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MODERATION OF DOXORUBICIN-INDUCED DYSLIPIDAEMIA IN WISTAR RATS BY AQUEOUS EXTRACTS OF *Pleurotus tuberregium* SCLEROTIA AND *Cnidoscolus aconitifolius* LEAVES

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Abstract

This study investigated the ability of aqueous extracts of *Pleurotus tuberregium* sclerotia and *Cnidoscolus aconitifolius* leaves to prevent or attenuate dyslipidaemia, and adverse alterations in cardiac cholesterol and triglyceride levels, weight gain and heart weight indices, and atherogenic indices, which was induced by intra-peritioneal administration of doxorubicin (15 mg/kg body weight) to Wistar rats. Metformin was administered daily orally at 250 mg/kg; the extracts were administered daily orally at 50, 75 and 100 mg/kg (for 14 days). In both the ameliorative and protective studies, the heart weight, cardiac cholesterol, triglyceride, and plasma triglyceride, apolipoprotein B, total-, non-HDL-, VLDL- and LDL cholesterol concentrations, as well as the atherogenic coefficient, cardiac risk ratio, atherogenic index of plasma and Castelli's risk index II of the Negative control was significantly (p<0.05) higher than those of all the other groups. However, the plasma HDL cholesterol concentration of the Negative control was significantly (p<0.05) lower than those of all the other groups. Therefore, the administration of the extracts and metformin safeguarded (in the protective study) or restored (in the ameliorative study) the doxorubicin induced dyslipidaemia, as well as the cardiac cholesterol and triglyceride levels. These results suggest that, at least in part, due to hypolipidaemic properties, aqueous extracts of *Pleurotus tuberregium* sclerotia and *Cnidoscolus aconitifolius* leaves may potentially reduce the risk of doxorubicin-induced cardiovascular disorders.

INTRODUCTION

High myocardial lipid build-up and plasma lipid levels commonly accompany doxorubicin-induced cardiomyopathy [1-4]. Along with the dyslipidaemic effect, doxorubicin treatment reduces body weight or prevents body weight gain [5-8]. It is presumed that the weight loss is a sequel to doxorubicin-induced loss of adipose tissue [9,10].

Hyperlipidaemia and build-up of cholesterol in cardiac tissues are linked to cardiovascular damage and the development of cardiovascular disease [2,3,11,12]. Therefore, induction of dyslipidaemia may be one of the mechanisms for the cardiotoxic effect of doxorubicin [1,4,13]. Dyslipidaemia is one of the established risk factors for developing cardiovascular diseases [14-18]. It is, however, a modifiable risk factor [19]. Doxorubicin-induced dyslipidaemia is characterized by elevated plasma levels of triglycerides, total, low density (LDL) and very low density (VLDL) lipoproteins cholesterol and a low level of high density lipoprotein (HDL) cholesterol [1-4,8,13,20]. The increases in the levels of plasma

triglycerides, total, LDL and VLDL cholesterol, as well as a decrease in the level of HDL cholesterol that accompany doxorubicin treatment, indicates that it may interfere with cholesterol metabolism and enhance membrane degradation [3,13,20,21].

Therefore, since lipids play important roles in cardiovascular disease complications [22,23], drugs with lipid-lowering effects may be able to protect the myocardium from doxorubicin-induced cardiotoxicity [1,20]. Recall that the sclerotia of *Pleurotus tuberregium* and leaves of *Cnidoscolus aconitifolius* have been shown to contain a wide array of bioactive phytochemicals (e.g. allicins, carotenoids, glycosides, simple phenolics, flavonoids, tannins, phytosterols, terpenes and saponins), minerals and vitamins [24-38].

Most of these compounds have been reported to possess anti-dyslipidaemic activities [29,36,39-47]. They may account for the hypolipidemic effect of the extract of the sclerotia of *P. tuberregium* on salt-loaded rats and alloxan-induced diabetic rabbits that were reported by Ikewuchi and colleagues [28,35]. They may also be responsible for that of *C. aconitifolius* leaf-extract, reported by Achi *et al*. [48] in streptozotocin-induced diabetic rats; Olaniyan *et al*. [49], in egg yolk induced-hypercholesterolemic rabbits; and Somade *et al*. [47] in dimethylnitrosamine treated rats. Sequel to this, this study investigated the ability of aqueous extracts of the sclerotia of *P. tuberregium* and leaves of *C. aconitifolius* to prevent or attenuate doxorubicin-induced dyslipidaemia, and adverse alterations in cardiac cholesterol and triglyceride levels, weight gain, heart weight, and atherogenic indices in Wistar rats.

MATERIALS AND METHODS

Materials

Fresh samples of the sclerotia of *Pleurotus tuberregium* were purchased from Mile 1 Market in Port Harcourt; while fresh leaves (*Cnidoscolus aconitifolius*) were collected from Farm Gardens in Aluu Community of Rivers State, Nigeria; and were duly identified as previously reported [25,27-30,33,35,36,38]. Ninety Wistar rats (weight 80 - 130 g) were obtained from the Animal House of Department of Physiology, University of Port Harcourt, Nigeria. The triglyceride, total and HDL cholesterol kits were products of Randox Laboratories Ltd., County Antrim, UK. All other chemicals used were of analytical grade and products of Sigma-Aldrich, St Louis, MO, USA.

Preparation of Extracts

The sclerotia and leaves were rid of dirt and dried before grinding into powder. The powders (5.3 kg of *P. tuberregium* sclerotia and 5 kg of *C. aconitifolius* leaves) were separately soaked in hot (boiled) water for 12 h. The resultant mixtures were filtered using a sieve cloth. Then, the filtrates were concentrated with the aid of a rotary evaporator before freeze-drying, yielding 145 g and 131 g of *P. tuberregium* sclerotia and *C. aconitifolius* leaves extracts, respectively. The resultant extracts of *P. tuberregium* sclerotia and *C. aconitifolius* leaves (herein called PTSE and CALE, respectively) were weighed, reconstituted in distilled water and administered to the experimental animals, according to their weights and dosages of their groups.

Experimental Design

All experimental procedures in this study were performed in accordance with the ethical guidelines for investigations using laboratory animals and complied with the guide for the care and use of laboratory animals [50]. The animals were weighed and sorted into eighteen groups of five animals each so that the differences in their mean weights were ≤ 3 g [51]. The animals were weighed weekly, housed in cages at the Department of Physiology, and allowed water and feed ad libitum. After one week of acclimatization, both the ameliorative and protective studies commenced. The doxorubicin dosage was adopted from Song *et al*. [52]. The *P. tuberregium* sclerotia extract dosages was adopted and modified from Ikewuchi *et al*. [33] and Ifeanacho *et al*. [35,38]; that of *C. aconitifolius* extract was adopted and modified from Oyagbemi and Odetola [53], and Adaramoye and Aluko [54]; while that of metformin was adopted from Zilinyi *et al*. [55].

Ameliorative (or Doxorubicin Pre-treatment) Study

Nine groups were used for this study. Doxorubicin was dissolved in normal saline and intra-peritioneally injected (15 mg/kg body weight), into all the groups, except the Normal, which was administered with normal saline instead. One week post-administration of doxorubicin, the administration of the extracts and metformin commenced and lasted for 14 days. Diabetmin^{TM} (metformin HCl) was dissolved in distilled water and orally administered daily at 250 mg/kg to the Metformin group. The extracts were administered via the same route at 50 mg/kg to PTSE-50mg (PTSE) and CALE-50mg (CALE); 75 mg/kg to PTSE-75mg (PTSE) and CALE-75mg (CALE); and 100 mg/kg to PTSE-100mg (PTSE) and CALE-100mg (CALE). The Normal and Negative control received distilled water in place of the extract.

Protective (or Extract Pre-treatment) Study

Nine groups were used for this study. Diabetmin^{TM} (metformin HCl) was dissolved in distilled water and orally administered daily at 250 mg/kg body weight to the Metformin group. The extracts were administered via the same route at 50 mg/kg to PTSE-50mg (PTSE) and CALE-50mg (CALE); 75 mg/kg to PTSE-75mg (PTSE) and

CALE-75mg (CALE); and 100 mg/kg to PTSE-100mg (PTSE) and CALE-100mg (CALE). The Normal and Negative control received distilled water in place of the extract. On day 12, doxorubicin was dissolved in normal saline and intra-peritioneally injected (15 mg/kg), into all the groups, except the Normal, which received normal saline instead.

Sample Collection

After administering the extracts and metformin for 14 days, the animals were weighed and sacrificed under chloroform anaesthesia and blood samples were collected into heparin sample bottles. Then, their hearts were excised and weighed, and their weights recorded. The blood samples were centrifuged at 1000 rpm for 10 min, and their respective plasma were collected and stored. The heart samples were weighed and homogenized in distilled water (at 0.4 g per 5 mL), and the resultant homogenates were stored. The heart weight indices were determined according to the following formula [35].

$$\text{Heart weight index} = \frac{Heart\ weight\ (g)}{Body\ weight\ (g)} \times 100$$

Measurement of Cardiac and Plasma Lipid Profiles

The procedures for the assay of triglyceride and cholesterol contents of the homogenates, as well as the plasma triglyceride, total and HDL cholesterol concentrations, were in accordance with the kit manufacturer's instruction. The Lowry method [56] was used to estimate the protein contents of the homogenates.

Estimation of Plasma VLDL-, LDL- and non-HDL Cholesterol and ApoB Concentrations

Plasma VLDL- and LDL-cholesterol concentrations were calculated using the Friedewald formula [57] as follows:

$$\text{i. [LDL cholesterol](mmol/L)} = [\text{Total cholesterol}] - [\text{HDL cholesterol}] - [\text{Triglyceride}]/2.2$$

$$ii.\ [\text{VLDL cholesterol}]\ (\text{mmol/L}) = [\text{Triglyceride}]/2.2$$

NonHDL cholesterol concentrations were determined as follows [58].

$$[NonHDL\ cholesterol] = [Total\ cholesterol] - [HDL\ cholesterol]$$

Apolipoprotein B (apoB) concentration was calculated using the formulae developed by Hwang and colleagues [59,60].

For [Triglyceride] ≤ 270; Plasma apolipoprotein B concentration (mg/dL)

$$= 0.65 \times [\text{Total cholesterol}] - 0.59 \times [\text{HDL cholesterol}] + 0.01 \times [\text{Triglyceride}]$$

For [Triglyceride] > 270; Plasma apolipoprotein B concentration (mg/dL)

$$= 25.6 + 0.58 \times [\text{Total cholesterol}] - 0.3 \times [\text{HDL cholesterol}] - 0.06 \times [\text{Triglyceride}]$$

Determination of Atherogenic Indices

The atherogenic indices were calculated using the following formulae, where all concentrations were in mmol/L:

$$Cardiac\ risk\ ratio = [Total\ cholesterol]/[HDL\ cholesterol]$$ [61]

$$Atherogenic\ coefficient = ([NonHDL\ cholesterol])/([HDL\ cholesterol])$$ [28]

$$Castelli's\ risk\ index\ II = [LDL\ cholesterol]/[HDL\ cholesterol]$$ [62]

$$Atherogenic\ index\ of\ plasma = log([Triglyceride]/[HDL\ cholesterol])$$ [63]

Determination of per cent Recovery or Protection

The level of restoration or safeguard of plasma lipid indices, denoted as per cent recovery/protection, was calculated as follows [33, 64].

$$\text{Per cent recovery (or protection)} = \frac{Parameter_{test\ control} - Parameter_{treatment}}{Parameter_{test\ control} - Parameter_{normal\ control}} \times 100$$

Statistical Analysis of Data

Statistical calculations were carried out with the Excel 2010 (Data Analysis Add-in) software. All data are expressed as mean ± standard error of the mean (SEM), and were analysed using one-way analysis of variance. Significant difference of the means was determined using least significant difference test. In all, $p<0.05$ was considered statistically significant.

RESULTS

The effects of aqueous extracts of *Pleurotus tuberregium* sclerotia and *Cnidoscolus aconitifolius* leaves on body weight and heart weight indices of doxorubicin treated rats are shown in Table 1. In the ameliorative study, the negative control's percent weight gain was neither significantly higher than those of PTSE-50mg, PTSE-75mg and CALE-75mg; nor lower than the others. The heart weight of Negative control was significantly (p<0.05) higher than those of Metformin, PTSE-100mg, CALE-75mg and CALE-100mg; but not significantly higher than those of the others. The heart weight index of the Negative control was significantly (p<0.05) higher than that of PTSE-75mg, but not significantly higher than those of the others. In the protective study, the negative control's percent weight gain was significantly (p<0.05) lower than that of Normal, but not significantly lower than those of the others. The heart weight of Negative control was significantly (p<0.05) higher than those of Metformin and PTSE-100mg, but not significantly higher than those of the others. The heart weight index of the Negative control was significantly (p<0.05) higher than those of Metformin and PTSE-50mg, but not significantly higher than the others.

Table 1. Effects of aqueous extracts of sclerotia of *P. tuberregium* and leaves of *C. aconitifolius* on body weight and heart weight indices of doxorubicin treated rats

Treatment	Ameliorative			Protective		
	% Weight gain	Heart weight (g)	Heart weight index (%)	% Weight gain	Heart weight (g)	Heart weight index (%)
Normal	13.82±1.05^{a,c}	0.74±0.06^{a}	0.37±0.02^{a,b}	13.82±1.05^{a}	0.74±0.06^{a,c}	0.37±0.02^{a,b}
Negative control	9.72±1.22^{a,b,c}	0.78±0.04^{a}	0.42±0.03^{a}	-2.25±4.23^{b}	0.80±0.08^{a}	0.42±0.04^{a}
Metformin	19.98±4.25^{c}	0.60±0.03^{b,c}	0.36±0.03^{a,b}	5.18±0.66^{a,b}	0.58±0.06^{b,c}	0.33±0.03^{b}
PTSE-50mg	1.46±2.46^{b}	0.65±0.05^{a,b}	0.38±0.03^{a,b}	8.33±3.18^{a,b}	0.68±0.04^{a,b}	0.34±0.01^{b}
PTSE-75mg	3.33±4.19^{a,b,d}	0.63±0.02^{a,b}	0.32±0.01^{b}	7.27±14.06^{a,b}	0.68±0.05^{a,b}	0.37±0.02^{a,b}
PTSE-100mg	14.16±8.86^{a,c}	0.60±0.06^{b,c}	0.42±0.03^{a}	6.89±1.17^{a,b}	0.56±0.04^{b}	0.35±0.02^{a,b}
CALE-50mg	15.31±3.80^{c}	0.70±0.03^{a,c}	0.37±0.01^{a,b}	6.03±1.03^{a,b}	0.64±0.05^{a,b}	0.40±0.03^{a,b}
CALE-75mg	2.48±4.07^{a,b}	0.60±0.03^{b,c}	0.39±0.03^{a,b}	3.54±6.25^{a,b}	0.66±0.09^{a,b}	0.41±0.04^{a,b}
CALE-100mg	14.75±1.51^{c,d}	0.58±0.04^{b}	0.39±0.03^{a,b}	-5.50±2.73^{b}	0.65±0.05^{a,b}	0.38±0.03^{a,b}

Values are mean ± SEM, n = 5 animals, per group. Values in the same column with different superscript letters differ significantly at p<0.05.

In both studies, the cardiac and plasma triglyceride concentrations of Negative control were significantly (p<0.05) higher than those of all the other groups, except the cardiac triglyceride levels of PTSE-50mg and CALE-50mg in the ameliorative study (Table 2). Also, the cardiac and plasma total cholesterol concentrations of Negative control were significantly (p<0.05) higher than those of all the other groups (Table 3). In the ameliorative and protective study, the plasma HDL cholesterol concentrations of Negative control were significantly (p<0.05) lower than those of all the other groups (Table 4). However, the plasma non-HDL-, VLDL- and LDL cholesterol, and apolipoprotein B concentrations of Negative control were significantly (p<0.05) higher than those of all the other groups (Table 4). The atherogenic coefficient, cardiac risk ratio, atherogenic index of plasma and Castelli's risk index II of Negative control were significantly (p<0.05) higher than those of all the other groups (Table 5).

The administration of the extracts and metformin prevented (in the protective study) or attenuated (in the ameliorative study). The doxorubicin induced increases in cardiac and plasma lipid profiles atherogenic indices and caused subsequent protection or recovery towards normalization. These recoveries and protections are presented in Tables 6a and 6b in the form of percent recovery/protection of these lipid indices by the various treatments.

Table 2. Influence of the extracts on cardiac and plasma triglyceride concentrations

Treatment	Heart (mmol/mg protein)		Plasma (mmol/L)	
	Ameliorative	Protective	Ameliorative	Protective
Normal	0.332±0.014[a]	0.332±0.014[a]	0.465±0.015[a,f]	0.465±0.015[a]
Negative control	0.488±0.017[c]	0.918±0.032[c]	1.910±0.022[c]	1.536±0.071[c]
Metformin	0.402±0.013[d]	0.726±0.016[d]	0.567±0.006[d]	0.633±0.024[a,b]
PTSE-50mg	0.446±0.020[c]	0.522±0.026[e]	0.553±0.018[d]	0.499±0.063[a,d]
PTSE-75mg	0.327±0.015[a]	0.647±0.031[b]	0.485±0.012[a,f]	0.616±0.093[a,b]
PTSE-100mg	0.368±0.008[a,d]	0.580±0.019[e]	0.788±0.044[e]	0.638±0.052[b,d]
CALE-50mg	0.470±0.023[c]	0.361±0.013[a]	0.425±0.027[b,f]	0.579±0.086[a,b]
CALE-75mg	0.250±0.006[b]	0.370±0.018[a]	0.522±0.018[a,d]	0.548±0.049[a,b]
CALE-100mg	0.331±0.010[a]	0.765±0.028[d]	0.369±0.014[b]	0.690±0.038[b]

Values are mean ± SEM, n = 5. Values in the same column with different superscript letters differ significantly at $p<0.05$

Table 3. Effects of the extracts on cardiac and plasma total cholesterol concentrations

Treatment	Heart (µmol/mg protein)		Plasma (mmol/L)	
	Ameliorative	Protective	Ameliorative	Protective
Normal	0.821±0.019[a]	0.821±0.019[a]	3.098±0.053[a]	3.098±0.053[a]
Negative control	2.126±0.078[c]	3.135±0.115[c]	4.550±0.044[c]	8.088±0.219[c]
Metformin	1.727±0.020[d]	1.269±0.043[d]	3.169±0.077[a]	4.130±0.111[d,e]
PTSE-50mg	1.431±0.053[e]	1.685±0.044[e]	3.064±0.044[a]	3.886±0.138[e]
PTSE-75mg	1.182±0.033[f]	2.710±0.115[f]	3.749±0.062[d]	3.872±0.133[e]
PTSE-100mg	0.963±0.026[g]	2.335±0.114[g]	3.697±0.073[d]	4.976±0.094[f]
CALE-50mg	1.519±0.062[e]	1.111±0.024[d]	2.442±0.061[e]	4.365±0.151[d]
CALE-75mg	1.433±0.052[e]	1.620±0.028[e]	3.457±0.091[f]	2.072±0.048[b]
CALE-100mg	0.654±0.018[b]	1.903±0.050[b]	3.162±0.073[a,b]	3.984±0.118[e]

Values are mean ± SEM, n = 5. Values in the same column with different superscript letters differ significantly at $p<0.05$

Table 4. Effects of aqueous extracts of sclerotia of *P. tuberregium* and leaves of *C. aconitifolius* on plasma HDL, non-HDL, VLDL and LDL cholesterol and apolipoprotein B concentrations of doxorubicin treated rats

Treatment	HDL cholesterol (mmol/L)		NonHDL cholesterol (mmol/L)		VLDL cholesterol (mmol/L)		LDL cholesterol (mmol/L)		Apolipoprotein B (mg/dL)	
	Ameliorative	Protective	Ameliorative	Protective	Ameliorative	Protective	Ameliorative	Protective	Ameliorative	Protective
Normal	1.18±0.03^{a}	1.18±0.03^{a}	1.90±0.08^{a,f}	1.90±0.08^{a}	0.21±0.01^{a}	0.21±0.01^{a}	1.70±0.08^{a}	1.70±0.08^{a}	50.96±1.82^{a,f}	50.96±1.82^{a}
Negative control	0.17±0.02^{c}	0.14±0.01^{c}	4.38±0.05^{c}	7.95±0.23^{c}	0.87±0.01^{c}	0.70±0.03^{c}	3.51±0.05^{c}	7.25±0.20^{c}	112.20±1.29^{c}	201.44±5.69^{c}
Metformin	0.34±0.02^{d}	0.61±0.02^{d}	2.83±0.06^{d}	3.48±0.10^{d}	0.26±0.00^{d}	0.28±0.01^{b,d}	2.57±0.06^{d}	3.20±0.09^{d}	72.45±1.55^{d}	89.47±2.47^{d}
PTSE-50mg	0.24±0.01^{c}	1.37±0.05^{e}	2.80±0.05^{d}	2.47±0.17^{e}	0.26±0.01^{d}	0.23±0.03^{a,d}	2.54±0.04^{d}	2.25±0.16^{e}	71.40±1.14^{d}	65.65±4.09^{e}
PTSE-75mg	0.55±0.03^{e}	0.91±0.04^{f}	3.20±0.09^{e}	2.96±0.14^{f}	0.22±0.01^{a,e}	0.28±0.04^{b,d}	2.98±0.09^{e}	2.68±0.12^{f}	82.04±2.20^{e}	77.05±3.51^{f}
PTSE-100mg	1.91±0.04^{f}	0.68±0.03^{b,d}	1.78±0.04^{f}	4.30±0.11^{g}	0.36±0.02^{f}	0.29±0.02^{b,d}	1.43±0.05^{f}	4.01±0.13^{g}	49.98±1.0^{a}	110.23±2.74^{g}
CALE-50mg	1.00±0.06^{b}	0.65±0.04^{b,d}	1.44±0.09^{b}	3.71±0.16^{d}	0.19±0.01^{a,b}	0.26±0.04^{a,b}	1.25±0.08^{f}	3.45±0.13^{d}	38.91±2.17^{b}	95.33±4.10^{d}
CALE-75mg	1.69±0.04^{g}	0.72±0.03^{b}	1.87±0.08^{f}	1.32±0.03^{b}	0.25±0.01^{d,e}	0.28±0.01^{a,b}	1.63±0.07^{a}	1.05±0.03^{b}	51.49±1.93^{a,f}	35.37±0.71^{b}
CALE-100mg	0.99±0.03^{b}	1.21±0.03^{a}	2.08±0.01^{a}	2.77±0.10^{e,f}	0.17±0.01^{b}	0.31±0.02^{b}	1.91±0.00^{b}	2.46±0.12^{e,f}	54.89±0.10^{f}	73.15±2.57^{e,f}

Values are mean ± SEM, n = 5. Values in the same column with different superscript letters differ significantly at $p<0.05$.

Table 5. Influence of the extracts on the atherogenic indices of doxorubicin treated rats

Treatment	Atherogenic coefficients		Cardiac risk ratios		Atherogenic index of plasma		Castelli's risk index II	
	Ameliorative	Protective	Ameliorative	Protective	Ameliorative	Protective	Ameliorative	Protective
Normal	2.62±0.10^{a}	2.62±0.10^{a}	1.62±0.10^{a}	1.62±0.10^{a}	-0.42±0.02^{a,b}	-0.42±0.02^{a}	1.45±0.10^{a}	1.45±0.20^{a}
Negative control	28.73±3.51^{c}	60.24±6.36^{b}	27.73±3.51^{c}	59.24±6.36^{b}	1.07±0.05^{c}	1.05±0.05^{c}	22.28±2.90^{c}	54.05±5.83^{b}
Metformin	9.48±0.27^{b,d}	6.91±0.22^{a}	8.48±0.27^{b,d}	5.91±0.22^{a}	0.23±0.02^{d}	0.02±0.02^{b}	7.71±0.24^{d}	5.43±0.20^{a}
PTSE-50mg	12.85±0.65^{b}	2.77±0.16^{a}	11.85±0.65^{b}	1.77±0.16^{a}	0.37±0.03^{e}	-0.19±0.31^{a,b}	10.77±0.58^{b}	1.62±0.15^{a}
PTSE-75mg	6.96±0.44^{d}	4.37±0.23^{a}	5.96±0.44^{d}	3.37±0.23^{a}	-0.05±0.03^{f}	-0.18±0.08^{a,b}	5.55±0.41^{d}	3.05±0.19^{a}
PTSE-100mg	1.93±0.01^{a}	7.44±0.39^{a}	0.93±0.01^{a}	6.44±0.39^{a}	-0.39±0.03^{a}	-0.03±0.02^{a,b}	0.74±0.01^{a}	6.01±0.40^{a}
CALE-50mg	2.47±0.17^{a}	6.78±0.48^{a}	1.47±0.17^{a}	5.78±0.48^{a}	-0.37±0.05^{a}	-0.07±0.09^{a,b}	1.28±0.15^{a}	5.37±0.41^{a}
CALE-75mg	2.12±0.07^{a}	2.91±0.07^{a}	1.12±0.07^{a}	1.91±0.07^{a}	-0.49±0.02^{b}	-0.06±0.00^{a,b}	0.97±0.06^{a}	1.52±0.07^{a}
CALE-100mg	3.10±0.07^{a}	3.30±0.08^{a}	2.10±0.07^{a}	2.30±0.08^{a}	-0.42±0.03^{a,b}	0.01±0.26^{b}	1.93±0.06^{a}	2.03±0.08^{a}

Values are mean ± SEM, n = 5. Values in the same column with different superscript letters differ significantly at $p<0.05$.

Table 6a. Per cent recovery of cardiac and plasma lipid indices

Treatment	Metformin	PTSE-50mg	PTSE-75mg	PTSE-100mg	CALE-50mg	CALE-75mg	CALE-100mg
Cardiac cholesterol	30.6±1.5^{a}	53.3±4.0^{c}	72.4±2.5^{d}	89.2±2.0^{e}	46.6±4.8^{c}	53.1±4.0^{c}	112.8±1.3^{b}
Cardiac triglyceride	55.2±8.1^{a}	26.9±12.6^{c}	103.3±9.9^{d}	76.6±5.3^{a,d}	11.6±14.7^{c}	152.6±3.8^{b}	100.2±6.5^{d}
Plasma triglyceride	92.9±0.4^{a}	93.9±1.3^{a}	98.6±0.9^{c,e}	77.7±3.1^{d}	102.7±1.9^{b,c}	96.1±1.2^{a,e}	106.7±1.0^{b}
Plasma total cholesterol	95.1±5.3^{a}	102.3±3.0^{a}	55.2±4.3^{d}	58.8±5.0^{d}	145.2±4.2^{e}	75.3±6.3^{b}	95.6±5.0^{a}
Plasma HDL cholesterol	16.7±1.8^{a}	6.9±1.1^{a}	37.5±3.2^{c}	172.2±3.5^{d}	82.2±5.7^{b}	150.0±3.5^{e}	80.6±2.8^{b}
Plasma non-HDL cholesterol	62.6±2.4^{a}	63.9±1.8^{a}	47.8±3.6^{c}	104.8±1.5^{d}	118.6±3.6^{e}	101.1±3.1^{d}	92.9±0.2^{b}
Plasma VLDL cholesterol	92.2±0.4^{a}	92.5±1.4^{a}	98.1±0.9^{c,e}	77.1±3.0^{d}	101.9±1.9^{b,c}	93.9±0.9^{a,e}	105.2±0.8^{b}
Plasma LDL cholesterol	51.7±3.2^{a}	53.4±2.2^{a}	29.5±4.7^{c}	114.9±2.9^{d}	124.7±4.4^{d}	103.8±4.1^{e}	88.4±0.0^{b}
Plasma apolipoprotein B	64.9±2.5^{a}	66.6±1.9^{a}	49.2±3.6^{c}	101.6±1.6^{b}	119.7±3.5^{d}	99.1±3.1^{b,e}	93.6±0.2^{e}
Atherogenic coefficients	73.7±1.0^{a}	60.8±2.6^{c}	83.4±1.7^{d}	102.6±0.0^{b}	100.6±0.6^{b,e}	101.9±0.2^{b}	98.2±0.3^{e}
Cardiac risk ratios	73.7±1.0^{a}	60.8±2.5^{c}	83.4±1.7^{d}	102.6±0.0^{b}	100.6±0.6^{b,e}	101.9±0.2^{b}	98.2±0.3^{e}
Atherogenic index of plasma	56.5±1.4^{a}	46.9±2.3^{c}	75.6±2.2^{d}	98.2±2.0^{b}	97.1±3.2^{b}	105.3±1.3^{e}	100.3±1.7^{b,e}
Castelli's risk index II	70.0±1.1^{a}	55.3±2.8^{c}	80.3±2.0^{d}	103.4±0.1^{b}	100.8±0.7^{b,e}	102.3±0.3^{b}	97.7±0.3^{e}

Values are mean ± SEM, n = 5 animals, per group. Values in the same row with different superscript letters differ significantly at $p<0.05$.

Table 6b. Per cent protection of cardiac and plasma lipid indices

Treatment	Metformin	PTSE-50mg	PTSE-75mg	PTSE-100mg	CALE-50mg	CALE-75mg	CALE-100mg
Cardiac cholesterol	80.7±1.9^{a}	62.7±1.9^{c}	18.4±5.0^{d}	34.6±4.9^{e}	87.5±1.0^{a}	65.5±1.2^{c}	53.2±2.2^{b}
Cardiac triglyceride	32.7±2.8^{a}	67.5±4.4^{c}	46.2±5.2^{d}	57.6±3.3^{c}	95.1±2.2^{b}	93.5±3.1^{b}	26.1±4.8^{a}
Plasma triglyceride	84.3±2.3^{a,b}	96.8±5.9^{a}	85.9±8.7^{a,b}	83.8±4.8^{a,b}	89.4±8.0^{a,b}	92.2±4.6^{a,b}	79.0±3.6^{b}
Plasma total cholesterol	79.3±2.2^{a,c}	84.2±2.8^{c}	84.5±2.7^{c}	62.4±1.9^{d}	74.6±3.0^{a}	120.6±1.0^{b}	82.2±2.4^{c}
Plasma HDL cholesterol	44.9±2.0^{a}	118.4±4.7^{c}	74.1±4.0^{d}	51.4±2.4^{a,b}	49.2±3.7^{a,b}	55.7±3.2^{b}	102.7±2.7^{e}
Plasma non-HDL cholesterol	73.8±1.6^{a}	90.7±2.7^{c}	82.5±2.3^{d}	60.3±1.9^{e}	70.1±2.7^{a}	109.6±0.5^{b}	85.6±1.7^{c,d}
Plasma VLDL cholesterol	84.5±2.0^{a,b}	95.8±5.8^{a}	84.7±8.6^{a,b}	83.0±4.8^{a,b}	88.5±8.0^{a,b}	86.0±1.0^{a,b}	78.2±3.5^{b}
Plasma LDL cholesterol	72.9±1.6^{a}	90.0±2.9^{c}	82.3±2.2^{d}	58.3±2.4^{e}	68.4±2.3^{a}	111.7±0.6^{b}	86.2±2.1^{c,d}
Plasma apolipoprotein B	74.4±1.6^{a}	90.2±2.7^{c}	82.7±2.3^{d}	60.6±1.8^{e}	70.5±2.7^{a}	110.4±0.5^{b}	85.3±1.7^{c,d}
Atherogenic coefficients	92.6±0.4^{a}	99.7±0.3^{c}	97.0±0.4^{b}	91.6±0.7^{a}	92.8±0.8^{a}	99.5±0.1^{c}	98.8±0.1^{c}
Cardiac risk ratios	92.6±0.4^{a}	99.7±0.3^{c}	97.0±0.4^{b}	91.6±0.7^{a}	92.8±0.8^{a}	99.5±0.1^{c}	98.8±0.1^{c}
Atherogenic index of plasma	70.1±1.3^{a}	84.3±21.5^{a}	84.0±5.6^{a}	73.6±1.6^{a}	76.5±6.1^{a}	75.6±0.1^{a}	70.7±17.8^{a}
Castelli's risk index II	92.4±0.4^{a}	99.7±0.4^{c}	97.0±0.4^{b}	91.3±0.8^{a}	92.5±0.8^{a}	99.9±0.1^{c}	98.9±0.2^{c}

Values are mean ± SEM, n = 5 animals, per group. Values in the same row with different superscript letters differ significantly at $p<0.05$.

DISCUSSION

In this study, the doxorubicin treatment caused a significant reduction in body weight gain and increases in cardiac triglyceride and cholesterol levels. This result corroborated previous reports of doxorubicin-induced body weight loss [5-8] and increased myocardial lipid accumulation [1-4], in experimental animals. However, treatment with the extracts reversed these, towards Normal values, by lowering the cardiac cholesterol and triglycerides levels and increasing the body weight. Since the accumulation of cholesterol in cardiac tissue predisposes to cardiovascular damage and the development of cardiovascular disease [2,3,11], the reduction produced by the extracts in this study portends cardioprotective ability.

The doxorubicin treatment also significantly raised the plasma apolipoprotein B, triglyceride, total, VLDL, LDL and nonHDL cholesterol concentration and lowered plasma HDL cholesterol levels. This is in agreement with the results from several other studies [1-4,8,13,20]. Doxorubicin treatment causes significant increases in the plasma/serum levels of triglyceride, total, LDL and VLDL cholesterol, and a significant decrease in HDL cholesterol concentration.

In this study, the extracts significantly raised plasma HDL cholesterol (PTSE-100mg and CALE-75mg), and lowered plasma triglyceride (PTSE-50mg), apolipoprotein B (PTSE-50mg and CALE-75mg), and total-, nonHDL-, LDL- and VLDL cholesterol (PTSE-50mg, CALE-50mg and CALE-75mg). This indicates the ability of the extracts to protect against or reduce the risk of development of cardiovascular diseases [22,23,65-67]. This is because low plasma HDL cholesterol [23,68-71], and high plasma triglyceride [68-74], total cholesterol [69,70], nonHDL cholesterol [75-79], LDL cholesterol [19,69,79,80] and VLDL cholesterol [65,81,82] are all risk factors/and biomarkers for cardiovascular diseases. Studies have shown that plasma apolipoprotein B is a powerful and reliable risk indicator of cardiovascular disease and treatment efficacy [60,66,67,83-86]. Similar effects were earlier reported by Ikewuchi and colleagues for the *Pleurotus tuberregium* sclerotia aqueous extract on salt-induced hypertensive rats [28] and alloxan-induced diabetic rabbits [35]. Analogous effects were also reported for *Cnidoscolus aconitifolius* leaf extracts in streptozotocin-induced diabetic rats [48]; egg yolk induced-hypercholesterolemic rabbits [49], and dimethylnitrosamine treated rats [47]. Therefore, since treatment with the extracts significantly reverted the lipid parameters (plasma triglyceride, and total, LDL, VLDL and HDL cholesterol) to near-normal levels, it is safe to presume that they may be lowering the lipids by impeding cholesterol biosynthesis and enhancing LDL uptake from blood by the liver [3,21]. This hypolipidaemic effect of the extracts may be due to the presence of allicins, ascorbic acid, carotenoids, flavonoids, glycosides, phytosterols, saponins, tannins and terpenes [24-38], all of which are potent anti-dyslipidaemic agents.

Atherogenic indices or lipid ratios are reliable pointers of the risk of cardiovascular diseases [87,88] and have greater predictive capability than the isolated parameters [88-91]. This is because they express the imbalance between atherogenic and anti-atherogenic lipoproteins, and their values increase, with a heightened risk of developing cardiovascular disease [87-95]. Therefore, lowering atherogenic indices protects against the development of cardiovascular diseases [28,61,63,87,88,96]. Palem and Abraham [87] and Nimmanapalli *et al*. [97] reported a correlation between atherogenic indices and nitric oxide (a marker of endothelial dysfunction), thereby providing a possible link between the indices and cardiovascular disorders. Although they are good indicators of cardiovascular risk, they differ in their sensitivity due to the differences in the spectrum of lipoprotein that is incorporated in their determination. Cardiac risk ratio and atherogenic coefficient reflect the atherogenic potential of the entire spectrum of lipoprotein fractions (the total and nonHDL cholesterol); Castelli's risk index II reflects those of the low-density lipoprotein fraction (LDL cholesterol); while an atherogenic index of plasma reflects the presence of atherogenic small LDL and small HDL particles, and is associated with HDL, LDL, and VLDL particle sizes [94,98,99,100]. Evidence indicates that atherogenic index of plasma is the most sensitive marker compared with atherogenic coefficient, cardiac risk ratio and Castelli's risk index II, and has the closest association with cardiovascular disease risk [87,88,89,91,99,101]. Its values are often graded into three categories: those associated with low cardiovascular risk (<0.1); those associated with intermediate or medium risk (≥ 0.1 or ≤ 0.24); and those associated with high risk (>0.24) [69,88,89,94,102]. Therefore, the higher the values of the atherogenic index of plasma, the higher the predisposition to the development of cardiovascular diseases [86,87,88,90]. In this study, the extracts lowered atherogenic indices of the treated animals. Similar effects were previously reported for *P. tuberregium* sclerotia aqueous extract on salt-induced hypertensive rats [28] and alloxan-induced diabetic rabbits [35]. This, therefore, implies that aqueous extracts of *P. tuberregium* sclerotia and *C. aconitifolius* leaves can prevent/or manage cardiovascular complications.

From the foregoing, it can be said that the administration of the extracts reversed the doxorubicin-induced hypertriglyceridemia, hypercholesterolemia and weight loss in the experimental animals. This, therefore, lends credence to the ability of sclerotia of *Pleurotus tuberregium* and leaves of *Cnidoscolus aconitifolius* to alleviate/prevent doxorubicin-induced dyslipidaemia and by extension, highlights their potential to reduce the risk of cardiovascular disorders/complications usually associated with doxorubicin administration.

DATA ACCESSIBILITY STATEMENT

All relevant data are within the paper.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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