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Original Article

Terminalia catappa Leaf Abrogates Diabetes-induced Dyslipidaemia in Type 2 Diabetic Rats by Upregulating Lipid Metabolic Genes

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Abstract

Background and objectives: Insulin resistance and hyperinsulinemia in type 2 diabetes mellitus induced dyslipidemia. This study aims to investigate the abrogative role of *Terminalia catappa* (*T. catappa*) leaf aqueous extract (TCLAE) on diabetes-induced dyslipidaemia in type 2 diabetic rats.

Methods: Diabetic rats were induced by fat-rich feed for eight weeks and intraperitoneal streptozotocin (STZ, 30 mg/kg) injection, while glibenclamide (10 mg/kg) and TCLAE-graded doses were orally administered for four weeks. Then, the biomarkers for diabetes, liver function, lipid profile, cardiovascular indices, and liver histology were measured, in addition to the hepatic expression of some lipid metabolic genes.

Results: TCLAE reduced the diabetes-induced fasting blood glucose, weight loss, plasma insulin, alanine transaminase, bilirubin, cholesterol (CHOL), triglyceride (TRIG), low-density lipoprotein-CHOL and low-density lipoprotein-TRIG. TCLAE also decreased the abnormal cardiovascular indices. TCALE significantly enhanced the high-density lipoprotein-CHOL, the expression of peroxisome proliferator-activated receptor alpha (PPAR-α), PPAR delta (PPAR-δ), and carnitine palmitoyltransferase 1a, and decreased the hepatic expression of C-reactive protein of type 2 diabetic rats.

Conclusion: TCLAE alleviates diabetes-induced dyslipidaemia in type 2 diabetic rats by ameliorating the altered expression of lipid metabolic genes.

Keywords: *Terminalia catappa*; Streptozotocin; Lipid metabolic genes; Type 2 diabetes; Dyslipidaemia; Cardiovascular indices.

Abbreviations: AI, atherogenic index; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; AUC, area under the curve; BIL, bilirubin; CHOL, cholesterol; CRI, coronary risk index; CRP, C-reactive protein; CVD, cardiovascular disease; DM, Diabetes mellitus; FBG, fasting blood glucose; FFA, free fatty acid; GLB, Glibenclamide; GLUC, glucose; HDL, high-density lipoprotein; HFD, high-fat feeding; HTR, HDL/TRIG ratio; INS, insulin; IR, insulin resistance; LDL, low-density lipoprotein; PPAR, peroxisome proliferator-activated receptor; STZ, streptozotocin; TCL, *T. catappa* leaves; TCLAE, *Terminalia catappa* leaf aqueous extract; TRIG, triglyceride; TyG, triglyceride-glucose index; T2DM, type 2 diabetes mellitus.

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Introduction

Diabetes mellitus (DM) is a metabolic abnormality majorly characterised by chronic hyperglycaemia, as a result of insulin secretion dysfunction by β-cells in the pancreatic islets and/or defects in insulin action on peripheral target tissues. Polydipsia, polyuria and polyphagia are some of the accompanying symptoms that result from persistent high blood sugar.[1] DM presently affects 537 million adults worldwide, and 3 of 4 adults in middle- and low-income countries live with diabetes. This disease remains as a recurring public health challenge, and almost a trillion US dollars have been spent on health expenditures. In Africa, 24 million adults (one of 22 adults) presently have diabetes, and this figure would likely increase by 129% (increase to 55 million) in the next 20 years.[2] Type 2 diabetes mellitus (T2DM) is the most common diabetes that particularly affects Sub-Saharan Africa and Nigeria, due to the increase in obesity incidence.[1] Cardiovascular disease (CVD) is one of the major diabetic

Table 1. Experimental design

Group	Animals (Feed)	Treatment
Normal	Normal rats (Normal fat diet)	Distilled water (1 mL/kg bw)
DB	Diabetic rats (High fat diet)	Distilled water (1 mL/kg bw)
GLB	Diabetic rats (High fat diet)	Glibenclamide (10 mg/kg bw)
TCLAE4	Diabetic rats (High fat diet)	TCLAE (400 mg/kg bw)
TCLAE8	Diabetic rats (High fat diet)	TCLAE (800 mg/kg bw)

DB, diabetic; GLB, glibenclamide; TCLAE, *T. catappa* leaf aqueous extract.

complications that arise in type 2 diabetics, and contributes to diabetes pathophysiology with dyslipidaemia as the driver.[3]

Insulin plays an important role in glucose homeostasis, lipid metabolism, and circulating serum lipids by regulating some of the energy-metabolising tissues (liver, adipose, and skeletal muscle tissue), and the transcriptional induction of lipogenic genes via the AKT2 phosphorylation of SREBP1 and FOXO1.[4,5] During T2DM, insulin resistance (IR) and hyperinsulinemia are the prolonged features that occur before β-cell destruction and low insulin production, causing lipid metabolism dysfunction and dyslipidaemia.[6] In T2DM, the hepatic regulation of lipid metabolism is impaired due to hyperglycaemia and hyperinsulinemia, through the increase in *de novo* lipogenesis,[7] overproduction of hepatic low-density lipoprotein,[8] and poor clearance of free fatty acid (FFA) and lipoprotein from circulation.[9] In addition, the transcription factors that activate lipogenic genes are highly expressed during T2DM onset and hepatic impairment.[7,10] This leads to increased citrate translocation into the cytoplasm from the mitochondria, inducing the synthesis of fatty acid to yield fatty acids, triglycerides, phospholipids, acylglycerols, ceramides, and other metabolic end products.[11,12] This tilts the balance between systemic lipid delivery and uptake, causing hepatic hypertriglyceridemia and excessive lipid species accumulation, while activating pathways that progress T2DM.[13,14] Furthermore, this alters the hepatic secretory and membrane integrity, increasing the plasma concentration of hepatic enzymes, which may lead to liver damage.[15]

T2DM-induced lipogenesis occurs in the adipose, muscles and pancreas, with the most effect on the liver, since this affects the distribution and production of lipid metabolites.[16] This interrupts the energy homeostasis via dysfunctional glucose and lipid metabolism, interorgan crosstalk disturbance, and hepatic homeostasis disruption, which leads to increased toxicant exposure, liver damage, and non-alcoholic fatty liver disease.[17,18] Lipid dysfunction during T2DM concomitantly induces interleukin-6 and tumour necrosis factor-alpha expression, promoting lipolysis, inhibiting insulin receptor substrate 1, and downregulating the expression of peroxisome proliferator-activated receptors (PPARs).[19–21] Clinically prescribed therapies for managing T2DM and preventing CVD scourge are becoming less effective, with numerous side effects. Hence, identifying novel therapeutic strategies that truncate dyslipidaemia might be useful in preventing CVD development, and further metabolic derangement.[22–24] A previous study revealed that by improving the impaired antioxidant system and downregulating proinflammatory genes, *Terminalia catappa* L. palliates oxidative stress.[25] Thus, this might play a role in dyslipidaemia management in T2DM. Hence, the present study determined the abrogative role of *Terminalia catappa* (*T. catappa*) leaf aqueous extract (TCLAE) on diabetes-induced dyslipidaemia in obese diabetic (DB) rats, and investigated some of the hepatic genes involved in lipid metabolism.

Materials and methods

Reagents and chemicals

The streptozotocin (STZ) and one-step EasyScript RT-PCR kit were procured from Solarbio Life Sciences (China) and TransGen Biotech (China), respectively. The insulin and adiponectin ELISA kits were purchased from Solarbio Life Sciences (China) and Easthangzhou Biopharm (China), respectively. The molecular primers and agarose gel were sourced from Integrated DNA Technologies (USA) and Sigma Aldrich (Germany), respectively, while the biochemical diagnostic kits were purchased from Randox Laboratories (UK). All other analytical-grade organic chemicals and reagents were purchased from relevant vendors.

Collection, authentication and plant extract preparation

The *T. catappa* leaves (TCL) were sourced from fruiting trees in Covenant University, Ota, Nigeria, which were authenticated and specimen deposited (FHI 112775) in FRIN, Nigeria. The aqueous crude extract of the leaf (TCLAE) was concocted by concentrating the aqueous filtrate using a Stuart RE 300/MS rotary evaporator (Staffordshire, UK).[26] Then, the leaves were shade dried for two weeks, pulverised, macerated (5% w/v) in distilled water for three days, and filtered to obtain the aqueous filtrate. The ethical guidelines/regulations on plant usage pertinent to local and national jurisdictions were adhered to in the present study.

Study animals and determination of dosage

The male Wistar rats (n = 30, 200 ± 20 g, approximately seven weeks of age) used for the present study were purchased from the University of Lagos Medical College, and acclimatised for two weeks before experimentation. *Ad libitum* provision of food and water was put in place with optimal husbandry conditions (humidity, 50 ± 5%; room temperature, 23 ± 2°C; day/night cycle). The experimental protocol followed the institutional animal care and handling guidelines documented in the National Institutes of Health (NIH) and Animal Research: Reporting of *In vivo* Experiments (ARRIVE) guidelines, and was approved by the Health Research Ethics Committee of Covenant University, with Approval no. CHREC/031/2018. The TCLAE dose used for the present study was determined, as previously established.[27]

Diabetes induction and experimental design

T2DM was induced by high-fat feeding (HFD) for eight weeks and intraperitoneal injection of STZ (30 mg/kg bw), as previously reported.[27] Then, the animals (fasting blood glucose [FBG] ≥250 mg/dL) were randomly divided into five groups (n = 6, Table 1), and orally treated for four weeks. The normal and DB group comprised non-DB and DB animals, respectively, and were administered with distilled water (1 mL/kg bw). The glibenclamide (GLB), TCLAE4

Table 2. Primer-specific gene sequence and annealing temperature

Gene	Primer sequence (5′-3′)	Annealing temperature (°C)	Reference
PPAR-α	AATCCACGAAGCCTACCTGA (F)	58	NM_013196.2
	GTCTTCTCAGCCATGCACAA (R)		
PPAR-δ	AGGCCTCAGGCTTCCACTAC (F)	56	NM_013141.2
	TTGCGGTTCTTCTTCTGGAT (R)		
CRP	TGTCTCTATGCCCACGCTGATG (F)	54	NM_017096.4
	GGCCCACCTACTGCAATACTAAAC (R)		
CPT-1a	AAGTCAACGGCAGAGCAGAG (F)	60	NM_031559.2
	ACGCCCAAGTATTCACAGGG (R)		
AdipoR2	ACATGCTCCAAGAGATCTCCAG (F)	55	NM_139192.2
	GTACTCCAGCTTGGGCGG (R)		
GAPDH	CTGACATGCCGCCTGAAAC (F)	51	Iheagwam *et al.*[27]
	CCAGCATCAAAGGTGGAAGAA (R)		

AdipoR2, adiponectin receptor 2; CPT-1, carnitine palmitoyltransferase 1; CRP, C-reactive protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPAR-δ, peroxisome proliferator-activated receptor delta; PPAR-α, peroxisome proliferator-activated receptor alpha.

and TCLAE8 groups comprised of DB rats, and were administered with glibenclamide (10 mg/kg bw) and TCLAE (400 and 800 mg/kg bw, respectively). The weight and FBG of the animals were monitored throughout the study period. These rats underwent overnight fasting for approximately 15 hours before anaesthesia (xylazine/ketamine 1:10 v/v), and were sacrificed at the end of the experiment.

Sample preparation

The animals were sacrificed using the cardiac puncture method, with blood collected in heparinised bottles, and separated into erythrocytes and plasma. Then, the hepatic and renal tissues were removed, primed and stored, while a section of the excised liver was immersed in 10% formal saline for histological evaluation, according to a previous procedure.[28]

Biochemical evaluation

The activity of plasma aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), plasma bilirubin (BIL), albumin (ALB), insulin (INS), and glucose (GLUC) was assessed using the Randox diagnostic and Hangzhou Eastbiopharm ELISA kits, according to the instruction manual. The plasma glucose area under the curve (AUC) was calculated using Equation 1, as described by Sakaguchi *et al.*[29]

$$\mathrm{AUC\,(mg\cdot H/dL)} = \frac{(\mathrm{BG}_{0\ \mathrm{mins}}) + (\mathrm{BG}_{30\ \mathrm{mins}} \times 2) + (\mathrm{BG}_{60\ \mathrm{mins}} \times 3) + (\mathrm{BG}_{120\ \mathrm{mins}}) \times 2}{4} \quad (1)$$

Next, the low-density lipoprotein (LDL), cholesterol (CHOL), triglyceride (TRIG), and high-density lipoprotein (HDL) concentrations were evaluated in the erythrocytes, liver, plasma, and kidneys using the Randox diagnostic kit, according to the manual. The concentration of adiponectin in plasma was assessed using the Solarbio ELISA kit, according to the instructions in the manual. The FFA plasma concentration was analysed using the method described by Soloni and Sardina.[30]

Cardiovascular indicators

The HDL/TRIG ratio (HTR) and atherogenic index (AI) were evaluated according to the method described by Sheela and Augusti,[31] while the coronary risk index (CRI), triglyceride-glucose index (TyG), and Disse index were evaluated, as explained by Mohammed *et al*.,[32] Liu *et al.*,[33] and Antuna-Puente *et al.*[34] These indices were calculated, as follows (Equations 2–6):

$$\mathrm{HTR} = \frac{\text{High-density lipoprotein cholesterol}}{\text{Triglyceride}} \quad (2)$$

$$\mathrm{AI} = \frac{\text{Total cholesterol} - \text{High-density lipoprotein cholesterol}}{\text{High density lipoprotein cholesterol}} \quad (3)$$

$$\mathrm{CRI} = \frac{\text{Total cholesterol}}{\text{High-density lipoprotein cholesterol}} \quad (4)$$

$$\mathrm{TyG} = \ln \frac{\text{fasting triglycerides} \times \text{fasting glucose}}{2} \quad (5)$$

$$\mathrm{Disse} = 12 \times \left[2.5 \times \left(\frac{\text{High density lipoprotein cholesterol}}{\text{Total cholesterol}} - \mathrm{FFA} \right) \right] - \text{Fasting Insulin} \quad (6)$$

Gene expression analysis

The total hepatic RNA was extracted using the trizol method before examining the expression of C-reactive protein (*CRP*), peroxisome proliferator-activated receptor alpha (*PPAR-α*), carnitine palmitoyltransferase 1a (*CPT-1a*), PPAR delta (*PPAR-δ*), and adiponectin receptor 2 (*AdipoR2*), and primers specific to these genes and appropriate parameters for the synthesis of cDNA were used, with GAPDH as the reference gene (Table 2). The amplicons were run on 1.5% agarose gel dyed with ethidium bromide, and viewed under the UVP bioimaging system (CA, USA).[25]

Hepatic histology

The fixed portion of the excised liver was histologically assessed, as previously described by Chinedu *et al.*[35] The dehydration of the

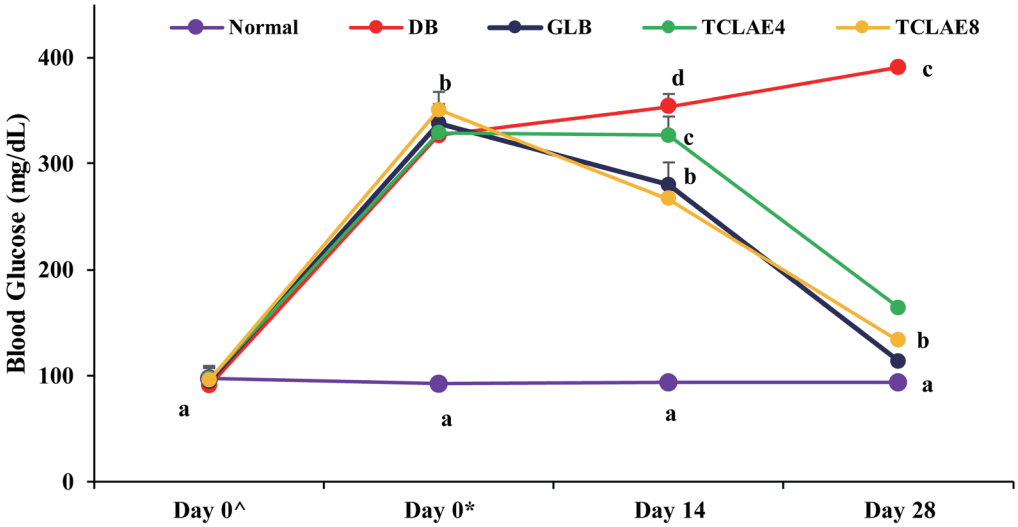

Fig. 1. Effect of TCLAE treatment on the daily fasting blood glucose of T2DM rats. The points refer to the mean ± standard error of the mean (SEM) of six animals. The points with different superscripts (a,b,c,d) per day are significantly different, while those with the same superscript are not significantly different at 95% CI. DB, diabetic; GLB, glibenclamide; TCLAE, *T. catappa* leaf aqueous extract; T2DM, type 2 diabetes mellitus; ∧, before induction; *, after induction.

liver was facilitated by graded ethanol concentration before this was cleaned with xylene, infused and immersed in paraffin wax, placed on glass slides, sectioned (5 µm), and dyed using the H&E stain. Then, the slides were viewed using the Leica SCN 4000 scanner (Wetzler, Germany). The assessing pathologist was blinded to the grouping of samples, in order to prevent bias.

Statistical analysis of the data

The results were presented as the mean ± standard error of the mean (SEM) of six animals after undergoing two-way ANOVA, and the mean differences between groups were considered at a 95% confidence level using Duncan's multiple range test on the SPSS version 25 (IBM, NY, USA).

Results

TCLAE treatment restores abnormal diabetic parameters

The induction of T2DM significantly ($p < 0.05$) increased the monitored FBG, with a dose-dependent decrease ($p < 0.05$) observed by the 14th day in TCLAE-administered rats, when compared to the DB group. On the last day of TCLAE treatment, both doses significantly reduced the monitored FBG, when compared to rats administered with GLB (Fig. 1). Figure 2 presents austere weight loss in DB animals after STZ induction. The DB rats administered with TCLAE began to significantly gain weight ($p < 0.05$) from the 14th day, until the 28th day. The most observed weight gain effect was after the 800 mg/kg bw TCLAE treatment, when com-

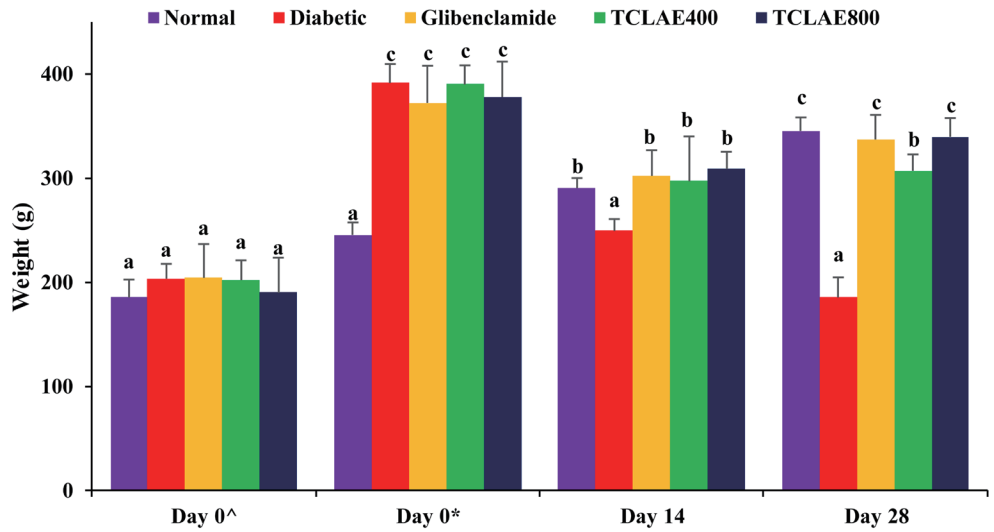

Fig. 2. Effect of TCLAE treatment on the body weight of T2DM rats. The bars refer to the mean ± standard error of the mean (SEM) of six animals. The bars with different superscripts (a,b,c) per day are significantly different, while those with the same superscript are not significantly different at 95% CI. The same superscripts per day refer to no significance at 95% CI. TCLAE, *T. catappa* leaf aqueous extract; T2DM, type 2 diabetes mellitus; ^, before induction; *, after induction.

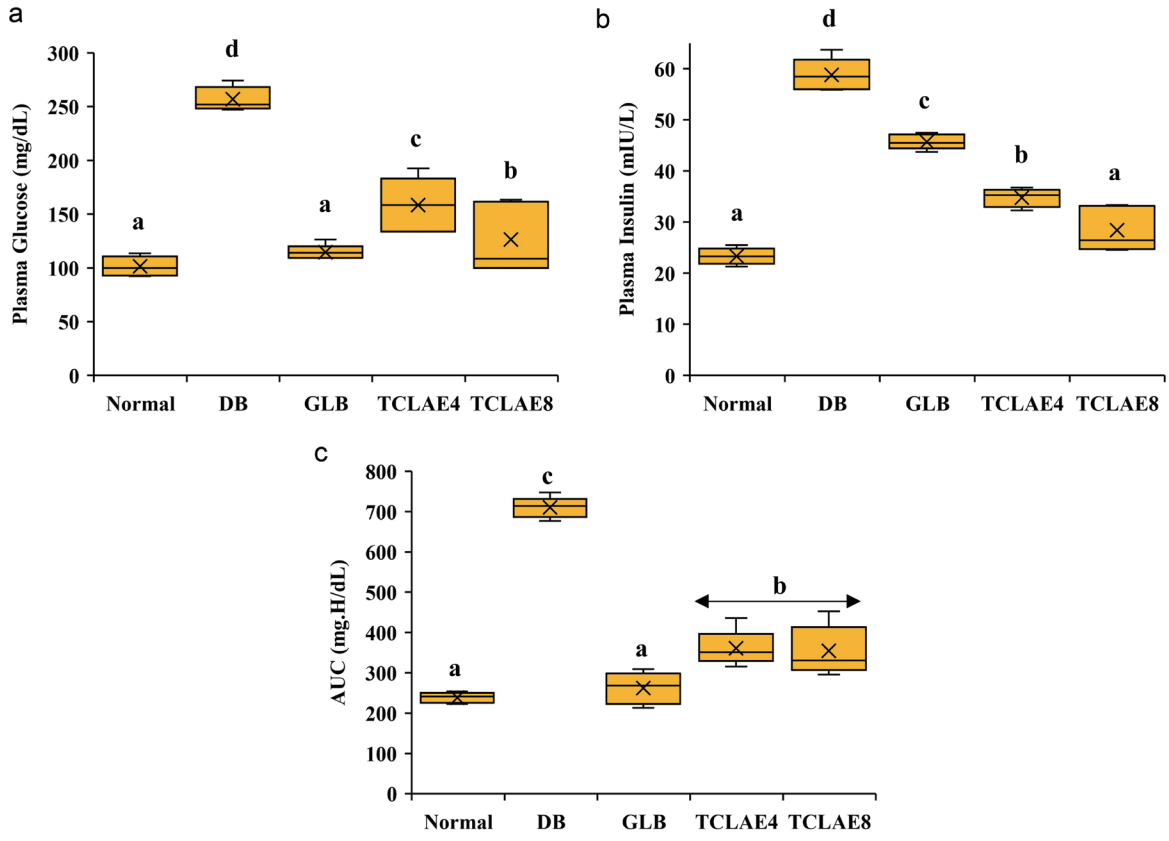

Fig. 3. Effect of TCLAE treatment on the plasma (a) glucose, (b) insulin and (c) glucose area under the curve of T2DM rats. The box plots present the mean ± standard error of the mean (SEM) of six animals. The box plots with different superscripts (a,b,c,d) are significantly different, while those with the same superscript are not significantly different at 95% CI. DB, diabetic; GLB, glibenclamide; TCLAE, *T. catappa* leaf aqueous extract; T2DM, type 2 diabetes mellitus.

pared to normal and GLB-treated rats. In Figure 3, the T2DM increased ($p < 0.05$) the plasma glucose (from 101.42 to 257.06 mg/dL), INS (from 23.33 to 58.79 mIU/L), and AUC (from 283.65 to 710.20 mg.H/dL) in the experimental animals. However, the GLB and TCLAE treatment (in both experimental doses) reduced ($p < 0.05$) the plasma glucose (111.46, 158.51, and 126.32 mg/dL, respectively), INS concentration (45.72, 34.76, and 28.44 mIU/L, respectively), and AUC (262.30, 360.75, and 354.35 mg.H/dL, respectively) in DB rats.

Effect of TCLAE treatment on diabetes-induced liver dysfunction

The data in Table 3 illustrates the increase of 11.9% and 259.8% in plasma ALT activity and BIL concentration of T2DM rats, respectively, when compared to normal rats. However, the ALT activity and BIL concentration significantly decreased in a dose-dependent manner after treatment with TCLAE at 400 mg/kg bw (4.4% and 12.7%, respectively) and 800 mg/kg bw (57.4% and 65.3%, respectively), when compared to the untreated experimental and normal groups. Furthermore, the plasma ALB concentration decreased by 13.9% in T2DM rats, but the TCLAE treatment at both doses did not change ($p > 0.05$) this level, when compared to normal rats. Moreover, the induction of T2DM and TCLAE treatment did not alter ($p > 0.05$) the AST and ALP plasma activity, when compared to normal rats.

Table 3. Effect of TCLAE treatment on liver function parameters in T2DM rats

	Normal	DB	GLB	TCLAE4	TCLAE8
ALT (U/l)	112.38 ± 15.53^{a}	125.71 ± 18.47^{c}	118.13 ± 8.74^{b}	120.16 ± 28.28^{b}	109.72 ± 33.59^{a}
AST (U/l)	293.65 ± 25.93	287.12 ± 52.21	304.96 ± 76.47	307.21 ± 72.63	297.93 ± 82.37
ALP (U/l)	525.58 ± 122.51	577.34 ± 190.33	368.26 ± 90.15	607.38 ± 177.17	499.98 ± 154.08
ALB (g/dL)	3.89 ± 0.13^{b}	3.35 ± 0.08^{a}	3.25 ± 0.04^{a}	3.11 ± 0.02^{a}	3.16 ± 0.15^{a}
BIL (mg/L)	0.92 ± 0.36^{a}	3.31 ± 0.85^{b}	1.24 ± 0.25^{a}	1.41 ± 0.26^{a}	1.15 ± 0.26^{a}

The values present the mean ± standard error of the mean (SEM) of six animals. The values with different superscripts (a,b,c) across each row are significantly different, while those with the same superscript are not significantly different at 95% CI. ALB, albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BIL, bilirubin; DB, diabetic; GLB, glibenclamide; T2DM, type 2 diabetes mellitus; TCLAE, *T. catappa* leaf aqueous extract.

Effect of TCLAE treatment on diabetes-induced systemic dyslipidaemia

The plasma CHOL, TRIG, HDL-TRIG, LDL-CHOL, LDL-TRIG, and FFA concentrations increased ($p < 0.05$) in untreated DB animals, when compared to control animals. After four weeks of TCLAE treatment with both experimental dosages, the CHOL, HDL-TRIG, LDL-CHOL, LDL-TRIG, and FFA plasma concentrations significantly decreased ($p < 0.05$). The plasma concentrations of TRIG, LDL-CHOL, LDL-TRIG, and FFA in the TCLAE groups were comparable ($p > 0.05$) with those in both the GLB and normal groups. Furthermore, an increase ($p < 0.05$) in plasma HDL-C and adiponectin concentration was observed after TCLAE treatment, in contrast to the decrease ($p < 0.05$) induced by the diabetes induction (Fig. 4).

Compared to normal rats, the CHOL, TRIG, HDL-TRIG, LDL-CHOL, and LDL-TRIG concentrations significantly increased ($p < 0.05$) in the liver of untreated DB rats. However, this increase was reduced ($p < 0.05$) by the GLB, TCLAE4 and TCLAE8 intervention, when compared to the untreated group. Furthermore, the hepatic LDL-CHOL concentration in the treatment groups was comparable ($p > 0.05$) to that in the normal group (Fig. 5). However, the diabetes induction and subsequent treatment with GLB, TCLAE4 and TCLAE8 did not alter ($p > 0.05$) the hepatic level of HDL-C, when compared to the normal group (Fig. 5).

There were no differences in renal CHOL, TRIG, HDL-CHOL, and LDL-CHOL levels in the normal and treatment groups ($p > 0.05$). However, the induction of diabetes significantly increased ($p < 0.05$) the renal HDL-TRIG and LDL-TRIG concentrations. Furthermore, the GLB and TCLAE treatment induced a reduction ($p < 0.05$) in renal HDL-TRIG and LDL-TRIG concentrations, when compared to the respective levels in normal rats (Fig. 6).

The erythrocyte concentration of CHOL, TRIG, HDL-TRIG, LDL-CHOL, and LDL-TRIG in untreated DB rats significantly increased ($p < 0.05$), when compared to normal rats. After the administration of TCLAE, the erythrocyte CHOL, TRIG, HDL-TRIG, LDL-CHOL, and LDL-TRIG concentrations decreased ($p < 0.05$), when compared to untreated DB rats. The reduction in CHOL, LDL-C and LDL-T erythrocyte concentrations in the TCLAE4 and TCLAE8 groups were comparable with the normal group (Fig. 7).

Effect of TCLAE treatment on abnormal cardiovascular indices

In Figure 8, the AI, CRI, HTR and TyG significantly increased ($p < 0.05$) in DB rats, when compared to normal rats. After the experimental regimen, the indices decreased ($p < 0.05$) after the TCLAE treatment, when compared to the GLB and normal groups, especially at the highest dose. Compared to normal rats, the opposite was observed for the Disse index, since this significantly decreased ($p < 0.05$) in untreated DB rats. However, this increased ($p < 0.05$) in GLB- and TCLAE-treated rats, when compared to DB rats (Fig. 8).

Effect of TCLAE treatment on the diabetes-induced interference of gene expression

In Figure 9, the hepatic expression of *PPAR-α*, *PPAR-δ*, *AdipoR2* and *CPT-1a* was downregulated ($p < 0.05$), while *CRP* was upregulated ($p < 0.05$), in DB rats, when compared to normal rats. After treatment with GLB and TCLAE, the hepatic expression of *PPAR-α*, *PPAR-δ*, *AdipoR2* and *CPT-1a* was upregulated ($p < 0.05$), while *CRP* was downregulated ($p < 0.05$), in DB rats. Nonetheless, GLB was unable to change ($p > 0.05$) the diabetes-induced alteration in *CPT-1a,* while the TCLAE treatments non-significantly increased the *AdipoR2* hepatic expression at the highest dose.

Effect of TCLAE treatment on altered liver histology

The hepatic histopathology revealed distinct centrioles and hepatocytes, with a pyknotic nucleus and well-fenestrated sinusoids in the control group (Fig. 10a). The diabetes onset led to the distortion of centrioles, which were surrounded by focal inflammatory cells with fatty changes and steatosis (Fig. 10b). In the GLB group, a prominent portal vein with mild fatty hydropic change was observed (Fig. 10c). In hepatic tissues obtained from 400 mg/kg bw TCLAE-treated rats, moderate inflammation was observed in the hepatic lobes (parenchyma) and centrioles, with focal microvesicular steatosis and visible fatty changes (Fig. 10d). Furthermore, mild Kupffer cell activation, prominent central veins and hepatocytes were observed in hepatic tissues obtained from TCLAE-treated rats (800 mg/kg bw, Fig. 10e).

Discussion

HFD and intraperitoneal STZ injection can experimentally induce hyperglycaemia and DB metabolic profiles in a manner similar to the T2DM clinical progression in humans.[36] Significant weight loss and hyperglycaemia are usually associated with experimental animals. Thus, the improvement in body weight and daily fasting blood glucose of DB rats after TCLAE treatment can be attributed to improved glucose homeostasis control via increased insulin sensitivity and decreased hyperglycaemia. This finding suggests that TCLAE has the potential to improve diabetes-related weight defects, and possess hypoglycaemic activity, which are the core features of T2DM. Stimulating glucose uptake, utilisation and storage through insulin action is critical in maintaining optimal glucose levels in blood.[37,38] The observed increase in blood glucose and plasma INS concentrations is a common occurrence in HFD/STZ-induced diabetes, similar to the reports of other studies.[39–43] The increase in hepatic and extrahepatic INS sensitivity by TCLAE may be responsible for the reversal of increased blood glucose and plasma INS, which was verified by the decrease in glucose AUC. The increase in ATP generation may also be a consequence, leading to the induction of insulin's anabolic effect, in addition to TCLAE's ability to improve glucose metabolism.[44,45]

The reduction of plasma ALT activity and plasma BIL concentration in DB rats after TCLAE may be attributed to the radical scavenging ability that concomitantly thwarted the lipid peroxidation, and preserved the integrity of the hepatocyte cell wall, in order to prevent further leakage of the enzyme.[46] A similar finding was previously reported by other studies on STZ-induced DB rats.[47–49] However, contrary to these present findings, Soliman[50] reported an increase in BIL after treatment with *Paracentrotus lividus* extract. The decrease in plasma ALB levels in untreated DB animals further corroborated the decline in body weight, suggesting evidence of tissue wasting. This decline may also be due to liver degeneration, since organ pathology has been reported in prolonged diabetes.[51] The selective GLUT-2 uptake of STZ by the liver may also destroy hepatocytes.[43] Despite the inability of TCLAE to improve the ALB levels, the hepatoprotective property of *T. catappa* in DB rats has been previously reported.[52] This suggests that the time frame is not enough to restore the liver's synthetic ability, which has been recorded with some medicinal plants.[53,54]

Dyslipidaemia is an accompanying symptom of T2DM onset,

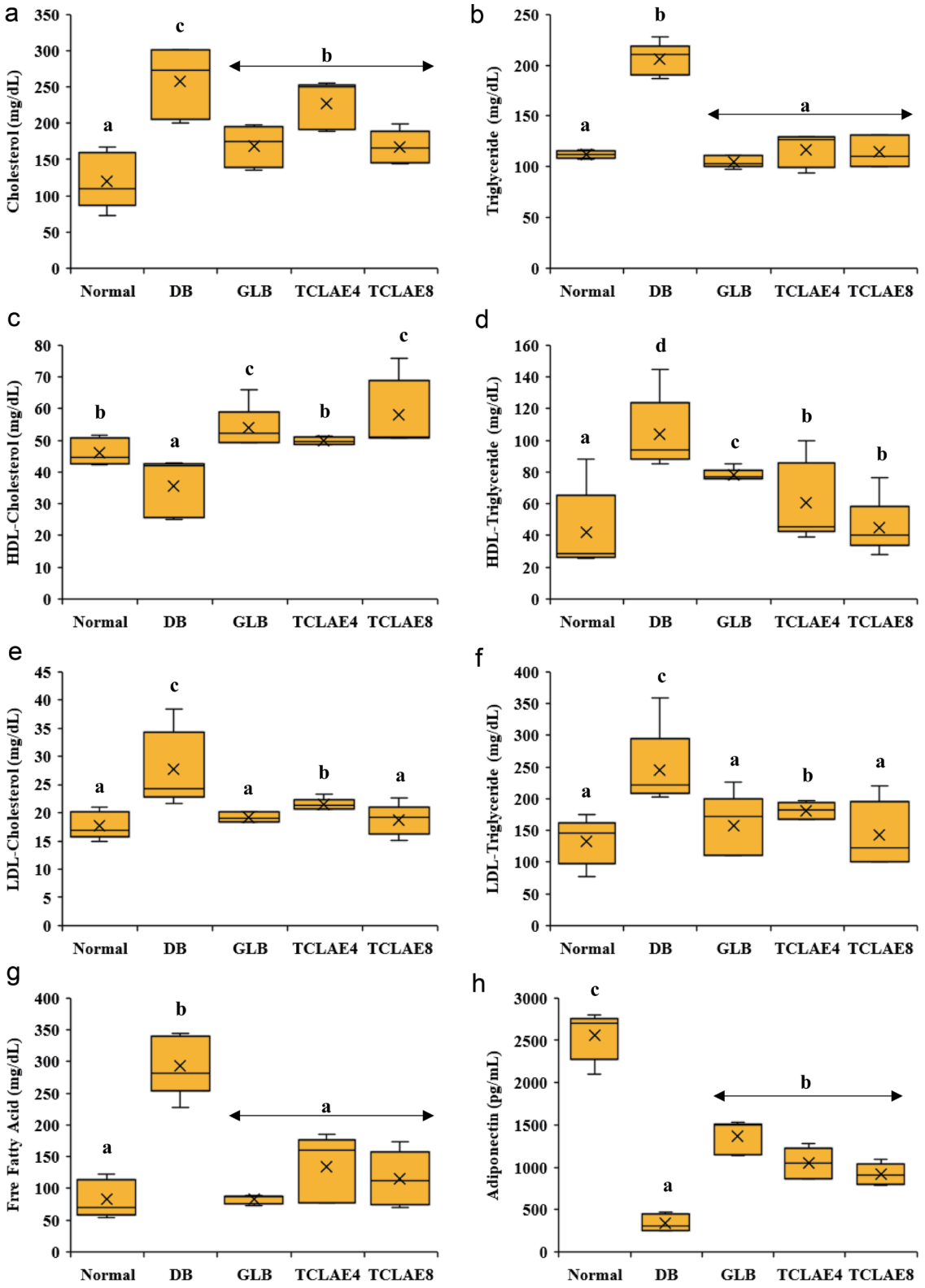

Fig. 4. Effect of TCLAE treatment on the plasma (a) cholesterol, (b) triglyceride, (c) HDL-cholesterol, (d) HDL-triglyceride, (e) LDL-cholesterol, (f) LDL-triglyceride, (g) free fatty acid concentrations, and (h) adiponectin concentrations of T2DM rats. The box plots with different superscripts (a,b,c,d) are significantly different, while those with the same superscript are not significantly different at 95% CI. DB, diabetic; GLB, glibenclamide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCLAE, *T. catappa* leaf aqueous extract; T2DM, type 2 diabetes mellitus.

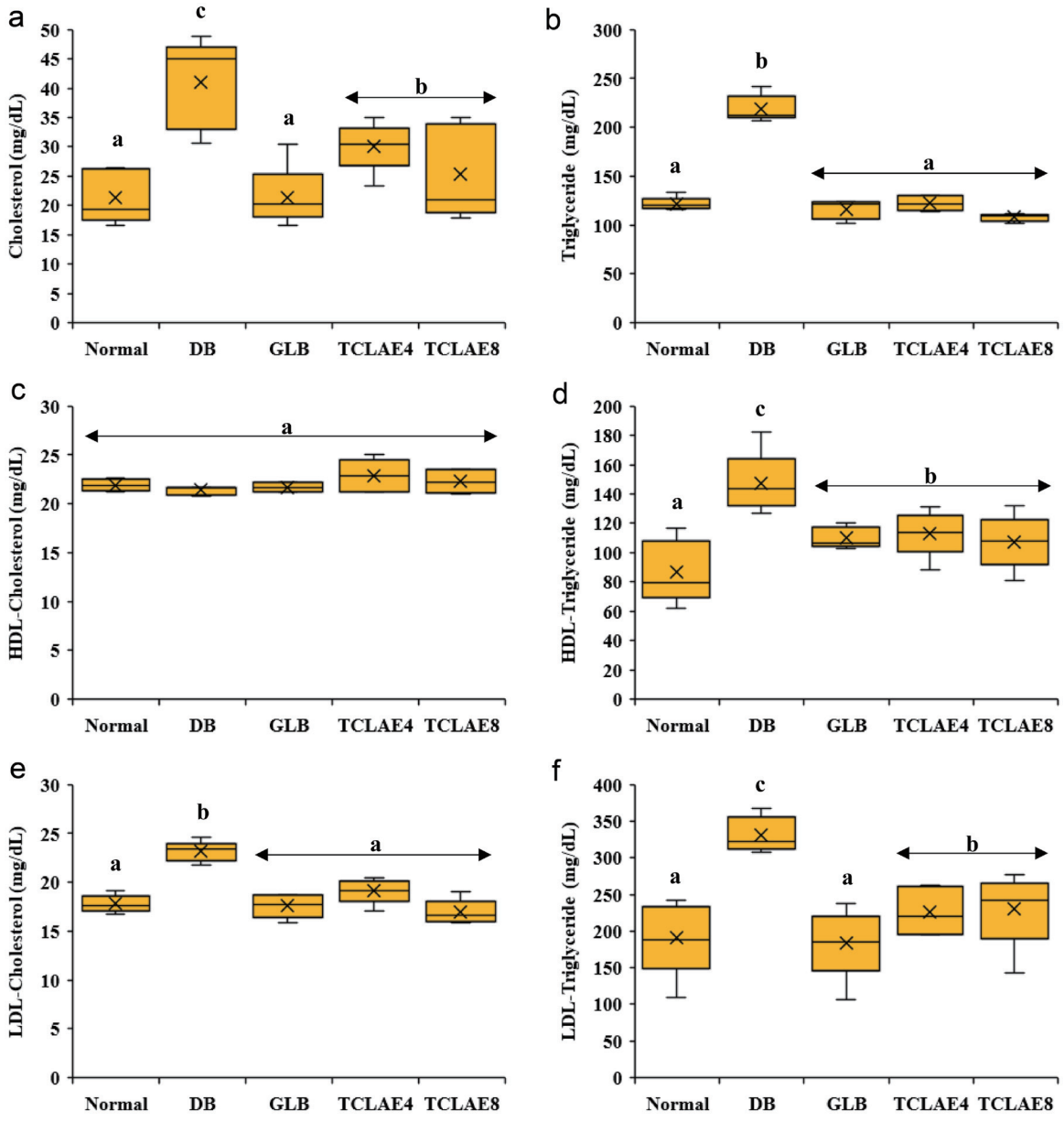

Fig. 5. Effect of TCLAE treatment on the hepatic (a) cholesterol, (b) triglyceride, (c) HDL-cholesterol, (d) HDL-triglyceride, (e) LDL-cholesterol, and (f) LDL-triglyceride concentrations of T2DM rats. The box plots with different superscripts (a,b,c) are significantly different, while those with the same superscript are not significantly different at 95% CI. Box plots with similar superscripts refer to the significant difference at 95% CI. DB, diabetic; GLB, glibenclamide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCLAE, *T. catappa* leaf aqueous extract; T2DM, type 2 diabetes mellitus.

in addition to hyperglycaemia. The inability of tissues to utilise blood glucose for energy purposes can lead to the use of fatty acids for energy generation.[55] The increase in concentration of plasma and organ CHOL, TRIG, LDL-CHOL, HDL-TRIG, LDL-TRIG and FFA, with the concomitant decrease in HDL-CHOL after the induction of T2DM using STZ/HFD in the present study, was synonymous with the findings reported by Xu *et al*.[56] and Alam *et al*.[57] This occurrence was due to the increase in catabolism of peripheral fat depots, which led to the upsurge in the mobilisation of FFA from adipose tissues, with the concomitant accumulation of excess fatty acids in the liver, and the subsequent conversion to triglycerides.[54] In the normal systemic maintenance of intermediary metabolism, insulin activates lipoprotein lipase, which is an enzyme responsible for the removal and degradation of circulatory TRIG. However, when defects in insulin action or secretion arise during DB conditions, this enzyme is inactivated, inducing the continuous action of HMG-CoA reductase, extracellular lipolytic hormones, and lipases on fat depots without inhibition.[58] These actions lead to a high concentration of fatty acid in blood, stimulating hepatic CHOL, TRIG, and phospholipid synthesis.[55,59] The resultant observation is the discharge of these formed macromolecules (CHOL, TRIG, and phospholipid) in blood, with hypercholesterolemia, hypertriglyceridemia, and hyperlipidaemia as the concomitant

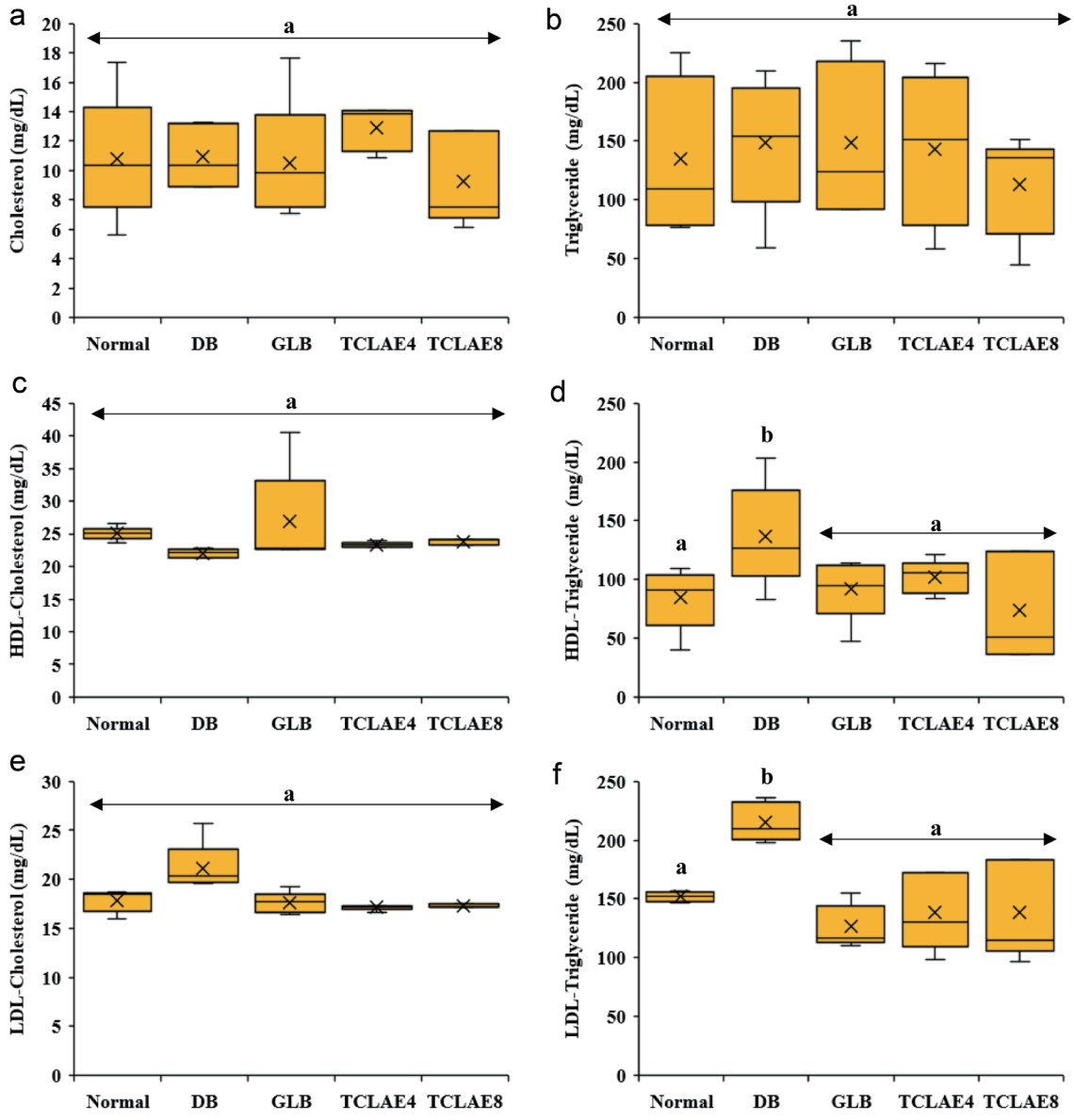

Fig. 6. Effect of TCLAE treatment on the renal (a) cholesterol, (b) triglyceride, (c) HDL-cholesterol, (d) HDL-triglyceride, (e) LDL-cholesterol, and (f) LDL-triglyceride concentrations of T2DM rats. The box plots ppresent the mean ± standard error of the mean (SEM) of six animals. The box plots with different superscripts (a,b) are significantly different, while those with the same superscript are not significantly different at 95% CI. DB, diabetic; GLB, glibenclamide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCLAE, *T. catappa* leaf aqueous extract; T2DM, type 2 diabetes mellitus.

conditions.[60] Advanced glycosylated end products usually occur in unregulated hyperglycaemia. The accelerated systemic formation has been attributed to the reduction in HDL-CHOL.[54] The observed decrease in these biomarkers of dyslipidaemia by TCLAE can also be attributed to the presence of flavonoids and phenolics, which have been identified to inhibit CHOL and bile synthesis.[50] The inhibition of lipolytic enzymes, and key CHOL and TRIG synthesis enzymes, the reversal of IR, proper energy metabolic control, and reversing hyperinsulinemia are other mechanisms by which the hypolipidaemic effect of TCLAE was attained.[50,61]

Hyperinsulinemia plays a role in T2DM dyslipidaemia at the molecular level via TRIG hydrolysis inhibition and acetyl CoA carboxylase activation, thereby increasing malonyl CoA production. The malonyl CoA in the subsequent reactions inhibited the *CPT-1* expression, hindering the mitochondria fatty acid transfer from undergoing β-oxidation, and causing hepatic cytosolic accumulation and increased circulation of FFA, as observed in the present study.[42] The accumulation of FFA aggravates IR, and induces lipotoxicity and steatosis, in addition to the upregulation of the *CRP* gene expression in the liver. This induces proinflammation, and inhibits the expression of *PPAR-α* and *PPAR-δ*, which further truncates the regulation of *CPT-1*, as observed in the present study.[62,63] The upregulation of *CPT-1a, PPAR-α*, and *PPAR-δ* genes by TCLAE in the liver might imply the improvement of hepatic fatty acid oxidation regulation, and the decrease in circulat-

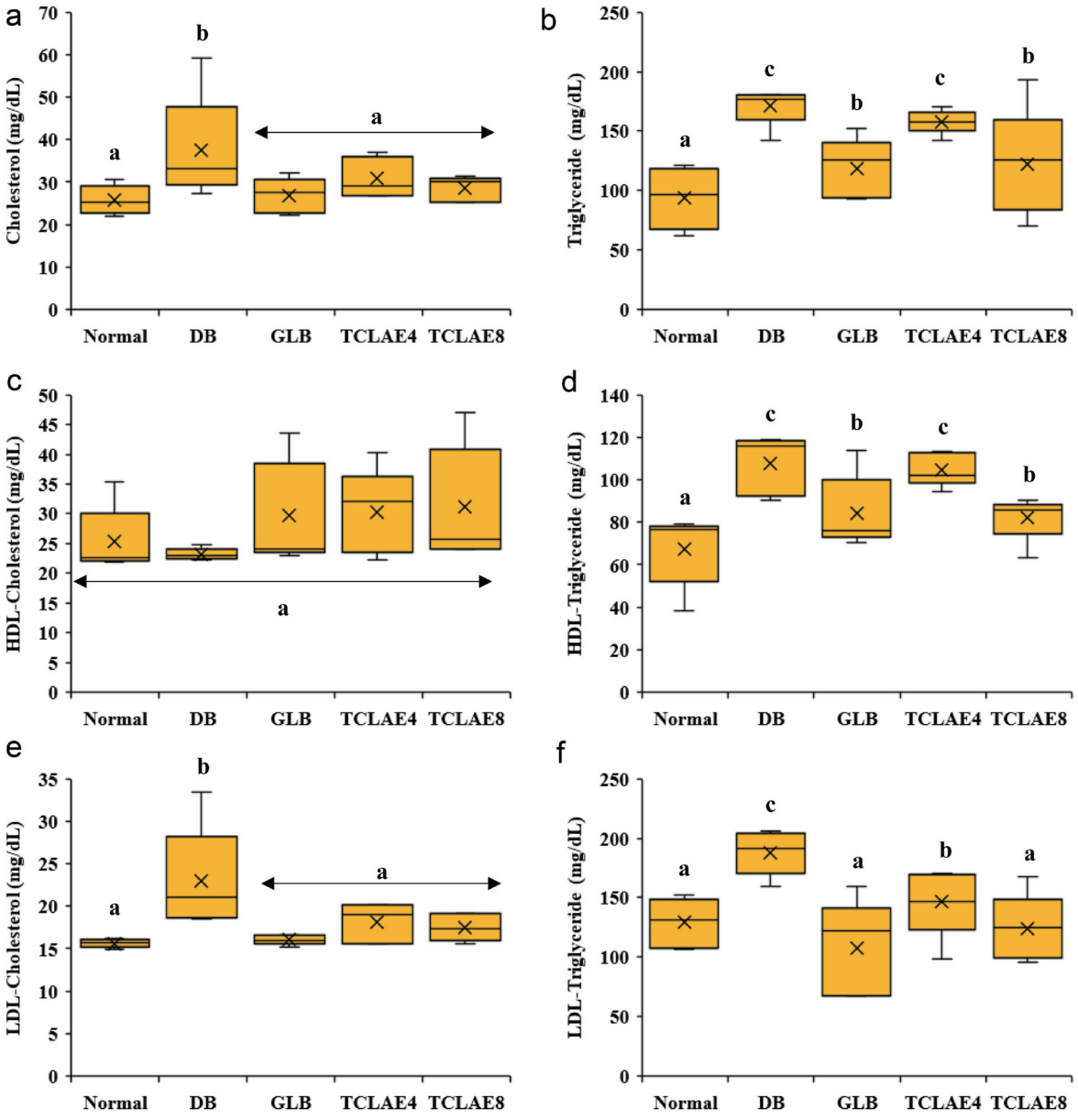

Fig. 7. Effect of TCLAE treatment on the erythrocytes (a) cholesterol, (b) triglyceride, (c) HDL-cholesterol, (d) HDL-triglyceride, (e) LDL-cholesterol, and (f) LDL-triglyceride concentrations of T2DM rats. The box plots present the mean ± standard error of the mean (SEM) of six animals. The box plots with different superscripts (a,b,c) are significantly different, while those with the same superscript are not significantly different at 95% CI. DB, diabetic; GLB, glibenclamide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCLAE, *T. catappa* leaf aqueous extract; T2DM, type 2 diabetes mellitus.

ing FFA observed in the present study, which is similar to other reports.[64–66] Hence, increasing the mitochondrial fatty acid transfer and desaturation pathway of fatty acid might be the biomolecular hypolipidaemic mechanism of TCLAE, which induced fatty acid uptake, trafficking and esterification in DB rats, as observed in a previous study.[67]

The cardiovascular indices viz., AI, HTR and CRI were consistent with the lipid profile results, in which dyslipidaemia was abrogated after TCLAE intervention. These cardiovascular indices are of great importance in evaluating the predisposition of diabetics to developing diabetes-related secondary cardiovascular complications. Patients with increased cardiovascular indices are prone to developing various cardiovascular complications, as observed in previous studies.[54,68] The return of these indices to normal levels may be due to the increase in HDL levels, preventing the continuous deposition of cholesterol in the circulatory system, and thereby reducing the risk of atherosclerosis.[38] The increase in hepatic *AdipoR2* expression can be hypothesised to induce hepatic fatty acid oxidation, and enhance glucose uptake. This was further corroborated by the ability of TCLAE to increase circulating adiponectin in the present study. This suggests that the increase in TCLAE treatment duration might have further induced the expression of *AdipoR2*. These findings are in tandem with those of previous studies that reported a decrease in adi-

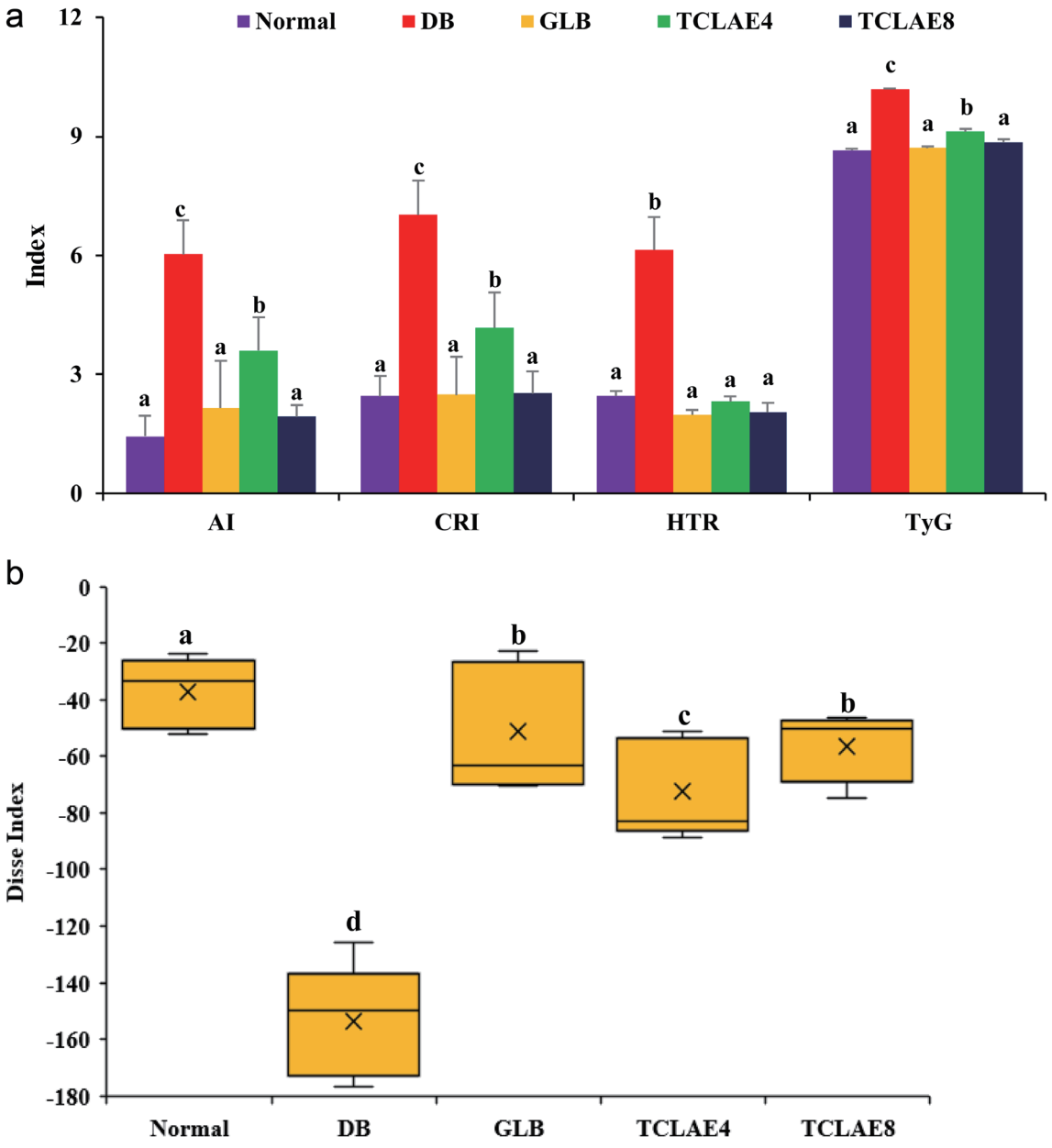

Fig. 8. Effect of TCLAE treatment on the (a) cardiovascular and (b) Disse indices of T2DM rats. The bars and box plots present the mean ± standard error of the mean (SEM) of six animals. The box plots and bars on each index with different superscripts (a,b,c,d) are significantly different, while those with the same superscript are not significantly different at 95% CI. AI, atherogenic index; CRI, coronary risk index; HTR, HDL/TRIG ratio; TCLAE, *T. catappa* leaf aqueous extract; TyG, triglyceride-glucose index; T2DM, type 2 diabetes mellitus.

ponectin and its *AdipoR2* gene in DB patients and models,[15,69–72] since reduced plasma adiponectin concentration and *AdipoR2* hepatic expression indicates increased triglyceride concentration and lipid oxidation in peripheral tissues.[69,73] The increase in expression of hepatic *CRP* in diabetics signifies not only chronic subclinical inflammation and diabetic induced-dyslipidaemia, but also endothelial dysfunction and vascular remodelling, as reported by other studies.[74–76] The elevated level of hepatic *CRP* expression in diabetic rats might be due to the increased circulation of blood glucose, adipokines and FFA concentration, signifying the T2DM progression.[77] The reduction in hepatic *CRP* expression in TCLAE-treated animals further authenticates its hypoglycaemic and dyslipidaemic abrogative properties, since hepatocytes release very low amounts of CRP under normal conditions.[78,79]

The histopathological reports further lend credence to the results of the present study, since TCLAE remarkably improved the architectural structure of hepatic cells by clearing the diabetes-induced fatty alterations and steatosis, thereby reducing diabetic complications.[43] Furthermore, TCLAE was able to improve the STZ-induced pathological damage caused by the induction of Kupfer and mononuclear cell activation, corroborating the ob-

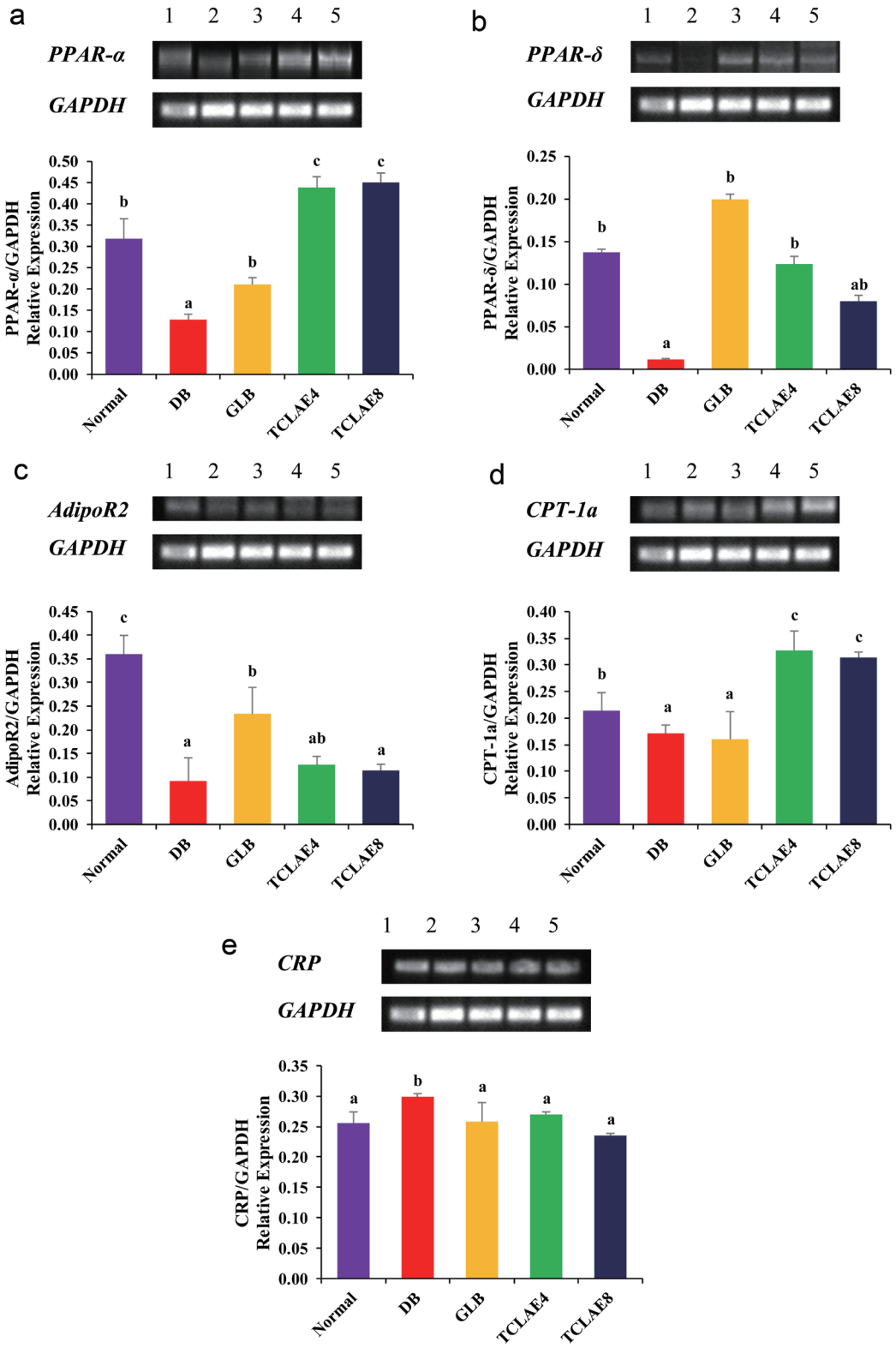

Fig. 9. Effect of TCLAE treatment on the hepatic expression of (a) *PPAR-α*, (b) *PPAR-δ*, (c) *AdipoR2*, (d) *CPT-1a*, and (e) *CRP* genes in T2DM rats. The bars present the mean ± standard error of the mean (SEM) of six animals. The bars with different superscripts (a,b,c) are significantly different, while those with the same superscript are not significantly different at 95% CI. CPT-1, carnitine palmitoyltransferase 1; CRP, C-reactive protein; DB, diabetic; GLB, glibenclamide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TCLAE, *T. catappa* leaf aqueous extract; T2DM, type 2 diabetes mellitus; PPAR, peroxisome proliferator-activated receptor.

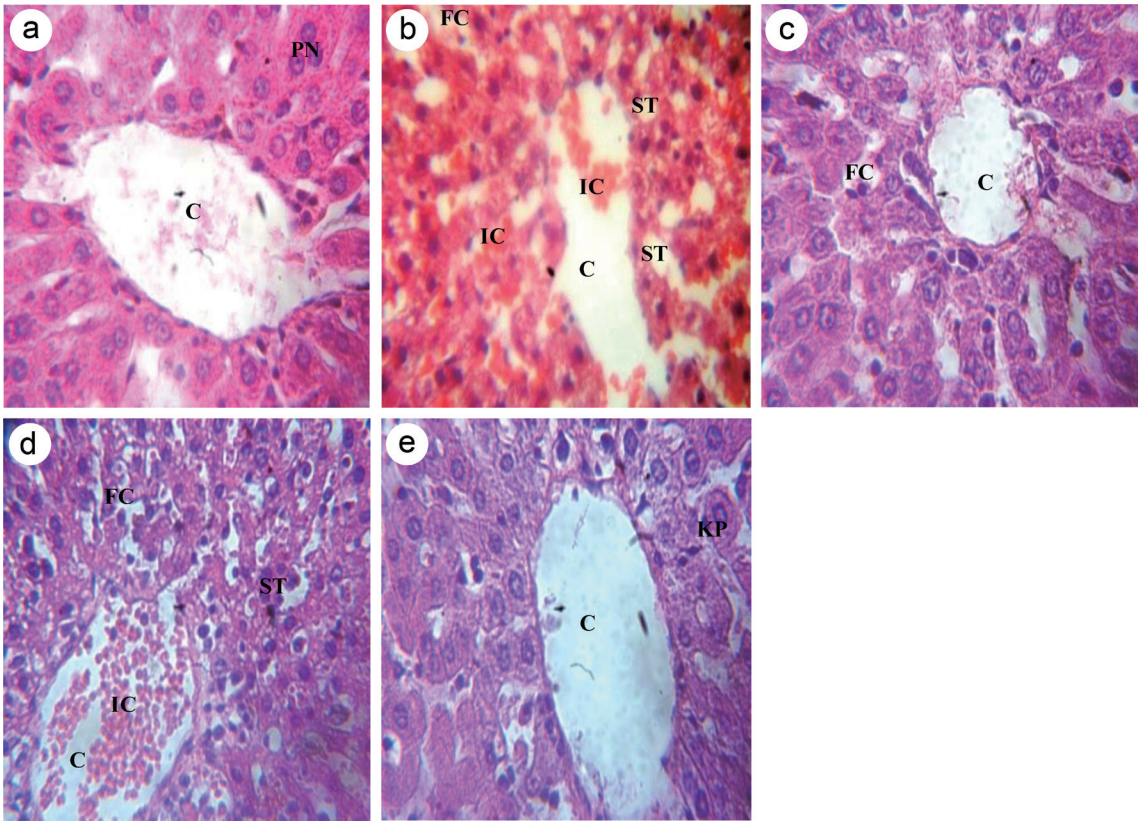

Fig. 10. Histopathological examination of (a) normal, (b) diabetic, (c) glibenclamide, (d) 400 mg/kg bw TCLAE-treated, and (e) 800 mg/kg bw TCLAE-treated hepatic tissues (×400, scalebar = 50 µm). C, centriole; PN, pyknotic nucleus; ST, steatosis; IC, inflammatory cells; FC, fatty changes; KP, Kupffer cells; TCLAE, *T. catappa* leaf aqueous extract.

served antidiabetic effect and hepatic *CRP* downregulation by the extract. These cells have been reported to work synergistically and efficiently in the capture and phagocytosis of targeted damaged cell components, which is important for liver regeneration.[80,81]

Conclusion

In conclusion, TCLAE abrogates dyslipidaemia in T2DM diabetic rats to improve dysregulated lipid metabolism. This was achieved by upregulating *PPAR-α, PPAR-δ, CPT-1a*, and *AdipoR2*, while downregulating *CRP* genes in the liver. This molecular effect reduces circulating FFA, hepatic proinflammation, and lipid accumulation, while increasing adiponectin levels, concomitantly activating β-oxidation, alleviating dyslipidaemic downstream processes, and inducing triglyceride hydrolysis and clearance (Fig. 11). Hence, TCLAE may be used as an adjuvant to ameliorate diabetes-induced hyperlipidaemia, and its associated complications. Some of the limitations of the present study were the lack of lipid metabolic gene protein expression assessment, and TCLAE bioactive(s) identification. Further studies need to be conducted to standardise the crude extract and isolate bioactive principles, allowing for its further development for therapeutic use in the clinic. In addition, the evaluation of the protein expression of more lipid metabolic genes might reveal a new molecular mechanism, through which TCLAE may modulate diabetes-induced dyslipidaemia in type 2 DB rats.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

FNI, OOO and SNC: study concept and design; FNI and OTI: acquisition of data; FNI: analysis and interpretation of data; FNI: drafting of the manuscript. All authors have made a significant contribution to the study, critically revised the manuscript for important intellectual content, and approved the final manuscript.

Ethics approval

The experimental protocol followed the institutional animal care and handling guidelines documented in the NIH and ARRIVE guidelines, and was approved by the Health Research Ethics Committee of Covenant University, with Approval no. CHREC/031/2018.

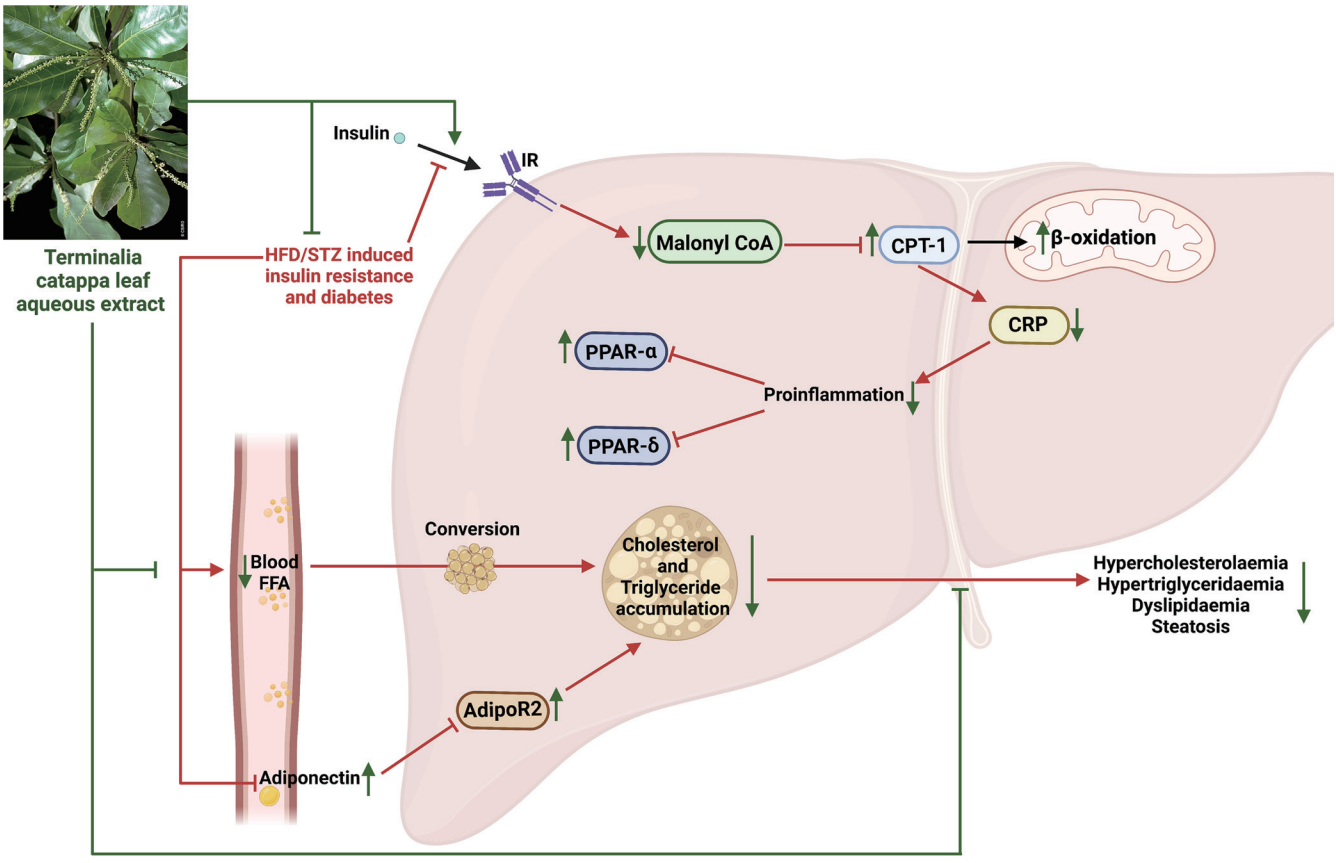

Fig. 11. The proposed *Terminalia catappa* dyslipidaemia abrogative molecular mechanism. The red arrows show the diabetes and insulin resistance-induced pathways. The green arrows show the *T. catappa* leaf aqueous extract's effect on the pathways, and molecular elements. AdipoR2, adiponectin receptor 2; CPT-1, carnitine palmitoyltransferase 1; CRP, C-reactive protein; FFA, free fatty acid; HFD, high-fat feeding; IR, insulin receptor; PPAR-δ, peroxisome proliferator-activated receptor delta; PPAR-α, peroxisome proliferator-activated receptor alpha; STZ, streptozotocin.

Data sharing statement

No additional data are available.

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