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Effect of Theobroma Cacao Polyphenol on Isoproterenol-Induced Myocardial Infarction in Wistar Rats

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ABSTRACT

The effect of *Theobroma cacao* (cocoa) polyphenol on isoproterenol-induced myocardial infarction in Wistar rats was studied. Polyphenol was extracted from cocoa using hydroalcohol solvent. Twenty four rats divided into six groups of four rats each were used for the study. Groups 3, 4, 5 and 6 were pretreated with the extract (300, 500 and 700 mg/kg) and 100 mg/kg of atenolol respectively before administration with isoproterenol. Group 1 served as normal control and group 2 was administered with isoproterenol without treatment. The activities of marker enzymes, aspartate amino transferase (AST), alanine amino transferase (ALT) and lactate dehydrogenase (LDH) in both serum and heart tissue homogenate were assayed. Cocoa polyphenol ameliorated the leakage of marker enzymes from the heart into the serum and also restored the levels of these enzymes in the cardiomyocytes. Histological examination of heart tissues revealed marked ability of the extract to reduce the progression of infarct lesions.

INTRODUCTION

Cocoa (*Theobroma cacao*) is one of the major cash crops in Nigeria (Awe *et al.,* 2012). Cocoa and cocoa derivatives are recognized as major dietary sources of antioxidants because of their high phenolic content (Tomas-Barberan *et al.,* 2007). Plant-derived flavanol-rich foods and beverages include wine, tea, various fruits and berries, as well as cocoa and cocoa products. The three main groups of polyphenols in cocoa are catechins or flavan-3-ols (37%), anthocyanins (4%) and proanthocyanidins (58%) (Belscak *et al.,* 2009). The main catechin is (−)-epicatechin, which constitutes approximately 35% of the total polyphenol content of cocoa.

Numerous dietary intervention studies in humans and animals indicate that flavanol-rich foods and beverages exert cardioprotective effects with respect to vascular function and platelet reactivity (Carl *et al.,* 2005). An epidemiological long-term study reported a lowering effect of cocoa on cardiovascular mortality in elderly men (Buijsse *et al.,* 2006) and postmenopausal women (Mink *et al.,* 2007). The consumption of flavanol-rich cocoa has been reported to improve endothelial function (Wang-Polagruto *et al.,* 2006) and reduce the incidence of atherosclerotic diseases (McCullough *et al.,* 2006). These results are in agreement with data from human studies that showed the antihypertensive properties of cocoa polyphenols (Taubert *et al.,* 2007). The improvement of the cardiovascular function in humans upon cocoa consumption has been specifically linked to the presence of flavan-3-ol derived metabolites in plasma, especially epicatechin glucuronide (Tomas-Barberan *et al.,* 2007). Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (Boudina *et al.,* 2002). Ischemic tissues generate oxygen derived free radicals which have been implicated in cardiac diseases and metabolic disorder (Prabhu *et al.,* 2006). The model of isoproterenol-induced myocardial ischemia is considered as one of the most widely used experimental model to study the beneficial effects of many drugs on cardiac function (Grimm *et al.,* 1998). The pathophysiological changes following isoproterenol administration are comparable to those taking place in human myocardial ischemia/infarction (Wexler, 1978). Increases in the formation of reactive oxygen species during ischemia and the adverse effects of oxyradicals on myocardium have been well established by both direct and indirect measurements.

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Many epidemiological studies associate an increased consumption of foods and beverages rich in flavonoids, with a reduced risk of cardiovascular diseases and death (Kris-Etherton and Keen 2002; Perez-Vizcaino and Duarte, 2010). Myocardial cell protection and prevention of cell ischemia have been therapeutic targets for a long time. New therapies, especially from natural products such as cocoa with minimal side effects are continually being sought and researched for, for myocardial cell protection and prevention of cell necrosis.

MATERIALS AND METHODS

Plant material

Mature fruits of *Theobroma cacao* were harvested from local farms in Njaba Local Government Area of Imo State.

Animals

Twenty four male albino rats of average weight 100g used for the biochemical and histological studies and eighteen albino mice average weight 32g used for the acute toxicity (LD_{50}) study were purchased from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The rats were fed with rat pellets (Grand Cereals and Oil Mills Ltd, Jos, Nigeria) and water *ad libitum*.

Chemicals and reagents

The chemicals and reagents used were of analytical grade. Isoproterenol hydrochloride (ISO) and Atenolol were purchased from Sigma-Aldrich, USA and Bristol Labs LTD, Berkhamsted, Herts, UK respectively.

Preparation of plant material

The seeds of *T. cacao* were removed from the fresh fruits, depulped and blanched immediately for 5 minutes at a temperature of 95 °C, and air dried under a shed. The husks from the dried seeds were removed and the seeds milled to a fine powder.

Extraction of Plant Material and Preparation of Cocoa Polyphenol Extract (CPE)

The preparation of the cocoa polyphenol was as described by Schinella *et al.* (2010). The cocoa powder (500 g) was defatted with petroleum ether for three days and filtered using a muslin cloth. The residue obtained was air dried and extracted using a magnetic stirrer in hydroalcohol solvent of 70 % ethanol for two hours. The mixture was further filtered with Whatman no. 4 filter paper and the filtrate concentrated to a semi-solid residue in a water bath at 60 ºC.

Preparation of Drug Solutions

Standard drugs were weighed and dissolved in appropriate volume of distilled water. All solutions were kept in tightly closed sterile bottles and were made use of on the same day. Leftovers were discarded.

Induction of Myocardial Infarction

Myocardial infarction was induced by subcutaneous injection of isoproterenol hydrochloride (ISO, 150 mg/kg body weight), dissolved in physiological saline, for 2 consecutive days (Seth *et al*., 1998).

Acute toxicity test

The method of Lorke (1983) was used. Eighteen (18) albino mice were grouped into six (6) groups of three mice each. The animals were administered orally with 10, 100, 1000 1600, 2900 and 5000 mg/kg body weight of the extract respectively. The animals were then observed for nervousness, dullness, in-coordination and or mortality for 24 h.

Determination of Polyphenol Content

The total polyphenol content in *T. cacao* was determined using Folin-Ciocalteu's Reagent as described by Velioglu *et al.*, (1998). The extract was prepared at a concentration of 1 mg/mL using ethanol. The sample (100 µL) was mixed with 750 µL of Folin-Ciocalteu's Reagent (previously diluted 10-fold with distilled water) and allowed to stand for 5 min at a temperature of 25 ºC; Na_2CO_3 (0.57 M) solution (750 µL) was then added to the mixture. After 90 min, the absorbance was measured using JENWAY 640 UV/VIS Spectrophotometer (Beckman/Instruments Inc., Huston Texas) at 725 nm. Results were expressed as gallic acid equivalents (GAE) in milligram per 100g dry weight of sample. The range of the calibration curve was from 0.01-0.1 mg/mL with $R^2 = 0.9588$.

Experimental Design

Twenty four male Wistar rats of average weight 100g procured from the animal house, Faculty of Biological Sciences, University of Nigeria, Nsukka were randomly divided into 6 groups of 4 rats each. The animals were housed in metal cages, maintained at light and dark cycle at 28^{o}C in a well ventilated animal house and acclimatized to laboratory conditions for 7 days prior to the commencement of the experiment. Groups 3, 4, 5 and 6 were pre-treated orally with graded doses (300, 500 and 700 mg/kg body weight) of CPE and atenolol respectively for 14 days. On the 15th and 16th day, groups 2-6 received ISO subcutaneously at an interval of 24 h. The animals were then sacrificed after a 12 h fast. All animal experiment was performed according to the ethical guidelines laid down by the institutional animal ethics committee (IAEC).

The groups and doses administered were:

Group 1 (normal control): no induction of myocardial infarction
Group 2: no treatment + induction with ISO
Group 3: 300 mg/kg b.w of CPE + induction with ISO
Group 4: 500 mg/kg b.w of CPE + induction with ISO
Group 5: 700 mg/kg b.w of CPE + induction with ISO
Group 6: 100 mg/kg b.w of standard drug (atenolol) + induction with ISO

Sample Collection

The animals were sacrificed by medial decapitation along the stomach. Blood from the heart was collected into sample bottles, centrifuged to obtain the serum component which was used for biochemical analysis. The heart tissues were harvested, washed in ice-cold saline and used for histological examination.

Biochemical Estimations

Total cholesterol concentration was determined using the method of Allain *et al.,* (1974) as contained in QCA commercial kits. HDL-cholesterol concentration and triglyceride concentration were determined using the method of Albers *et al.* (1978) as contained in Randox commercial kit.

LDL-cholesterol concentration was determined using the method of Assmann *et al.,* (1984) while ALT and AST activities were determined using the method of Reitman and Frankel (1975). LDH was determined using the method of Weisshaar *et al*. (1975) and lipid peroxidation products were determined by the method of Varshney and Kale (1990).

Histological Studies

Samples from the heart tissue were collected in 10 % buffered formalin. The necropsy samples in formalin were trimmed, dehydrated in 4 grades of alcohol, cleared in 3 xylene grades and embedded in molten wax. On solidifying, they were sectioned 5µm thick with a microtome, floated in water bathe and incubated at 60˚C for 30 minutes. Sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 2 grades of alcohol. The sections were then stained with hematoxylin for 15 minutes. Blueing was done with ammonium chloride, differentiation in 1% acid alcohol before counterstaining with eosin. Permanent mounts were made on glass slides using DPX (a mountant). Histological examination of the slides were done using x40 objective of the light microscope.

Statistical Analysis

Data were presented as mean of 3 replicates ± SD. Statistical analysis was carried out using Statistical Product and Service Solutions (SPSS) version 20. One way analysis of variance was adopted for comparison, and the results were subjected to Post Hoc Test using least significant difference (LSD). $P \leq 0.05$ was considered significant.

RESULTS

Polyphenol Yield at Different Extraction Stages

Table 1 shows the polyphenol yield at different extraction stages of cocoa expressed as gallic acid equivalents (GAE) in milligram per 100g dry weight of sample.

Acute toxicity (LD_{50}) tests

The acute toxicity test of cocoa polyphenol extract showed no death of the animals up to 5000 mg/kg body weight of the extract.

Table 1: Polyphenol Yield at Different Extraction Stages .

Purification stage	Polyphenol content (mg GAE/100g dry sample)
Undefatted sample	594.0281±0.60[a]
Defatted sample	606.2249±0.58[a]
Hydroalcoholic extract	751.4568±0.50[b]

Mean values with different alphabets as superscripts are considered significant ($p < 0.05$)

Result of Marker Enzymes and MDA concentrations in the serum of control and cocoa polyphenol extract treated rats

Table 2 shows mean values for marker enzymes and MDA concentration in the serum of rats administered with graded doses of cocoa polyphenol extract and atenolol before induction with isoproterenol.

A significant increase ($p < 0.05$) in MDA concentration and the activities of the enzymes (AST, ALT and LDH) in the serum of the untreated rats was observed when compared with the normal rats. Dose-dependent significant reductions ($p < 0.05$) in MDA, ALT and LDH, comparable to that obtained for atenolol, was observed in the extract treated groups when compared with the untreated group.

A significant decrease ($p < 0.05$) in AST activity was observed in the serum of the groups treated with atenolol and 700 mg/kg b.w of the extract while a non-significant decrease was observed in groups treated with 300 and 500 mg/kg b.w of the extract when compared with the untreated group.

Result of Marker Enzymes and MDA concentrations in heart homogenate of control and cocoa polyphenol extract treated rats

Table 3 shows mean values for marker enzymes and MDA concentration in the heart tissue homogenates of rats administered with graded doses of cocoa polyphenol extract and atenolol before induction with isoproterenol.

The untreated group showed significant decrease ($p < 0.05$) in the activities of the enzymes (AST, ALT and LDH) and significant increase ($p < 0.05$) in the MDA concentration compared to the normal (control) group. A significant increase ($p < 0.05$) in LDH activity was observed in the heart tissue homogenate of groups treated with atenolol, 500 and 700 mg/kg b.w of the extract compared to the untreated group. A non-significant increase ($p > 0.05$) in the activity of all the enzymes assayed was observed in the group treated with 300 mg/kg b.w of extract when compared with the untreated group. There was a significant decrease ($p < 0.05$) in MDA concentration in the heart tissue homogenate of the atenolol treated group only, and non-significant decreases ($p > 0.05$) in the extract treated groups when compared with the untreated group.

Table 2: Marker enzymes activities and MDA concentrations in the serum of control and cocoa polyphenol extract treated rats .

Groups	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	MDA (mg/ml)
Normal	90.00±8.00a	33.00±4.58a	116.33±17.04a	1.82±0.14a
Untreated	125.76±9.02b	62.00±5.57b	212.33±15.70b	2.77±0.20b
300 mg/kg b.w Extract	113.33±14.05b	53.00±3.61c	175.33±25.90c	2.64±0.20b
500 mg/kg b.w Extract	107.00±11.14b	47.33±5.13c	151.67±12.04c	2.18±0.22a
700 mg/kg b.w Extract	95.00±12.00a	38.33±4.51a	131.33±19.01a	1.91±0.33a
100 mg/kg b.w Atenolol	90.33±9.50a	47.00±4.58c	134.33±4.04a	2.06±0.40a

Results are expressed as mean ± SD. Mean values with different alphabets as superscripts are considered significant ($p < 0.05$) while mean values with the same alphabets as superscripts are considered non-significant ($p > 0.05$)

Table 3: Activities of marker enzymes and MDA concentrations in heart tissue homogenates of control and cocoa polyphenol extract treated rats.

Groups	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	MDA (mg/ml)
Normal	190.67±13.65a	68.33±9.50a	108.67±11.93a	2.84±0.43a
Untreated	155.68±26.86b	45.00±7.94b	63.33±11.37b	4.26±0.75b
300 mg/kg b.w Extract	178.33±19.55b	49.67±6.43b	72.58±12.10b	3.87±0.59b
500 mg/kg b.w Extract	184.33±11.68b	57.33±7.77b	90.67±13.05a	3.44±0.64b
700 mg/kg b.w Extract	189.67±15.57a	64.33±4.51a	98.67±14.22a	3.37±0.50b
100 mg/kg b.w Atenolol	182.00±21.52b	61.33±7.10a	99.33±17.16a	3.13±0.60a

Results are expressed as mean ± SD. Mean values with different alphabets as superscripts are considered significant ($p < 0.05$) while mean values with the same alphabets as superscripts are considered non-significant ($p > 0.05$)

Table 4: Levels of CHOL, LDL, HDL and TAG in the serum of control and cocoa polyphenol extract treated rats .

	CHOL (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	TAG (mmol/L)
Normal	3.73±0.68a	2.23±0.12a	1.96±0.21a	1.33±0.25a
Untreated	4.53±0.46a	3.40±0.27b	1.26±0.12b	1.90±0.20b
300 mg/kg b.w Extract	4.10±0.40a	2.66±0.21a	1.46±0.15b	1.66±0.25b
500 mg/kg b.w Extract	4.00±0.72a	2.56±0.21a	1.60±0.30b	1.60±0.30b
700 mg/kg b.w Extract	3.93±0.38a	1.73±0.32c	1.83±0.32a	1.30±0.20a
100 mg/kg b.w Atenolol	3.46±0.45a	2.46±0.40a	1.70±0.27a	1.43±0.25a

Results are expressed as mean ± SD. Mean values with different alphabets as superscripts are considered significant ($p < 0.05$) while mean values with the same alphabets as superscripts are considered non-significant ($p > 0.05$. n=3.

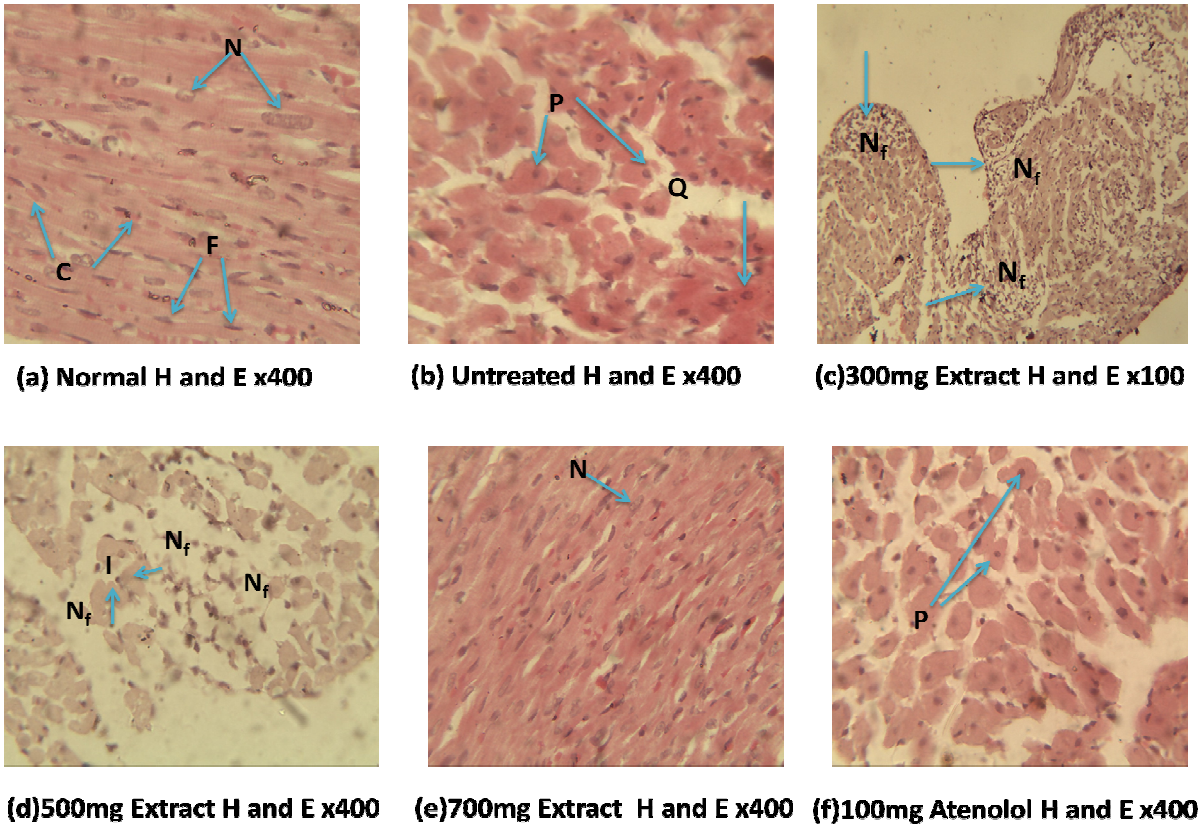

Fig. 1: Result of histological examination of the heart tisues.

Result of Lipid profile parameters

Result in table 4 show mean values of cholesterol, LDL, HDL and TAG of rats administered with graded doses of cocoa polyphenol extract and atenolol before induction with isoproterenol. The untreated group showed significant increases ($p < 0.05$) in the levels of LDL and TAG and a significant decrease ($p < 0.05$) in the level of HDL when compared with the normal (control) group. No significant difference ($p > 0.05$) was observed in the cholesterol level of all the extract and atenolol treated groups when compared with the untreated group. A dose-dependent significant decrease ($p < 0.05$) in the level of LDL was observed in all extract treated groups when compared with the untreated control. Groups treated with atenolol and 700 mg/kg b.w of extract showed significant increase ($p < 0.05$) in HDL when compared to the untreated control group while the 300 and 500 mg/kg b.w treated groups showed non-significant increase ($p > 0.05$) in HDL compared to that of the untreated group. Groups treated with 300 and 500 mg/kg b.w of extract showed non-significant decrease ($p > 0.05$) while the groups treated with 700 mg/kg b.w of extract and atenolol showed a significant decrease ($p < 0.05$) in TAG when compared with the untreated control group.

HISTOLOGICAL RESULTS

From the result of the histological examination of heart tissue sections of control and extract treated animals, *(a)* Heart tissue from control rat showed normal cross striated myocardial fibres with faintly stained nucleus (**N**), fibrocytes (**F**) and blood capillaries (**C**). *(b)* Heart section of untreated and ISO-myocardial infarcted rat showed severe multifocal myocardial degeneration and necrosis, varying degrees of fragmentation, separation of myocardial fibres (**Q**) with loss of normal striation of the myocardium and pyknosis of the nuclei (**P**). *(c)* Heart section of group treated with 300 mg/kg b.w of extract showed multifocal areas of myocardial necrosis with mild leukocytic infiltration of the necrotic foci ($\mathbf{N_f}$). *(d)* Heart section of group treated with 500 mg/kg b.w of extract showed multifocal areas of myocardial necrosis with leukocytic infiltration (**I**) of the necrotic foci ($\mathbf{N_f}$). *(e)* Heart section of the group treated with 700 mg/kg b.w of extract showed no observable evidence of myocardial degeneration or necrosis, ie. normal shaped nucleus (**N**). *(f)* Group treated with 100 mg/kg b.w of atenolol, showed mild necrosis and pyknosis (**P**) of the cardiomyocyte nuclei.

DISCUSSION

The present investigation was aimed at determining the effect of polyphenol-rich extract of *Theobroma cacao* treatment on cardiovascular health. Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction such as; lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and once metabolically damaged, releases its contents into the extracellular fluid (Suchalatha and Shyamala, 2004). There was increased activity of the serum marker enzymes (Table 2) of the untreated animals with concomitant decrease in the activities of the same enzymes assayed in the heart homogenate (Table 3) when compared with the normal group; thus inferring development of myocardial necrosis. This could be attributed to formation of lipid peroxides and degeneration of membrane lipids which compromised membrane integrity causing leakage of these enzymes into the serum. One of the mechanisms by which ISO causes myocardial infarction is through the formation of free radicals which initiate and stimulate lipid peroxidation causing irreversible damage to the myocardium (Senthil *et al.*, 2001). However, the marker enzymes in the serum of the extract treated groups showed decreased activity, with the group treated with the highest dose (700 mg/kg b.w) giving the highest significant decrease ($p < 0.05$) when compared with the untreated and other groups. This could be attributed to the antioxidant and membrane stabilizing effects of the polyphenols, thereby preventing further leakage of these enzymes into the blood stream. The reverse was observed in the heart tissue homogenate of the treatment groups whereby the activities of these enzymes increased compared to that of the untreated group. This agrees with the work of Prabhu *et al.* (2006), who reported a dose dependent significant cardioprotection by mangiferin, a non-nutrient phytochemical extracted from *Mangifera indica*, treatment in isoproterenol myocardial infarcted rats. The standard drug; atenolol, acts by antagonizing the stimulation of β-1-adrenergic receptors by catecholamines such as epinephrine, norepinephrine and isoproterenol. However, polyphenols, in particular, flavanols in cocoa products, have been shown to increase the formation of endothelial nitric oxide (NO), which promotes vasodilation and consequently lower blood pressure (Fisher and Hollenberg, 2006). During the first seconds to minutes of reperfusion, the myocardium produces a large concentrated burst of NO. At the same time that nitric oxide is being produced, large amounts of superoxide anion are also generated. NO and superoxide anion rapidly react during early reperfusion to form peroxynitrite, a potent oxidant (Yasmin *et al*., 1997). Polyphenols sustain the concentration of nitric oxide through their antioxidant effect by scavenging free radicals (e.g. superoxide anion) that may react with NO forming peroxynitrites. Cocoa polyphenols therefore, maintain cardiovascular tone by inducing the formation of NO and sustaining it, thus producing consistent peripheral vasodilation (Bayard *et al*., 2007), enhanced flow mediated dilation (FMD) of conduit arteries and augmentation of microcirculation (Schroeter *et al*., 2006) and increased blood flow (Neukam *et al.*, 2007). It has been shown that endothelial secretion of NO counterbalances the direct vasoconstrictive effects of norepinephrine, serotonin, angiotensin II and endothelin on the vascular smooth muscle (Rubanyi, 1993). NO has also been shown to reduce oxygen consumption (Shen *et al*., 1994) and plays a critical role in the pathogenesis of atherosclerosis due to its inhibitory effects on platelet aggregation (Radomski *et al*., 1987), leukocyte adhesion (Kubes *et al*., 1991), DNA synthesis (Nakaki *et al*., 1990) and vascular smooth muscle cell proliferation (Garg and Hassid, 1989). Cocoa polyphenols may be involved in cholesterol control and

TAG homeostasis. The levels of LDL and TAG (Table 5) were significantly increased ($p < 0.05$) in the untreated group when compared with the normal group. With cocoa extract treatment, the levels of these parameters were decreased. Waterhouse *et al*. (1996) reported that polyphenols from chocolate inhibited LDL oxidation by 75%, compared to 37–65% of red wine (Cooper *et al*., 2008). Hamed *et al*. (2008) reported 6% decrease of LDL cholesterol after 7-day consumption of regular dark chocolate. In addition, Vinson *et al*. (1999) reported that dark chocolate had higher quality of phenol antioxidants expressed as IC_{50} for LDL + VLDL oxidation compared to red wine and black tea, with high lipoprotein bound antioxidant activity, which is very important in prevention of heart diseases. Reports have shown that total and LDL cholesterol were decreased after 15- day consumption of polyphenol-rich dark chocolate by 6.5% and 7.5%, respectively (Grassi *et al*., 2008). In this study, HDL concentration was significantly reduced ($p < 0.05$) in the untreated group when compared to the normal group; however significant increases ($p < 0.05$) were observed in the 700 mg/kg b.w extract and atenolol treated groups when compared to the untreated group. Mursu *et al*. (2004) reported increase in HDL after a 3-week consumption of dark and polyphenol-rich dark chocolate. Hamed *et al*. (2008) also reported a 9% increase of HDL after 7-day consumption of regular dark chocolate. Mellor *et al*. (2010) reported that atherosclerotic cholesterol profile (cholesterol: HDL ratio) in patients with diabetes was improved after 8-week chocolate consumption without affecting weight, inflammatory markers, insulin control, or glycaemic control. However, Kurlandsky and Stote (2006) reported no significant difference in HDL and LDL cholesterol levels between "chocolate consuming" and control group. Almoosawi *et al*. (2010) also observed no significant change in total cholesterol level after consumption of dark chocolate. There was increase in the level of malondialdehyde concentration in the untreated group, both in the serum and heart homogenate, compared to the normal group. Increased levels of TBARS (MDA) indicate excessive formation of free radicals and activation of lipid peroxidation system resulting in irreversible damage to the heart in animals subjected to ISO stress (Prabhu *et al*., 2006). There were non-significant decreases in the level of malondialdehyde assayed in heart tissue homogenates of extract treated groups. However, the level of malondialdehyde concentration in the serum showed significant decreases in the treatment groups when compared with the untreated group. This corroborates the work of Schinella *et al*. (2010) which stated that cocoa powder inhibited lipid peroxidation in both brain homogenates and human plasma. Verstraeten *et al*. (2005) studied the antioxidant and membrane effects of procyanidin B2 (dimer) and procyanidin C1 (trimer) from cocoa in phosphatidyl choline liposomes. Both procyanidins inhibited lipid oxidation of liposomes in a concentration dependent manner and also increased the membrane surface potential. This they did by decreasing membrane fluidity, an effect that extended into the hydrophobic region of the bilayer, protecting the lipid bilayer from disruption by Triton X-100. Procyanidins can thus interact with membrane phospholipids, probably through their polar head group, to provide protection against the attack of oxidants and other molecules that challenge the integrity of the bilayer (Andujar *et al*., 2012). Microscopic examination of the heart tissue of the normal group showed normal cross striated myocardial fibres with faintly stained nucleus, fibrocytes and blood capillaries, while in the untreated group, there were severe multifocal myocardial degeneration and necrosis, varying degrees of fragmentation, separation of myocardial fibres with the loss of normal striation of the myocardium. Heart sections from the group administered the highest dose of the extract (700 mg/kg) showed no evidence of myocardial degeneration or necrosis. This agrees with the works of Muruganandan *et al*. (2002) and Prabhu *et al*. (2006) who reported that treatment with the polyphenol, mangiferin, protected the cardiac tissues of diabetic and ISO myocardial infarct rats. In this study, cocoa extract exhibited antioxidant activity, antihyperlipidemic and membrane stabilizing effect. The *T. cacao* extract may exert its protective effect by radical scavenging amongst other mechanisms; as intervention studies have suggested that cocoa has several beneficial effects on cardiovascular health, which includes the lowering of blood pressure, the improvement of vascular function and glucose metabolism, the reduction of platelet aggregation and adhesion (Sudano *et al*., 2012a) Cocoa and its flavanols increase nitric oxide (NO) bioavailability, activate nitric oxide synthase (NOS), and exert antioxidative, anti-inflammatory, and anti-platelet effects, which in turn improves vascular function, reduces blood pressure and therefore, explains the positive impact on clinical outcome proposed by epidemiological studies (Corti *et al*., 2009; Shrime *et al*., 2011; Hooper *et al*., 2012; Sudano *et al*., 2012b). More so, there is some evidence that flavanols and flavanol-rich foods including cocoa inhibit angiotensin-converting enzyme (ACE) activity *in vitro* (Actis-Goretta *et al*., 2003; Actis-Goretta *et al*., 2006). ACE regulates the renin−angiotensin system; it cleaves angiotensin-I into angiotensin-II, which stimulates the release of vasopressin or aldosterone and antidiuretic hormone, increasing sodium and water retention. The inhibition of the ACE system will therefore increase the amount of sodium and water excreted thus reducing blood pressure and improving CVD treatment outcome. It also inactivates the vasodilators bradykinin and kallidin, (Sudano *et al*., 2012b) thereby enhancing vasodilatation and perfusion.

CONCLUSION

Results from this study have shown that cocoa and its derivatives processed to retain high polyphenol content may confer resistance of cardiomyocytes to toxicant and free radical-induced injury and could be used as a nutraceutical and functional food in the prevention and management of myocardial infarction and perhaps other forms of cardiovascular diseases.

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