

***Sterculia tragacantha* Lindl Aqueous Leaf Extract Ameliorate Cardiomyopathy in Streptozotocin-induced Diabetic Rats via Urotensin II and FABP3 Expressions**

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Abstract: *Sterculia tragacantha* (ST) Lindl leaf is commonly used locally in the management of diabetes mellitus (DM) and its complications. This study was aimed at assessing the valuable effects of ST leaf on streptozotocin-diabetic cardiomyopathy (DCM). Streptozotocin was administered intraperitoneally to the experimental animals to induce DM, and hence, placed on different doses of ST for 14 days. Thereafter, on the 15th day of the experiment, the animals were euthanized, and a number of cardiomyopathy indices were investigated. The diabetic rats exhibited a momentous increase in hyperlipidemia, lipid peroxidation as well as a significant ($p < 0.05$) decline in antioxidant enzyme activities. The serum creatine kinase MB (CK-MB), C-reactive protein (CRP), cardiac troponin I, tumour necrosis factor- α (TNF- α) and urotensin II expression revealed a significant ($p < 0.05$) upsurge in diabetic rats. Also, the expression of GLUT4 and fatty acid-binding protein 3 (FABP3) were significantly ($p < 0.05$) reduced in diabetic rats. However, at the conclusion of the experimental trial ST significantly ($p < 0.05$) attenuated hyperlipidemia, oxidative stress biomarkers by augmenting the antioxidant enzyme activities and decrease in lipid peroxidation, ameliorated CK-MB, CRP, cardiac troponin I, TNF- α , and urotensin-II levels, and improved GLUT4 and FABP3 expressions. Similarly, the administration of ST prevented histological alterations in the heart of diabetic animals. Therefore, the obtained results suggest that ST could mitigate DCM in streptozotocin-induced diabetic rats.

Key words: hyperglycaemia, oxidative stress, hyperlipidemia, diabetic, cardiomyopathy, gene expression

1 Introduction

Diabetes mellitus (DM) is a metabolic malady tagged with persistent hyperglycemia resulting in instabilities of carbohydrate, protein, and lipid metabolisms because of flaws in insulin secretion, insulin action, or both¹. This triggers a series of clinical signs such as polyuria, polydipsia, polyphagia, and abnormal weight loss, amongst others². Currently, more than 422 million people are suffering from this disease globally, with a death rate of over 4 million each year².

Furthermore, diabetic cardiomyopathy (DCM) is one of

the clinical complications of diabetes mellitus connected to cardiac irregularities³. This affects more than 12% of diabetic individuals worldwide, which could cause heart malfunction and later death. The main attributes of DCM include oxidative stress, fatigue, and cardiac hypertrophy amongst others⁴.

Likewise, Bhattacharjee *et al.*⁵ reported that continuous hyperglycemia in DM patients inflames the production of reactive oxygen species (ROS) as well as inflammation; these perform an important function in DCM. In addition, accumulation of ROS activates overexpression of urotensin

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II with downregulation of fatty acid-binding protein 3 (FABP3) which negatively regulates the cell cycle and inhibits proliferation of myocardial cells leading to heart disease (e.g. cardiomyopathy)^{6, 7}. Urotensin II is a vasoconstrictor and even more potent than endothelin-1 and is one of the several neurohormonal systems activated in heart-related diseases. FABP3 is known as a 14 to 15 kDa protein mainly expressed in the heart and some other organs⁸. FABP3 has the ability to binds long-chain fatty acids and then transports them to specific cell organelles for lipid storage and metabolism as well as signaling transduction and transcriptional regulation⁷. These abnormalities encourage inflammation biomarkers (e.g. C-reactive protein) as well as the activities of other biomarkers that are paramount to the heart tissue (such as Creatin kinase MB, serum lipid profiles, etc.).

In order to mimic what happens in human beings, DM was induced by introducing a single dose of streptozotocin (diabetogenic chemical) into the experimental animals. This triggers selective destruction of pancreatic beta cells which are insulin-producing cells¹.

Currently, there are no perfect medications that can cure DM, and they are also associated with series of drawbacks such as fatty liver, anorexia, insulin resistance amongst others⁹. However, medicinal plants have been endowed with different phytochemicals which are useful as a remedy for human diseases. Locally, various herbs have been endorsed in the management of diabetes mellitus and its complications especially DCM. In this regard, an example is *Sterculia tragacantha*; the family of *Malvaceae* called Uhobo and Abalo among Eastern and Western people of Nigeria. *Sterculia tragacantha* has been documented in the treatment of boils, diarrhea, dyspepsia, gout, fever, gonorrhea, snake bite, syphilis, tapeworm, gout, whitlow, etc. especially in rural communities^{10, 11}. Added to this, without proper scientific barking, the leaf of this plant is used locally in some communities in the Western and Eastern parts of Nigeria in the management of diabetes mellitus and its complications. Hence, there is little/no information on the effectiveness of this plant in the treatment/management of diabetic cardiomyopathy which is the focus of this study.

2 Materials and Methods

2.1 Plant and its preparation

The leaves of *Sterculia tragacantha* were acquired from a local farm in Osogbo, Osun State, Nigeria, and scientifically defined at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria with voucher number FHI:112856. Hence, the leaves were shade dried for three weeks before being pulverized into a powder form via an electric blender. Subsequently, five hundred grams of the

powder sample was extracted in 5 liters of distilled H₂O₂ and occasionally stirred at room temperature, sieved via Whatman No. 1 filter paper. Then a freeze-dryer was used to concentrate the obtained filtrate and the acquired yield was weighed and named ST (*Sterculia tragacantha*). This was scrapped into a sample bottle and used for various analyses.

2.2 Chemicals/Reagents

Sigma-Aldrich, Inc. (St Louis MO) provided the chemicals and reagents employed in this study (St Louis MO). In addition, the primers were procured from Inqaba Biotech (South Africa). Furthermore, enzyme kits were acquired from Randox (USA).

2.3 Experimental animals and induction of diabetes mellitus

A known number (50) of male albino rats of six to eight weeks old were acquired from Ikere-Ekiti (Suramos farm), Ekiti State, Nigeria of 130–146 g in weight. The animals were acclimatized for a week and permitted to food and drinking water. The protocol of Afe Babalola University Animal Ethical Committee (ABUAD/2020/SCI/BCH/011) was strictly abided throughout the experimental duration.

The animals' fasting blood glucose levels were studied after overnight fasting. Following that, the rats were injected intraperitoneally via a single dose (35 mg/kg) of freshly prepared streptozotocin (STZ) [using 0.1 M citrate buffer at pH 4.5]. At exactly 48 hours after induction, each rat fasting blood glucose level was measured by employing a Fine testTM glucometer. Surprisingly, all the induced animals had ≥ 200 mg/dL fasting blood glucose levels and are diabetic as previously reported by Ajiboye et al.¹².

2.4 Animal grouping

Five groups of animals (ten animals in each group) based on the body weight were grouped as follows:

- Group A: Non-diabetic rats given distilled water (normal control)
- Group B: Diabetic rats without treatment (diabetic control)
- Group C: Diabetic rats given a low dose (150 mg/kg body weight) of ST (LDST) (DR + LDST)
- Group D: Diabetic rats given a high dose (300 mg/kg body weight) of ST (HDST) (DR + HDST)
- Group E: Diabetic rats given 200 mg/kg body weight of metformin (ME) (DR + MET)

Note that the ST and MET were given to the respective animals via syringe and canular (with a round ball-like bead at the tip). Also, the doses employed was established from the earlier report by Ajiboye et al.¹³.

2.5 Sacrifice, collection of blood and hearts

At the end of the intervention period of two weeks, the

rats were sacrificed by cervical dislocation as previously documented by Ajiboye *et al.*¹³⁾. Blood was collected from rats into plain bottles and centrifuged at 1500 rpm for 30 minutes. Then the hearts were harvested and washed using 0.9% NaCl. The remaining parts of the heart were homogenized in phosphate buffer (pH 7.5) and centrifuged at 3000 rpm for ten minutes. The supernatants were collected and used for different analyses while the pellets were discarded.

2.6 Biochemical parameters studied

2.6.1 Biochemical parameters evaluated

The oxidative stress biomarkers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione, Glutathione-S-transferase (GST), and malondialdehyde (MDA) were carried out on the rat heart homogenates, serum lipid profiles, serum cardiac troponin I, and creatine kinase MB were estimated via RX Daytona Automated Analyser (Randox, UK) in line with the manufacturer's guidelines. Also, tumor necrosis factor (TNF)- α and C-reactive protein were evaluated in heart homogenate using ELISA kits (Biosource, USA) at 450 nm.

2.6.2 Quantitative real-time PCR analysis

A known gram (0.5 μ g) of the newly harvested heart was inserted into 20 μ L of Trizol[®]. Then, the total RNA of the organ was extracted through a cold solution of TRI reagent (Zymo Research, USA) by following the manufacturer's protocol. A spectrophotometer (Jen-way UV-VIS spectrophotometer model 6305, UK) was used to estimate the concentration of RNA (at 260 nm). This was subsequently converted into cDNA via M-MuLV Reverse Transcriptase Kit by following manufacturer procedure. Hereafter, 25 μ L of the solution comprising 2 μ L cDNA, 2 μ L primer, 12.5 μ L Ready Mix Taq PCR master mix and 8.5 μ L nuclease-free water were mixed.

The primer sequences used were indicated in Table 1.

Additionally, the quantification of the band images captured on the gel electrophoresis using 1.5% agarose was evaluated using Image-J software¹³⁾.

2.6.3 Histological examination of the heart

A small portion (1 gram) of the heart tissue obtained from each group was stored in a 10% formalin buffer. The tissues were then washed overnight, dehydrated with

graded alcohols, and embedded in paraffin wax. For histological evaluation, 5 μ m thick sequential section was deparaffinized and stained with hematoxylin and eosin (H&E)¹⁴⁾.

2.7 Data analysis

A graph pad prism 5 (GPP) software was employed in this study. This was done through One-way ANOVA as well as Tukey multiple range *post-hoc* test. The data were articulated as the means of ten (10) replicates $\pm$ standard deviation (SD), and a significant difference was fixed at $p < 0.05$.

3 Results

3.1 Aqueous extract of *Sterculia tragacantha* leaf suppresses oxidative stress biomarkers in the diabetic rats

Diabetic control animals exhibited a remarkable decrease in SOD, CAT, GPx, GST and GSH levels when compared to other groups. However, diabetic rats placed on the two doses (LDST and HDST) of aqueous extract of *Sterculia tragacantha* leaf as well as MET revealed a significant ($p < 0.05$) upsurge in SOD, CAT, GPx, GST and GSH levels. Also, there was no significant ($p > 0.05$) difference in SOD, CAT, GPx, GST and GSH levels in normal control and diabetic rats administered HDST. In addition, diabetic control rats demonstrated a significant ($p < 0.05$) increase in MDA levels when compared to other groups, but at the end of the experimental trial, diabetic rats administered LDST, HDST, and MET exhibited no momentous difference compared to normal control (Fig. 1). Moreover, there was a substantial increase in activities of CAT, SOD, GPx, GST and GSH in diabetic rats administered HDST when compared to diabetic rats administered LDST.

3.2 Aqueous extract of *Sterculia tragacantha* leaf ameliorates hyperlipidemia in diabetic rats

Diabetic control rats exhibited a significant ($p < 0.05$) rise in serum total cholesterol, triacylglycerols, and low-density lipoproteins (LDL) compared to other groups. There was no significant ($p > 0.05$) difference in total cholesterol, triacylglycerols, and low-density lipoproteins (LDL) of normal control and diabetic rats placed on HDST and MET. Diabetic control rats demonstrated a significant ($p < 0.05$) decline in

Table 1 Primers list.

S/No	Primer names	Forward primers (5' - 3')	Reversed primers (5' - 3')
1	GLUT 4	TCTCCGGTTCCTTGGGTTGT	TTCCCCATCTTCAGAGCCGAT
2	Fatty acid-binding protein 3 (FABP3)	ACCAAGCCGACCACAATCAT	CGACCGACTTGACCTTCCTG
3	Urotensin II	CTCTGGAGGAGCTGGAGAGG	CTTGCCCAGTGAGAGCCTTC
4	Actin, beta (Actb)	CTGGCTCCTAGCACC	ATGAACGCAGCTCAGTAACAGTCCG

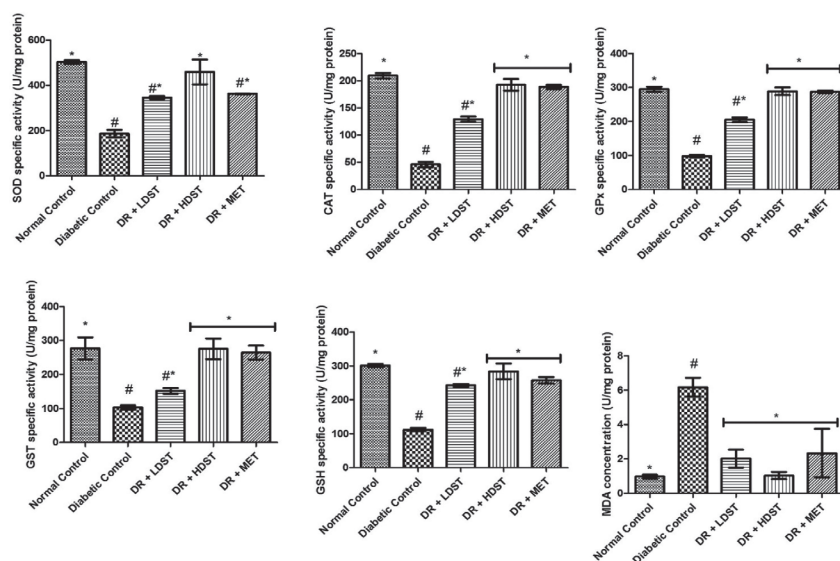


Fig. 1 Aqueous extract of *Sterculia tragacantha* leaf suppresses oxidative stress biomarkers in the heart of STZ-induced diabetic rats.

Data expressed as mean $\pm$ SD, n = 10. * Mean is significantly different compared to Diabetic Control at $p < 0.05$; # Mean is significantly different compared to Normal Control

Legend: DR: Diabetic rats; LDST: Low dose of *Sterculia tragacantha* leaves (150 mg/kg); HDST: High dose of *Sterculia tragacantha* leaves (300 mg/kg); MET: metformin (200 mg/kg); SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GST: Glutathione S-transferase; GSH: Reduced Glutathione; and MDA: Malondialdehyde

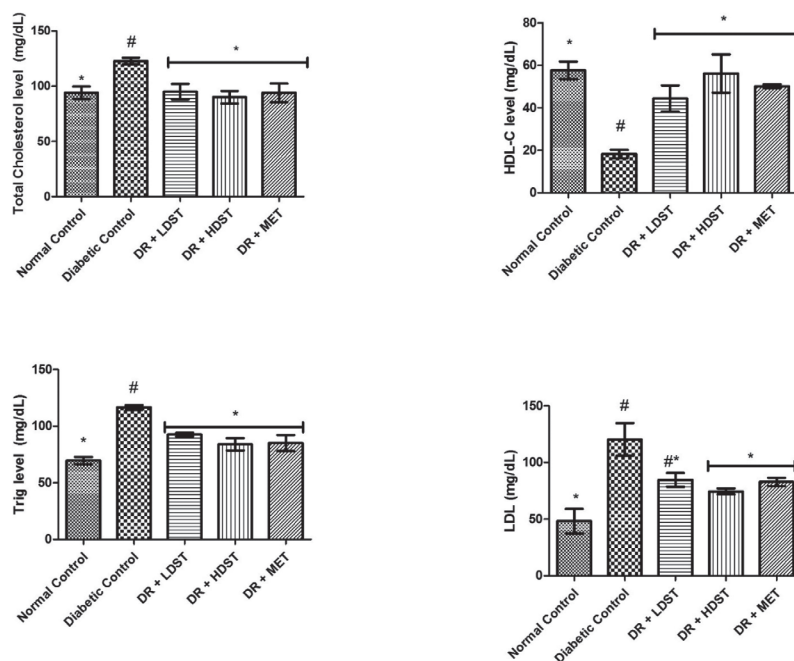


Fig. 2 Aqueous extract of *Sterculia tragacantha* leaf ameliorates hyperlipidemia in STZ-induced diabetic rats.

Data expressed as mean $\pm$ SD, n = 10. * Mean is significantly different compared to Diabetic Control at $p < 0.05$; # Mean is significantly different compared to Normal Control

Legend: DR: Diabetic rats; LDST: Low dose of *Sterculia tragacantha* leaves (150 mg/kg); HDST: High dose of *Sterculia tragacantha* leaves (300 mg/kg); MET: metformin (200 mg/kg); HDL-C: High density lipoprotein-cholesterol; Trig: Triacylglycerols; and LDL: Low density lipoprotein

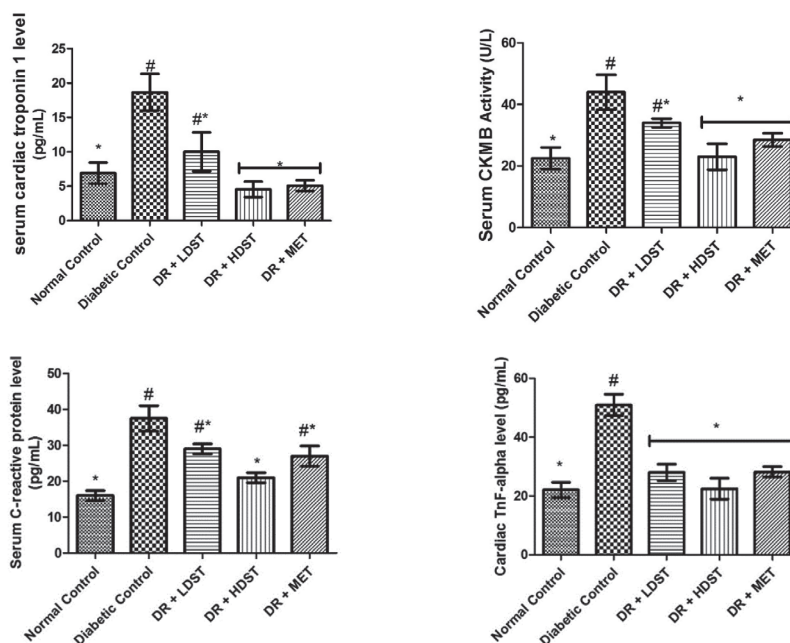


Fig. 3 Aqueous extract of *Sterculia tragacantha* leaf ameliorates inflammation and apoptosis in STZ-induced diabetic rats. Data expressed as mean $\pm$ SD, n = 10. * Mean is significantly different compared to Diabetic Control at $p < 0.05$; # Mean is significantly different compared to Normal Control

Legend: DR: Diabetic rats; LDST: Low dose of *Sterculia tragacantha* leaves (150 mg/kg); HDST: High dose of *Sterculia tragacantha* leaves (300 mg/kg); MET: metformin (200 mg/kg); CKMB: Creatine kinase-MB; TNF- α : Tumour Necrosis Factor alpha

HDL levels compared to both normal control and diabetic treated groups. But diabetic rats placed on LDST, HDST and MET exhibited no significant ($p > 0.05$) difference in HDL levels compared to the normal control group (Fig. 2).

3.3 Aqueous extract of *Sterculia tragacantha* leaf inhibits inflammation and apoptosis in diabetic rats

Diabetic control rats demonstrated a significant ($p < 0.05$) rise in the level of cardiac troponin I and CKMB levels compared to other groups. Hence, diabetic rats treated with LDST, HDST, and MET revealed a significant ($p < 0.05$) reduction in cardiac troponin I and CKMB levels. Although diabetic rats treated with HDST and MET presented no significant ($p > 0.05$) difference with normal control rats as indicated in Fig. 3. Also, in diabetic control animals, there was a significant ($p < 0.05$) increase in the levels of C-reactive protein when compared to other groups. Whereas, diabetic rats that received HDST indicate no significant ($p > 0.05$) difference compared with normal control rats. Furthermore, diabetic control rats show a significant ($p < 0.05$) increase in the level of TNF- α compared to other groups. This was drastically reduced in all the treated groups with no significant ($p > 0.05$) difference in diabetic rats given LDST, HDST and MET when linked to normal control rats.

3.4 Aqueous extract of *Sterculia tragacantha* leaf on relative gene expression of glucose transport 4 (GLUT-4), fatty acid-binding protein 3 (FABP 3), and urotensin II in diabetic rats

There was a substantial reduction in GLUT-4 relative gene expression in the diabetic control group when linked to other groups. There was no significant ($p > 0.05$) difference in GLUT-4 relative gene expression of diabetic animals treated with HDST and MET when related to normal control rats (Fig. 4).

In Fig. 4, there was a noticeable reduction in relative gene expression of FABP3 in diabetic control animals when related to other groups. Also, there was a substantial difference in relative gene expression of FABP3 in diabetic rats treated with LDST, HDST and MET linked with both normal and diabetic control groups.

There was a noticeable upsurge in the relative gene expression of urotensin II in diabetic control rats over other groups. Also, the diabetic rats treated with LDST and HDST exhibited a significant ($p < 0.05$) difference when related to both normal and diabetic control rats (Fig. 4).

3.5 Aqueous extract of *Sterculia tragacantha* leaf on heart histopathology examination in diabetic rats

Figure 5 shows the histological examinations in the heart of diabetic rats administered aqueous extract of *S. tragacantha* leaves. There were severe degradation, congestion,

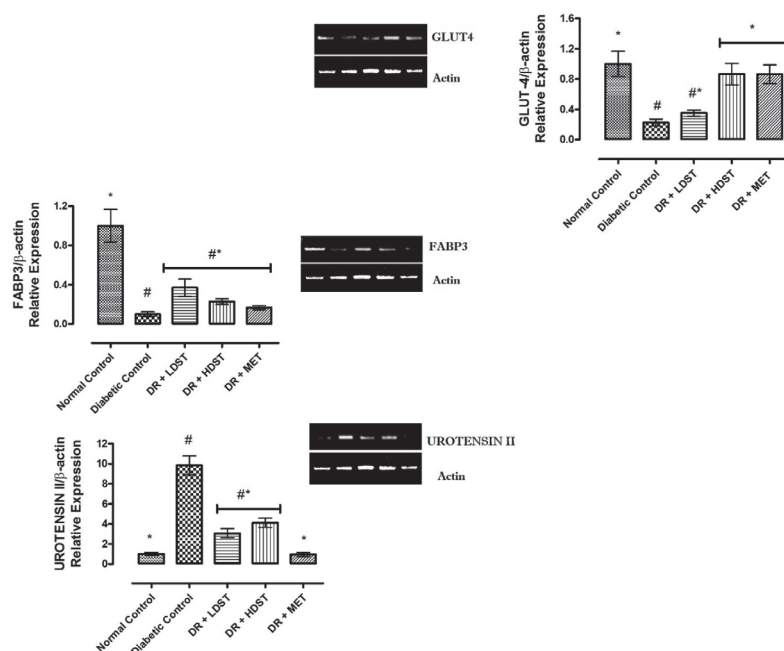


Fig. 4 Aqueous extract of *Sterculia tragacantha* leaf on relative gene expression of glucose transport 4 (GLUT-4), fatty acid-binding protein 3 (FABP 3), and urotensin II in STZ-induced diabetic rats.

Data expressed as mean $\pm$ SD, n = 10. * Mean is significantly different compared to Diabetic Control at $p < 0.05$; # Mean is significantly different compared to Normal Control

Legend: DR: Diabetic rats; LDST: Low dose of *Sterculia tragacantha* leaves (150 mg/kg); HDST: High dose of *Sterculia tragacantha* leaves (300 mg/kg); MET: metformin (200 mg/kg); GLUT-4: Glucose transport 4; FABP 3: Fatty acid-binding protein 3

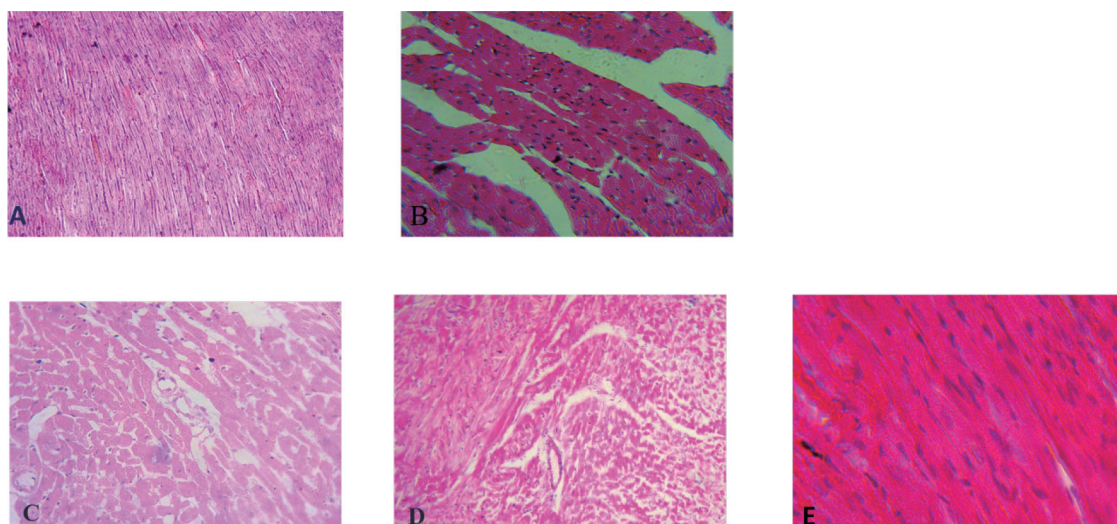


Fig. 5 Aqueous extract of *Sterculia tragacantha* leaf on heart histopathology in STZ-induced diabetic rats. Magnification: X 400

Legend: A- Normal control, B- Diabetic control, C- Diabetic rats administered LDST [Low dose of *Sterculia tragacantha* leaves (150 mg/kg)], D- Diabetic rats administered HDST [High dose of *Sterculia tragacantha* leaves (300 mg/kg)], E- Diabetic rats administered MET [Metformin (200 mg/kg)]

necrosis and inflammation in diabetic control heart tissues linked to other groups. However, at the end of the experimental trial, this was ameliorated in diabetic rats especially

in those that received HDST with normal degradation, congestion, necrosis and inflammation like the normal control group.

4 Discussion

The major approach of controlling diabetes mellitus is by lowering fasting blood glucose levels as well as all the associated complications. In this study, streptozotocin (STZ) was employed to mimic diabetes mellitus in experimental animals. A low dose of STZ was used to cause partial beta cell destruction, resulting in mild insulin deficiency¹⁵⁾. This exhibited hyperglycemia in the animals, probably linked to the partial destruction of pancreatic beta-cell due to abnormal production of reactive oxygen species (ROS) by the diabetogenic agent used¹²⁾. Hence, the administration of aqueous extract of *Sterculia tragacantha* (ST) leaf possibly demonstrated normoglycaemic potential on those diabetic animals as demonstrated in Figs. 1 to 5. This could be attributed to the antioxidant properties of ST leaf in protecting pancreatic β -cells against ROS, which encourages an upsurge in insulin secretion¹⁶⁾.

One of the proposed mechanisms of action of this plant extract as anti-diabetic cardiomyopathy was its ability to counteract oxidative stress as indicated in Fig. 1. Persistent hyperglycemia stimulates the generation of ROS and reactive nitrogen species (RNS) in the heart mitochondria¹⁷⁾. Surplus ROS support rises in lipid peroxidation (MDA) and subsequently changes membrane structure and enzyme activities¹⁸⁾. In this study, diabetic rats presented momentarily raised in MDA probably due to accumulation of free radicals which might lead to DNA fragmentation and protein damage of the heart tissue. This is in accordance with the previous study carried out by Alabi *et al.*¹⁷⁾. However, this was ameliorated in diabetic rats that received ST leaf (LDST and HDST) probably due to its antioxidant ability. In another word, diabetic animals showed a reduction in cardiac GSH, designating its excessive usage in the redox-tackled cellular vicinity¹⁹⁾. SOD, an enzyme involved in the dismutation of superoxide, CAT and GPx are enzymes that catalyze the breakdown of hydrogen peroxide (free radical) to water and oxygen²⁰⁾, and GST is a detoxification enzyme that functions in safeguarding cellular macromolecules from attack by reactive electrophiles²⁾. The levels of all these (enzymatic and non-enzymatic) were noticeably increased in diabetic animals treated with the two doses of ST leaf, which were drastically reduced in diabetic rats, supporting their roles in scavenging the generated ROS due to STZ. This may be associated with ROS mopping up the ability of the extract. Thus, this is in accordance with the previous report by Al-Rasheed *et al.*⁴⁾.

In addition, hyperlipidemia is an additional element of cardiac dysfunctions that can cause death in individuals with diabetes mellitus²¹⁾. The findings obtained (Fig. 2) showed that diabetic rats developed hyperlipidemia because of elevated levels of their lipid profiles with a reduction in HDL-C, presumably because of increment in the activation of free unsaturated fats from the fringe terminals. Hence, diabetic rats placed on LDST and HDST ame-

liorate hyperlipidemia by increasing HDL-C, which makes it beneficial in the management of diabetic cardiomyopathy.

According to Frati *et al.*²²⁾, oxidative stress and inflammation are acknowledged in promoting diabetic cardiomyopathy via cardiac hypertrophy. In this study, persistent of hyperglycemia motivate cardiomyocyte damage as evidence in elevated serum CK-MB and cardiac troponin I (Fig. 3). Creatine kinase-MB (CK-MB) is an enzyme present mainly in the heart muscle cells, as one of the isoenzymes of creatine-kinase-CK. On the other hand, cardiac troponin I play an important role in the binding of actin in thin myofilaments to hold the actin-tropomyosin complex in order, it also averts myosin from binding to actin in the relaxed muscle²³⁾. One of the ways of detecting myofibrillar degeneration is by increased circulating CK-MB and cardiac troponin I²⁴⁾ as noticed in this study. This may be linked to an increase in the level of inflammation as indicated in Fig. 1. However, diabetic rats administered LDST and HDST revealed a significant decline in the levels of these biomarkers probably connected to the anti-inflammatory ability of the extract as illustrated in Fig. 1. This is in line with the previous report by Safhi *et al.*²⁵⁾.

Suzuki *et al.*²⁶⁾ stated that cardiac inflammation, apoptosis, and fibrosis are all important factors in DCM. The persistent increase in ROS generation in STZ-induced diabetes animals may be responsible for an increase in the level of circulating CRP and TNF- α (Fig. 3). C-reactive protein (CRP) serves as innate immunity, as well as an early defense system against infections in the human body system²⁷⁾. Tumour Necrosis Factor-alpha (TNF- α), is an inflammatory cytokine produced by macrophages during acute inflammation (trigger by ROS) leading to necrosis²⁸⁾. Remarkably, diabetic rats maintained on LDST and HDST showed a significant reduction in serum CRP and TNF- α levels. Therefore, this corroborates the anti-inflammatory ability of the plant extract used as previously reported in Fig. 1.

Thorens and Mueckler²⁹⁾ reported that mammalian cells consume glucose, mainly for energy generation, via glucose transporter proteins. GLUT-4 is a glucose transporter responsible for glucose uptake into the cardiac muscles. In the diabetes mellitus condition, the level of GLUT-4 is always hindered in cardiac muscles which encourages hyperglycemia in the bloodstream³⁰⁾. This was witnessed in this study (Fig. 4), however, the administration of LDST and HDST into the diabetic animals was able the increase the level of GLUT-4 gene expression and possibly promote glucose assimilation into the cardiac muscles.

Urotensin-II gene (U-II) is a well-known factor in vascular contraction in mammals, especially in the heart³¹⁾. The U-II plays an important role in insulin resistance, impaired glucose tolerance, impaired glucose transport to muscles through GLUT-4, etc.³²⁾. Therefore, over-expression of the

cardiac U-II gene is an indicator of DCM as shown in Fig. 4, also, its over-expression can be linked to its noticeable decreased in GLUT-4 levels as indicated in (Fig. 4). Interestingly, diabetic animals maintained on LDST and HDST were able to normalize the level of U-II, probably due to an increase in GLUT-4 relative gene expression as demonstrated by this extract. This is in accordance with the report of Jafria *et al.*³⁰⁾.

According to Kusudo *et al.*³³⁾, fatty acid-binding proteins 3 (FABP3) are known as small cytosolic proteins, belonging to a superfamily of lipid-binding proteins. FABP3 expressions are present in series of cells, particularly the heart cells. FABP3 is involved in fatty acid (FA) uptake, transport, esterification, and β -oxidation for energy generation, particularly in the heart muscles³⁴⁾. Hence, in this study, the significant decrease in FABP3 expression in diabetic control animals may be associated with over usage of this protein in FA uptake due to non-useful of the available glucose for the body system. But at the end of the experimental trial, the LDST and HDST were able to boost the expression of this protein, which may be associated with their ability to make glucose available into the cells and render a decrease in FA uptake (Fig. 4).

Moreover, the histological examination of the diabetic control heart (Fig. 5) showed myocardium degeneration as

well as improved collagen deposition. This may be linked to oxidative stress, due to persistent hyperglycaemia experienced in this study. This is in line with the early work described by Al-Rasheed *et al.*¹⁶⁾. In another vein, cardiomyocytes, as well as inflammatory cells, encourage fibrosis in the animal's diabetic control heart due to the release of cytokines and profibrotic factors²⁵⁾. However, administration of LDST and HDST noticeably reduced the synthesis of collagen and fibrosis, presumably via its antioxidant booster ability which led to a reduction in heart inflammation in diabetic animals. Therefore, the administration of ST inhibited histological alterations and deposition of collagen in the heart of diabetic animals. Added to this, the proposed mechanism of action of ST is illustrated in Fig. 6.

5 Conclusion

It can be presumed from this study that aqueous extract of *Sterculia tragacantha* leaf ameliorated hyperlipidemia, oxidative stress biomarkers by increasing the antioxidant enzyme activities and decrease in lipid peroxidation, enhanced CK-MB, CRP, cardiac troponin I, TNF- α , and urotensin-II levels, and improved GLUT4 and FABP3 expressions, supported by histological examination. However,

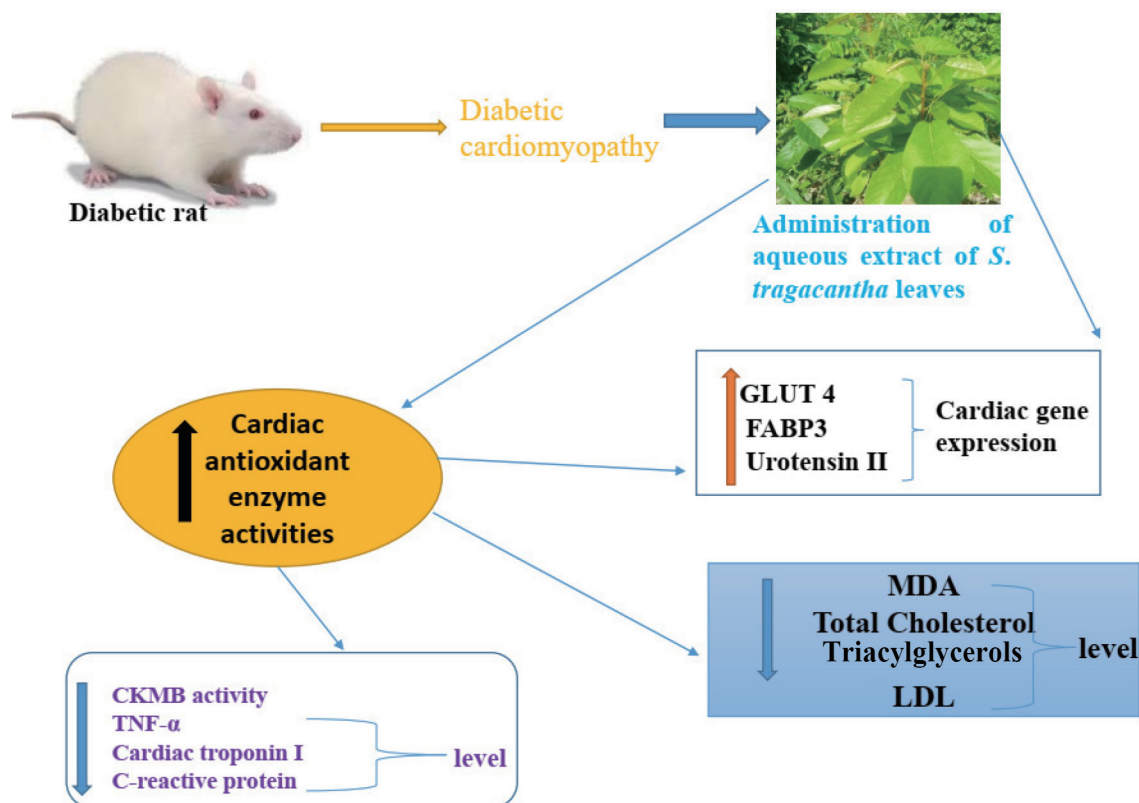


Fig. 6 Proposed mechanism of action of diabetic cardiomyopathy rats administered aqueous extract of *S. tragacantha* leaves.

more research into the isolation of different phytochemicals from this plant is required to elucidate the proper mechanism of action as an anti-cardiomyopathy agent.

Declarations

Author Contributions

This research was designed by BOA, BEO, OAO, and FAB; BOA, BEO, OAO, FAB, SAO, and OEL performed the research; BOA, BEO, OAO, FAB, SAO, and OEL contributed to the analytic tools and analyzed the data; and the manuscript was written by BOA, BEO, OAO, FAB, SAO, and OEL.

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Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

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Competing Interests: The authors declare no conflict of interest.

Availability of data and materials: Only on request from the corresponding author.

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