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Doxorubicin induced neuro- and cardiotoxicities in experimental rats: Protection against oxidative damage by *Theobroma cacao* Stem bark☆

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ABSTRACT

80 rats, randomly selected, were divided into 3 treatment groups: pre-, co- and post-treatment; consisting of 6 sub-groups each (5 rats per sub-group): baseline, normal saline (2 mL), α-lipoic acid (20 mg/kg body weight), 200 mg/kg, 400 mg/kg or 800 mg/kg body weight *Theobroma cacao* stem bark aqueous extract (TCAE). All rats except for baseline group were intoxicated with 20 mg/kg body weight doxorubicin (DOX) intraperitoneally. The animals in pre- or post-treatment group received a single dose of DOX (20 mg/kg body weight) intraperitoneally 24 h before or after 7 days' oral administration with TCAE respectively while those in co-treatment group were co-administered 2.86 mg/kg body weight of DOX with either normal saline, α- lipoic acid or TCAE orally for 7 days. Animals were sacrificed (pre- and post- treatment groups were sacrificed on the ninth day while the co-treatment group sacrificed on the 8th day). Brain and heart tissue samples were harvested for enzyme markers of toxicity, oxidative stress and histopathological examinations. DOX intoxication caused significant decrease in activities of LDH and ACP, and increase in γGT and ALP activities in brain tissues while causing a significant increase in LDH, ACP, γGT activities and decrease in ALP activity in the cardiac tissues. DOX intoxication caused a significant increase in concentrations of H_2O_2 generated, MDA and PC, XO, MPx and NOX activities with concomitant decrease in CAT, SOD, GPx and GST activities, and in concentrations of GSH, AsA and α-Toc in brain and cardiac tissues. Pre-, co- and post-treatment with TCAE at either 200 mg/kg, 400 mg/kg or 800 mg/kg body weight significantly reversed the oxidative damage to the organs induced by DOX-intoxication. The result affirmed that *T. cacao* stem bark aqueous extract protected against DOX induced oxidative damage in brain and cardiac tissues of experimental rats.

1. Introduction

Doxorubicin (DOX) obtained from soil actinomycetes *Streptococcus peucetius* is a powerful drug used for the treatment of solid tumors such as those arising in the breast, bile ducts, endometrial tissue, esophagus and liver, osteosarcomas, soft-tissue sarcomas and non-Hodgkin's lymphoma [47]. DOX is known as a powerful anthracycline antibiotic widely used to treat many human cancers, but significant cardiotoxicity and brain damage [24], hepatotoxicity [37], nephrotoxicity [32] and testicular toxicity [48] limits its clinical application. A number of studies were conducted for antioxidants screening from the natural medicine aiming to minimize oxidative injury by DOX. Several natural antioxidants have been shown to alleviate the DOX-induced cell damage without compromising its anti-tumor efficacy in the animal studies [52]. Over the past few years, the antioxidant and health-promoting properties of cocoa (***Theobroma cacao***) and cocoa-related products have been thoroughly investigated. Polyphenols, widely distributed in plant foods, are the main antioxidant-active fraction of cocoa, and within polyphenols, flavanols and procyanidins have been identified as the active antioxidant agents of cocoa and dark plain chocolate [28]. More than 200 studies have reported that various parts of the cocoa plant, e.g., cocoa beans (prepared as chocolate), the bark, flower, pulp, and leaf, and cocoa butter have been used for medicinal purposes. The phenolic compounds in cocoa contain bioactive compounds that have potential health benefits for chronic diseases such as inflammation, cardiovascular illness, neurodegenerative disorders, and cancer [43]. **α-Lipoic acid (ALA)** also known as thioctic acid (TA) and 1,2 dithiolane –3-pentanoic acid, is a naturally occurring substance, that is essential for the function of different enzymes of oxidative metabolism. It is believed that ALA or its reduced form, dihydrolipoic acid (DHLA) have many biochemical functions acting as biological antioxidants, as metal chelators, reducing the oxidized forms of other antioxidant agents such as vitamin C and E and glutathione (GSH), and modulating the

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

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

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

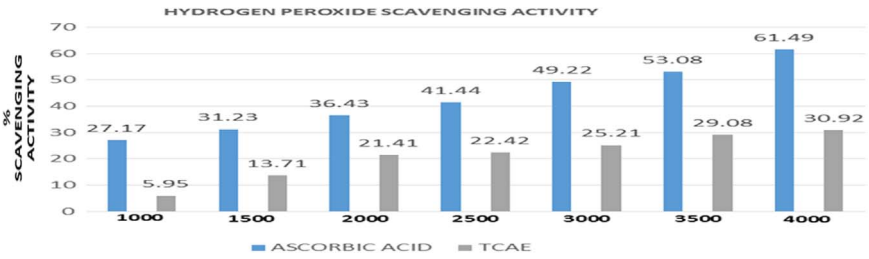

Fig. 3.1.1. Hydrogen peroxide scavenging activity of TCAE.

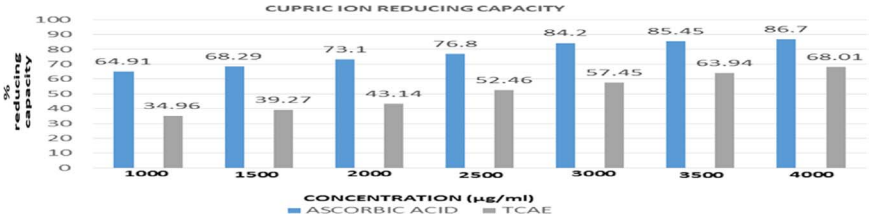

Fig. 3.1.2. Cupric ion reducing capacity activity of TCAE.

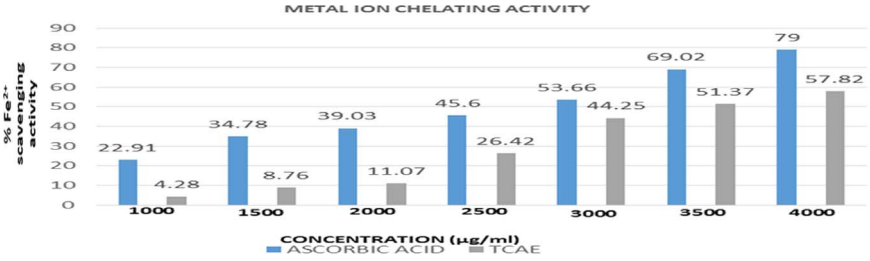

Fig. 3.1.3. Metal ion chelating activity of TCAE.

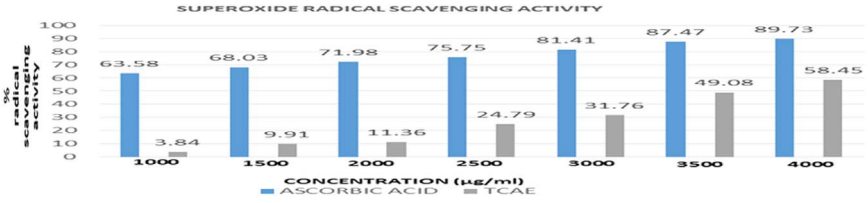

Fig. 3.1.4. Superoxide radical scavenging activity of TCAE.

signaling transduction of several pathways, like insulin and nuclear factor kappa B (NF-kB) [15]. **Brain** is the main organ of the human nervous system. It is located in the head, protected by the skull. It has the same general structure as the brains of other mammals, but with a more developed cerebral cortex. Despite being protected by the thick bones of the skull, suspended in cerebrospinal fluid, and isolated from the bloodstream by the blood–brain barrier, the human brain is susceptible to damage and disease [9]. **Heart** is a muscular organ in humans and other animals, which pumps blood through the blood vessels of the circulatory system and also assists in the removal of