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TRICHOSANTHES CUCUMERINA FRUIT EXTENUATES DYSLIPIDEMIA, PROTEIN OXIDATION, LIPID PEROXIDATION AND DNA FRAGMENTATION IN THE LIVER OF HIGH-FAT DIET-FED RATS

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ABSTRACT

The effect of *Trichosanthes cucumerina* fruit pulp extract on dyslipidemia, protein oxidation, lipid peroxidation and DNA fragmentation in the liver of high-fat diet-fed rats was investigated. High-fat diet-mediated alterations in liver and serum total cholesterol, triacylglycerol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol, alkaline phosphatase, and alanine and aspartate aminotransferase were significantly ($P < 0.05$) reversed by the *T. cucumerina* pulp extract. The extract increased the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose 6-phosphate dehydrogenase in the mitochondria and post-mitochondria fractions of rat liver. The increase significantly ($P < 0.05$) attenuated high-fat diet-mediated reductions in antioxidant enzyme activities. High-fat diet-mediated elevation in the levels of conjugated dienes, lipid hydroperoxides, malondialdehyde, protein carbonyl and DNA fragmentation in rat liver was dose-dependently lowered by the extract. The data obtained from this study showed that the *T. cucumerina* fruit pulp extract palliated high-fat diet-mediated dyslipidemia, protein oxidation, lipid peroxidation and DNA fragmentation in rats.

PRACTICAL APPLICATIONS

Trichosanthes cucumerina fruit pulp can be developed into a nutraceutical product to treat liver disorders. It can also be consumed at home and as substitute to *Lycopersicon esculentum* (L.) Mill. The fruit pulp of *T. cucumerina* is sweet tasting, aromatic, deep red in color and does not go sour as quickly as paste of *L. esculentum*.

INTRODUCTION

Metabolic syndrome is a cluster of cardiovascular risk factors that is characterized by obesity, central obesity, insulin resistance, atherogenic dyslipidemia and hypertension (Deedwania and Gupta 2006). The underlying cause of metabolic syndrome continues to challenge experts, but both insulin resistance and central obesity are considered significant factors (Carr *et al.* 2004; Hu *et al.* 2004). The prevalence of dyslipidemia resulting from excess energy

intake and physical inactivity is increasing worldwide (Yang *et al.* 2008). Dyslipidemia, a component of metabolic syndrome, is characterized by elevated triglycerides (TGs) and low concentrations of high-density lipoprotein cholesterol (HDLc) together with elevated apolipoprotein B and small amounts of low-density lipoprotein cholesterol (LDLc), all of which are independently atherogenic (Brunzell and Ayyobi 2003).

In addition to the characteristics of dyslipidemia, high levels of fat decrease antioxidant enzymes, leading to increased oxidative stress (Slim *et al.* 1996), which results from the generation of reactive oxygen species (ROS) such as superoxide anion radicals, hydrogen peroxides and hydroxyl radicals. The generated ROS in dyslipidemia often overwhelms the endogenous antioxidants (catalase [CAT], superoxide dismutase [SOD], glutathione peroxidase [GSH-Px], glutathione reductase [GSH-red] and reduced glutathione), resulting in oxidative stress (Roberts and Sindhu 2009). Fruits, vegetables and medicinal plants are widely used and are prospects for nutraceutical and phytomedicines (Oloyede *et al.* 2013). Among the large number of underutilized indigenous edible plant species that are important for the livelihoods of local populations in West and Central Africa is *Trichosanthes cucumerina*.

T. cucumerina L., also known as snake tomato and member of the Cucurbitaceae family, is an indigenous and underexploited leaf and fruit vegetable in Southwest Nigeria (Adebooye, 2008). The plant is also widely distributed in India and Srilanka, tropical Himalaya, Malaysia, Polynesia and Northern Australia. *T. cucumerina* is used in the treatment of infective hepatitis, hepatomegaly and hepatic dyspepsia (Panda, 2000). The juices of leaves and fruits are also used to treat liver disorders (Rahman *et al.*, 2006). It is gaining prominence in Southwest Nigeria because the fruit pulp paste of *T. cucumerina* has been shown to be a suitable substitute for the Solanaceous tomato (*Lycopersicon esculentum* [L.] Mill.), especially during the periods when the latter is scarce in Nigeria. The fruit pulp is sweet, aromatic, deep red in color and does not go sour as quickly as paste of the *L. esculentum* (Adebooye *et al.* 2005; Adebooye and Oloyede 2006). The pulp contains ascorbic acid, lycopene, phenolics, flavonoids and antioxidant power that are comparable to those of Solanaceous tomato and are higher than those of most members of the Cucurbitaceae family (Adebooye, 2008).

Despite the inherent antioxidant components of *T. cucumerina* fruit pulp, the nutraceutical potential of the plant remains untapped. This study investigated the effects of the fruit pulp on dyslipidemia, protein oxidation, lipid peroxidation and DNA fragmentation in the liver of high-fat diet-fed rat.

MATERIALS AND METHODS

Experimental Animal

Two-month-old healthy male albino rats (*Rattus norvegicus*) of Wistar strain, weighing 173 ± 1.65 g, were obtained from the animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. They were kept in clean plastic cages in well-ventilated house with free access to feed (Ace Feeds Ltd., Osogbo, Nigeria) and tap water. The animals were used according to the Guidelines of National Research Council Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and in accordance with the principles of Good Laboratory Procedure (WHO, 1998).

Plant Materials

T. cucumerina fruits were obtained from Igbona market, Osogbo, Nigeria. Identification of the fruit was carried out at the Forestry Research Institute of Nigeria, Ibadan, Nigeria.

Chemicals and Assay Kits

Diphenylamine, 5,5′-dithiobis(2-nitrobenzoic acid), guanidine hydrochloride and *N*-ethylmaleimide (NEM) were procured from Research Organics, Cleveland, OH. Total cholesterol (TC), triacylglycerol (TAG), HDLc, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), SOD, GSH-Px, GSH-red and glucose 6-phosphate dehydrogenase (Glc 6-PD) were products of Randox Laboratories Ltd., Co. Antrim, U.K. All other reagents used were supplied by Sigma-Aldrich, Inc., St. Louis, MO.

METHODS

Preparation of *T. cucumerina* Pulp Extract

Ripe pulps of *T. cucumerina* fruit were extracted and freeze-dried using lyophilizer (LTE SCIENTIFIC LTD., Greenfield, Oldham, U.K.). The dried material was then extracted in 80% methanol for 48 h. Methanol was evaporated under reduced pressure using Rotavapor (R-200, BUCHI, Flawil, Switzerland) and the resulting extract was freeze-dried using lyophilizer and kept frozen at –4C until used.

Experimental Design

Sixty male rats were completely randomized into six groups (A–F) of 10 animals each. Rats in group A, the control, were fed with normal chow (4% fat; Table 1), throughout the

TABLE 1. FEED COMPOSITION AND FORMULATION

Feed components	Control diet (g/kg)	High-fat diet (g/kg)
Corn starch	506	406
Casilan 90*	250	250
Lard	40	140
Sucrose	100	100
Rice husk	40	40
DL-methionine	4	4
Lysine	10	10
Vitamin mix†	10	10
Mineral mix‡	40	40

* Casilan 90 (g/100 g), energy (1,572 kg/100g), protein (90 g), carbohydrate (0.3 g), fat (1.0 g), fiber (trace), sodium (0.03 mg), calcium (1,400 mg).

† Vitamin mix (per kilogram of diet): thiamine hydrochloride (6 mg), pyridoxine hydrochloride (7 mg), nicotine acid (30 mg), calcium pantothenate (16 mg), folic acid (2 mg), biotin (0.2 mg), cyanocobalamin (0.01 mg), retinol palmitate (4,000 IU), cholecalciferol (100 IU), α-tocopherol acetate (50 IU), menadine (0.05 mg) and choline chloride (2 g).

‡ Mineral mix (g/kg): $CoCl_2{\cdot}6H_2O$ (0.001), $CuSO_4{\cdot}5H_2O$ (0.079), $MnSO_4{\cdot}7H_2O$ (0.178), KI (0.033), NaCl (3.573), $ZnCO_3$ (1.60), $CaSO_4$ (11.61), $MgSO_4{\cdot}7H_2O$ (2.292), K_2HPO_4 (10.559) and $FeSO_4{\cdot}7H_2O$ (1.075).

experimental period. Group B and group D–F rats were maintained on high-fat diet (14% animal fat, Table 1), whereas group C–F rats were administered 100, 25, 50 and 100 mg/kg body weight of the extract orally once daily at 24-h interval for 4 weeks, respectively.

Preparation of Serum and Tissue Homogenates

Serum and tissue homogenates were prepared after the rats had been sacrificed using the procedure described by Yakubu *et al.* (2009) and Ajiboye *et al.* (2014), respectively.

Preparation of Mitochondria and Post-Mitochondria Fraction

Freshly excised liver was homogenized in sucrose–Tris buffer (0.25 M sucrose, 10 mM Tris–HCl, pH 7.4). The homogenate was centrifuged at $600 \times g$ at 4C for 10 min to remove the debris and the supernatant was centrifuged at $7{,}000 \times g$ at 4C for 10 min to obtain a mitochondrial pellet. The pellet (mitochondria fraction) suspended in sucrose–Tris buffer solution and the supernatant (post-mitochondria fractions) were stored at −80C until further analysis.

Lipid Profile

Serum TC, TAG and HDLc were estimated using the procedure outlined in commercial kits (Randox Laboratories Ltd.). LDLc and very-low-density lipoprotein cholesterol (VLDLc) were calculated using the following formula (Friedewald *et al.* 1972):

$$\text{VLDLc} = 0.2 \times \text{TAG}$$

$$\text{LDLc} = \text{TC} - (\text{HDLc} + \text{VLDLc})$$

Cardiac index was calculated using the formula: TC/HDLc (Kang *et al.* 2004). Atherogenic index (AI) was calculated using the following formula (Kayamori and Igarashi 1994): AI = (Total cholesterol – HDLc)/HDLc. Coronary artery index was calculated using the expression: LDLc/HDLc.

Hepatic lipids were extracted using the procedure described by Tzang *et al.* (2009), by extracting with chloroform and methanol (2:1, v/v). The extract was dried under N_2 and resuspended in isopropanol. The extract was used for determination of hepatic cholesterol and TAG concentrations as outlined in commercial kits (Randox Laboratories Ltd.).

Oxidative Stress Biomarkers

The activities of ALP, ALT and AST in the liver homogenates were determined as described by Wright *et al.* (1972) and Bergmeyer *et al.* (1986a,b), respectively. SOD, CAT, GSH-Px, GSH-red and Glc 6-PD activities were assayed in the mitochondria and post-mitochondria fractions according to the procedures described by Misra and Fridovich (1972), Beers and Sizer (1952), Rotruck *et al.* (1973), Mavis and Stellwagen (1968), and Kornberg and Horecker (1955), respectively. The levels of reduced glutathione (GSH) and glutathione disulfide (GSSG) in the mitochondria and post-mitochondria fractions were determined using the procedures described by Ellman (1959) and Hissin and Hilf (1976), respectively. The concentration of protein carbonyl in the liver homogenates was determined according to the procedure described by Levine *et al.* (1990). The concentrations of conjugated dienes, lipid hydroperoxides and malondialdehyde (MDA) were assessed according to the procedures described by Reilly and Aust (2001). The fragmented DNA was quantified according to the procedure described by Burton (1956).

Statistical Analysis

Results were expressed as the mean of five determinations ± SD. Analysis of variance (ANOVA) followed by

Tukey–Kramer test for differences between means was used to detect any significant differences ($P < 0.05$) between the treatment groups in this study using StatPlus, 2011 (AnalystSoft, Inc., Alexandria, VA).

RESULTS

Lipid Profile

The levels of serum TC, TAG, VLDLc and LDLc of high-fat diet-fed rats increased significantly ($P < 0.05$) compared with the control (Table 2). Similar increase in the levels of TC and TG of high-fat diet-fed rat liver was recorded (Table 2). Conversely, high-fat diet resulted in decreased serum HDLc level of rats (Table 2). The changes in the lipid profile were dose-dependently attenuated in high-fat diet-fed rats after the administration of 25, 50 and 100 mg/kg body weight of the pulp extract. Furthermore, atherogenic, cardiac and coronary artery indices were significantly ($P < 0.05$) increased in high-fat diet-fed rats when compared to the control (Table 2). However, administration of the extract (25–100 mg/kg body weight) dose-dependently reversed this increase (Table 2).

Hepatocellular Enzymes

Liver ALP, ALT and AST activities of high-fat diet-fed rats decreased significantly with corresponding increase in the serum (Table 3). Although there was no change in the activities of these enzymes (ALP, ALT and AST) for rats treated with only *T. cucumerina* pulp extract when compared to the control, the changes mediated by high-fat diet were dose-dependently annulled by the extract (Table 3).

TABLE 2. LIPID PROFILE OF HIGH-FAT DIET-FED RAT AFTER 4-WEEK ADMINISTRATION OF 25, 50 AND 100 MG/KG BODY WEIGHT OF *TRICHOSANTHES CUCUMERINA* PULP EXTRACT

	Control	High-fat diet	100 mg/kg body weight extract	High-fat diet + 25 mg/kg body weight extract	High-fat diet + 50 mg/kg body weight extract	High-fat diet + 100 mg/kg body weight extract
Serum						
Total cholesterol (mg/dL)	47.52 ± 1.23^a	66.13 ± 3.10^b	46.09 ± 2.00^a	58.46 ± 0.39^c	55.72 ± 2.36^c	49.10 ± 1.78^a
Total triglycerides (mg/dL)	56.97 ± 2.42^a	75.44 ± 0.77^b	54.21 ± 2.84^a	70.32 ± 1.16^c	64.86 ± 1.43^d	60.01 ± 3.21^a
HDL-cholesterol (mg/dL)	30.71 ± 1.06^a	19.13 ± 2.01^b	30.32 ± 0.12^a	22.95 ± 1.11^c	27.42 ± 0.37^d	29.11 ± 0.58^a
LDL-cholesterol (mg/dL)	5.42 ± 0.09^a	31.91 ± 1.39^b	5.08 ± 0.10^a	21.45 ± 0.70^c	15.33 ± 0.26^d	7.99 ± 1.23^e
VLDL-cholesterol (mg/dL)	11.39 ± 0.53^a	15.09 ± 0.54^b	11.63 ± 0.16^a	14.06 ± 1.48^c	12.97 ± 1.17^a	12.00 ± 0.68^a
Atherogenic index	0.55 ± 0.01^a	2.46 ± 0.23^b	0.57 ± 0.01^a	1.55 ± 0.05^c	1.03 ± 0.06^d	0.69 ± 0.09^e
Cardiac index	1.55 ± 0.01^a	3.46 ± 0.08^b	1.43 ± 0.02^a	2.55 ± 0.20^c	2.03 ± 0.16^d	1.69 ± 0.24^e
Coronary artery index	0.18 ± 0.01^a	1.67 ± 0.10^b	0.20 ± 0.01^a	0.93 ± 0.07^c	0.56 ± 0.08^d	0.27 ± 0.04^a
Liver						
Total cholesterol (mg/dL)	123.41 ± 2.35^a	190.45 ± 5.89^b	125.20 ± 5.23^a	167.53 ± 2.62^c	150.40 ± 7.31^d	135.64 ± 7.01^a
Total triglycerides (mg/dL)	158.33 ± 4.87^a	208.52 ± 3.68^b	160.33 ± 3.43^a	180.97 ± 1.01^c	171.28 ± 2.65^d	169.14 ± 5.18^a

Data are mean of five determinations ± SD. Values with superscripts different for the liver and serum parameters are significantly different ($P < 0.05$). HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

TABLE 3. HEPATOCELLULAR ENZYMES ACTIVITIES OF HIGH-FAT DIET-FED RAT AFTER 4-WEEK ADMINISTRATION OF 25, 50 AND 100 MG/KG BODY WEIGHT OF *TRICHOSANTHES CUCUMERINA* PULP EXTRACT

	Alkaline phosphatase		Alanine aminotransferase		Aspartate aminotransferase	
	Liver	Serum	Liver	Serum	Liver	Serum
Control	7.30 ± 0.54^a	0.076 ± 0.001^a	64.70 ± 2.86^a	4.62 ± 0.42^a	80.24 ± 0.44^a	6.82 ± 0.15^a
High-fat diet	2.86 ± 0.42^b	0.260 ± 0.001^b	21.64 ± 1.76^b	13.20 ± 0.43^b	33.24 ± 0.21^b	21.23 ± 0.17^b
100 mg/kg body weight extract	7.47 ± 0.84^a	0.078 ± 0.002^a	63.05 ± 1.26^a	4.20 ± 0.48^a	79.08 ± 2.15^a	6.01 ± 0.02^a
High-fat diet + 25 mg/kg body weight extract	4.24 ± 0.21^c	0.192 ± 0.001^c	35.62 ± 1.43^c	8.76 ± 0.29^c	50.31 ± 1.21^c	16.74 ± 0.12^c
High-fat diet + 50 mg/kg body weight extract	5.86 ± 0.64^a	0.102 ± 0.002^c	47.86 ± 2.21^d	6.03 ± 0.83^c	62.72 ± 0.62^c	12.92 ± 0.62^c
High-fat diet + 100 mg/kg body weight extract	7.03 ± 0.30^a	0.065 ± 0.001^a	60.83 ± 0.64^a	4.54 ± 0.14^a	77.19 ± 2.23^a	7.15 ± 0.85^a

Data are mean of five determinations ± SD. Specific enzyme activities are expressed as nmol/min/mg protein. Values with superscripts different for the liver and serum of each enzyme are significantly different ($P < 0.05$).

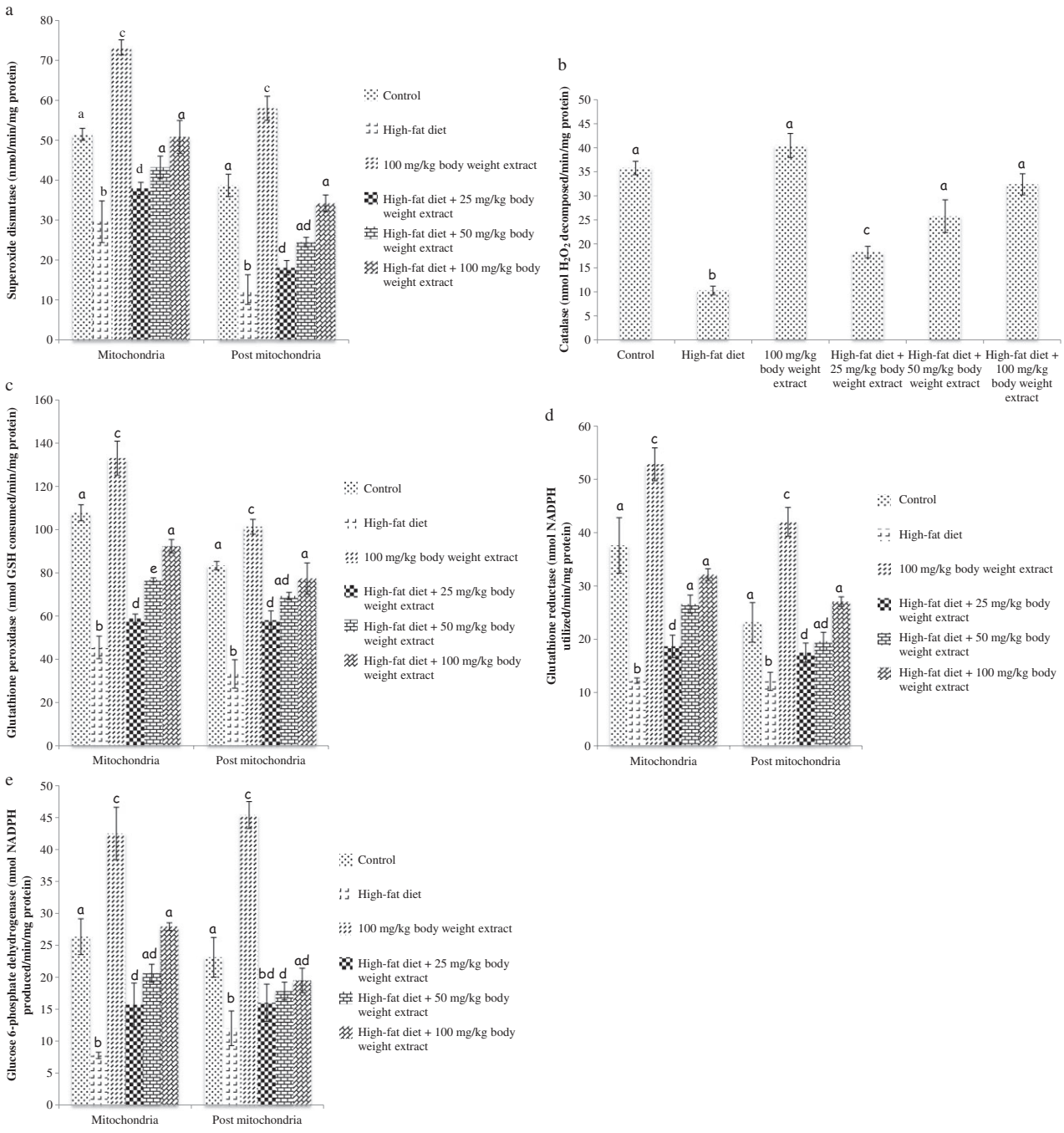

FIG. 1. SPECIFIC ACTIVITY OF (A) SUPEROXIDE DISMUTASE, (B) CATALASE, (C) GLUTATHIONE PEROXIDASE, (D) GLUTATHIONE REDUCTASE AND (E) GLUCOSE 6-PHOSPHATE DEHYDROGENASE IN THE MITOCHONDRIA AND POST-MITOCHONDRIA FRACTIONS OF HIGH-FAT DIET-FED RATS LIVER AFTER 4-WEEK ADMINISTRATION OF 25, 50 AND 100 MG/KG BODY WEIGHT OF *TRICHOSANTHES CUCUMERINA* PULP EXTRACT
Bars with different superscripts for mitochondrial or post-mitochondrial fractions are significantly different from each other.

Oxidative Stress Biomarkers

Antioxidant Enzymes and Nonenzymatic Antioxidants. SOD, GSH-Px, GSH-red and Glc 6-PD activities in mitochondria and post-mitochondrial fractions decreased ($P < 0.05$) in high-fat diet-fed rat liver (Fig. 1a,c–e). Similar decrease ($P < 0.05$) in the activity of CAT was also recorded Fig. 1b). Administration of the pulp

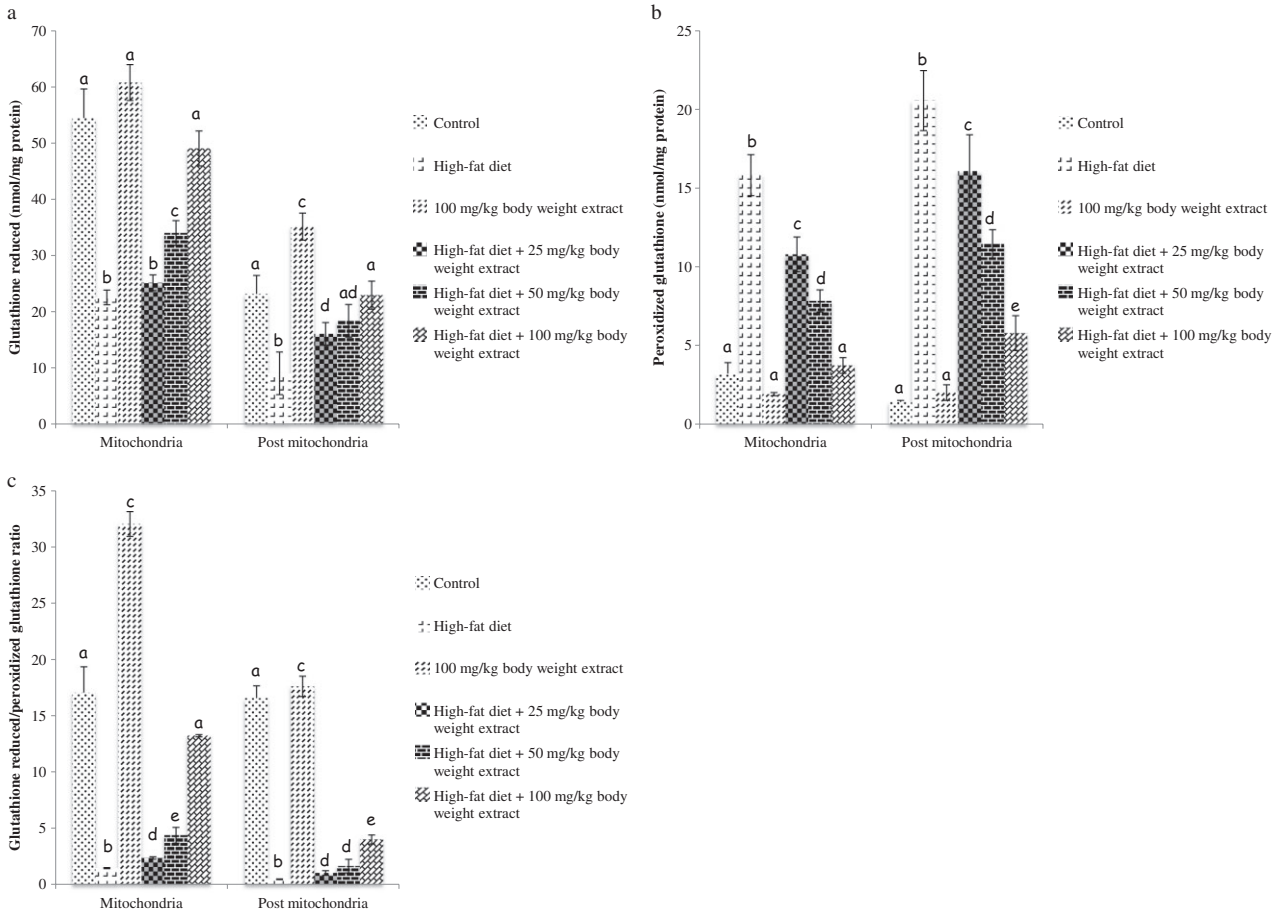

FIG. 2. LEVELS OF (A) GLUTATHIONE REDUCED, (B) PEROXIDIZED GLUTATHIONE AND (C) REDUCED GLUTATHIONE/PEROXIDIZED GLUTATHIONE IN THE MITOCHONDRIA AND POST-MITOCHONDRIA FRACTIONS OF HIGH-FAT DIET-FED RATS LIVER AFTER 4-WEEK ADMINISTRATION OF 25, 50 AND 100 MG/KG BODY WEIGHT OF *TRICHOSANTHES CUCUMERINA* PULP EXTRACT
Bars with different superscripts for mitochondrial or post-mitochondrial fractions are significantly different from each other.

extract increased the activities of these antioxidant enzymes.

GSH level and GSH : GSSG ratio in the liver mitochondria and post-mitochondria fractions of high-fat diet-fed rats decreased significantly ($P < 0.05$) with corresponding increase in GSSG level when compared to control (Fig. 2). Treatment of high-fat diet-fed rats with the extract significantly reversed these alterations (Fig. 2).

Protein Carbonyl and Fragmented DNA. Compared with the control, there was a significant ($P < 0.05$) increase in the protein carbonyl level of liver mitochondrial and post-mitochondrial fractions of high-fat diet-treated rat (Fig. 3a). The increase was attenuated by administration of the pulp extract in a dose-dependent manner (Fig. 3a).

There was similar increase in fragmented DNA (%) in the liver mitochondria and post-mitochondria fractions of high-fat diet-fed rats (Fig. 3b). The pulp extract ameliorated the high-fat diet-mediated increase in the percentage of fragmented DNA (Fig. 3b).

Lipid Peroxidation Products. Levels of microsomal-conjugated dienes, lipid hydroperoxides and MDA increased significantly ($P < 0.05$) in the liver of high-fat diet-fed rat (Table 4). The changes were reversed after treatment with the pulp extract (Table 4).

DISCUSSION

Dyslipidemia

In addition to rapid weight gain associated with high-fat diets (Akbay *et al.* 2004), dyslipidemia, oxidation of proteins and lipids, as well as DNA fragmentation have been reported (Chang *et al.* 2013; Peng *et al.* 2013).

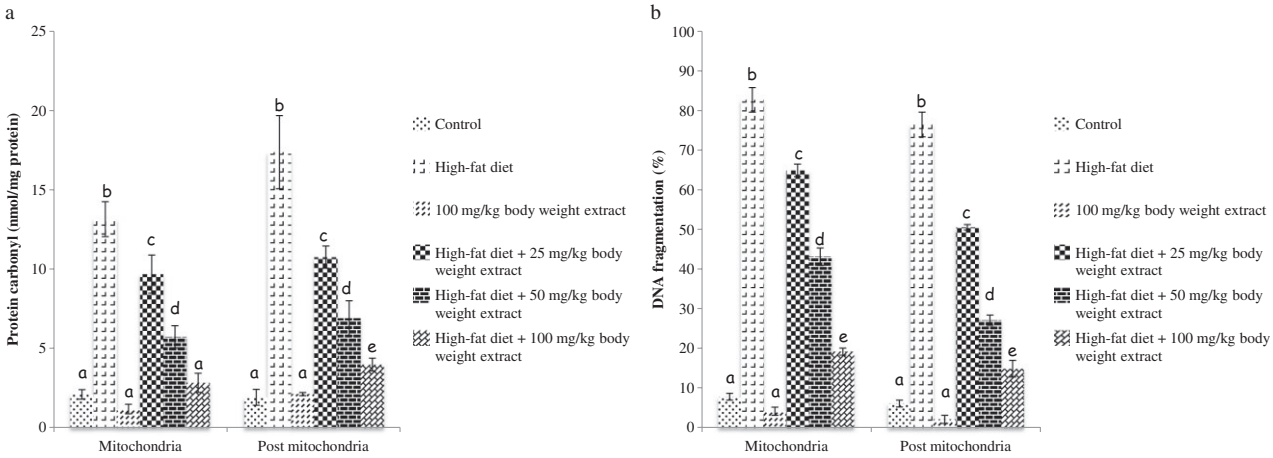

FIG. 3. LEVELS OF (A) PROTEIN CARBONYL LEVEL AND (B) FRAGMENTED DNA IN THE MITOCHONDRIA AND POST-MITOCHONDRIA FRACTIONS OF HIGH-FAT DIET-FED RATS LIVER AFTER 4-WEEK ADMINISTRATION OF 25, 50 AND 100 MG/KG BODY WEIGHT OF *TRICHOSANTHES CUCUMERINA* PULP EXTRACT

Bars carrying different superscripts for mitochondrial or post-mitochondrial fractions are significant different from each other.

Changes in the lipid metabolism have been implicated in the pathogenesis of atherosclerosis and cardiovascular disease, which is characterized by elevated LDLc and TAGs and decreased HDLc (Hutcheson and Rocic 2012). Thus, levels of TC, HDLc, LDLc and TAGs are important indicators of disorders in lipid metabolism and predisposition to atherosclerosis (Yakubu *et al.* 2009). LDLc and VLDLc have also been implicated in heart diseases because they clog artery walls and set the stage for heart disease (Oloyede, 2005). However, HDLc is considered to inhibit these diseases (Masuzaki *et al.* 2001).

The increased LDLc could be oxidized in the endarterium, leading to cholesterol accumulation in phagocytes and formation of foam cells, promoting the development of atherosclerosis (Chen *et al.* 2011). Elevations in liver TC and TAGs (Table 2) indicate that absorbed free fatty acids were directed toward TAG synthesis in the liver of high-fat diet-fed rats (Botham and Mayes, 2006). It also suggests impairment of mitochondrial ß-oxidation of free fatty acids, hence compromising ATP production in these tissues.

The reversal of high-fat diet-mediated decrease in HDLc shows the capability of the extract to prevent development of atherosclerosis (Tall *et al.* 2008; McGillicuddy *et al.* 2011). Since elevated VLDLc is associated with increased risk of coronary disease, the decreased level of VLDLc by the extract could prevent coronary artery disease.

Atherogenic, cardiac and coronary artery changes are strong and reliable indicators of cardiovascular, coronary and ischemic diseases (Bonaa and Arnesen, 1992; Ansell *et al.* 2005; Yang *et al.* 2008). Thus, the higher ($P < 0.05$) calculated indices of high-fat diet-fed rats could predispose these diseases (Yang *et al.* 2010).

TABLE 4. LEVELS OF LIPID PEROXIDATION PRODUCTS OF HIGH-FAT DIET-FED RAT LIVER AFTER 4-WEEK ADMINISTRATION OF 25, 50 AND 100 MG/KG BODY WEIGHT OF *TRICHOSANTHES CUCUMERINA* PULP EXTRACT

	Conjugated dienes	Lipid hydroperoxides	Malondialdehyde
Control	23.59 ± 0.36^{a}	18.11 ± 0.63^{a}	3.62 ± 0.06^{a}
High-fat diet	42.63 ± 2.06^{b}	30.87 ± 2.57^{b}	10.98 ± 0.12^{b}
100 mg/kg body weight extract	18.62 ± 0.57^{a}	17.99 ± 0.23^{a}	2.06 ± 0.03^{a}
High-fat diet + 25 mg/kg body weight extract	37.35 ± 0.34^{c}	26.43 ± 0.62^{c}	8.52 ± 0.10^{c}
High-fat diet + 50 mg/kg body weight extract	30.18 ± 0.23^{d}	23.21 ± 0.43^{d}	6.01 ± 0.72^{d}
High-fat diet + 100 mg/kg body weight extract	25.14 ± 0.59^{a}	19.05 ± 0.54^{a}	3.18 ± 0.36^{a}

Data are mean of five determinations ± SD. Concentrations are expressed as nmol/mg protein. Values carrying superscripts different for each parameter are significantly different ($P < 0.05$).

Hepatocellular Enzymes

Hepatocellular enzymes such as ALP, ALT and AST have been shown to increase in the serum of high-fat diet-treated rats (Chang *et al.* 2013; Peng *et al.* 2013). Thus, the decrease in activities of liver ALP, ALT and AST of high-fat diet-fed rats with corresponding increase in the serum indicates compromised hepatocellular integrity. Reversal of these effects by the pulp extract demonstrate its capability to scavenge and prevent peroxidation of membrane lipids (Ajiboye *et al.* 2010).

Enzymatic Antioxidants

High-fat diets have been reported to generate free radicals and ROS, leading to redox imbalance, oxidative stress, morphological changes and tissue injury (Muthulakshmi and Saravanan 2013). This is evident from the reduction of the antioxidant enzymes (SOD, CAT, GSH-Px, GSH-red and Glc 6-PD) in high-fat diet-fed rats (Malheiros *et al.* 2003). Also, high-fat diets have been reported to reduce the activity of manganese-SOD, which may diminish the capability of mitochondria to eliminate O_2 (Yang *et al.* 2008). These observations were in line with Kim *et al.* (2008) and Yang *et al.* (2008) who reported that hypercholesterolemia caused an increase in MDA and SOD, whereas GSH was decreased.

The decreased antioxidant enzyme (SOD, CAT, GSH-Px, GSH-red and Glc 6-PD) activities in liver mitochondria and post-mitochondrial fractions of high-fat diet-fed rats could lead to mitochondrial dysfunction, impaired bioenergetics leading to uncontrolled generation of ROS, tissue damage and cell death (Ajiboye *et al.* 2010). This might have resulted in the increased ALP, ALT and AST activities in the serum. The attenuation of high-fat diet-mediated alterations of antioxidant enzyme activities in liver mitochondria and post-mitochondria fractions could be attributed to the capability of the inherent antioxidant components (such as ascorbic acid, phenolics and flavonoids) of the extract to enhance the antioxidant enzymes (Adebooye and Oloyede 2006; Ajiboye *et al.* 2011).

Nonenzymatic antioxidants

The GSH : GSSG ratio is widely used as an indicator of the mitochondrial or cellular redox state (Cardoso *et al.* 2012). Previous studies have shown that total glutathione level is significantly lower than GSSG level in high-fat diet groups, suggesting that greater oxidative stress occurs in dietary fat supplemented groups than that in normal diet groups (Anderson *et al.* 2009). The amelioration of high-fat diet-mediated increase in GSSG and GSSG:GSH ratio in liver mitochondria and post-mitochondria fractions indicates protective potential of *T. cucumerina* against ROS generation and oxidative stress.

Lipid Peroxidation

Lipid peroxidation distorts membrane organization and induces functional loss, modification of proteins and DNA bases (Niki 2009). Studies have shown elevated levels of lipid peroxidation in high-fat diet-fed rats (Ming *et al.* 2009; Ling *et al.* 2012). Thus, the high-fat diet-mediated elevation of conjugated dienes, lipid hydroperoxides and MDA levels in mitochondria and post-mitochondria fractions could have resulted from peroxidation of the liver polyunsaturated fatty acids by accumulated ROS (Ming *et al.* 2009). The capability of the extract to attenuate these effects may be attributed to its antioxidant activity and elevated levels of antioxidant enzymes.

Protein Oxidation

Protein carbonyl, an indicator of irreversible oxidative damage to cellular proteins, may have lasting detrimental effects on cells and tissues (Dalle-Donne *et al.* 2003). Studies have reported persistent elevation of protein carbonyl level in high-fat diet-fed rats (Matsuzawa-Nagata *et al.* 2008; Noeman *et al.* 2011; Yuzefovych *et al.* 2013). Thus, the significant elevation of protein carbonyl level in the liver mitochondria and post-mitochondria fractions of high-fat diet-fed rats indicates oxidative degeneration of proteins. This protein degeneration was prevented by the extract likely due to the elevated levels of antioxidant enzymes in the liver, thus preventing the oxidation of protein by detoxifying ROS.

DNA Fragmentation

High-fat diets have been reported to induce mitochondrial DNA damage (Bonnard *et al.*, 2008; Yuzefovych *et al.* 2013). Thus, the elevated level of mitochondrial and post-mitochondrial fragmented DNA could have resulted from increased generation of ROS particularly hydroxyl radical (Yuzefovych *et al.* 2013). The capability of the extract to assuage the high-fat diet-mediated increase in fragmented DNA may be attributed to the antioxidant components (such as ascorbic acid, lycopene, phenolics and flavonoids), which are capable of scavenging ROS and free radicals (Adebooye and Oloyede 2006).

CONCLUSION

It is evident from the data obtained in this study that *T. cucumerina* fruit palliates high-fat diet-mediated

dyslipidemia, protein oxidation, lipid peroxidation and DNA fragmentation. This could be due to the antioxidant components of the fruits.

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