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Dietary *Theobroma cocao* Prevents Hepatotoxicity Secondary to Myocardial Injury

Justin Atiang Beshel [a*], Francisca Upekiema Adie [b], Idara Asuquo Okon [c], Mercy Okon Ekong [b] and Daniel Udofia Owu [a]

[a] Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Nigeria.
[b] Department of Microbiology, Faculty of Biological Sciences, Cross River State University, Calabar, Nigeria.
[c] Department of Physiology, PAMO University of Medical Sciences, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Dietary products like cocoa with high flavonoids may play a role to boost the integrity of the liver against insults resulting from myocardial injury. Twenty-four male Wistar rats, divided into four groups of 6 rats were used for the study. Group 1 was control and received 0.9% normal saline via oral gavage. Group 2 was the acute myocardial injury group, and received two doses of subcutaneous injection of isoproterenol (100 mg/kg body weight) at an interval of 24 hours between doses. Group 3 was administered *Theobroma cacao* (100 mg/kg body weight orally) only for 2 weeks. Group 4 was pretreated with *Theobroma cacao* for 2 weeks and then followed by injection of

**Corresponding author: E-mail: justinbeshel@unical.edu.ng, atiangjustbesh@yahoo.com;*

isoproterenol (100 mg/kg body weight) on day 15 and 16. At the end of the experimental period, the rats were euthanized and serum collected for laboratory investigations of lactate dehydrogenase, troponin, alanine aminotransferase, aspartate transaminase, and alkaline phosphatase. Administration of isoproterenol resulted in a significant ($p<0.001$) elevation in the serum concentrations of the cardiac biomarkers, troponins and LDH when compared with the control group. Pretreatment with *Theobroma cacao* before myocardial infarction caused a significant ($p<0.01$) reduction in the concentrations of LDH and troponins with values similar to the control group. The serum liver enzymes AST, ALT, and ALP levels were also significantly ($p<0.01$) but were reversed in the *Theobroma cacao* cocoa treatment groups. It is concluded that *Theobroma cacao* prevents hepatic and heart damage thus providing a support for the prophylactic use of dietary *Theobroma cacao* against hepatotoxicity.

Keywords: Cocoa; flavonoid; serum; cardiac biomarkers.

1. INTRODUCTION

"Acute myocardial infarction (AMI) is one of the leading cardiovascular diseases with a high mortality rate" [1]. "In the emergency unit, It has been estimated that one percent of patients on admission is diagnosed of heart attack" [2]. "In developing countries, a change in lifestyle is considered a predisposing factor for the increased mortality rate due to AMI" [3]. "AMI causes distortion in the structural, mechanical, electrical, and biochemical properties of the heart" [4]. "It can be due to ischaemic heart disease and/or in conjunction with coronary artery disease with a resultant deterioration of ventricular function and myocardial necrosis" [5,6]. "Serum enzymes such as lactate dehydrogenase (LDH), creatine kinase (CK), Aspartate aminotransferase (AST), malondialdehyde (MDA), and troponins are biomarkers used for the diagnosis of AMI" [7,8]. "This is because when there a decreased coronary blood flow and a consequent deterioration of ventricular function due to myocardial necrosis, the serum concentrations of LDH, CK, AST, MDA, and troponins will significantly increase, indicating tissue damage" [9].

"There is a correlation between myocardial injury and hepatic insufficiency" [10]. "The liver plays a vital role in metabolism, detoxification and excretion. It metabolizes substance via hydration, condensation, oxidation, reduction, hydrolysis or conjugation. An alteration in any of these processes may result in liver cell injury" [11]. Blockage of blood flow and congestion can manifest in liver damage [12], and the damaging effect of the myocardial infarction on the liver are multifactorial including decrease in blood flow to the liver, reduced arterial saturation and increased hepatic vein pressure [13]. Liver disease possibly may be inflammatory, non-inflammatory and degenerative.

"Nutraceuticals in the form of antioxidants, dietary fibers, plant-based metabolites such as polysaccharides, polyphenols, polysterols, and vitamins also play a preventive and curative role in cardiovascular diseases, so do plants with antioxidant properties" [14,15,16]. One such plant with high antioxidant properties is *Theobroma cacao* [17]. *Theobroma cacao* is reported to suppress the development of atherosclerotic lesions [18], decrease platelet hyperactivity [19], increase dermal blood flow [20], decrease oxidation of LDL cholesterol and promote normal lipid profile [21]. It also inhibits the proliferation of human breast cancer cells [22]. There is however paucity of reports on the hepatoprotective potentials of *Theobroma cacao* secondary to myocardial injury [23].

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

Dry Trinitario variety of *Theobroma cacao* seeds were obtained from Cross River State, Nigeria. The variety was identified at the Herbarium unit of the Department of Botany, University of Calabar, Nigeria, and assigned a voucher number TCB/990123. 3kg de-coated dry cocoa seeds were ground into coarse powder yielding 1.65 Kg of the powder. "This was suspended in two liters of ethanol (BDH Ltd Poole, England) and left to percolate for 24 hours at room temperature. The suspension was thereafter filtered with Whatman No. I filter paper. The filtrate was evaporated by hot air oven treatment at 40-45ºC to a thick dark gummy crude extract giving a yield of 66g (4.8%). The extract was refrigerated at -4ºC until required for use" [24].

2.2 Experimental Animals/Design

Approval was sought and the consent granted by the Faculty of Basic Medical sciences Animal Research Ethics Committee, the University of Calabar with Approval No: 019PY20401. "Male Wistar rats (200-250g, n = 24) were divided into four groups of six rats and used for the study. The animals were kept in plastic cages and controlled environment (12h light/dark cycles at 27 ± 2°C) one week for acclimatization before the commencement of the study. Group 1 was the control that received 0.9% normal saline via oral gavage. Group 2 was the acute myocardial injury group, and received two doses of subcutaneous injection of isoproterenol (100 mg/kg body weight) at an interval of 24 hours between doses. Group 3 was administered *Theobroma cacao* (100 mg/kg body weight orally) only for 2 weeks. Group 4 was pretreated with *Theobroma cacao* for 2 weeks and then followed by injection of isoproterenol (100 mg/kg body weight) on day 15 and 16. All animals had free access to rat chow and tap water throughout the duration of the experiment" [24].

2.3 Induction of Acute Myocardial Injury

Myocardial infarction was induced by subcutaneous injection of 100 mg/kg isoproterenol once for two days with a 24 hours interval following a method described previously [24]. The drug was dissolved in normal saline before injection.

2.4 Determination of biochemical biomarkers

Myocardial injury and liver function were assessed in rats after 24 h of second injection of isoproterenol by estimating the level of specific biomarkers in serum. Blood samples were obtained from the rats by cardiac puncture after intraperitoneal injection of ketamine anesthesia (80 mg/kg body weight). The blood was allowed to clot for two hours and centrifuged at 200 g for 10 minutes to obtain serum. ALT, ALP LDH and cardiac troponin were measured in serum using their respective enzyme kits obtained from Cell Biolabs Inc, San Diego, CA, USA.

2.5 Data Analysis

Results are expressed as mean ± standard error of mean (SEM). Data were analyzed using the GraphPad Prism software (version 7.0). Analysis of variance (ANOVA) was followed by Tukey comparison test where F value was significant. Probability level of $p<0.05$ was accepted as significant.

3. RESULTS

3.1 Troponin and Lactate Dehydrogenase Concentrations in Isoproterenol-induced Myocardial Injury Treated with Cocoa

Serum concentrations of troponin and lactate dehydrogenase were measured to evaluate myocardial damage. The serum concentration of troponin in the control, MI, *Theobroma cacao* only, and *Theobroma cacao* + MI groups was 0.04 ± 0.00 ng/ml, 0.06 ± 0.00 ng/ml, 0.05 ± 0.00 ng/ml, and 0.04 ± 0.00 ng/ml respectively. The result showed a significant ($p<0.01$) increase in the serum concentration of troponin in the MI group compared to the control group. Administration of *Theobroma cacao* before MI induction significantly ($p<0.01$) decreased troponin concentration when compared with the MI group. Similarly, the mean serum concentration of LDH in control, MI, *Theobroma cacao* only, and *Theobroma cacao* + MI groups was 1465 ± 3.9 IU/L, 1653 ± 3.5 IU/L, 1430 ± 8.3 IU/L, and 1422 ± 18. IU/L, respectively. This shows a significant ($p<0.01$) increase in the serum concentration of LDH in the MI group when compared to the control. This increase was attenuated ($p<0.01$) by pretreatment with *Theobroma cacao* before MI induction. This is presented in Table 1.

3.2 Serum Aspartate Aminotransferase (AST) Concentrations in Isoproterenol-induced Myocardial Injury Treated with *Theobroma cacao*

The liver enzymes activity was measured to evaluate the integrity of the hepatocytes following isoproterenol-induced myocardial injury. Serum aspartate aminotransferase (AST) concentrations in the control, MI, *Theobroma cacao* only, and *Theobroma cacao* + MI groups was 50.9 ± 0.1 IU/L, 82.7 ± 0.2 IU/L, 46.7 ± 0.2 IU/L, and 57.9 ± 0.3 IU/L respectively. The result showed a significant ($p<0.001$) increase AST concentration in the MI group when compared with the control. However, pretreatment with *Theobroma cacao* before MI induction resulted in a decrease serum

level of AST, with values similar to the control group. This is presented in Fig. 1.

3.3 Serum Alanine Aminotransferase Concentrations in Isoproterenol-induced Myocardial Injury Treated with *Theobroma cacao*

Serum alanine aminotransferase (ALT) was also measured to evaluate the integrity of the hepatocytes. The serum ALT concentrations in the control, MI, *Theobroma cacao* only, and *Theobroma cacao* + MI groups was 21.6 ± 0.2 IU/L, 38.1 ± 0.3 IU/L, 20.5 ± 0.3 IU/L, and 29.8 ± 0.3 IU/L respectively. The MI group had an increased ($p<0.001$) ALT concentrations compared with the control. Pretreatment with *Theobroma cacao* prevented increased in serum ALT (Fig. 2).

3.4 Serum Alkaline Phosphatase Concentrations in Isoproterenol-induced Myocardial Injury Treated with *Theobroma cacao*

Serum alkaline phosphatase (ALP) was also measured to evaluate the integrity of the hepatocytes. The serum ALP concentrations in the control, MI, *Theobroma cacao* only, and *Theobroma cacao* + MI groups was 57.3 ± 0.2 IU/L, 125.2 ± 0.6 IU/L, 53.5 ±0.3 IU/L, and 71.4 ± 0.4 IU/L respectively. The result followed a similar trend with other liver enzymes with a significantly higher ($p<0.001$) ALP concentration in the MI group when compared with the control. The *Theobroma cacao* pretreatment group showed a lower concentration of ALP concentration when compared with the MI group. ALP values in the pretreated groups are similar with the control group. This is presented in Fig. 3.

Table 1. Serum troponin and lactate dehydrogenase concentration in isoproterenol induced myocardial injury treated with *Theobroma cacao*

Cardiac Markers	Control Group	Myocardial injury (MI) Group	*Theobroma cacao* Only	*Theobroma cacao* + MI
Troponin (ng/ml)	0.04 ± 0.00	0.06 ± 0.00**	0.04 ± 0.00	0.04 ± 0.00^{c}
Lactate dehydrogenase (IU/L)	1465 ± 3.9	1653 ± 3.5**	1430 ± 8.3	1422 ± 18.0^{c}

*** = p<0.01 compared with control; **c** = p<0.01 compared with MI group*

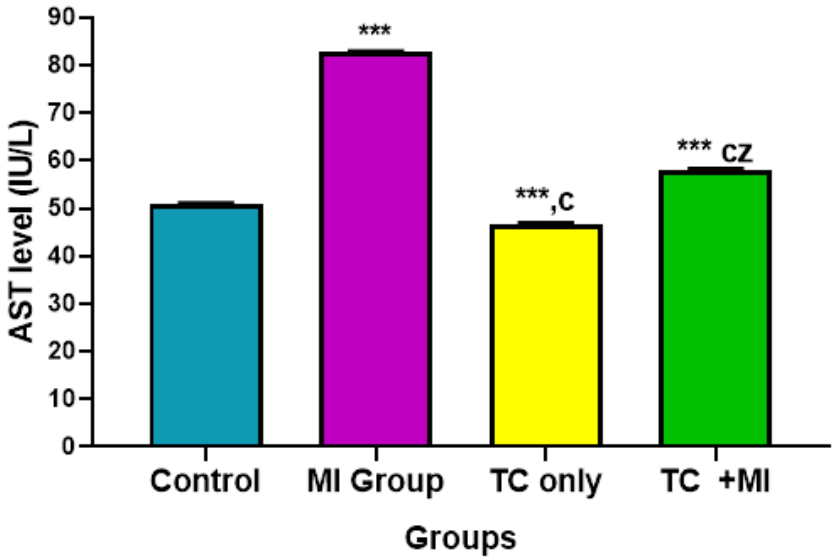

Fig. 1. Serum aspartate aminotransferase activity in control and myocardial injury rats treated with *Theobroma cacao* seed extract

*Values are expressed as mean ± standard error of mean (SEM). *** = p<0.001 versus control; c = p< 0.001 versus myocardial injury (MI) group, z = p< 0.001 versus Theobroma cacao (TC) only group; n = 6.*

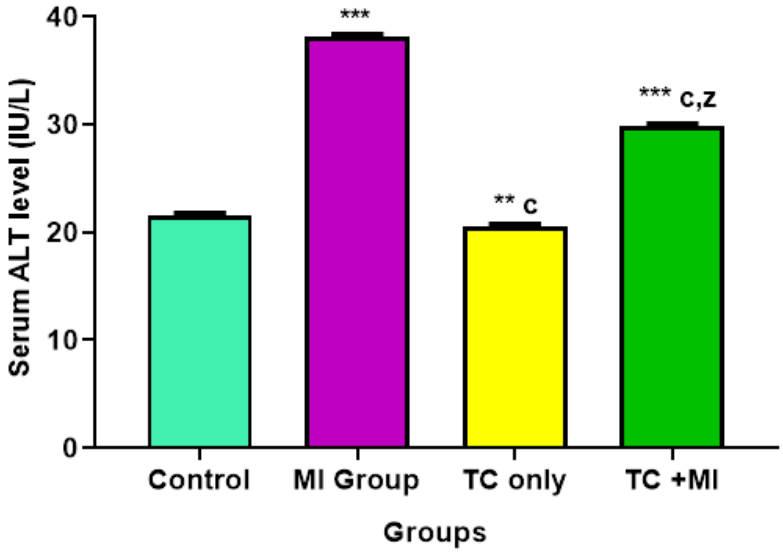

Fig. 2. Serum alanine aminotransferase levels in the different experimental groups

*Values are expressed as mean ± standard error of mean. n = 6. ** = p<0.01, *** = p<0.001 versus control; c = p<0.001 versus myocardial injury (MI); z = p<0.001 vs Theobroma cacao (TC) only group.*

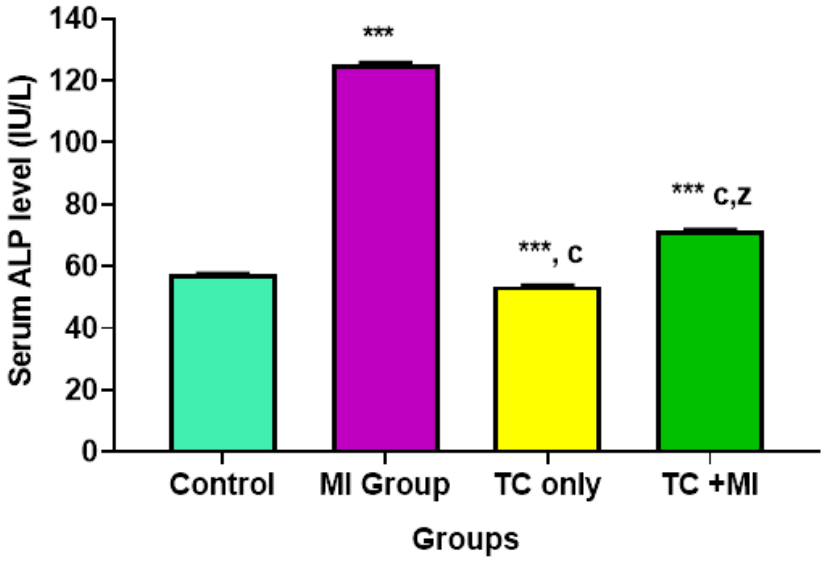

Fig. 3. Serum alkaline phosphatase activity in the different experimental groups

*Values are expressed as mean ± standard error of mean. n = 6. *** = p<0.001 vs control; c= p<0.001 vs myocardial injury (MI) group; z = p<0.001 vs Theobroma cacao (TC) only group*

4. DISCUSSION

The present study evaluated the hepato-protective potentials of *Theobroma* cacao secondary to isoproterenol-induced myocardial injury in rats. “Isoproterenol acts by decreasing the blood flow to the myocardium with consequent hypoxia. The hypoxic state causes a fall in mitochondrial ATP, hence depleting cellular ATP. There is a generation of reactive oxygen species, calcium overload, and phospholipid depletion with attendant lipid peroxidation, tissue inflammation, and structural membrane damage. These result in an irreversible damage to the myocardium” [24]. “Myocardial infarction induced by isoproterenol is reported to show many

metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction" [25].

Reports have shown "a significant link between myocardial infarction, liver dysfunction, and risk of ischemic hepatitis; a condition that occurs as a result of decrease total hepatic blood flow secondary to low cardiac output, shock or cardiac arrest" [26,27]. "Blockage of blood flow and congestion can manifest in liver damage" [12]. "The damaging effect of the myocardial infarction on the liver includes decrease in blood flow to the liver, reduced arterial saturation and increased hepatic vein pressure" [13]. "Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time" [20]. "Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), measure the level of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatase (AP), γ-glutamyl transpeptidase (GGT), and bilirubin act as markers of biliary function and cholestasis. Increased serum levels of liver enzymes such as AST, ALT and ALP are indicators of hepatocellular injury. Elevations of aminotransferases greater than eight times the upper limit of normal reflect either acute viral hepatitis, ischemic hepatitis, or drug- or toxin-induced liver injury" [20]. Following the myocardial injury caused by ISO administration, the serum liver enzymes AST, ALT, and ALP concentrations were significantly elevated above the normal control, indicating hepatotoxicity in the ISO group. The increased serum liver enzymes were reversed in the cocoa treatment groups, indicating the hepatoprotective potentials of *T. cacao.* This result is in agreement with a previous study that reported that *Theobroma cacao* preserved normal liver function by reducing the levels of liver enzymes [28] and amelioration of non-alcoholic fatty liver diseases [29]. The observed effect could be due to the abundant presence of flavonoids, epicatechin and procyanidin present in cocoa.

From the study, administration of ISO resulted in a significant elevation in the serum concentrations of the cardiac biomarkers, troponins and LDH when compared with the normal control rats, depicting myocardial injury [30]. Pretreatment with cocoa before MI induction preserved the myocardial cells to withstand the insult, with a consequent reduction in the concentrations of LDH and troponins, with values similar to the control group. This is in agreement with a previous report that *Theobroma cacao* protects the heart from myocardial injury with a reduction of cardiac biomarkers [24].

There are few limitations in this study. Though the study has demonstrated the hepatic dysfunction in terms of biochemical alteration of the key enzymes due to myocardial injury, the histological changes could not be carried out. Also, the pathway that results in amelioration of hepatic damage by active phytochemicals could not be assessed due to technical constrains. Future studies are suggested in this direction. Oxidative stress and inflammation are in the core of mechanisms of liver damages induced by viruses, drugs, or chemical agents [31] which studies have documented the anti-inflammatory and antioxidant of this cocoa seed.

5. CONCLUSION

Theobroma cacao seed extract prevents hepatic damage arising from myocardial injury. The result of this study has further strengthened the link between myocardial injury and the liver and also provided a support for the prophylactic use of dietary *Theobroma cacao* against hepatotoxicity.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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