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Original Research

Short-term capacities of ethanolic *Theobroma cacao* bean extract to ameliorate oxidative stress, hyperglycemia, and dyslipidemia in alloxan-induced diabetic rats

Paul Chidoka Chikezie

Department of Biochemistry, Imo State University, Owerri, Nigeria

Address for correspondence: Paul Chidoka Chikezie, Department of Biochemistry, Imo State University, Owerri, Nigeria. E-mail: p_chikezie@yahoo.com

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ABSTRACT

Objectives: The present study ascertained the capacities of ethanolic *Theobroma cacao* bean extract to ameliorate hyperglycemia and dyslipidemia in Type I diabetic rats (T1-DR) following the short-term treatment for 64 h. In addition, erythrocyte hemolysate reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio and malondialdehyde (MDA) concentration were measured to establish erythrocyte antioxidant status of T1-DR. **Materials and Methods:** Diabetes mellitus (DM) was induced in the experimental rats by a single intraperitoneal injection of 0.1 mol/L alloxan monohydrate in phosphate-buffered saline solution (pH = 7.4) at a dosage of 140 mg/kg body weight. At the end of the treatment period, blood samples were drawn from the orbital sinus and measured for fasting plasma glucose concentration (FPGC), erythrocyte hemolysate GSH/GSSH ratio, MDA concentration, and serum lipid profile (SLP) using standard methods. **Results:** Blood samples of T1-DR treated with *T. cacao* bean extract showed a substantial reduction in FPGC but were hyperglycemic. Erythrocyte hemolysate GSH/GSSG ratio and MDA concentrations of T1-DR treated with *T. cacao* bean extract were significantly different ($P < 0.05$) from that of the untreated group. In general, the administration of *T. cacao* bean extract caused readjustments in perturbed SLP of T1-DR, which tended toward normalcy within the 64 h treatment period. Calculated AI of the experimental rats was within the range of 5.35 ± 0.51 to 0.50 ± 0.08. **Conclusion:** Short-term administration of *T. cocoa* bean extract caused substantial reduction in blood glucose concentration but did not obliterate hyperglycemia. In addition, *T. cocoa* bean extract, in the present form and doses exhibited comparatively limited capacities to reduce oxidative stress and ameliorate dyslipidemia in T1-DR.

KEY WORDS: Diabetes mellitus, glutathione, hyperglycemia, malondialdehyde, serum lipid profile

INTRODUCTION

Diabetes mellitus (DM) describes a collection of endocrine metabolic disorders of multifactorial etiologies and physiochemical peculiarities, which serve as bases for its classifications and diagnosis. Regardless of the complexities of DM pathology, the disease is primarily characterized by hyperglycemia and glucosuria [1]. The absence or low circulating plasma levels of insulin as a result of failure of the β-cells of islet of Langerhans to synthesize and secrete insulin is often associated with insulin-dependent DM (Type I DM or juvenile-onset DM). Epidemiological survey and experimental evidence tends to suggest that Type I DM is a hereditary autoimmune disorder elicited by auto-reactive $CD4^+$ and $CD8^+$ T-cells that recognize pancreatic insulin as an antigen and consequently causes damage to insulin producing β-cells [2,3]. Studies have also suggested that viral actions are associated with molecular events that trigger Type I DM autoimmune disorders [2].

The role of reactive oxygen and nitrogen species (RONS) in the pathogenesis of Type I DM is well established [4], which is demonstrable by the administration of alloxan and streptozotocin (STZ) to induce Type I DM in experimental animal systems [5-7]. Cytotoxicity and oxidative damage to insulin producing pancreatic β-cells is an outcome of the generation of nitric oxide and alkylation of DNA by STZ metabolites, whereas alloxan and its metabolic product, dialuric acid, undergo redox cycle associated with the generation of superoxide radicals that are subsequently converted to hydrogen peroxide and hydroxyl radicals through dismutation and Fenton reaction pathways, respectively [8,7].

The generation of overwhelming levels of RONS as a result of the metabolic fallout of diabetogenic agents causes fragmentation of pancreatic DNA as well as a precipitate hindrance in Ca^{2+} mediated secretion of insulin by the islet

cells [1]. The pathophysiology of non-insulin-dependent DM (Type II DM and sub-Type II DM or maturity-onset diabetes of the young have been described elsewhere) [8,9].

Chronic hyperglycemia in DM causes protein glycation and auto-oxidation of glucose; along with substantial diversion of glucose metabolism via the polyol pathways, which elicits overwhelming levels of cytotoxic RONS. Studies concerning the pathogenesis and complications of DM have shown that the most important sources of RONS, under hyperglycemic conditions, are mitochondrial metabolic events, nicotinamide adenine dinucleotide phosphate oxidase activity, and glycated protein molecules [4]. Because elevated cellular RONS levels lead to damage to organelles and obliteration of enzyme activities, most of the complications arising from DM are associated with RONS induced injuries to body tissues [1]. Furthermore, the raised levels of RONS elicit lipid peroxidation, of which membrane lipid components are mostly affected. The accumulation of malondialdehyde (MDA) and other products of lipid peroxidation advanced glycation end products such as hemoglobin A_{1c} and damaged DNA fragments eventually cause DM complications such as nephropathy, retinopathy, cardiomyopathy, cataract, and neuropathy [3].

Several types of dyslipidemia are associated with DM, of which hypertriglyceridemia and low plasma high-density lipoprotein cholesterol (HDL-C) levels are most common [10,11] because of low activity of insulin activating lipoprotein lipases lining the endothelial surface. Noteworthy, the characteristic small dense nature of low-density lipoprotein cholesterol (LDL-C), observable in Type II DM, renders this class of lipoprotein more susceptible to glycation and oxidative changes, which promotes greater atherogenic outcome [10]. In addition, under the prevailing pathophysiologic state of DM, increase the availability of plasma free fatty acids and its metabolic products (ketone bodies) provoke ketoacidosis with exacerbated hyperglycemia due to interference in glucose utilization.

Cocoa bean tree: *Theobroma cacao* (Linnaeus), originated from Latin America about 500 years ago and thereafter was propagated in Europe, from where the crop was introduced to other regions of the world. This cash crop is now grown all over the humid tropical regions in about 6.5 million hectares of cultivated land, which covers over 57 countries. The nutritional benefits and energy value of cocoa products are largely hinged on their overall chemical composition, the quantity of proteins, carbohydrates, fats, and corresponding digestibility coefficient [12]. The nutraceutical usefulness of *T. cacao* decoctions has been reported [13]. Furthermore, the phytochemical compositions of cocoa beans have been reported by previous study [14]. The present study ascertained the capacities of ethanolic *T. cacao* bean extract to ameliorate hyperglycemia and dyslipidemia in alloxan-induced Type I diabetic rats (T1-DR) following the short-term treatment for 64 h. In addition, levels of erythrocyte MDA and reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio were measured to establish erythrocyte antioxidant status of the experimental T1-DR.

MATERIALS AND METHODS

Collection and Preparation of Bean Sample

Dried and fermented cocoa beans (*T. cacao*) cultivated in Nigeria were purchased from a local market in Oka-Akoko, Ekiti State, Nigeria. The cocoa pods were harvested between July and October, 2012. A 50 g part of the cocoa beans were weighted using a triple beam balance (OHAU 750-50: Burlington, NC, USA) and pulverized using Thomas-Willey milling machine (ASTM D-3182, India), after which the ground samples were stored in air-tight plastic bottles with screw caps pending extraction.

Extraction of Bean Sample

The extraction procedure was carried out as previously described by Ojiako *et al.*, [15]. Portion of 10 g of the pulverized cocoa beans was subjected to repeated soxhlet extraction cycles for 2 h using 96% C_2H_5OH (BDH, U.K) as solvent to obtain final volume of 50 mL of the extract. The volume of the extract was concentrated and recovered in a rotary evaporator (BÜCHI, R-3000 Switzerland) for 12 h at 60°C under reduced pressure. Next, the extract was dried in a desiccator for 24 h, wrapped in aluminum foil and stored in air-tight plastic bottles with screw caps at ≤4°C. The yield was calculated to be 20.11% (w/w). The extract was reconstituted in phosphate buffered saline (PBS) solution (extract vehicle), osmotically equivalent to 100 g/L PBS (90.0 g NaCI, 17.0 g $Na_2HPO_4.2H_2O$, and 2.43 g $NaH_2PO_4.2H_2O$), before appropriated doses were administered to the experimental animals.

Experimental Animals

Male albino (Wistar) rats weighing between 150 and 160 g were maintained at room temperatures of 24 ± 5°C, 30-55% of relative humidity on a 12 h light/12 h dark cycle, with access to water and standard commercial feed (Ewu Feed Mill, Edo State, Nigeria) *ad libitum* for 2 weeks acclimatization period. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

Induction of Diabetes/Experimental Design

The experimental design was according to the methods previously described [16] with modifications on the dose of extracts administered to the rats. DM was induced in the experimental rats by a single intraperitoneal (i.p.) injection of 0.1 mol/L alloxan monohydrate in PBS solution (pH = 7.4) at a dosage of 140 mg/kg body weight. The animals with fasting plasma glucose concentration (FPGC) > 110 mg/dL, after 5 consecutive days of alloxan treatment, were considered to be T1-DR and selected for the study [15]. The animals were deprived of food and water for additional 12 h before the commencement of treatment at regular intervals of 16 h for 64 h.

A total of thirty six rats were divided into six groups of six (n = 6) rats each as follows:

- Group C1: Control-normal rats received PBS only (Vehicle; 1.0 mL/kg body weight; i. p.).
- Group C2: C-T1-DR received PBS only (Vehicle; 1.0 mL/kg body weight; i. p.).
- Group T1: T1-DR received *T. cacao* (100 mg/kg body weight; i.p.).
- Group T2: T1-DR received *T. cacao* (200 mg/kg body weight; i.p.).
- Group T3: T1-DR received *T. cacao* (400 mg/kg body weight; i.p.).
- Group T4: T1-DR received glibenclamide (5.0 mg/kg body weight; i.p.).

At the end of the treatment period, blood samples were drawn from the orbital sinus from 12 h post-fasted animals and measured for FPGC, serum lipid profile (SLP). Preparation of erythrocyte hemolysate was according to methods previously described [16]. The erythrocyte hemolysate was measured for MDA concentration and GSH/GSSH ratio.

Fasting Plasma Glucose Concentration

Measurement of FPGC was by the glucose oxidase method according to Randox® kit manufacturer's procedure (Randox® Laboratories Ltd. Ardmore, United Kingdom).

Erythrocyte Hemolysate Reduced GSH/GSSG Ratio

Erythrocyte hemolysate GSH concentration was measured according to the methods of Moron *et al.*, [17] with modifications. A 100 μL aliquot of the hemolysate was mixed with 25% of $CHCl_3$ and centrifuged at 2000 × *g* for 15 min to precipitate proteins. The supernatant was aspirated and diluted to 1.0 mL with 0.2 M Na_2PO_4/$NaHPO_4$ buffer (pH = 8.0). Next, 2.0 mL of 0.6 mM 5,5'-dithiobis-(2-nitrobenzoic acid) was added. The absorbance of the developed yellow-color complex maintained at 24 ± 5°C was measured at a maximum wavelength (λ_{max}) =405 nm after 10 min. A standard curve was obtained with GSH standards. Protein concentration was measured according to the methods of Lowry *et al.*, [18]. Erythrocyte hemolysate GSH concentration was expressed as μg GSH/mg protein.

Erythrocyte hemolysate GSSG concentration (μg GSSG/mg protein) was measured using Bioxytech-412 kits according to manufacturer's procedure (Oxis International Inc., Foster City, CA, USA).

$$C_{GSSG} = \frac{Abs_{blank} - Abs_{sample} \times C_{std}}{Abs_{blank} - Abs_{std}} \quad \text{Equation 1}$$

Accordingly, erythrocyte hemolysate GSH/GSSG ratio was evaluated.

Erythrocyte Hemolysate MDA

Measurement of erythrocyte hemolysate MDA concentration was by the methods of Tjahjani *et al.*, [19]. A mixture of 20% trichloroacetic acid and 0.67% thiobarbituric acid in a ratio of 2:1 were introduced into a test tube. A volume of 0.2 mL of erythrocyte hemolysate was added to the mixture and boiled for 10 min in a water bath. After cooling to 24°C, the mixture was centrifuged at 3,000 × *g* for 10 min. The absorbance of the supernatant was read with a spectrophotometer (Spectronic 20, lab tech - digital blood analyzer®) at λ_{max} = 532 nm. The values of absorbance of the samples were converted to MDA concentrations using the MDA standard curve [20].

Serum Lipid Profile

Blood samples were obtained from the various experimental animal groups and measured for SLP according to the methods previously described [21]. Serum total cholesterol (TC), triacylglycerol (TAG), and HDL-C concentrations were measured using commercial kits (Randox Laboratory Ltd., UK). LDL-C concentration was estimated according to the formula of Friedewald *et al.*, [22].

$$LDL\text{-}C = TC - (HDL\text{-}C) - (\frac{TAG}{5}) \quad \text{Equation 2}$$

Atherogenic index (AI) was calculated [23] thus:

$$AI = \frac{TC - (HDL\text{-}C)}{HDL\text{-}C} \quad \text{Equation 3}$$

Statistical Analysis

The results were expressed as mean ± standard error of the mean, and statistically analyzed by one-way ANOVA followed by Dunnett test, with the level of significance set at $P < 0.05$.

RESULTS

At the commencement of animal treatment (t = 0 h), FPGC of the T1-DR was within a relatively narrow range of 251.70 ± 3.01 mg/dL - 258.10 ± 3.27 mg/dL; $P > 0.05$. Similarly, at the end of the experimental time, alteration in the blood glucose concentration of Group C2 corresponded to 4.06% increase in FPGC; $P > 0.05$. Furthermore, within the experimental 76 h period; FPGC of Group C1 was relative constant and did not indicate profound variations.

Conversely, blood samples of T1-DR treated with *T. cacao* bean extract showed substantial reduction in FPGC. Specifically, at t = 76 h, relative reduction in FPGC was in the following order: $T4_{[FPGC]}$ = 63.48% > $T3_{[FPGC]}$ = 55.30% > $T2_{[FPGC]}$ = 53.42% > $T1_{[FBGD]}$ = 46.53%.

At the end of the 76 h experimental time, FPGC of Group C1 and T4 were normoglycemic, whereas Group C2 showed evidence of hyperglycemia [Table 1]. Furthermore, the results presented in Table 1 showed that the FPGC of T1-DR treated with *T. cacao* bean extract (T1-T3) were significantly lower ($P < 0.05$) than that of Group C2 but were hyperglycemic.

Specifically, at t = 76 h, FPGC of Group T3 was not significantly different ($P > 0.05$) from that of Group T2; whereas FPGC of

Group T4 was significantly lower ($P < 0.05$) than Groups T1-T3. In addition, Group T4 was normoglycemic.

Figure 1 showed that the erythrocyte hemolysate GSH/GSSG ratio of Group C1 was 3.65 folds greater than that of Group C2; $P < 0.05$. Erythrocyte hemolysate GSH/GSSG ratio of Groups T1-T3 were significantly ($P < 0.05$) lower than that of Group C1, but significantly higher ($P < 0.05$) than that of Group C2. In addition, variations in erythrocyte hemolysate GSH/GSSG ratios of Groups T1-T3 showed no significant difference ($P > 0.05$) among the experimental animal groups. The erythrocyte GSH/GSSG ratio of Group T4 was significantly higher ($P < 0.05$) than that of Groups T1-T3 as well as Group C2.

The results presented in Figure 2 showed that among the various experimental groups, Group C2 gave the highest erythrocyte hemolysate MDA concentration. Furthermore, erythrocyte hemolysate MDA concentrations of Groups T1-T3 were significantly lower ($P < 0.05$) than that of Group C2; except Group T1 ([MDA] = 2.45 ± 0.03 mmol/mL). Erythrocyte hemolysate MDA concentration of Groups C1 and T4 showed no significant difference ($P > 0.05$) and were comparatively lower than that of other experimental Groups (C2, T1-T3).

Figure 3 showed that the serum TAG concentration of Group C2 was significantly ($P < 0.05$) higher than that of Group C1 and the treated animal Groups (T1-T4). Specifically, variations in serum TAG concentrations of Groups T1-T4 showed no significant difference ($P > 0.05$) among the experimental animal groups. Furthermore, serum TAG concentrations of Groups T1-T4 were significantly higher ($P < 0.05$) than that of Group C1.

Serum very LDL-C (VLDL-C) concentration of Group C2 was higher than that of Group C1, which represented ≈ 2.3 folds elevation in serum VLDL-C concentration; $P < 0.05$. Serum VLDL-C concentration of Groups T2-T4 were significantly lower ($P < 0.05$) than that of Group C2. In addition, the comparatively higher serum VLDL-C concentration of Groups T2-T4 was not significantly different ($P > 0.05$) from serum VLDL-C concentration of Group C1. Serum VLDL-C concentration of Group T1 gave significantly higher ($P < 0.05$) value than that of Group C1.

Serum LDL-C concentrations of Groups T1-T3 were significantly higher ($P < 0.05$) than that of Group C1 in a dose-dependent manner: T1 > T2 > T3. However, serum LDL-C concentration of Group T4 was not significantly different ($P > 0.05$) from that of Group T3. Worthy of note, serum LDL-C concentration of Group C2 was ≈ 3.68 folds greater than that of Group C1.

Serum HDL-C concentration of Group C2 was not significantly different ($P > 0.05$) from that of Group C1. However, serum HDL-C concentrations of Groups T2-T4 were significantly higher ($P < 0.05$) than that of Group C2. Specifically, Group C2 gave the lowest level of serum HDL-C concentration. Serum TC concentrations of Groups T1-T4 were elevated when compared with Group C1; $P > 0.05$, whereas serum TC concentration of Group C2 represented 18.79% elevation in serum TC concentration; $P < 0.05$. A general overview of Figure 3 showed evidence of the tendency of *T. cacao* bean extract to readjust perturbed SLP of T1-DR toward normalcy within the experimental 76 h period.

Calculated AI of the experimental rats was within the range of 5.35 ± 0.51 to 0.50 ± 0.08, which was in the increasing order of C1 > T4 > T3 > T2 > T1 > C2. Figure 4 showed that AI of Group T1 was not significantly different ($P > 0.05$) from Group T2, whereas AI of T3 and T4 were comparable with that of Group C1.

Table 1: FPGC of normal, diabetic, and treated rats

Group	FPGC (mg/dL)	
	t=0 h	*t*=76 h
C1	89.42 ± 0.73^{f}	85.32 ± 0.73^{f}
C2	$251.70 \pm 3.01^{a,b,c,d,e}$	261.92 ± 5.40^{a}
T1	258.10 ± 3.27^{a}	138.00 ± 3.27^{b}
T2	$254.58 \pm 2.01^{a,b}$	118.58 ± 2.69^{c}
T3	$253.94 \pm 2.13^{a,b,c}$	$113.50 \pm 3.55^{c,d}$
T4	$253.78 \pm 2.00^{a,b,c,d}$	92.69 ± 1.70^{e}

The mean (X)±SD of six (n=6) determinations. Means in the column with the same letter are not significantly different at P>0.05 according to LSD. FPGC>110 mg/dL=Hyperglycemia, SD: Standard deviation, FPGC: Fasting plasma glucose concentrations

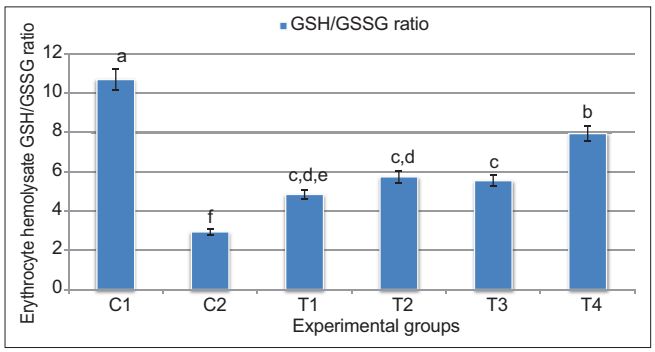

Figure 1: Erythrocyte hemolysate reduced glutathione/oxidized glutathione ratios of normal, diabetic and, treated rats means denoted by the same letter (a,b,c,d,f) are not significantly different at $P > 0.05$

DISCUSSION

The application of bioactive principles, sourced from plant materials, as agents of glycemic control in chemically induced diabetic animals following short- and long-term treatment schemes have been widely reported [3,5,9,24]. The findings of the present study showed that short-term administration of ethanolic extract of *T. cacao* bean caused significantly ($P < 0.05$) reduction of FPGC in alloxan-induced T1-DR. However, within the experimental time of 76 h, the doses and constituted form in which the extract was administered to the experimental T1-DR did not obliterate hyperglycemia [Table 1]. Several studies have described the diverse mechanisms by which specific phytochemicals act to

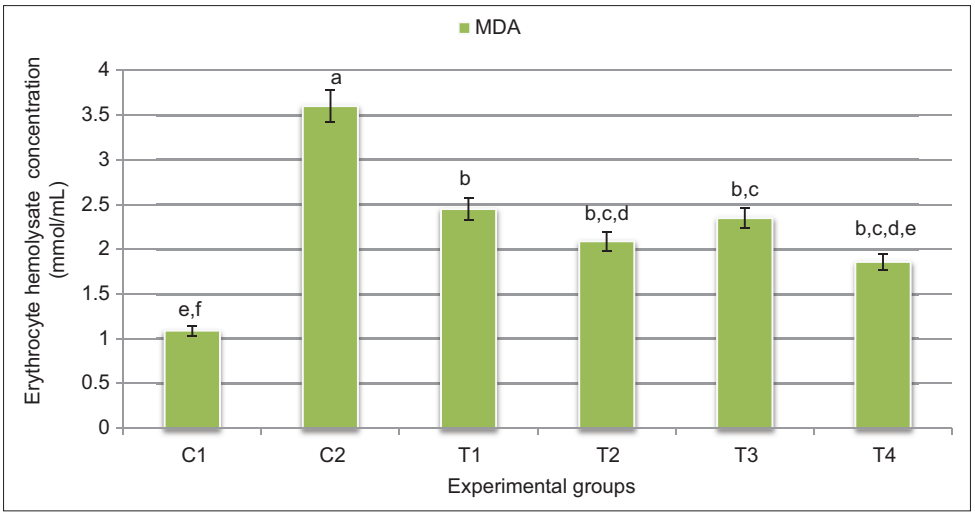

Figure 2: Erythrocyte hemolysate malondialdehyde of normal, diabetic, and treated rats means denoted by the same letter (a,b,c,d,f) are not significantly different at $P > 0.05$

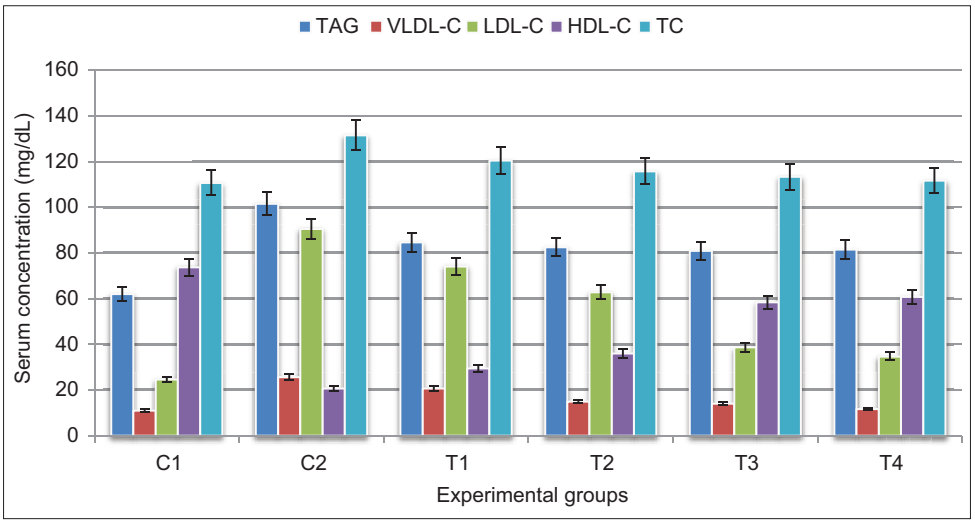

Figure 3: Serum lipid profile of normal, diabetic, and treated rats

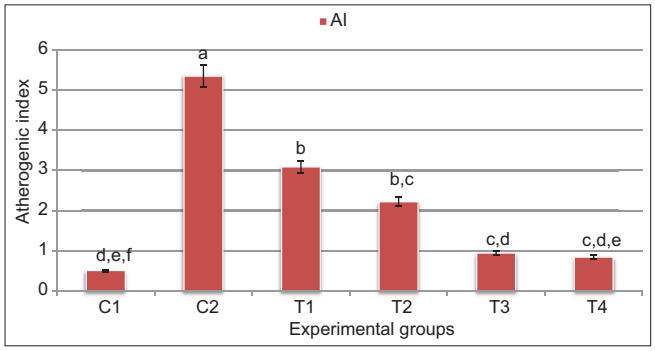

Figure 4: Atherogenic indices of normal, diabetic, and treated rats means denoted by the same letter (a,b,c,d,f) are not significantly different at $P > 0.05$

alleviate hyperglycemia [24-26]. In connection to the present findings, reports according to Ruzaidi *et al.*, [27] stated that the metallothionein, nicotinamide, and (−)-epicatechin contents of *T. cacao* bean extract suppressed diabetogenesis *in vivo*. Furthermore, earlier findings have also shown that the major flavonoids in *T. cacao* beans such as the catechin, epicatechin, the dimers epicatechin-(4β → 8)-catechin (procyanidin B-l) and epicatechin-(4β → 8)-epicatechin (procyanidin B-2), the trimer [epicatechin-(4β → 8)] 2-epicatechin (procyanidin C-l) as well as the proanthocyanidins ameliorated hyperglycemia and prevented complications arising from DM pathology [28].

To a large extent, the thiol-redox status of the cell is defined by cellular levels of GSH/GSSG ratio and decreased GSH content is related to increase oxidative stress [5]. In the event of failure of appropriate compensatory endogenous antioxidant antagonist against glucotoxicity and lipotoxicity, engendered by DM associated hyperglycemia and hyperlipidemia, overwhelming levels of oxidative stress ensue [4,29], as exemplified by relatively low GSH/GSSG ratio of the untreated T1-DR (Group C2) [Figure 1]. Accordingly, researchers have opined that alleviation of DM and its complications can be achieved by the introduction of control measures that assuage high cellular levels of oxidative

stress and attenuate stress-sensitive signaling pathways [5,8]. The improvement in erythrocyte hemolysate GSH/GSSG ratio of T1-DR following the administration of *T. cacao* bean extract was consistent with previous findings of Ngozi *et al.*, [30]. They noted that ethanolic extract of *Gongronema latifolium* administered to T1-DR effectively increased cellular GSH/GSSG ratio, which ameliorated oxidative stress and, by extension, promoted low levels of membrane lipid peroxidation associated with DM. The capability of *T. cocoa* to ameliorate oxidative stress in T1-DR is connected with the presence of a high quantity of antioxidant phytochemicals in the bean extract as previously established by related investigations [14].

From another perspective, cellular GSH concentration inversely parallels the concentration of by-product of lipid peroxidation-MDA [31]. The relatively raised level of erythrocyte hemolysate MDA concentration of Group C2 rats [Figure 2] was an indication of raised oxidative stress associated with DM. The reduction in erythrocyte MDA concentrations following the administration of *T. cocoa* bean extract was consistent with the antioxidant potency of the herbal extract, which was comparable with that of the standard anti-DM drug-glibenclamide and in concord with the findings of Dewanjee *et al.*, [32], in which they noted that the presence of flavonoids in matured fruits extract of *Diospyros peregrine* Gurke (Ebenaceae) caused reduction in level of lipid peroxidation in alloxan-induced diabetic rats. Furthermore, the capability of *T. cocoa* bean extract to impede lipid peroxidation in diabetic rat corroborates earlier findings following the administration of *Azadirachta indica* to STZ-induced severe diabetic rats [33]. However, the seemingly poor corresponding capacity of 400 mg/kg body weight of *T. cocoa* bean extract to ameliorate DM associated oxidative stress, as typified by the non-significant difference ($P > 0.05$) between erythrocyte hemolysate GSH/GSSG ratio and MDA concentrations at the various experimental doses [Figures 1 and 2], could have been the outcome of the presence of interfering substances that neutralized the antioxidant potency of *T. cocoa* bean extract as previously proposed [3].

For the fact that hormone sensitive lipoprotein lipases are inactive in the absence or low circulating levels of insulin, T1-DR showed evidence of dyslipidemia, as exemplified by disarrangement in their SLPs [Figure 3]. According to Silva *et al.*, [34], abnormal lipid metabolism plays a crucial role in the pathogenesis of DM and elevated plasma cholesterol concentration represents a risk factor for coronary artery disease. In addition, Subash *et al.*, [35] previously noted that chemically induced T1-DR exhibited hypercholesterolemia, which was an outcome of increased intestinal cholesterol absorption and biosynthesis. The present investigations showed that *T. cocoa* bean extract lowered blood TC concentrations in T1-DR in a dose-dependent manner, which by implication was as a result of impeded intestinal absorption and probably interfered with cholesterol metabolism by the actions of the herbal extract as previously reported [23]. The abundant bioactive principles in *T. cocoa* bean extract and other plant materials, notably, the β-sitosterol and saponins, have been described to be involved in ameliorating hypercholesterolemia in animal models [21,36]. Findings of the present investigations showed that the reduction in serum TC concentration following the administration of *T. cocoa* bean extract was accompanied by a corresponding increase in serum HDL-C concentration in T1-DR [Figure 3]. In connection with the present report, previous studies on pathophysiology and management of DM induced hyperlipidemia reported that elevation in serum HDL-C concentration was intertwined with increased catabolism of VLDL-C and substitution of TAG in the core of HDL-C with cholesterol [14]. By implication, HDL-C is cardio-protective, in the sense that it facilitates the uptake of cholesterol by the hepatocytes for catabolism and eventual excretion [37], which engenders low serum TC concentration.

Insulin insufficiency is associated with diminished levels of LDL-C receptors in peripheral tissues and poor tissue uptake of LDL-C particles. Evidence showed that these receptors re-appeared following DM therapy [14]. Levels of LDL-C particles are expected to be raised in plasma of diabetic animals. Paradoxically, studies have shown that individuals who present DM do not exhibit increase plasma levels of LDL-C per se [10,38]. Rather, LDL-C particles become small and dense in DM. Small and dense LDL-C particles are more atherogenic than their larger ones because they are more susceptible to non-enzymatic glycation and oxidation [38]. Reports according to Ibero-Baraibar *et al.*, [13] stated that plasma level of oxidized LDL-C was lowered following the consumption of diet supplemented with cocoa extract, which was in consonance with the outcome of dosing T1-DR with *T. cocoa* bean extract as reported here. The relatively high phenolic content of *T. cocoa* bean extract [14] obviously contributed to the delay in LDL-C oxidation and plasma LDL-C lowering effect as previously described [39].

The associated reduction in serum VLDL-C and TAG, along with comparative normalization of other blood lipid components, further confirmed the beneficial effects of *T. cocoa* bean extract on T1-DR following short-term treatment. Earlier reports have equally established the capability of extract of some medicinal plants and multitude of natural products [25,28,39] to ameliorate dyslipidemia and improve AI in diabetic animal models.

Short-term administration of *T. cocoa* bean extract caused substantial reduction in blood glucose concentration in alloxan-induced T1-DR but did not obliterate hyperglycemia. In addition, *T. cocoa* bean extract, in the present form and doses, exhibited comparative limited capacities to reduce oxidative stress and ameliorate dyslipidemia in the experimental rats. However, from the findings and interpretations of the present study, it is envisaged that fractionation and other purification measures would serve to improve the nutraceutical potentials of *T. cocoa* bean extract in T1-DR.

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