Ameliorative Effects of Ethanolic leaf extract of *Diodia sarmentosa* on high fat induced cardiac tissue injury in male wistar albino rats

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Research Article

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Abstract

Background

Diodia sarmentosa is a native straggling herb found in Nigeria, especially in evergreen forest, bushland, and riverine areas. In folklore medicine, it has been used to treat ailments like diabetes, ulcers, etc. This study investigated the ameliorative effects of ethanolic leaf extract of *Diodia sarmentosa* on high-fat diet-induced cardiac tissue injury in male albino Wistar rats.

Methods

Thirty (30) male Wistar rats (150g-200g) were divided into five (5) groups namely; Negative control (NC) group which was not induced nor treated, Positive control (PC) was induced but not treated, Low dose extract (LDE) was treated with 250mg/kg of the extract, High dose extract (HDE) was treated with 500mg/kg of the extract and SIM group treated with 5mg/kg of Simvastatin. Cardiac tissue injury was induced by feeding the rats with high-fat diet (Ghee and Coconut oil in the ratio of 3:1) for six (6) weeks. The administration of the treatments (extract and drug) at the appropriate doses started from the 4th week till the 6th week. Parameters like Total protein concentration, Lactate dehydrogenase (LDH) activity, Malondialdehyde level, Superoxide dismutase (SOD) activity, Catalase (CAT) activity, Glutathione S-transferase (GST) activity, and Glutathione peroxidase (GPx) activity were carried out in the cardiac tissue of the test rats to determine the ameliorative effects of *Diodia sarmentosa*.

Results

Results obtained showed that high-fat diet reduced antioxidant enzymes, protein levels, and caused lipid peroxidation indicating injury in the cardiac tissue of the test rats. However, the extract at high dose significantly reduced ($p < 0.05$) lipid peroxidation and high GST activity in the cardiac tissue caused by high-fat diet. Protein levels and antioxidant enzyme activities in SOD, CAT, GPx, and LDH were all significantly increased ($p < 0.05$), when compared to the positive group. The extract at high dose possesses similar efficacy to the standard drug simvastatin used to compare the efficacy of the extract.

Conclusions

Thee evidence from this study shows *Diodia sarmentosa* has the potential to improve and reverse the injury in the cardiac tissue caused by high-fat diet administration in the test rats, by reducing lipid peroxidation, and increasing antioxidant enzymes and total protein concentration. This makes the plant a potential new natural product for the treatment of cardiovascular diseases.

Introduction

Diets containing fat play a vital role in the energy needs, growth, and healthy state of the body [1]. High consumption of dietary fat on the other hand has been reported to cause various health conditions such as oxidative stress, obesity, dyslipidaemia, and hyperlipidaemia. When these health conditions are not properly managed or treated, they lead to the development of cardiovascular diseases like Hypertension, Stroke, Arteriosclerosis, heart attack, and ultimately death [2, 3]. These conditions arise when the high fat intake induces oxidative stress in the body, by blocking the hepatic production of the antioxidant glutathione and triggering the oxidation of fatty acids, leading to series of reactions that can alter the immune system [4].

Oxidative stress arises when the ratio of free radicals generated is more than the level of antioxidants used to neutralize those free radicals [5]. This insufficient level of antioxidants is caused by the overproduction of free radical species (reactive oxygen or nitrogen species) thereby weakening the defense system [6]. An extended weakening of the immune system causes oxidative damage to the cells, tissues, and organs leading to the development of health problems like cancers, coronary heart diseases, high blood pressure, stroke, etc [7]. The body experiences oxidative stress when there is an increase in lipid peroxidation products like Malondialdehyde, and a decline in antioxidant enzymes such as Superoxide dismutase, and Catalase that helps in the defense mechanism of the body [5]. Cardiovascular diseases are characterized by high levels of lipid peroxidation products and deficiency in antioxidants such as Glutathione, Superoxide dismutase, Glutathione peroxidase, Glutathione s-transferase, Catalase, etc [8]. Apart from the consumption of diets containing high fat, other activities like high alcohol consumption, use of drugs (anti-inflammation, anti- analgesic, anti-cancer, and anti-depressants), environmental pollution by pollutants, and pesticides can cause oxidative stress in the system [5].

Diodia Sarmentosa (Sw) also known as Zimbabwe flora or tropical buttonweed is from the family of RUBIACEAE. It is a procumbent perennial herb that grows in open riverine vegetation, bushland, and on rocky places close to rivers [9]. Various authors have reported that *Diodia sarmentosa* possesses a wide range of pharmacological properties like anti-ulcer, anti-inflammatory and analgesic, anti-diabetic, and anti-cancer potentials against ethyl nitrosamine induced hepatocellular carcinoma albino rats [9–12]. It was also reported to scavenge free radicals and possess antioxidant potentials in high-fat-fed rats [13]. A study by Ezejiofor & Korie [14] reported the antihyperlipidaemic potentials of high-fat-fed rats. This study demonstrated the ameliorative effects of the ethanolic leaf extract of *Diodia sarmentosa* on cardiac tissue injury caused by high-fat diet in male albino Wistar rats.

Materials And Methods

Plant materials

Fresh samples of *Diodia sarmentosa* (Sw) leaves were collected from farmlands and natural vegetation within Ihiagwa environment.

Chemicals and reagents

Analytical grade chemicals and reagents were used for this study.

Experimental animals

Male Wistar albino rats weighing between 150-200g were used for this study. The animals were purchased from the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria.

Preparation of plant extract

Fresh leaves of *Diodia sarmentosa* (Sw) were air-dried at room temperature and then ground into a fine powder using laboratory mortar and pestle. These leaves now in the fine powder were soaked in 80% ethanol for one week and then filtered using Whatman filter paper No. 42 to get the plant extract.

Experimental site

The animals were acclimatized in an animal house at the Biochemistry department at the Federal University of Owerri, under room temperature and relative humidity of 40-65% with a 12h natural light-dark cycle. The animals were granted free access to water and rats chew in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments.

Experimental design

According to the Ezejiofor & Okoroafor [12], the median lethal dose (LD50) of the plant extract was established as 1600mg/kg, this led to the adoption of safe doses of 250mg/kg and 500mg/kg.

Thirty (30) male albino Wistar rats used for this experiment were grouped into five (5) groups, six (6) for each group;

Group NC, Negative control: Rats fed normal diet and water

Group PC, Positive control: Untreated rats fed with high-fat diet and water

Group LDE: High-fat diet fed rats treated with 250mg/kg body weight of ethanolic leaf extract of *Diodia sarmentosa*

Group HDE: High-fat diet fed rats treated with 500mg/kg body weight of ethanolic leaf extract of *Diodia sarmentosa*

Group SIM: High-fat diet fed rats treated with 5mg/kg body weight of Simvastatin. The efficacy of the extract was compared to Simvastatin (a statin-derived drug) used in reducing the risk of cardiovascular diseases.

Treatment and sample collection phase

The rats were placed on High-fat diet (Ghee and Coconut oil in the ratio of 3:1) for six (6) weeks adapted from a previous study by Munshi *et al.* [15] except for the negative control group. All animals were allowed access to food and water and the body weight of rats was taken weekly.

From the 4^{th} week till the end of the experiment, groups PC, LDE, HDE, and SIM continued with high-fat diet and their respective doses of extract and drug respectively, while group NC remained on normal rat chew. The appropriate dosages of the extract and drug were administered to the animals orally once daily by intubation using an intravenous cannula tube. The rats were allowed to fast for Twenty four hours, after the last treatment, they were anesthetized with chloroform vapour and sacrificed. The hearts from the sacrificed animals were removed and washed with ice-cold saline, blotted dry, and stored at -20^{o}C. Heart tissues were homogenized, and put inside labeled tubes for biochemical analyses.

Determination of Total Protein Concentration

Total protein concentration was determined using the Biuret method as described by Gornall *et al.* [16] as reported by Hassan *et al.* [17] was employed for the determination of protein concentration.

Determination of Lactate dehydrogenase (LDH) activity

Lactate dehydrogenase activity was determined according to the method of Wroblewski & La Due [18] as reported by Oloyede & Sunmonu [19].

Determination of Lipid peroxidation

Lipid peroxidation as expressed as malondialdehyde levels in the heart tissue was determined spectrophotometrically by assessing the concentration of thiobarbituric acid reactive substances (TBARS) described by Buege & Aust [20] as reported by Nair *et al.* [21].

Determination of antioxidant enzymes

The assay of the antioxidant enzyme Superoxide dismutase (SOD) activity was carried out according to the procedure of Das *et al.* [21]. Catalase (CAT) activity in the samples was assayed following the procedure of Aebi [23] as reported by Hassan *et al.* [24]. Glutathione-S-transferase (GST) in the samples was measured by the method of Habig *et al.* [25] as reported by Sasi Bhusana Rao *et al.* [26]. Glutathione peroxidase (GPx) in the samples was measured by the method of Rotruck *et al.* [27] as reported by Sajeeth *et al.* [28].

Statistical Analysis

Data were analyzed using appropriate software (Microsoft Excel 2013). Results were presented as mean ± Standard deviation of four determinations and statistically analyzed using a one-way analysis of variance on a statistical computer software program (SPSS 21). The degree of statistical difference was accepted as significant at $p < 0.05$.

Results

The results of superoxide dismutase (SOD) activities in the heart tissue across the study groups ranging from 19.37 ± 3.43 — 29.71 ± 2.77 (IU/mg Protein) is as presented in Table 1. The PC group showing the lowest activity, while the LDE showing the highest activity.

Table 1
Result of Superoxide dismutase activity in the various study groups

	Groups	Heart Tissue
SOD (IU/mg Protein)x10^-6	NC	26.29 ± 1.65^{b}
	PC	19.37 ± 3.43^{a}
	LDE	29.71 ± 2.77^{b}
	HDE	26.70 ± 2.56^{b}
	SIM	24.62 ± 2.19^{a}

Each value represents mean ± SD (n = 4). Groups with different alphabets are significantly different ($p < 0.05$), while groups with similar alphabets are not significantly different.

The results of catalase (CAT) activities in the heart tissue across the various study groups ranging from 5.03 ± 0.32 — 7.18 ± 0.62 (μMH2O2/min/mgprotein) is as presented in Table 2. Group PC showing the lowest activity, while the SIM group showing the highest activity.

Table 2
Result of Catalase activity in the various study groups

	Groups	Heart Tissue
CATALASE (µM H2O2/min/mgProtein)x10^-6	NC	$6.08 \pm 0.33^{a,b,c}$
	PC	5.03 ± 0.32^{a}
	LDE	$5.83 \pm 0.60^{a,b}$
	HDE	$6.84 \pm 0.57^{b,c}$
	SIM	7.18 ± 0.62^{c}

Each value represents mean ± SD (n = 4). Groups with different alphabets are significantly different ($p < 0.05$), while groups with similar alphabets are not significantly different.

Table 3 shows the results of glutathione s-transferase (GST) activities in the heart tissue across the various study groups ranging from 0.82 ± 0.05 — 1.66 ± 0.22 (µmol GSH-CDNB/min/mgprotein). The NC group shows the lowest activity, while group PC shows the highest activity.

Table 3
Result of Glutathione S-transferase activity in the various study groups

	Groups	Heart Tissue
GST (µmol GSH-CDNB /min/mgprotein)x10^-6	NC	0.82 ± 0.05^{a}
	PC	1.66 ± 0.22^{c}
	LDE	$1.43 \pm 0.24^{b,c}$
	HDE	$1.07 \pm 0.27^{a,b}$
	SIM	$1.16 \pm 0.20^{a,b}$

Each value represents mean ± SD (n = 4). Groups with different alphabets are significantly different ($p < 0.05$), while groups with similar alphabets are not significantly different.

The results of glutathione peroxidase (GPx) activities in the heart tissue across the various study groups ranging from 2.30 ± 0.20 — 3.85 ± 0.20 (mg GSH/min/mgprotein) is as presented in Table 4. Group PC shows the lowest activity, while group NC shows the highest activity.

Table 4
Result of Glutathione peroxidase activity in the various study groups

	Groups	Heart Tissue
GPx(mg GSH/min/mgprotein)	NC	3.85 ± 0.20^{d}
	PC	2.30 ± 0.20^{a}
	LDE	$3.54 \pm 0.08^{c,d}$
	HDE	$3.27 \pm 0.31^{b,c}$
	SIM	2.88 ± 0.20^{b}

Each value represents mean ± SD (n = 4). Groups with different alphabets are significantly different ($p < 0.05$), while groups with similar alphabets are not significantly different.

Table 5 shows the results of lipid peroxidation expressed as Malondialdehyde (MDA) levels in the heart tissue across the various study groups ranging from 0.25 ± 0.02 — 0.64 ± 0.06 (nmol/mgprotein). Group NC shows the lowest level, while group PC shows the highest level.

Table 5
Result of Malondialdehyde level in the various study groups

	Groups	Heart Tissue
MDA (nmol/mgprotein)	NC	0.25 ± 0.02^{a}
	PC	0.64 ± 0.06^{c}
	LDE	0.37 ± 0.03^{b}
	HDE	$0.32 \pm 0.02^{a,b}$
	SIM	0.28 ± 0.03^{a}

Each value represents mean ± SD (n = 4). Groups with different alphabets are significantly different ($p < 0.05$), while groups with similar alphabets are not significantly different.

The total protein level in the heart tissue across the various study groups ranging from 29.94 ± 3.44 — 65.44 ± 4.95 (g/l) is as presented in Table 6. The PC group shows the lowest level, while the SIM group shows the highest level.

Table 6
Result of Total Protein concentration in the various study groups

	Groups	Heart Tissue
Heart Protein (g/L)	NC	63.27 ± 3.66^{c}
	PC	29.94 ± 3.44^{a}
	LDE	53.13 ± 4.17^{b}
	HDE	$62.06 \pm 5.71^{b,c}$
	SIM	65.44 ± 4.95^{c}

Each value represents mean ± SD (n = 4). Groups with different alphabets are significantly different ($p < 0.05$), while groups with similar alphabets are not significantly different.

Table 7 shows the result of lactate dehydrogenase activity in the heart tissue across the various study groups ranging from 72.65 ± 8.45 — 97.50 ± 6.70 (U/L). The LDE group shows the lowest activity, while the SIM group shows the highest activity.

Table 7
Result of Lactate dehydrogenase activity in the various study groups

	Groups	Heart Tissue
Heart LDH (U/L)	NC	89.85 ± 5.65^{b}
	PC	73.28 ± 7.32^{a}
	LDE	72.65 ± 8.45^{a}
	HDE	$87.30 \pm 8.68^{a,b}$
	SIM	97.50 ± 6.70^{b}

Each value represents mean ± SD (n = 4). Groups with different alphabets are significantly different ($p < 0.05$), while groups with similar alphabets are not significantly different.

Discussion

Cardiac toxicity is an abnormal change in the biochemical and molecular structure damage of the heart tissue caused as a result of oxidative stress, which is characterized by an increase in lipid peroxidation and a decrease in antioxidant enzymes [29]. Cardiac toxicity in the heart tissue of the test rats was examined by estimating some biomarkers like Malondialdehyde (MDA) level, Glutathione peroxidase

(GPx) activity, Glutathione S- transferase (GST), Superoxide dismutase (SOD) activity, Catalase (CAT) activity, Lactate dehydrogenase (LDH) activity, and Protein concentration. The high-fat diet consumption by the rats caused a high concentration of fat deposition in the heart tissue, thus causing myocardial fibrosis in the positive control rats (Tables 1–7). Superoxide radicals were overproduced by NADPH oxidase in the myocardium of the positive control test rats, because of the decrease in the antioxidant superoxide dismutase (Table 1). This decline in SOD activity is due to the inability of superoxide radicals to be broken down into hydrogen peroxide and oxygen. This finding is similar to that of Bhandari *et al.* [2], who reported a decrease in SOD activity in the cardiac tissue of obese rats. Superoxide dismutase is an antioxidant enzyme that reduces superoxide radicals in the cell. Table 1 shows both doses of the extract significantly increased ($p < 0.05$) the antioxidant when compared to the positive control group. The activity of the antioxidant enzyme in the heart tissue increased in a dose-dependent manner. SOD activity in the extract groups was higher than that of the group treated with simvastatin. This increase reduced superoxide radicals in the myocardium of the rats, by the dismutation of superoxide radicals. The efficacy of the extract is similar to that of Vitamin D which elevated SOD activity in the cardiac tissue of an obese rat [30].

Catalase (CAT) is an antioxidant enzyme found in the heart and other tissues that protects the body from oxidative damage, by catalysing the conversion of hydrogen peroxide to water and oxygen [31]. Table 2 showed the CAT activity in the positive control group decreased, but this decrease was not significantly different ($p > 0.05$), when compared to the negative control group. This decrease in catalase activity maybe due to the depletion of catalase resulting from the consumption of high-fat diet by the test rats. This led to the overproduction of hydrogen peroxide radicals in the cardiac tissue of the test rats inducing oxidative stress. Both doses of the extract and the SIM group increased CAT activity, but only in the LDE group that it was not significantly different ($p > 0.05$) when compared to the positive control group (Table 2). This shows the higher the dose, the higher the efficacy of the extract in neutralizing hydrogen peroxide radicals in the heart. This catalase increasing potential is similar to the ability of *Naringin* and *Allium sativum* in increasing CAT activity in doxorubicin-induced and isoproterenol cardiac toxicity in rats respectively [28, 41].

Glutathione S-transferase (GST) is an antioxidant enzyme that catalyses the conjugation of reduced glutathione to xenobiotics or other range of substrates for the purpose of detoxification [32]. Glutathione s-transferase activity was significantly increased ($p < 0.05$) in the heart tissue of the positive control group when compared to the negative control group (Table 3). Farhangi *et al.* [30] reported that the body's response to oxidative stress markers and the antioxidant enzyme system is tissue specific; that is, various organs can respond to oxidative stress by selectively increasing or decreasing oxidative stress markers. This increase in GST activity in the cardiac tissue of the positive control group correlates with the high GST activity observed in the heart tissue of obese rats [33]. GST activity was reduced by both doses of the extract. At high dose, GST activity in the heart tissue was significantly reduced ($p < 0.05$) when compared to the positive control group and brought to normal levels with the NC and SIM groups (Table 3). This observation might be because the drugs slowed the expression of Glutathione S-transferase, avoiding overproduction of the enzyme in the cardiac tissue of the test rats. Glutathione

peroxidase (GPx) is an antioxidant enzyme that catalyses the oxidation of reduced glutathione into glutathione disulfide, breaking down hydrogen peroxide and lipid hydroperoxide into nontoxic forms. It helps in repairing cellular damage caused by lipid peroxidation [34]. Glutathione peroxidase activity was significantly reduced ($p < 0.05$) in the positive control group when compared to the negative control group (Table 4). This reduction of glutathione peroxidase activity in the positive control group caused cellular damage to the heart tissue, because of the inability of hydroxyl radicals and lipid hydroperoxide to be decomposed to harmless forms by the antioxidant enzyme. This effect of high-fat diet in reducing GPx activity in the cardiac tissue is similar to the effect of Aspartame in reducing GPx activity in the myocardium of the test rats [35]. Both doses of the extract significantly increased ($p < 0.05$) GPx activity when compared to the positive control group (Table 4). This increase in GPx activity by the extract reduced cellular damage, by scavenging and degrading hydroxyl and lipid hydroperoxide radicals. The increase in GPx activity by the extract is better than that of Simvastatin in the SIM group. The low dose of the extract was most favorable in increasing glutathione peroxidase activity in the heart tissue of the test rats. The efficacy of *Diodia sarmentosa* is similar to the efficacy of Ruzu herbal bitters [1] and Vitamin D [30] in increasing GPx activity in the heart tissue of test rats.

Lipid peroxidation is the oxidative decomposition of lipids. Malondialdehyde (MDA) is one of the final products of lipid peroxidation, it is produced as a result of the overproduction of free radicals in the cell. It is a major marker or a direct confirmation of oxidative stress [36]. In this study, Malondialdehyde level was significantly increased ($p < 0.05$) in the positive control group when compared to the negative control group (Table 5). This increase in MDA levels indicates cell injury or damage in the myocardium caused by the production of reactive oxygen species by tumour necrosis factor alpha (cytokine). Lipid peroxidation in the heart leads to cellular membrane damage due to oxidative modification of lipids, proteins, and bioavailability of free fatty acids that can ultimately lead to various cardiovascular diseases and sudden death. This result is in concordance with various authors which all reported that high levels of malondialdehyde cause oxidative stress which is toxic to the heart [2, 13, 29, 35 &37]. Both doses of the extract significantly reduced ($p < 0.05$) MDA levels, when compared to the positive control group (Table 5). The decrease in MDA level by the extract shows the inhibition of lipid peroxidation, by the regulation of cholesterol metabolism to reduce cellular damage. The extract at a high dose is comparable to the negative control and the SIM group because MDA levels were not significantly different ($p > 0.05$) amongst them (Table 5). This shows the extract possesses potential to reduce cardiotoxicity. Proteins are used as an indicator of liver injury and cellular damage. Total protein level in the heart of the positive control group was significantly reduced ($p < 0.05$) when compared to the negative control group (Table 6). The decrease showed cardiac injury caused by high lipid concentration in the test rats. The extract at both doses significantly increased ($p < 0.05$) protein levels, when compared to the positive control group. The extract at a high dose was more effective because protein levels were brought to levels comparable to the NC and SIM groups (Table 6). Lactate dehydrogenase (LDH) is a biomarker for cardiac disorders. It plays a role in the transfer of lactic acid to pyruvate and the formation of ATP from ADP in anaerobic systems. The deficiency of the enzyme lactate dehydrogenase is problematic and sometimes is increased as a result of prolonged exercise and some psychological disorders like liver disease, cancers, etc [38].

Lactate dehydrogenase activity was significantly reduced ($p < 0.05$) in the heart tissue of the PC group when compared to the NC group (Table 7). This was because the high-fat diet administration inhibited the expression of LDH activity, and deficiency of LDH activity affects the cardiac muscle of the test rats. These findings correspond with the findings of Poongkodi *et al.* [39] who reported low LDH activity in hypercholesterolemic rats. Only at the high dose of the extract was the increase in LDH activity significant, and brought to normal levels with the NC and SIM groups (Table 7). The potency of the extract at a higher dose is similar to Vitamin C and Agaricusbisporus in increasing LDH activity in the heart tissue of hypercholesterolemic rats and carbofuran induced rats [39, 40], while Seshadri [41] reported lower LDH activity in isoproterenol-induced male rats treated with *Allium sativum*.

Conclusions

The study showed that high-fat diet administration caused oxidative damage in the cardiac tissue of the male Wistar rats over time. This damage in the cardiac tissue caused an oxidative decomposition of lipid and protein accompanied by depletion of antioxidant enzymes. This is evidence of cardiac tissue injury or heart tissue damage in the rats. The ethanolic extract of *Diodia sarmentosa* reduced lipid peroxidation, restored proteins, and increased depleted antioxidant enzymes. When compared to simvastatin, the extract showed similar potency especially at the higher dose, thus showing it possesses therapeutic potential in the cardiac tissue. This adds to the pharmacological knowledge of the plant in being a possible remedy for cardiovascular diseases.

Abbreviations

CAT
Catalase
GPx
Glutathione peroxidase
GST
Glutathione S- transferase
HDE
High dose extract
LDE
Low dose extract
LDH
Lactate dehydrogenase
NC
Negative control
PC
Positive control
SIM

Simvastatin
SOD
Superoxide dismutase.

Declarations

Acknowledgment

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Ethical approval and consent to participate

All animals procedures were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments.

Consent for publication

Not applicable

Availability of data and material

All datasets generated in this current study are not publicly available because they are a part of the corresponding author's Masters of Science (MSc) thesis, however, they are available from the corresponding author on reasonable request.

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None

Authors' Contributions

This study was carried out in collaboration among all authors. Authors KSC and ETIN drafted the study and wrote the protocol. Author KSC carried out all literature searches, statistical analysis and wrote the first and final draft of the manuscript. Authors ETIN and EEU reviewed and corrected the final draft of the manuscript. All authors read and approved the final manuscript.

Competing Interests

All the authors firmly declare that they have no competing interests

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