.225 $^{\alpha\beta\gamma}$	1.312±0.058 $^{\alpha\beta\gamma}$	----------	3.67±0.16 $^{\alpha\beta\gamma}$	2.53±0.30$^{\alpha\beta\gamma}$	1.77±0.078$^{\alpha\beta\gamma}$	---------
400TCAE	1.796±0.076 $^{\alpha\beta\gamma}$	1.223±0.063 $^{\alpha\beta\gamma}$	0.877±0.044 $^{\alpha\beta\gamma}$	----------	2.42±0.10$^{\alpha\beta\gamma}$	1.65±0.08$^{\alpha\beta\gamma}$	1.18±0.060 $^{\alpha\beta\gamma}$	---------
800TCAE	1.082±0.027 $^{\alpha\beta\gamma}$	0.739±0.028 $^{\alpha\beta\gamma}$	0.531±0.011 $^{\beta\gamma}$	----------	1.46±0.03$^{\alpha\beta\gamma}$	1.00±0.03$^{\alpha\beta\gamma}$	0.72±0.016$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	0.238±0.007	----------	----------	----------	0.32±0.01

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

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.

The table above revealed that DOX intoxication caused a significant increase in PC concentration in the testes. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant reduction in PC concentration in the testes of experimental animals. Doxorubicin intoxication caused a significant increase in PC concentration in the lungs across the three modes of treatment. A dose dependent reduction in PC concentration was observed with the administration of TCAE compared with other groups across the three modes of treatment.

Table 3.10: Myeloperoxidase (MPX) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	26.57±0.903$^{\alpha\gamma}$	18.62±0.850$^{\alpha\gamma}$	13.22±0.438$^{\alpha\gamma}$	----------	36.69±0.01 $^{\alpha\gamma}$	25.10±1.14$^{\alpha\gamma}$	17.88±0.59 $^{\alpha\gamma}$	---------
α-lipoic acid	8.084±0.898$^{\alpha\beta}$	5.586±0.593$^{\alpha\beta}$	3.826±0.382$^{\alpha\beta}$	----------	10.90±1.21$^{\alpha\beta}$	7.53±0.80$^{\alpha\beta}$	5.16±0.52 $^{\alpha\beta}$	---------
200TCAE	4.942±0.221$^{\alpha\beta\gamma}$	3.407±0.409$^{\alpha\beta\gamma}$	2.384±0.105$^{\alpha\beta\gamma}$	----------	6.66±0.29$^{\alpha\beta\gamma}$	4.59±0.55$^{\alpha\beta\gamma}$	3.21±0.14$^{\alpha\beta\gamma}$	---------
400TCAE	3.263±0.138$^{\alpha\beta\gamma}$	2.221±0.114$^{\alpha\beta\gamma}$	1.593±0.080$^{\alpha\beta\gamma}$	----------	4.40±0.18$^{\alpha\beta\gamma}$	2.99±0.15$^{\alpha\beta\gamma}$	2.15±0.11$^{\alpha\beta\gamma}$	---------
800TCAE	1.965±0.050$^{\alpha\beta\gamma}$	1.342±0.051$^{\alpha\beta\gamma}$	0.965±0.020$^{\beta\gamma}$	----------	2.65±0.06$^{\alpha\beta\gamma}$	1.81±0.07$^{\alpha\beta\gamma}$	1.30±0.03$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	0.33±0.012	----------	----------	----------	0.58±0.01

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

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.

The table above revealed that DOX intoxication caused a significant increase in MPX activity in the testes. Pre-, co and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant reduction in MPX activity in the testes of experimental animals. Doxorubicin intoxication caused a significant increase in MPx activity in the lungs across the three modes of treatment. A significant dose dependent decrease in MPx activity was observed with the administration of TCAE compared with the other groups across the three modes of treatment.

Table 3.11: NADPH oxidase (NOX) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	24.44±0.879$^{\alpha\gamma}$	17.15±0.242$^{\alpha\gamma}$	12.22±0.403	----------	33.80±0.01$^{\alpha\gamma}$	23.12±1.05$^{\alpha\gamma}$	16.47±0.54$^{\alpha\gamma}$	---------
α-lipoic acid	7.447±0.827$^{\alpha\beta}$	5.146±0.546$^{\alpha\beta}$	3.524±0.352$^{\alpha\beta}$	----------	10.04±1.12$^{\alpha\beta}$	6.94±0.73$^{\alpha\beta}$	4.75±0.47$^{\alpha\beta}$	---------
200TCAE	4.552±0.203$^{\alpha\beta\gamma}$	3.138±0.377$^{\alpha\beta\gamma}$	2.196±0.097$^{\alpha\beta\gamma}$	----------	6.14±0.28$^{\alpha\beta\gamma}$	4.23±0.51$^{\alpha\beta\gamma}$	2.96±0.13$^{\alpha\beta\gamma}$	---------
400TCAE	3.005±0.127$^{\alpha\beta\gamma}$	2.046±0.105$^{\alpha\beta}$	1.467±0.074$^{\alpha\beta\gamma}$	----------	4.05±0.17$^{\alpha\beta\gamma}$	2.76±0.14$^{\alpha\beta\gamma}$	1.98±0.10$^{\alpha\beta\gamma}$	---------
800TCAE	1.810±0.046$^{\alpha\beta\gamma}$	1.237±0.047$^{\alpha\beta\gamma}$	0.889±0.019$^{\beta\gamma}$	----------	2.44±0.06$^{\alpha\beta\gamma}$	1.67±0.06$^{\alpha\beta\gamma}$	1.20±0.03$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	0.399±0.011	----------	----------	----------	0.54±0.02

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

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.

The table above revealed that DOX intoxication caused a significant increase in NOX activity in the testes. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant reduction in NOX activity in the testes of experimental animals. Doxorubicin intoxication caused a significant increase in NOX activity in the lungs across the three modes of treatment. A significant dose dependent decrease in NOX activity was observed with the administration of TCAE across the three modes of treatment.

Table 3.12: Xanthine oxidase (XO) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	26.28±0.896$^{\alpha\gamma}$	18.41±0.841$^{\alpha\gamma}$	13.12±0.433$^{\alpha\gamma}$	----------	36.29±0.02$^{\alpha\gamma}$	20.99±4.279$^{\alpha\gamma}$	17.69±0.58$^{\alpha\gamma}$	---------
α-lipoic acid	7.996±0.888$^{\alpha\beta}$	5.526±0.586$^{\alpha\beta}$	3.784±0.378$^{\alpha\beta}$	----------	10.78±1.19$^{\alpha\beta}$	7.45±0.79$^{\alpha\beta}$	5.10±0.51$^{\alpha\beta}$	---------
200TCAE	4.880±0.267$^{\alpha\beta\gamma}$	3.3700±0.327$^{\alpha\beta\gamma}$	2.358±0.104$^{\alpha\beta}$	----------	6.59±0.30$^{\alpha\beta\gamma}$	4.54±0.54$^{\alpha\beta\gamma}$	3.17±0.14$^{\alpha\beta\gamma}$	---------
400TCAE	3.226±0.136$^{\alpha\beta\gamma}$	2.197±0.113$^{\alpha\beta\gamma}$	1.575±0.079$^{\alpha\beta\gamma}$	----------	4.35±0.18$^{\alpha\beta\gamma}$	2.96±0.15$^{\alpha\beta\gamma}$	2.12±0.11$^{\alpha\beta\gamma}$	---------

800TCAE	1.994±0.049$^{\alpha\beta\gamma}$	1.328±0.051$^{\alpha\beta\gamma}$	0.955±0.020$^{\beta\gamma}$	----------	2.62±0.06 $^{\alpha\beta\gamma}$	1.79±0.07$^{\alpha\beta\gamma}$	1.29±0.03$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	0.428±0.012	----------	----------	----------	0.57±0.02

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

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.

The table above revealed that DOX intoxication caused a significant increase in XO activity in the testes. Pre-, co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant reduction in XO activity in the testes of experimental animals. Doxorubicin intoxication caused a significant increase in XO activity in the lungs across the three modes of treatment. A significant dose dependent decrease in XO activity was observed with the administration of TCAE across the three modes of treatment.

Table 3.13: Catalase (CAT) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				**LUNGS**			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	6.821±0.118$^{\alpha}$	12.48±1.011$^{\alpha}$	23.48±1.209$^{\alpha\gamma}$	----------	9.00±0.81$^{\alpha\gamma}$	16.32±1.68$^{\alpha\gamma}$	24.83±1.36$^{\alpha\gamma}$	---------
α-lipoic acid	10.82±0.636$^{\alpha}$	19.46±4.216$^{\alpha}$	60.64±4.637 $^{\alpha\beta}$	----------	14.58±0.86$^{\alpha\beta}$	28.09±7.38$^{\alpha\beta}$	37.74±6.25$^{\alpha\beta}$	---------
200TCAE	5.807±0.473$^{\alpha}$	25.29±5.482$^{\alpha\beta}$	78.83±6.028 $^{\alpha\beta\gamma}$	----------	8.83±0.63$^{\alpha\gamma}$	33.63±4.00$^{\alpha\beta\gamma}$	46.30±0.13$^{\alpha\beta\gamma}$	---------
400TCAE	8.097±0.306 $^{\alpha}$	32.37±2.968 $^{\alpha\beta\gamma}$	103.1±7.883$^{\alpha\beta\gamma}$	----------	17.91±0.41$^{\alpha\beta\gamma}$	49.29±0.16$^{\alpha\beta\gamma}$	52.80±1.62$^{\alpha\beta\gamma}$	---------
800TCAE	9.150±0.112 $^{\alpha}$	44.47±6.799 $^{\beta\gamma}$	128.2±10.40 $^{\alpha\beta\gamma}$	----------	26.33±0.15$^{\alpha\beta\gamma}$	57.65±1.63$^{\alpha\beta\gamma}$	64.10±1.03$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	51.09±1.881	----------	----------	----------	68.87±1.59

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

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.

The table above revealed that DOX intoxication caused a significant decrease in CAT activity in the testes. Pre-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused no significant change in CAT activity in the testes. Co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in CAT activity in the testes.

Doxorubicin intoxication caused a significant decrease in catalase activity in the lungs across the three modes of treatment. A significant dose dependent increase in catalase activity was observed with the administration of TCAE compared with other groups across the three modes of treatment.

Table 3.14: Superoxide dismutase (SOD) activity (units/mg protein) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	6.481±0.265$^{\alpha}$	13.08±1.011 $^{\alpha}$	24.60±1.267$^{\alpha\gamma}$	----------	8.64±0.48$^{\alpha\gamma}$	17.63±1.42$^{\alpha\gamma}$	23.16±1.70$^{\alpha\gamma}$	---------
α-lipoic acid	11.33±0.666$^{\alpha}$	20.38±4.417 $^{\alpha}$	11.33±0.666$^{\alpha}$	----------	15.28±0.89$^{\alpha\beta}$	27.48±0.79$^{\alpha\beta}$	38.63±6.54$^{\alpha\beta}$	---------
200TCAE	6.084±0.496 $^{\alpha}$	26.50±5.742 $^{\alpha\beta}$	82.59±6.315 $^{\alpha\beta\gamma}$	----------	8.20±0.66$^{\gamma}$	35.72±7.74$^{\alpha\beta\gamma}$	43.02±8.51$^{\alpha\beta\gamma}$	---------
400TCAE	8.097±0.320 $^{\alpha}$	33.91±3.110 $^{\alpha\beta\gamma}$	108.0±8.258 $^{\alpha\beta\gamma}$	----------	11.43±0.43$^{\alpha\beta\gamma}$	45.71±4.19$^{\alpha\beta\gamma}$	57.24±1.13$^{\alpha\beta\gamma}$	---------
800TCAE	9.586±0.117 $^{\alpha}$	47.64±7.123 $^{\beta\gamma}$	135.1±12.44 $^{\alpha\beta\gamma}$	----------	12.92±0.15$^{\alpha\beta\gamma}$	64.21±9.60$^{\alpha\beta\gamma}$	69.16±6.70$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	53.53±1.237	----------	----------	----------	72.15±1.67

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

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.

The table above revealed that DOX intoxication caused a significant decrease in SOD activity in the testes. Pre-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused no significant change in SOD activity in the testes. Co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in SOD activity in the testes.

Doxorubicin intoxication caused a significant decrease in SOD activity in the lungs across the three modes of treatment. A significant dose dependent increase in SOD activity was observed with the administration of TCAE compared with other groups across the three modes of treatment.

Table 3.15: Glutathione peroxidase (GSH consumed/ mg protein) activity in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	6.478±0.779$^{\alpha}$	13.78±1.117$^{\alpha\gamma}$	25.93±1.330$^{\alpha\beta}$	----------	8.36±0.15$^{\alpha\gamma}$	12.63±6.62$^{\alpha\gamma}$	18.59±1.80$^{\alpha\gamma}$	---------
α-lipoic acid	11.95±0.702$^{\alpha}$	21.49±4.656 $^{\alpha}$	66.97±5.120$^{\alpha\beta}$	----------	16.11±0.94$^{\alpha\beta}$	24.27±6.90$^{\alpha\beta}$	35.14±3.96$^{\alpha\beta}$	---------
200TCAE	6.413±0.502 $^{\alpha}$	27.93±6.053 $^{\alpha\beta}$	87.06±6.657 $^{\alpha\beta\gamma}$	----------	7.64±0.70$^{\alpha\beta\gamma}$	35.30±8.97$^{\alpha\beta\gamma}$	43.50±5.85$^{\alpha\beta\gamma}$	---------
400TCAE	8.941±0.338 $^{\alpha}$	35.75±3.278$^{\alpha\beta\gamma}$	113.8±8.700$^{\alpha\beta\gamma}$	----------	12.05±0.45$^{\alpha\beta\gamma}$	44.52±1.73$^{\alpha\beta\gamma}$	68.66±6.46$^{\alpha\beta\gamma}$	---------
800TCAE	10.10±0.124 $^{\alpha}$	50.21±7.50 $^{\beta\gamma}$	141.6±11.49 $^{\alpha\beta\gamma}$	----------	13.62±0.16$^{\alpha\beta\gamma}$	47.26±15.48$^{\alpha\beta\gamma}$	72.74±2.04$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	56.43±1.304	----------	----------	----------	76.06±1.75

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

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.

The table above revealed that DOX intoxication caused a significant decrease in GPX activity in the testes. Pre-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused no significant change in GPX activity in the testes. Co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in GPX activity in the testes. Doxorubicin intoxication caused a significant decrease in GPx activity in the lungs across the three modes of treatment. A significant dose dependent increase in GPx activity was observed with the administration of TCAE compared with other groups across the three modes of treatment.

Table 3.16: Glutathione S-transferase (µmol/min/mg protein) activity in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	6.594±0.468$^{\alpha}$	13.58±1.100 $^{\alpha}$	25.55±1.316$^{\alpha\gamma}$	----------	9.14±0.27$^{\alpha\gamma}$	18.31±1.48$^{\alpha\gamma}$	34.44±1.77$^{\alpha\gamma}$	---------
α-lipoic acid	11.77±0.692 $^{\alpha}$	21.17±4.588 $^{\alpha}$	65.98±5.045 $^{\alpha\beta}$	----------	15.87±0.93$^{\alpha\beta}$	28.54±0.18 $^{\alpha\beta}$	48.93±0.80$^{\alpha\beta}$	---------
200TCAE	6.318±0.515 $^{\alpha}$	27.52±5.960 $^{\alpha\beta}$	85.77±6.559 $^{\alpha\beta\gamma}$	----------	8.52±0.69$^{\alpha\beta\gamma}$	37.10±0.56 $^{\alpha\beta\gamma}$	52.21±0.84 $^{\alpha\beta\gamma}$	---------
400TCAE	8.809±0.333 $^{\alpha}$	35.22±3.230 $^{\alpha\beta\gamma}$	97.12±8.557 $^{\alpha\beta\gamma}$	----------	11.87±0.45$^{\alpha\beta\gamma}$	47.48±1.35 $^{\alpha\beta\gamma}$	65.40±1.56 $^{\alpha\beta\gamma}$	---------
800TCAE	8.809±0.333 $^{\alpha}$	38.21±1.140 $^{\alpha\beta\gamma}$	112.10±2.658 $^{\alpha\beta\gamma}$	----------	13.42±0.17$^{\alpha\beta\gamma}$	66.69±0.97$^{\alpha\beta\gamma}$	72.13±1.26$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	54.77±1.682	----------	----------	----------	74.94±1.73$^{\beta\gamma}$

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

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.

The table above revealed that DOX intoxication caused a significant decrease in GST activity in the testes. Pre-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused no significant change in GST activity in the testes. Co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in GST activity in the testes. Doxorubicin intoxication caused a significant decrease in GST activity in the lungs across the three modes of treatment. A significant dose dependent increase in GST activity was observed with the administration of TCAE compared with other groups across the three modes of treatment.

Table 3.17: Reduced glutathione concentration (µg/ml) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	1.392±0.012 $^{\alpha}$	2.713±0.219 $^{\alpha}$	5.104±0.262 $^{\alpha\gamma}$	----------	8.36±0.15$^{\alpha\gamma}$	12.63±6.62$^{\alpha\gamma}$	18.59±1.80$^{\alpha\gamma}$	---------
α-lipoic acid	2.352±0.138 $^{\alpha}$	4.229±0.916 $^{\alpha}$	13.18±1.007 $^{\alpha\beta}$	----------	16.11±0.94$^{\alpha\beta}$	24.27±6.90$^{\alpha\beta}$	35.14±3.96$^{\alpha\beta}$	---------
200TCAE	1.262±0.102 $^{\alpha}$	5.498±1.191 $^{\alpha\beta}$	17.13±1.310 $^{\alpha\beta\gamma}$	----------	7.64±0.70$^{\alpha\beta\gamma}$	35.30±8.97$^{\alpha\beta\gamma}$	43.50±5.85$^{\alpha\beta\gamma}$	---------
400TCAE	1.759±0.066 $^{\alpha}$	7.036±0.645 $^{\alpha\beta\gamma}$	22.40±1.713 $^{\alpha\beta\gamma}$	----------	12.05±0.45$^{\alpha\beta\gamma}$	44.52±1.73$^{\alpha\beta\gamma}$	68.66±6.46$^{\alpha\beta\gamma}$	---------
800TCAE	1.988±0.024 $^{\alpha}$	9.833±1.477 $^{\alpha\beta\gamma}$	27.87±2.261 $^{\alpha\beta\gamma}$	----------	13.62±0.16$^{\alpha\beta\gamma}$	47.26±1.54$^{\alpha\beta\gamma}$	72.74±2.04$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	30.05±0.694	----------	----------	----------	76.06±1.75

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

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.

The table above revealed that DOX intoxication caused a significant decrease in GSH concentration in the testes. Pre-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused no significant change in GSH concentration in the testes. Co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in GSH concentration in the testes. Doxorubicin intoxication caused a significant decrease in GSH concentration in the lungs across the three modes of treatment. A dose dependent increase in GSH concentration was observed with the administration of TCAE compared with other groups across the three modes of treatment.

Table 3.18: α-tocopherol concentration (µmol/L) in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	5.519±0.333 $^{\alpha}$	11.28±0.074 $^{\alpha}$	21.23±1.093 $^{\alpha\gamma}$	----------	6.62±1.61$^{\alpha\gamma}$	15.22±1.20$^{\alpha\gamma}$	28.62±1.47$^{\alpha\gamma}$	---------
α-lipoic acid	9.784±0.575 $^{\alpha}$	17.59±3.812 $^{\alpha}$	54.83±4.192 $^{\alpha\beta}$	----------	13.19±0.77$^{\alpha\beta}$	23.72±5.14$^{\alpha\beta}$	73.90±5.65$^{\alpha\beta}$	---------
200TCAE	5.251±0.428 $^{\alpha}$	22.87±4.956 $^{\alpha\beta\gamma}$	71.28±5.450 $^{\alpha\beta\gamma}$	----------	7.08±0.56$^{\alpha\beta\gamma}$	30.83±6.68$^{\alpha\beta\gamma}$	96.07±7.34$^{\alpha\beta\gamma}$	---------
400TCAE	7.321±0.276 $^{\alpha}$	29.27±2.684 $^{\alpha\beta\gamma}$	93.21±7.128 $^{\alpha\beta\gamma}$	----------	9.86±0.37$^{\alpha\beta\gamma}$	39.45±3.62$^{\alpha\beta\gamma}$	125.3±9.61$^{\alpha\beta\gamma}$	---------
800TCAE	8.273±0.101 $^{\alpha}$	41.11±6.148 $^{\beta\gamma}$	115.9±9.409 $^{\alpha\beta\gamma}$	----------	11.15±0.14$^{\alpha\beta\gamma}$	55.42±8.28$^{\alpha\beta\gamma}$	156.4±12.68$^{\alpha\beta}$	---------
BASELINE	----------	----------	----------	46.20±1.067	----------	----------	----------	62.27±1.44

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

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.

The table above revealed that DOX intoxication caused a significant decrease in α-TOC concentration in the testes. Pre-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused no significant change in α-TOC concentration in the testes. Co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in α-TOC concentration in the testes. Doxorubicin intoxication caused a significant decrease in α-tocopherol concentration in the lungs across the three modes of treatment. A dose dependent increase in α-Tocopherol concentration was observed with the administration of TCAE compared with other groups across the three modes of treatment.

Table 3.19: Ascorbic acid concentration (µmol/L) activity in doxorubicin-induced toxicity & the ameliorative role of TCAE

	TESTES				LUNGS			
	PRE-TREATMENT	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**	**PRE-TREATMENT**	**CO-TREATMENT**	**POST-TREATMENT**	**BASELINE**
Normal saline	6.176±0.069 $^{\alpha}$	12.21±0.989 $^{\alpha}$	22.97±1.183 $^{\alpha\gamma}$	----------	8.15±0.35$^{\alpha\gamma}$	15.22±1.23$^{\alpha\gamma}$	30.96±1.59$^{\alpha\gamma}$	---------
α-lipoic acid	10.58±0.622 $^{\alpha}$	19.03±4.124 $^{\alpha\gamma}$	59.31±1.180 $^{\alpha\beta}$	----------	14.27±0.84 $^{\alpha\beta}$	23.72±5.14$^{\alpha\beta}$	79.95±6.11$^{\alpha\beta}$	---------
200TCAE	5.680±0.463 $^{\alpha}$	24.74±5.361 $^{\alpha}$	91.70±1.24 $^{\alpha\beta\gamma}$	----------	7.65±0.62$^{\alpha\beta\gamma}$	30.83±6.68$^{\alpha\beta\gamma}$	104.1±7.95$^{\alpha\beta\gamma}$	---------
400TCAE	7.919±0.299 $^{\alpha}$	31.66±2.903 $^{\alpha\beta}$	100.8±7.710 $^{\alpha\beta\gamma}$	----------	10.67±0.40$^{\alpha\beta\gamma}$	39.45±3.618$^{\alpha\beta\gamma}$	136.3±10.39$^{\alpha\beta\gamma}$	---------
800TCAE	8.949±0.110 $^{\alpha}$	44.47±0.041 $^{\beta\gamma}$	125.4±10.17 $^{\alpha\beta\gamma}$	----------	12.06±0.15$^{\alpha\beta\gamma}$	55.42±8.287$^{\alpha\beta\gamma}$	169.1±3.72$^{\alpha\beta\gamma}$	---------
BASELINE	----------	----------	----------	49.97±1.155	----------	----------	----------	67.36±1.56

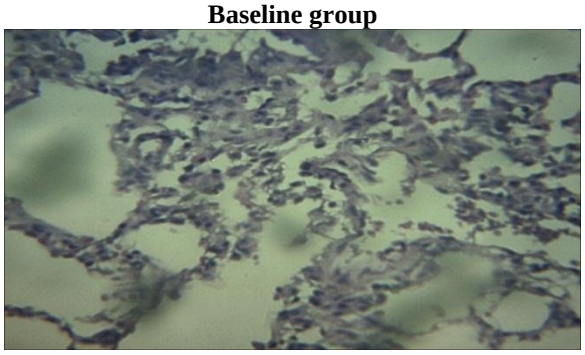
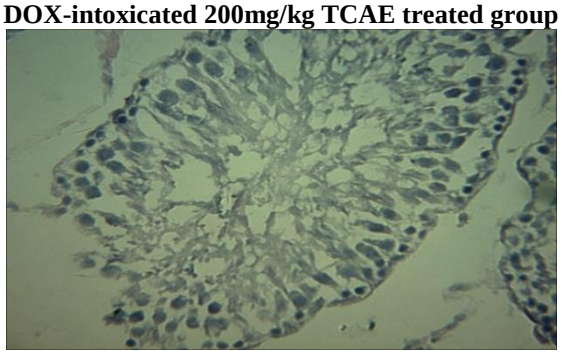
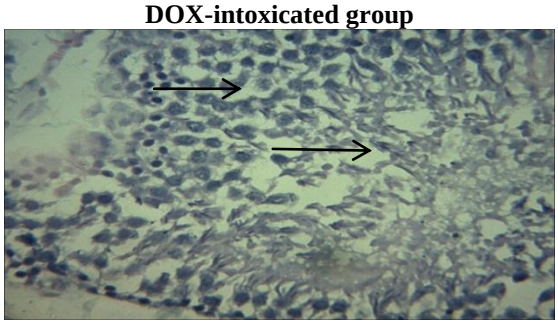
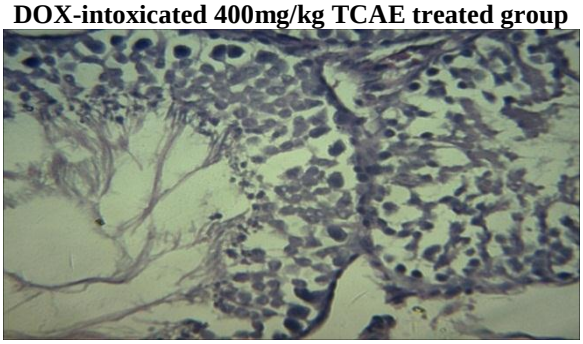
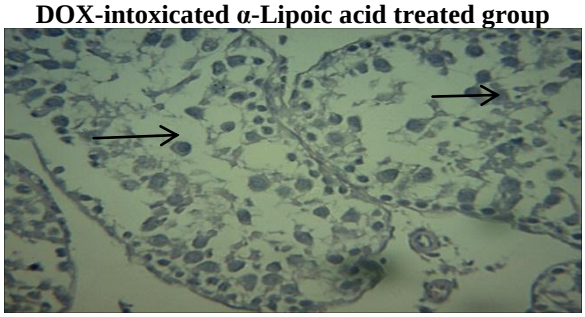
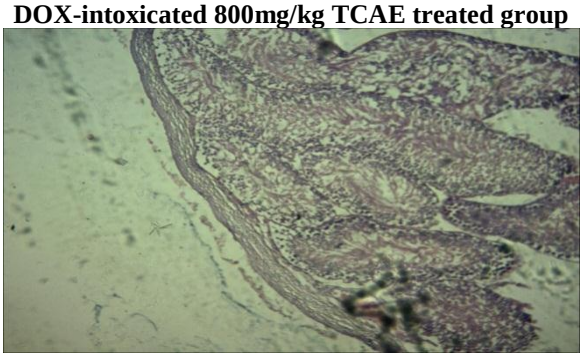
Values are expressed as mean±standard deviation (n=5). Significant at $p<0.05$
α= significant difference compared with baseline.
β= significant difference compared with normal saline
γ= significant difference compared with α – lipoic acid

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.

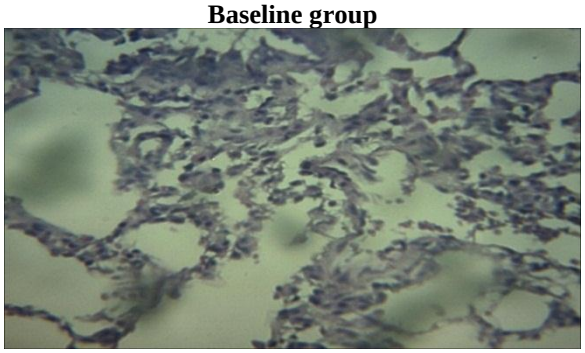
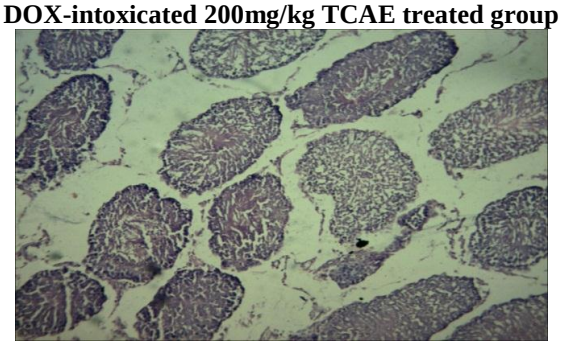
The table above revealed that DOX intoxication caused a significant decrease in AsA activity in the testes. Pre-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused no significant change in AsA activity in the testes. Co- and post-treatment of experimental animals with 200mg/kg, 400mg/kg or 800mg/kg body weight of TCAE caused a significant increase in ASA activity in the testes. Doxorubicin intoxication caused a significant decrease in ascorbic acid concentration in the lungs across the three modes of treatment. A dose dependent increase in ascorbic acid concentration was observed with the administration of TCAE compared with other groups across the three modes of treatment.

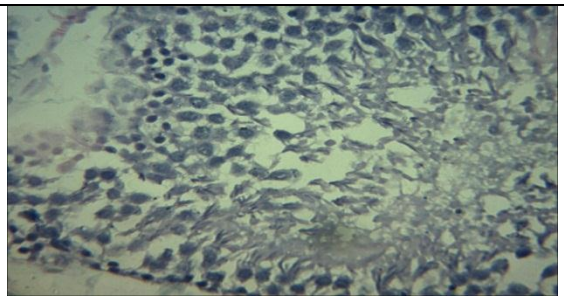
Photomicrograph of DOX-induced testicular oxidative damage and ameliorative role of TCAE

Pre-treatment group

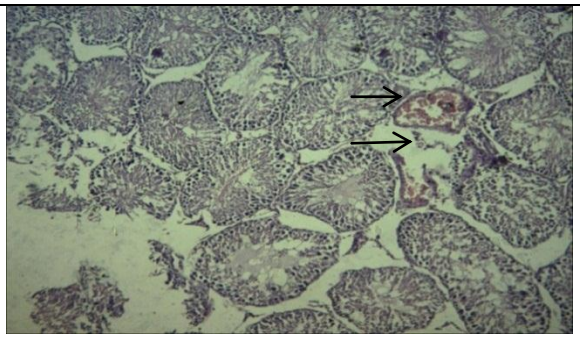
Baseline group  **No visible lesions seen.**	**DOX-intoxicated 200mg/kg TCAE treated group**  **No visible lesions seen.**
DOX-intoxicated group  **Some seminiferous tubules have immature germinal cells in their lumina**	**DOX-intoxicated 400mg/kg TCAE treated group**  **No visible lesions**
DOX-intoxicated α-Lipoic acid treated group  **Some seminiferous tubules have immature germinal cells in their lumina**	**DOX-intoxicated 800mg/kg TCAE treated group**  **No visible lesions seen**

Co-treatment group

Baseline group  **No visible lesions seen.**	**DOX-intoxicated 200mg/kg TCAE treated group**  **No visible lesions seen.**
DOX-intoxicated group	**DOX-intoxicated 400mg/kg TCAE treated group**

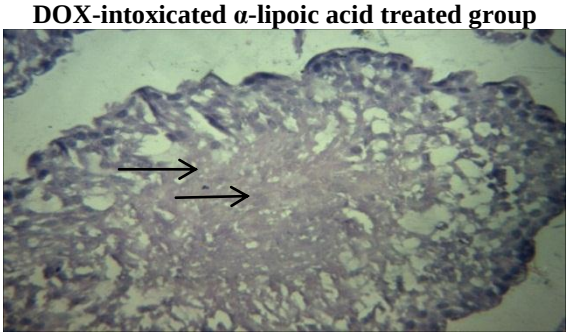


Some seminiferous tubules have immature germinal cells in their lumina



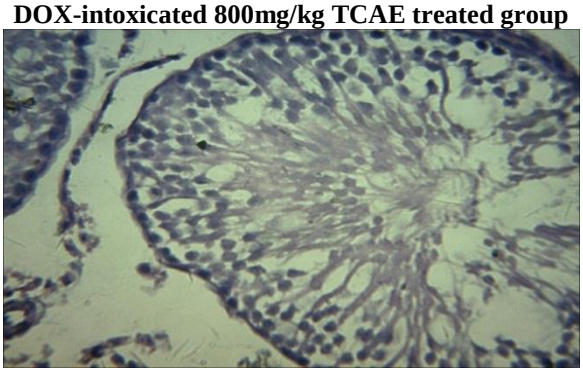
No visible lesions

DOX-intoxicated α-lipoic acid treated group



There are immature germinal cells in the lumen of some seminiferous tubules.

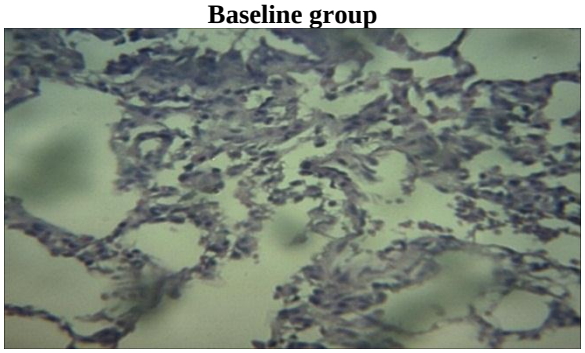
DOX-intoxicated 800mg/kg TCAE treated group



No visible lesions seen

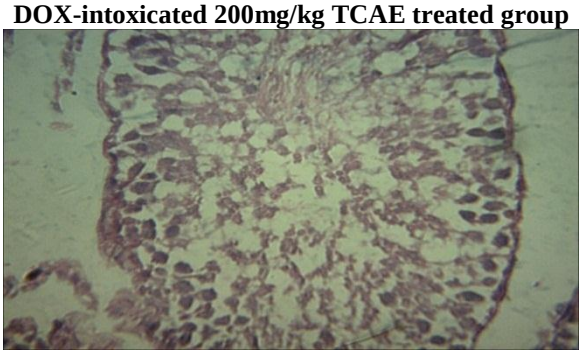
Post-treatment group

Baseline group



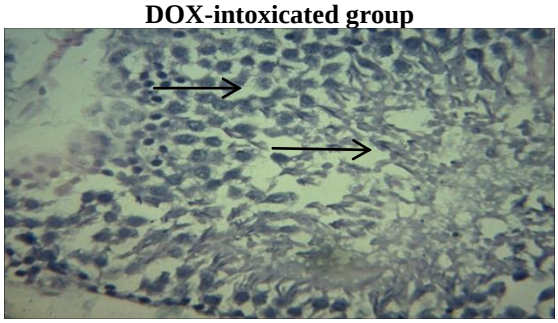
No visible lesions seen.

DOX-intoxicated 200mg/kg TCAE treated group



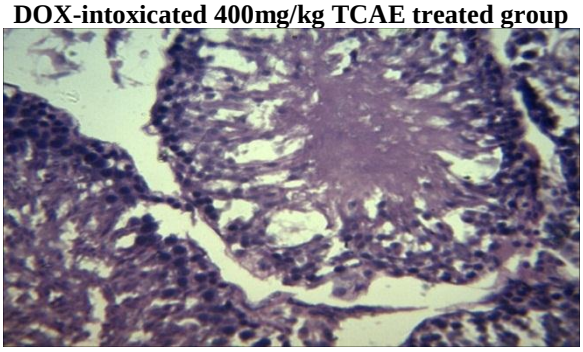
No visible lesions seen.

DOX-intoxicated group



Some seminiferous tubules have immature germinal cells in their lumina

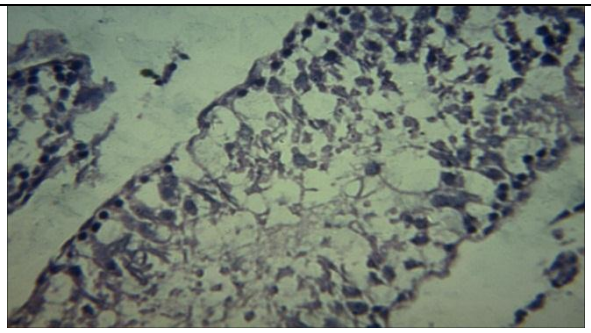
DOX-intoxicated 400mg/kg TCAE treated group



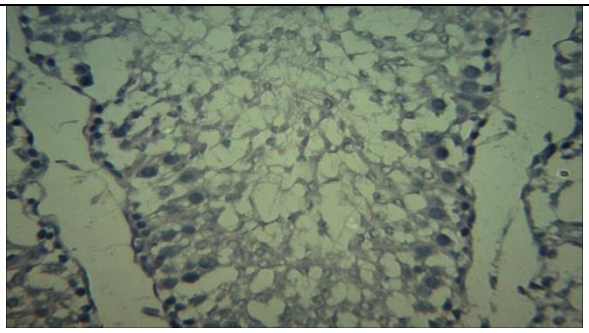
No visible lesions

DOX-intoxicated α-lipoic acid treated group

DOX-intoxicated 800mg/kg TCAE treated group



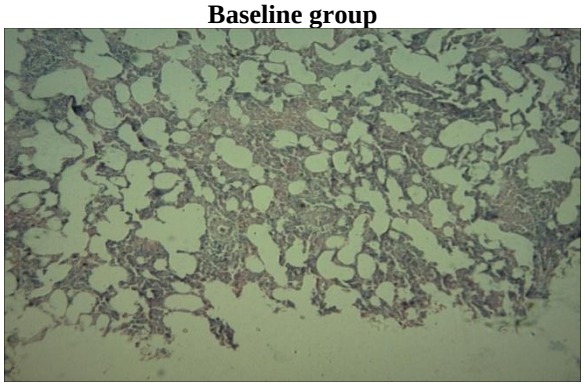
No visible lesions



No visible lesions

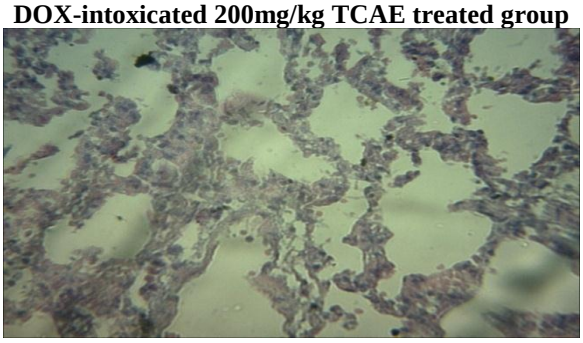
Photomicrograph of DOX-induced lungs oxidative damage and ameliorative role of TCAE Pre-treatment

Baseline group



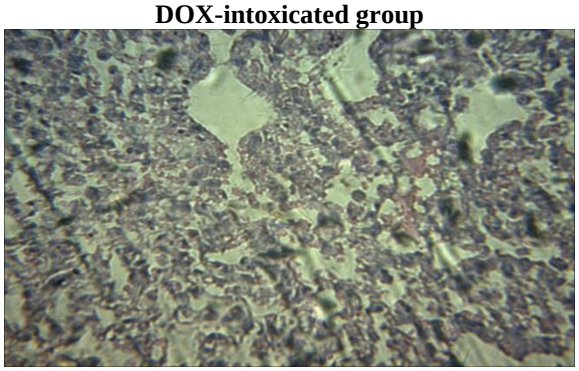
Mildly thickened interstitium

DOX-intoxicated 200mg/kg TCAE treated group



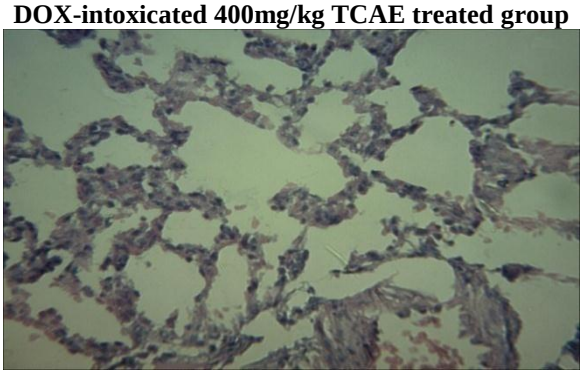
Moderate thickening of the alveolar walls which contain proliferated pneumocytes and congested interstitium

DOX-intoxicated group



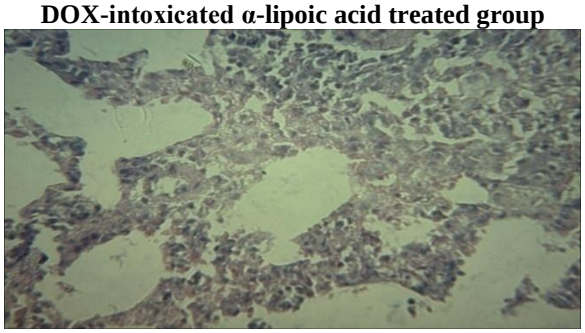
Severe alveolar wall thickening. Several alveolar spaces are filled with proliferated and inflammatory cells

DOX-intoxicated 400mg/kg TCAE treated group



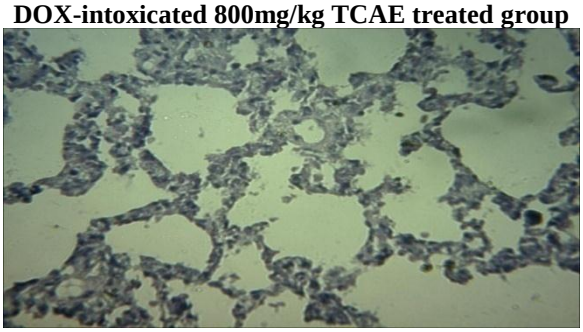
No visible lesions

DOX-intoxicated α-lipoic acid treated group



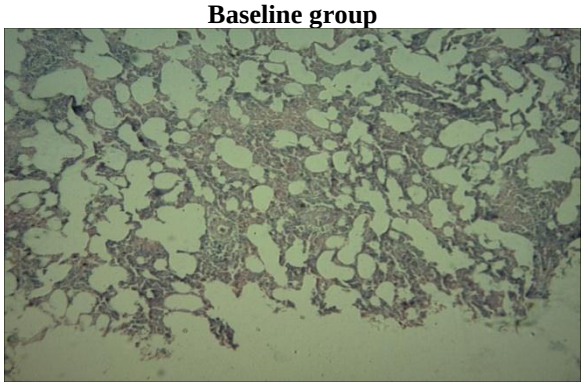
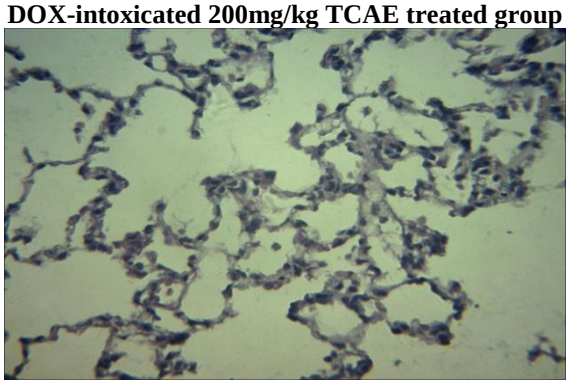
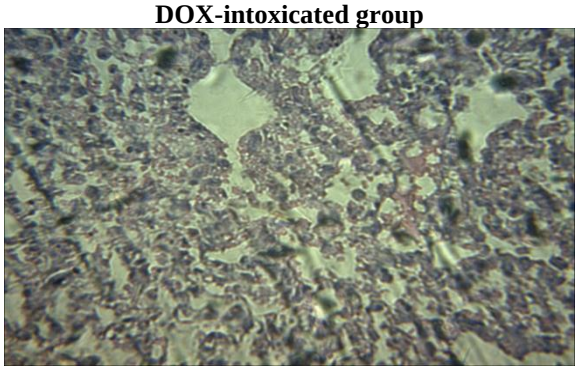
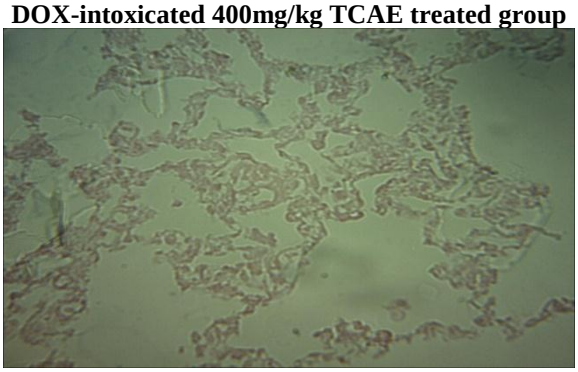
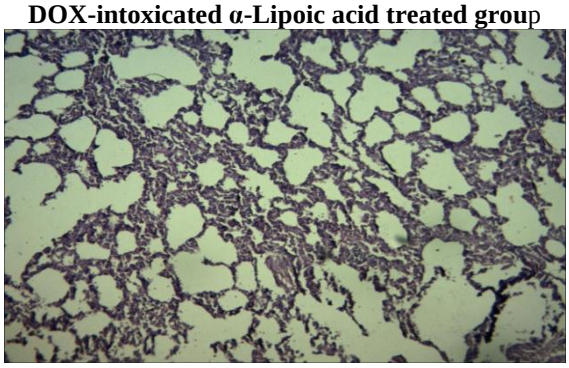
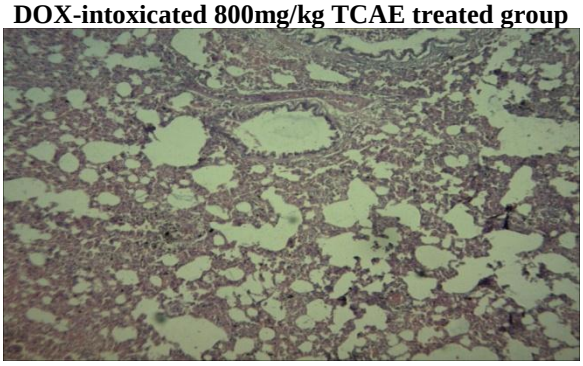
Diffused moderate proliferation of alveolar pneumocytes

DOX-intoxicated 800mg/kg TCAE treated group

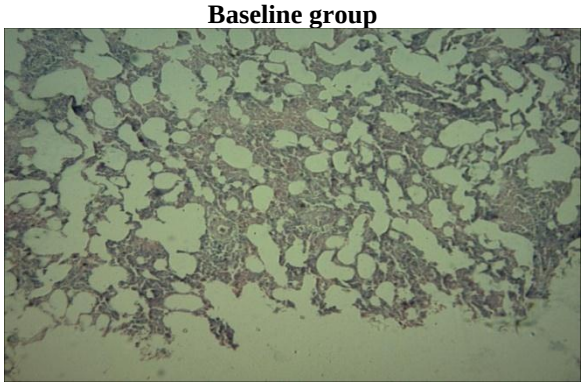
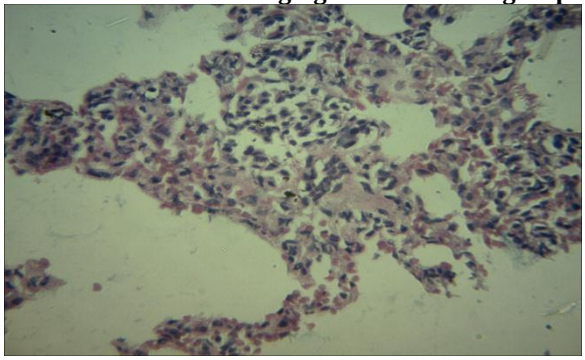


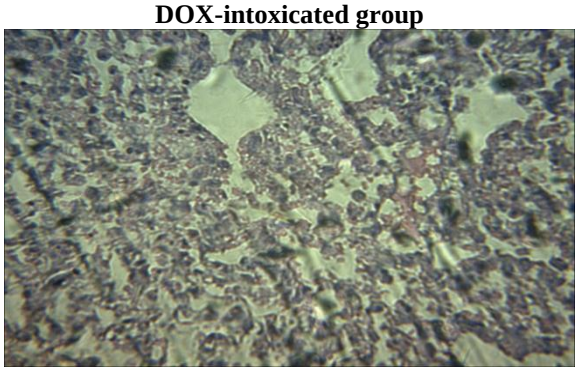
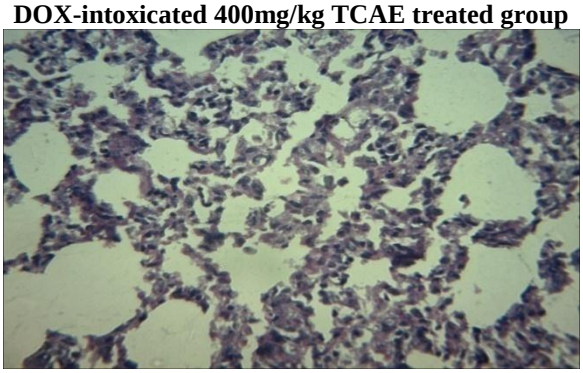
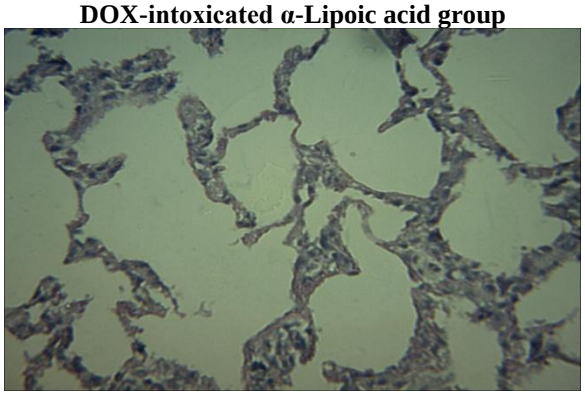
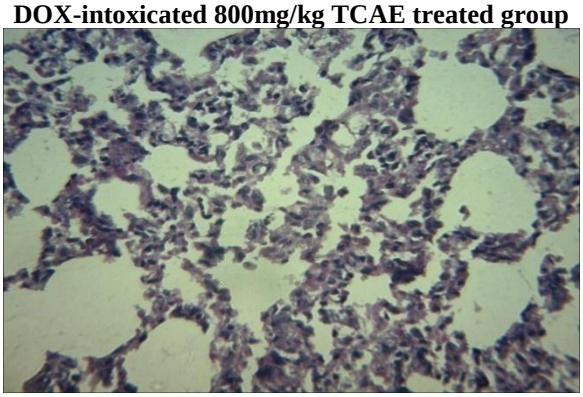
No visible lesions

Co-treatment

Baseline group  **Mildly thickened interstitium**	**DOX-intoxicated 200mg/kg TCAE treated group**  **No visible lesions**
DOX-intoxicated group  **Several alveolar spaces are filled with proliferated and inflammatory cells**	**DOX-intoxicated 400mg/kg TCAE treated group**  **No visible lesions**
DOX-intoxicated α-Lipoic acid treated group  **Mild diffuse proliferation of the interstitial pneumocytes**	**DOX-intoxicated 800mg/kg TCAE treated group**  **Mild diffuse proliferation of alveolar pneumocytes**

Post-treatment

Baseline group  **Mildly thickened interstitium**	**DOX-intoxicated 200mg/kg TCAE treated group**  **Mild proliferation of alveolar pneumocytes**

DOX-intoxicated group  **Severe alveolar wall thickening. Several alveolar spaces are filled with proliferated and inflammatory cells**	**DOX-intoxicated 400mg/kg TCAE treated group**  **Severe thickening of the alveolar wall, characterised by interstitial proliferation of pneumocytes and diffuse cellular infiltration**
DOX-intoxicated α-Lipoic acid group  **No visible lesions**	**DOX-intoxicated 800mg/kg TCAE treated group**  **Mild proliferation of alveolar pneumocytes.**

4.0 DISCUSSION

Doxorubicin (DOX)-based chemotherapy is used to treat some leukemias and Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma and also has severe deleterious effects on testicular integrity and spermatogenesis (Vijay *et al*., 2013). In addition, DOX-based chemotherapy causes chromosomal abnormalities in sperm, temporary or permanent azoospermia and oligospermia (Vijay *et al*., 2013) The principal mechanism of DOX is chelating DNA, inhibiting topoisomerase II and then producing free radicals to kill cells (Chen *et al*., 2013). The present study revealed the potential protective role of graded doses of Theobroma cacao stem bark aqueous extract (TCAE) against DOX-induced oxidative stress, testicular and lungs damage in experimental rats. Activities of some testicular biomarker enzymes (alkaline phosphatase, lactate dehydrogenase, acid phosphatase, γ- glutamyl transferase), markers for oxidative stress; free radical (hydrogen peroxide), products of free radical generation (malondialdehyde, protein carbonyl), enzymes implicated in free radical generation (myeloperoxidase, NADPH oxidase, xanthine oxidase), antioxidant enzymes (glutathione peroxidase, glutathione S-transferase, catalase, superoxide dismutase), non enzymic antioxidants (reduced glutathione, ascorbic acid, α-tocopherol), sperm morphometrics and testicular histopathology were investigated.

The results presented here showed that DOX cause an important decrease in testicular weight. This might be due to substantial reduction in the spermatogenic cell population (Zanetti *et al*., 2007). Cocoa had been considered from long time, as a food-rich polyphenols. Flavonoids and phenolic acids are mainly forming the type of polyphenols (Ranneh *et al*., 2013). Aqueous extract of Theobroma cacao stem bark, due to its high content of secondary metabolites such as polyphenols which according to Gülçin and Beydemir (2012) plays an antioxidative role in testes against DOX- induced testicular toxicity. The present result showed that treatment with graded doses of TCAE (200mg, 400mg or 800mg/kg body weight) markedly improved the biochemical and histological alterations induced by DOX intoxication. As an antioxidant, α-lipoic acid works as a direct inactivator of free radicals by electron donation, bolstering endogenous enzymatic defences against oxidative stress and by regenerating other antioxidants within the cell (Deng et al., 2013). Administration of 20 mg/kg body weight of α-lipoic acid in rats treated with doxorubicin resulted in a significant improvement in DOX-induced testicular damage. Lactate dehydrogenase (LDH) is involved in the catalysis of the reduction of pyruvate to lactate in the presence of NADH. This allows the glycolysis to proceed in the absence of oxygen, regenerating sufficient amount of NAD^+ for sustaining the glycolytic pathway and it is released during cell lysis (Sridevi, 2011). LDH activity in testes of DOX-treated experimental animals showed a significant decrease

compared to the treatment groups. Since the testes depend on the glucose for carbondioxide production, it is probable that the available glucose in the testes is metabolized via the Kreb's cycle, accounting for the reduced activity of LDH in the testes of DOX-intoxicated rats (Sridevi, 2011). Acid phosphatase (ACP) is a lysosomal enzyme and is used as a marker for lysosomal activity and it is also as a marker for prostate cancer. The testes showed a significant increase in the ACP activity in the DOX-intoxicated experimental group. This was reduced significantly in the treatment groups of ALA and TCAE. Increase in the size of the lysosome, principal and/ or clear cells occurred during ACP activity in the epididymis (Sridevi, 2011). From the present study, the increase in testicular ACP activity might indicate a probable increase in lysosomal size in the cells of the epididymis of the DOX-intoxicated group. Therefore, the testicular acid phosphatase activity in the present study may be attributed to the lysosomal hypertrophy (Sridevi, 2011). Alkaline phosphatase activity occurs in the plasma and it helps in the breakdown of glycogen to glucose in the glycolytic pathway (Sridevi, 2011). There was significant decrease in alkaline phosphatase activity in the testes of DOX-intoxicated rats. Activities of alkaline phosphatase in the plasma arise from the routine, normal destruction of erythrocytes, leucocytes and other cells; enter the circulation during accelerated cell death (Sridevi, 2011). Rahman *et al* (2000) suggested that the decrease in the activities of ALP and ACP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis. Doxorubicin is one of the most effective cytotoxic agents (Sridevi, 2011) and the values obtained in the present study might be attributed to the cytotoxic action of doxorubicin.

GGT is liver enzyme whose serum level serves as a biomarker for oxidative stress (Lee, 2008). The primary role of cellular gamma-glutamyl transferase is to metabolize extracellular reduced glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis. The testes of DOX-intoxicated experimental animals showed a significant increase in GGT activity. The increase in GGT activity in testes occurs at the time when sertoli cells are actively dividing and increasing (Lu and Steinberger, 1977). GGT exerts pro-oxidant effects due to increased metal-reducing ability of the GSH metabolite cysteinyl- glycine and extracellular production of reactive oxygen species (Paolicchi *et al*., 2002; Stark *et al*., 1993).

DOX is metabolically reduced to highly reactive free radicals, which generate superoxide radical and hydrogen peroxide. These highly toxic free radicals cause lipid peroxidation, peroxidation of polyunsaturated fatty acids and cause damage to cellular components (Khan *et al*., 2014).

DOX-intoxicated rats showed a significant increase in testicular hydrogen peroxide, malondialdehyde and protein carbonyl concentrations; myeloperoxidase, NADPH oxidase, xanthine oxidase activities and a significant decrease in concentrations of reduced glutathione, ascorbic acid, α-tocopherol; glutathione peroxidase, glutathione S-transferase, catalase, superoxide dismutase activities suggesting increased testicular oxidative stress and damage. Treatment with TCAE significantly counteracted the effects of DOX-induced testicular oxidative damage and toxicity by significantly reverting and improving the antioxidant status of the treated rats relative to DOX- intoxicated group. This findings further adds up to the facts suggesting TCAE might contain a potential and effective antioxidant molecules (Baharum *et al*., 2014; Cicerale *et al*., 2009; Okawa *et al*., 2001). H_2O_2 is decomposed into water and oxygen molecules by the enzyme catalase present in cytoplasm, thus protecting the cells from hydroperoxide mediated cellular damage (Hazell *et al*., 2001).

According to Vijay *et al* (2013), although DOX exposure in men can produce long-lasting azoospermia and testicular atrophy, animal studies of potential cellular targets and mechanisms of toxicity within the testes indicate that DOX has broad activity, targeting leydig cells, sertoli cells, and germ cells. In DOX-intoxicated control group, the sperm count was drastically decreased. This correlates with the findings of Vijay *et al* (2013) where epididymal sperm concentration, sperm motility, normal sperm rates, and plasma testosterone levels of animals administered DOX alone were found to be decreased. Motility is very much required for the sperm to swim from the cervix to fallopian tubes in order to fertilize the egg. Kato (2013) has done an intensified study on the pathological impact of DOX on sperm motility and proved that DOX treatment dramatically decreases the rapid and slow progressive sperm. The ALA and TCAE treated groups showed significant and dose dependent improvement in the sperm motion when compared to the DOX control group. It can be concluded that antioxidant compounds in TCAE have protective effect on the mitochondrial membrane (maintaining the energy levels in the sperm cell in a constant manner, providing ability for sperm to swim) which usually gets damaged in DOX-induced testicular oxidative stress (Vijay *et al*., 2013). Howell and Shaler (2001) showed that the occurrence of male infertility following DOX chemotherapy is due to alterations in the sperm parameters. Spermatogenic cells constitute one of the body tissues that are susceptible to DOX-induced testicular oxidative stress and apoptosis. This increased oxidative stress damages the sperm membranes, proteins and DNA (Kirsi and Timo, 2001).

Oxidative stress and lipid peroxidation (self-propagating) mechanism of DOX cytotoxicity could provide an explanation for the progressive worsening of the testicular histomorphometric parameters following DOX

intoxication. Thus, treatment with TCAE improved the testicular profiles relative to DOX- intoxicated untreated group.

The present study also revealed severe oxidative stress, biochemical changes and lungs damage resulting from single dose of DOX intoxication. This was manifested by increased activities of ACP, GGT and LDH and a decrease in ALP activity in DOX intoxicated rats compared to other test groups indicating lung injury. This effect of DOX on the lungs was supported by Rodney *et al*. (1988), who reported that DOX intoxication induced damage to the lungs. According to them, this lungs damage was effected by oxidative stress induced by DOX. This was in line with the present study where it was reported again oxidative stress is the mechanism of DOX-induced lung intoxication. Eser and Mustafa (2006) reported that doxorubicin induced significant increase in the activities of GGT, ACP and LDH and a decrease in the activity of ALP in rat lungs compared to the control. According to them, this increase in the activities of GGT, ACP and LDH was attributed to leakage of these enzymes from damaged cells of the lungs due to toxicity while the decrease in ALP activity was as a result of cell necrosis.

This present study shows the protective potential of *Theobroma cacao* stem back aqueous extract (TCAE) on oxidative stress and lung damage caused by doxorubicin intoxication. It was found that pre-, co- and post-treatment with TCAE caused a significant dose-dependent reduction in ACP, GGT and LDH activities and a significant increase in ALP activity in the lungs. This is in accordance with Osama *et al*. (2013) where they reported that Theobroma cacao extract, successfully improved the activities of LDH, ACP and GGT in the lungs of doxorubicin-intoxicated rats. They found that the extract of cocoa improved the functional status of the lungs as indicated by a decrease in activities of classical enzymes of the lungs. This improvement may be mediated via the antioxidant activity of cocoa. ALA and its reduced form DHLA, are considered as powerful natural antioxidant agents with a scavenging capacity for many reactive oxygen species. The present result showed that treating animal with alpha lipoic acid improved and reversed the biochemical changes induced in the lungs by DOX intoxication. This result correlates with the findings of Zhang *et al*., (2011) where it was reported that alpha-lipoic acid ameliorates oxidative stress by causing a significant fall in the lipid peroxide concentration.

The present result shows that doxorubicin induced an increase in hydrogen peroxide, malondialdehyde and protein carbonyl concentrations in the lungs of rats. Pre-, co-, and post-treatment with TCAE caused a significant dose-dependent reduction in hydrogen peroxide, malondialdehyde and protein carbonyl concentrations in the lungs. This is in accordance with Zhang *et al*., (2011). They reported a significant increase in hydrogen peroxide, malondialdehyde and protein carbonyl concentrations in the lungs of rats treated with doxorubicin. They evaluated the tissue protective effects of *Theobroma cacao* bean extract against the toxic effects of doxorubicin and found that administration of *Theobroma cacao* bean extract caused a significant decrease in the concentrations of hydrogen peroxide, malondialdehyde and protein carbonyl in rat lungs. They suggested that doxorubicin induces cellular toxicity by increasing free radical generation and that the protective effects of the extract may be due to its high phenolic content which has antioxidant activity.

Doxorubicin-induced toxicity caused an increase in activities of some enzymes implicated in free radical generation: myeloperoxidase (MPX), NADPH oxidase (NOX) and xanthine oxidase (XO) in the rat lungs. These enzymes are activated in the defense against oxidative cell injury. This is in accordance with the work of Hoffman *et al* (2004) who reported that the exposure of rat lungs to DOX led to an increase in the activities of MPX, NOX and XO due to ability of doxorubicin 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), who reported that the ameliorative effects of cocoa bean extract was as a result of its ability to inhibit the complex processes leading to carcinogenesis.

This study also investigated the effects of *T. cacao* on antioxidant defense system of the lungs, and our results showed that administration of doxorubicin induced a significant depletion in some enzymic antioxidants; catalase, superoxide dismutase, glutathione peroxidase, and glutathione-s-transferase activities in the lungs, which might be explained by their inactivation induced by excessive reactive oxygen species (ROS) generation by DOX. These results are in accordance with other authors (Abd El-Aziz *et al*., 2001, Kalendar *et al*., 2005) who reported significant depression in the concentrations of non-enzymic antioxidants as well as enzymic antioxidants in the lungs due to doxorubicin intoxication. They stated that one of the most prevailing hypothesis of doxorubicin-induced lung damage is the ability of the drug to produce reactive oxygen species (ROS) and suppress antioxidant defence mechanisms.

The biochemical alterations of the present study are associated with a marked elevation of lipid peroxidation in the lungs and a significant depression of non-enzymic antioxidants reduced glutathione, ascorbic acid and alpha-tocopherol. This is in agreement with Doroshow *et al* (2009) where it was reported that high concentration of DOX leads to a high redox reactivity and significant decline in reduced glutathione and α-tocopherol concentrations in the lungs. Pre-, co-, and post-treatment with TCAE significantly reversed the decrease and perturbations observed in the concentrations of reduced glutathione, ascorbic acid and α-tocopherol caused by

DOX intoxication. This finding correlates with the work of Golbidi *et al*, (2011), where cocoa was reported to act as biological antioxidants, as metal chelators, reducing the oxidized forms of other antioxidant agents such as vitamin C and E. The stem bark aqueous extract of *Theobroma cacao* significantly reversed and mitigate against deleterious damage caused by DOX intoxication.

5.0 CONCLUSION

The present study has therefore provided an addition to the body of evidence that doxorubicin intoxication induces oxidative damage, morphological and morphometric impairments in the testes of experimental rats. It was also shown that there is progressive worsening of testicular damage/injury with time, following a single dose of doxorubicin intoxication and affirms that administration of *Theobroma cacao* stem bark aqueous extract exerts protective effects against doxorubicin-induced lung toxicity in rats. This study has provided a solid foundation for the amelioration of DOX-induced oxidative stress, testicular and lung damage by T. cacao stem bark aqueous extract.

6.0 REFERENCES

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