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Impacts of trelagliptin and remogliflozin alone and in combination with Alpha Lipoic Acid on cardiac function in streptozotocin-induced diabetes mellitus in rats

Mohammed Abdullah Ali*, Ansam Naji Alhassani, Badraldin Kareem Hamad

Department of Pharmacology and Toxicology, College of Pharmacy, Hawler Medical University, Erbil, 44001, Iraq

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ABSTRACT

This study investigated the effects of trelagliptin and remogliflozin, alone and in combination with alpha lipoic acid (ALA), on cardiac biomarkers in diabetic cardiomyopathy (DCM). We aimed to assess the management of glucotoxicity consequences in streptozotocin-induced diabetic rats by measuring serum levels of pharmacologically active endogenous ligands. Forty-eight male rats were divided into different treatment groups, including negative control, positive control, and four experimental groups. After inducing diabetes, the rats were treated for 28 days, and serum levels of biomarkers associated with oxidative stress (malondialdehyde and thioredoxin-interacting protein), inflammation (nuclear factor NF-kappa-B p105 and lipoprotein-associated phospholipase A2), and myopathy (neprilysin and high selective cardiac troponin T) were measured. Immunohistochemical analysis of heart cells was also performed. The results showed that inducing hyperglycemia increased serum glucose levels and biomarkers associated with DCM. However, all treatment groups exhibited a significant decrease in these biomarkers and an increase in insulin levels compared to the diabetic control group. The groups receiving combination therapy with ALA showed greater improvements in cardiac biomarkers compared to the individual treatments. The immunohistochemical analysis supported these findings by demonstrating a reduction in the percentage area of cathepsin B, a protein involved in DCM pathophysiology. In conclusion, supplementing the base treatments with ALA showed promise in enhancing cardiac biomarkers associated with DCM. The combination of trelagliptin, remogliflozin, and ALA may have additional clinical value in managing DCM by targeting oxidative stress, inflammation, and glucotoxicity. However, further research is needed to validate these findings and explore their potential clinical applications.

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Introduction

Diabetes mellitus, a chronic diverse metabolic condition with complicated pathophysiology and many complications, has the highest morbidity and mortality rates of all chronic diseases. Hyperglycemia induces glucose auto-oxidation, protein glycation, and polyol activation. Reactive oxygen species (ROS) influence immune factor signaling. Inflammatory cytokines and free radicals can both rise with ROS (1). DCM is aberrant cardiac structure and function in the absence of additional cardiac risks factors such as coronary artery disease, hypertension, and severe valve disease and mostly is caused by metabolic changes, decreased calcium homeostasis and energy generation, increased inflammation, OS, advanced glycation end products (AGE) (2). Dipeptidyl peptidase-4 (DPP-4) inhibitors are a class of oral antidiabetic drugs that control hyperglycemia in patients suffering from type 2 diabetes mellitus (T2DM) that act on incretin hormones, mainly (glucagon-like peptide-1) GLP-1 and (gastric inhibitory peptide) GIP, which maintain glucose homeostasis by increasing insulin secretion and decreasing glucagon secretion (3) Trelagliptin, a once-weekly DPP-4 inhibitor, lowers fasting glucose and exhibits considerable glycemic efficacy in T2DM patients (4). SGLT2 inhibitors are used to treat T2D by limiting the reabsorption of glucose filtered through the kidney and so promoting glucose excretion in the urine (5). Remogliflozin is a novel low-cost SGLT-2 inhibitor that is safe and efficacious as dapagliflozin (6). Alpha-lipoic acid (ALA), a potent antioxidant used to treat diabetes, improves glucose metabolism, reduces oxidative stress, improves endothelial dysfunction, decreases platelet reactivity, inhibits nuclear factor kappa B (NFkB), chelates divalent transient metal ions, and induces adenosine monophosphate-activated protein kinase expression) (7). The present study goals are investigating the comparative effects of trelagliptin, trelagliptin plus ALA, remogliflozin, and remogliflozin plus ALA in the management of the negative repercussions of glucotoxicity including their direct effect on cardiac cells via measuring some of the cardiac biomarkers such as oxidative stress biomarkers (Malondialdehyde (MDA), thioredoxin-interacting protein (TXNIP), and inflammatory markers such as nuclear factor NF-kappa-B p105 (NF-Kb105), lipoprotein-associated phospholipase A2 (LPPLA2) besides myopathy biomarkers such as neprilysin (NEP) and high selective cardiac troponin T (Hs-CTnT).

Materials and Methods

Materials

ELK Biotechnology Co., Ltd. in Wuhan, China, sup-

* Corresponding author. Email: pharmacistnanakali@yahoo.com

plied rat (NFkB-p105), (TXNIP), (MDA, (LPPLA2), (NEP), and (Hs-CTnT) ELISA kits. Glentham Life Sciences Ltd. (UK) provided STZ. Trelagliptin (100 mg), remogliflozin (100 mg), and ALA (300 mg) were acquired from local pharmacies. Takeda (Japan) made trelagliptin, Glenmark (India) made remogliflozin, and Adipharm (Bulgaria) made ALA. Cathepsin B antibodies were bought from Wuhan's ELK Biotechnology Co., Ltd. for immunohistochemistry studies. Dako, Denmark supplied immunohistochemistry buffers, diluents, and chemicals. Additional chemicals originated from Sigma-Aldrich.

Experimental animals

Forty-eight male adult Sprague-Dawley albino rats aged 10-12 weeks (250-350 g) were donated by Jihan University's animal house. One week was provided to acclimate. Conventional rodent food and tap water were regularly supplied to the rats. The animals were maintained in an air-conditioned room (4 rats per cage) with a standard humidity level, temperature (22 ± 2 °C) and light-dark cycle (12 hours of light, 12 hours of darkness).

Study design

On the study's first day, all rats fasted for 12 hours before receiving STZ treatment. A pH 4.4-4.5 0.1 M sodium citrate buffer was prepared before injecting STZ. An ice-cold container had a citrate buffer that dissolved STZ. Each injection used a freshly dissolved STZ solution. Forty rats were intraperitoneally injected with STZ (42 mg/kg body weight (8). While the other eight normal control rats received citrate buffer solution (pH 4.4 to 4.5) intraperitoneally. Regular meals and 10% sucrose water were given to prevent hypoglycemia after the STZ injection. After the STZ injection, on the second day,10% sucrose suspension was replaced with water (9). After one week of STZ injection, animals' blood sugar levels were assessed after fasting for 6-8 hours. The Precicheck blood glucose monitoring gadget (Germany) measured glucose levels in the blood of the tail. STZ-induced diabetes was identified in rats with fasting glucose levels of 250 mg/dL or above. Six groups of eight rats were created after diagnosis. Non-diabetic rats make up group one (N). Diabetic control (D) group two received STZ without therapy. Group three was (T1) group diabetic rats got 20 mg/kg trelagliptin alone (10). Group four (T2) group received trelagliptin at the same dose plus 100 mg/kg ALA (11). Group five represented diabetic rats that received remogliflozin alone in a dose of 25mg/kg (12) and this group is (T3) group. Group six denoted diabetic rats given remogliflozin in the same dose in combination with ALA 100 mg/kg and this group was (T4) group. The oral drugs were dissolved in normal saline, and the appropriate dosage was determined based on weight. Animals received the medicines orally. All groups got unrestricted meals during the 28-day therapy after diagnosis.

Blood and serum collection

On the 28th day, animals were starved overnight, slaughtered, and blood samples were obtained. After intraperitoneally administering ketamine (75 mg/kg) and xylazine (5 mg/kg), rats were heart-punctured using a sterile disposable syringe to collect serum. A recheck glucometer measured blood glucose levels. Another quantity of blood was placed into labeled gel tubes and left to coagulate at room temperature for 10–20 minutes. After removing the clot, the serum was separated for biochemical examination. Rat-specific ELISA kits were processed per supplier instructions. After death, the hearts were removed and engrossed in 10% formaldehyde solution for immunohistochemistry analysis.

Immunohistochemical analysis.

After deparaffinization, rehydration in alcohol, and transfer to a diluted target-retrieved solution, heart sections were put in a PT Link (Dako North American Inc.) for one hour. Primary antibodies were applied to sections for an hour at room temperature. After washing, the samples received 20 minutes of secondary antibody treatment. After buffer-washing, the slide was exposed to chromogen and di-amino benzidine for six minutes to detect peroxidase activity. Cover-slipped and hematoxylin-stained slides were examined under a light microscope. Image J software (version 1.47v) from the National Institutes of Health, Bethesda, MD, USA, was used to evaluate immunostaining section pictures using an intensity threshold as close as possible to the visually detected staining spots For each group, the percentage area of brown color was calculated (13).

Statistical analysis

Mean and standard error mean represented all data. SPSS 26 from (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. A one-way analysis of variance (ANOVA) evaluated all parameters between control and medication-treated rats. The Tukey test for multiple comparisons was performed to compare groups. $P < 0.05$ indicated statistical significance.

Results

Table 1 demonstrated that D group rats had considerably higher blood glucose levels (483 ± 15.51 mg/dl) than N group rats (83.49±0.81 mg/dl) ($P < 0.001$). All therapies effectively lowered blood glucose compared to the D group. D group insulin levels were lower (28.11±1.02 pg/ml) than the N group (492.77±21.02 pg/ml) ($P < 0.001$). The treatment groups (T1, T2, T3, T4) had higher insulin levels than the D group, and by post hoc test, T4 and T2 had lower blood glucose and higher insulin than T1 and T3 ($P < 0.001$). Table 1 shows the parameter values after 28 days of therapy in all groups. MDA and TXNIP levels rose significantly in the D group compared to the N group (from 99.64±6.89 pg/ml and 2.38±0.25 pg/ml) to (973±11.71 and 11.02±0.26 pg/ml) respectively ($P<0.0001$). Four therapy groups significantly reduced blood MDA levels in diabetic rats ($P<0.0001$). The T4 group was the most powerful group in the reduction of oxidative stress biomarkers. The level of inflammatory biomarkers (NFkB-p105, LpPLA2) was significantly higher in the D groups than in the N group. A post hoc test was performed, and it revealed that those biomarkers levels were significantly reduced in all the treatment groups ($P < 0.0001$). The level of inflammatory biomarkers (NFkB-p105, LpPLA2) in D group rats was significantly higher (0.94±0.016 pg/ml, 41.99±0.79pg/ml) than in the N group (0.12±0.003pg/ml, 6.73±0.16pg/ml) respectively ($P < 0.0001$). Oxidative biomarker levels were significantly reduced in all treatment groups ($P < 0.05$). The T4 reduced them more than

Table 1. The effects of trelagliptin, trelagliptin plus alpha lipoic acid, Remogliflozin, and Remogliflozin plus alpha lipoic acid on serum biomarkers plus their impacts on cathepsin B antibody in immunohistochemical analysis.

Parameters	(N)	(D)	(T1)	(T2)	(T3)	(T4)
Hs-cTnT	18.59±2.56^{a}	117.03±5.26^{d}	33.11±0.83^{b}	12.1±0.67^{a}	48.16±4.09^{c}	14.5±1.01^{a}
Nep	44.68±1.27^{a}	319.32±23.17^{c}	127.82±7.27^{b}	50.13±2.27^{a}	111.34±16.73^{b}	38.42±1.81^{a}
Insulin	492.77±21.02^{c}	28.11±1.02^{a}	123.99±2.43^{b}	466.94±9.61^{c}	151.71±8.48^{b}	489.14±2.51^{c}
Blood glucose	83.49±0.81^{a}	483±15.51^{c}	181±5.07^{b}	79.62±9.53^{a}	157.5±4.33^{b}	90.25±18.96^{a}
MDA	99.64±6.89^{a}	973±11.71^{c}	342.96±28.21^{b}	126.63±2.06^{a}	326.6±3.10^{b}	121.15±12.58^{a}
TXNIP	2.38±0.25^{a}	11.02±0.26^{d}	5.79±0.07^{c}	2.24±0.17^{a}	4.4±0.23^{b}	1.79±0.13^{a}
NF-KBP105	0.12±0.003^{a}	0.94±0.016^{e}	0.63±0.038^{d}	0.19±0.032^{b}	0.54±0.043^{c}	0.19±0.038^{b}
LPPLA2	6.73±0.16^{a}	41.99±0.79^{c}	16.68±1.75^{b}	6.07±0.24^{a}	14.78±0.55^{b}	5.69±0.46^{a}
Cathepsin B	0.27±0.05^{a}	7.43±0.79^{d}	2.92±0.62^{b}	0.95±0.2^{a}	3.02±0.4^{c}	1.06±0.13^{a}

Similar letters specify non-significant differences and different letters are considered statistically significant at P ≤0.05. Values are expressed as mean ± SEM.

T3 and in comparison, with the D group they diminished this biomarker also, T2 was more powerful than T1 in reducing the level of mentioned biomarkers.

In Table 1, immunohistochemical staining against CTSB antibody indicated that the D group had a significantly greater brown-color percentage area than the N group, whereas all treatment groups had significantly reduced (CTSB) colored percentage area (P < 0.0001). T2, which included ALA to reduce myocardial cell damage and inflammation, reduced CTSB expression the highest. Figure 1 shows CTSB immunohistochemical staining under a 10x light microscope to show most of the damage. The brown-reddish color represents the percentage of (CTSB) released during inflammation and injured cardiac cells and scattered to most of the areas in D groups, which defines the extent of necrosis and damage to cardiac cell walls due to hyperglycemia, while in treatment groups those damaged areas have been reduced and restored the normal architecture of the heart and near to N control group. Also, Table 1 and Figure 1 show the changes in serum levels of cardiomyopathy biomarkers Hs-cTnT and NEP in all groups of our experimental animals. There was a significant difference between serum Hs-cTnT and NEP levels of group N rats with group D. Compared with group D, the serum level of both biomarkers was dramatically lowered in all treatment groups (P<0.0001). Both T4 and T2 groups due to the presence of ALA produced a significant reduction of both biomarkers in comparison with the T1 and T3 groups (P < 0.05).

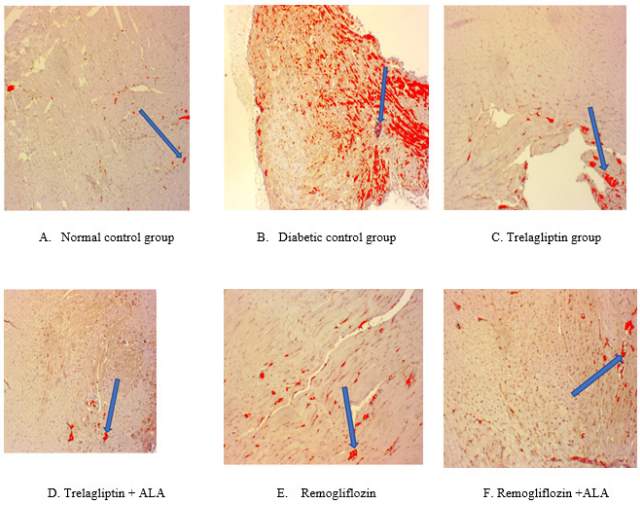

Figure 1. The effects of trelagliptin, trelagliptin plus alpha lipoic acid, Remogliflozin, and Remogliflozin plus alpha lipoic acid on cathepsin B antibodies expression in which tremendous scattering of (CTSB) found under the 10X light microscope

Discussion

Streptozotocin-induced DM produced heart hypertrophy and enlargement. Higher glucose levels diminish vascular endothelial growth factor A (VEGF-A) phosphorylation in coronary arteries, contributing to DCM (14). STZ is an antibiotic that destroys pancreatic islet β-cells and is used in experiments to imitate (T1DM). By dissociating into glucose and methyl nitrosourea, streptozotocin-induced beta-cell cytotoxicity. The beta cells are destroyed, DNA is fragmented, and the body develops insulin-dependent diabetes as a result of the latter's alkylating activities (9,15). The STZ-induced elevation of group D blood glucose levels above normal and considerably above the N group validated the diabetes state of the experimental rats. In the study, it has been shown that blood glucose in all the treatment groups significantly reduced the level of blood glucose in comparison with D groups. T 1 group reduced blood glucose because it was a novel and potent once-weekly DPP-4 inhibitor that presented sustained efficacy and increased endogenous GLP-1, which increased insulin secretion and suppressed glucagon release to control blood glucose (16) While the T3 groups reduced blood glucose by enhancing urine glucose excretion by preventing SGLT2, which reduced renal reabsorption of filtered glucose and lowered the renal glucose threshold and subsequently improved cell function through lowering glucotoxicity (17). While in both treatment T2 and T4 groups a Novel and powerful antioxidant drug have been added to both T1 and T3 groups respectively which were ALA which increases sugar absorption in insulin-sensitive and insulin-resistant muscle tissues to lower blood glucose (18). The ALA promotes glucose absorption by redistributing plasma membrane glucose transporters and tyrosine phosphorylating insulin receptor substrate-1 (19). Through our study, it has been shown that T2 and T4 reduced blood sugar more than T1 and T3 respectively because supplementation with ALA added a benefit mechanism in reducing hyperglycemia besides this finding was confirmed through raising insulin levels significantly (which was measured through

ELISA kits) more than D group and both T1, T3 groups respectively and no significant differences existed with the N group ($p > 0.05$) which indicated near complete regeneration of the beta cells of pancreas and restoring insulin levels and subsequently decreasing the blood glucose concentration.

Our investigation found that NF-β levels are higher in D groups than in N groups. All treatment groups showed substantial reductions in NF-β compared to the D groups. The best explanation is that NF-β, a DNA-binding protein factor, controls the transcription of pro-inflammatory and inflammatory substances like cytokines, chemokines, and enzymes (20). NF-β pathways modulate the pathogenesis of diabetes and its micro- and macrovascular consequences (21). Hyperglycemia promotes the development of AGEs as well as the overproduction of ROS (22) Both ROS and AGEs promote pro-inflammatory response and endothelial dysfunction via NF-κβ activation (23). AGEs attach to receptor RAGE on vascular smooth muscle cells and activate NF (24) According to the hypothetical mechanisms of DCM, several factors come into play, including autonomic dysfunction, insulin resistance, and myocardial fibrosis. Hyperglycemia stimulates the production of tumor necrosis factor-alpha (TNF-α) in myocardial muscles. These factors collectively contribute to the development and progression of diabetic cardiomyopathy (21). AGEs and oxidative stress regulate calcium influx, activating NF-β and decreasing myocardial contractility (25). NF-β activation in myocardial cells may cause myocardial hypertrophy via Toll-like receptors (TLRs) which leads to DCM (26). NF-β levels is declined significantly after using of different treatment groups such as T1 and T3 in comparison with D group. Those findings were parallel to the results of other studies that used different kinds of DPP4 inhibitors, such as sitagliptin and anagliptin. These inhibitors demonstrated a direct anti-inflammatory effect on various cytokines, leading to a reduction in the amount of NF-κB (27,28) Dapagliflozin showed similar outcomes by blocking the expression of TLR-4 and activation of NF-B, two key inflammatory pathways (29). ALA also controls high mobility group box 1 (HMGB1) translocation and the HMGB1/TLR4/NF-B signaling cascade, preventing apoptosis, oxidation, and inflammation (30). Based on the aforementioned points, both the T2 and T4 groups (combination groups) demonstrated a greater reduction in the level of NF-κB compared to the T1 and T3 groups. As a result, better improvements were observed. Regarding TXNIP, a metabolic, oxidative, and inflammatory marker generated in cardiovascular diseases, lower endothelial TXNIP expression is related to increased thioredoxin TRX and decreased Nicotinamide adenine dinucleotide phosphate NADPH oxidase expression, protecting the endothelium against metabolic diseases. TXNIP's function in OS is clear as it inhibits TRX's antioxidant action (31) In group D, excessive glucose caused TXNIP to produce ROS through mitochondria and NADPH oxidase (32), causing OS, endothelial dysfunction, poor vasorelaxation, and cardiac injuries (33) and this is why when the treatment groups were given they diminished the glucose level through increasing the insulin level and subsequently they could attenuate the effect of TXNIP activity (32). TXNIP is linked to TRX and inactive under unstressed situations because it does not interact with the nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3(NLRP3).Under oxidative stress, ROS dissociates TRX-TXNIP, enhancing NLRP3-TXNIP interaction (34). The TRX-TXNIP complex regulates the NLRP3 inflammasome (35). Our studies, consistent with other research, demonstrate that all treatment groups significantly reduce TXNIP levels such as attenuation effects of teneligliptin on TXNIP (36) Insulin represses TXNIP to prevent prolonged hyperglycemia from stimulating its expression (37) In our study, T3 treatment reduced TXNIP levels by raising insulin levels closer to normal, thereby attenuating glucotoxicity compared to the D group. By boosting insulin levels, SGLT2 inhibitors dramatically reduce NLRP3 inflammasome activation and IL-1β release in human macrophages (38). Reduced NLRP3 inflammasome activation leads to TXNIP remaining attached to TXP, resulting in decreased levels of free TXNIP and its proinflammatory role. This leads to improved cardiac cell function, as observed in the T3 group. T2 and T4 therapies, when coupled with ALA, inhibit the NF-kB pathway, which regulates NLRP3 inflammasome activation (39). When the NLRP3 inflammasome is not activated, there is a lower chance of it binding with TXNIP, thereby reducing TXNIP's proinflammatory effects. The combination treatment shows better improvement due to the synergistic effects of ALA with both T1 and T3. OS indicators like MDA are powerful independent predictors of cardiovascular disease and can lower the body's antioxidant defense system and harm cells (40). Compared to the D group, all therapy groups reduced MDA levels significantly. In DPP4 and SGLT2 inhibitor trials, MDA-LDL levels were dramatically reduced (41,42). Additionally, a study involving type 1 diabetic children and adolescents who received ALA supplementation (300 mg twice a day for 16 weeks) showed a substantial decrease in serum MDA levels compared to a placebo group (43). ALA supplementation decreases triglycerides (TG), total cholesterol (TC), and LDL levels (48), which can reduce lipid peroxidation caused by RO (44). Within our study, it was observed that T2 and T4 treatments resulted in a dramatic greater reduction in MDA levels compared to T1 and T3 treatments. This can be attributed to the addition of ALA to the standard treatment in both T1 and T3.

The serum lipoprotein-associated phospholipase A2 (LPPLA2) levels were significantly higher in the rats injected with STZ than in the N group. Similar to our findings, diabetic rats have higher LPPLA2 levels than nondiabetic rats (45) Inflammation and vascular dysfunction are linked to circulating LPPLA2 (46). Endothelial dysfunction and arterial stiffness are independent risk factors for stable coronary artery disease (CAD) patients with high LPPLA2 levels (47). LPPLA2 serum levels were considerably lower in the treated group T 1 and T3 than in the D group. This study suggests that diabetics may benefit from ALA in addition to regular anti-diabetic therapies to decrease cardiovascular problems. ALA as an antioxidant reduces macrophage and LPPLA2 synthesis by neutralizing free radicals in which LPPLA2 levels were considerably lower in treatment groups than D groups in our experiments (48,49). NLRP3 inflammasome is the best-recognized inflammasome, found largely on monocytes and macrophages (50) NLRP3 promotes inflammasomes and activates pro-caspase-1, which activates the pro-inflammatory and pro-fibrotic cytokines IL-1β and IL-18 (51) Lp-PLA2 hydrolyzes glycerophospholipids to produce bioactive

lipids with pro-inflammatory and pro-oxidative properties. Most Lp-PLA2-derived proinflammatory effects come from Lysophosphatidylcholine (LysoPC). Vascular disorders are linked to elevated plasma Lp-PLA2 (52) Our results provide further evidence that our therapy has therapeutic potential for reducing DCM-induced cardiac remodeling and dysfunction by inhibiting NLRP3 inflammasome activation in macrophages. As a result, blocking Lp-PLA2 might be a promising new treatment for reducing the risk of cardiac fibrosis and DCM. Researchers have shown that those with coronary artery disease or heart failure had greater levels of the protein cardiac troponin T (cTnT), a marker of myocardial injury (53). Diabetes patients may have high hs-cTnT levels and microvascular myocardial infarctions due to hyperglycemia and lipotoxicity, which cause microvascular dysfunction. In this study, D groups had higher biomarkers than N groups. Diabetes and CVD share risk factors such as inflammation, endothelial dysfunction, and platelet activation. Enhanced oxidative stress and AGE products may contribute to the development of diabetes and CVD by damaging β-cells and raising hs-cTnT (54). Due to anti-inflammatory effects of the treatment groups from both T1 and T3 in addition to their direct effect on ROS and oxidative stress biomarkers as explained before, so significant results were obtained in reduction of hs-cTnt in comparison with D group and the combination group treatment T2 and T4 provided significant reduction of hs-cTnt in comparison to treatment groups T1 and T3 due to presence of ALA which was a novel antioxidant and scavengers ROS and free radicals and provided additional benefit when combined with the treatment groups T1 and T3.

In heart failure with decreased ejection fraction, NEP, a metalloprotease that proteolyzes several peptides, including natriuretic peptides, is prognostic and therapeutic (55)s. NEP hydrolyzes natriuretic peptides (NP)s and modifies their structural and functional effects on the heart, kidney, and other organs (56) NEP is implicated in glucose homeostasis, and NEP inhibitors enhance protective NPs, which protect against diabetes consequences. Thus, inhibiting (NEP) in nutritional excess might boost GLP-1, natriuretic peptides, and bradykinin levels, which enhance glucose homeostasis (57). NEP boosts exogenous active GLP-1's insulinotropic action and glucose-mediated insulin production in vitro (58) In our studies T1 and T3 significantly decreased NEP in comparison with the D group because they could reduce glucose levels significantly in comparison with the D group by raising insulin levels and Inhibiting NEP, which is involved in the impairment of glucose homeostasis by influencing pancreatic cell mass and the development of pancreatic cell dysfunction, may have positive effects on insulin resistance and glycemic control by increasing endogenous GLP-1 activity and decreasing DPP4 activity Because NEP elevates dipeptidyl peptidase-4 (DPP4) activity, it also plays a role in boosting active GLP-1 proteolysis, which is why the D groups had a higher (NEP) value than the T3 group. In treatment combination groups T2 and T4 provided significant reduction in the level of NEP due to the presence of ALA which provided additional decline in the blood glucose through their antioxidant properties and significant reduction of NEP ($P<0.005$).

Immunohistochemistry with CTSB antibody showed that the D group had considerably more brown-colored CTSB percentage area than the N group. Lysosomal (CTSB) regulates cell autophagy and apoptosis (59) CTSB contributes to cell pyroptosis (60) Pyroptosis is a unique kind of programmed necrosis caused by inflammasomes like the NOD-like receptor (NLR) family (61). Inflammasomes are activated by numerous danger-associated molecular patterns (DAMPs) after cell damage and activate caspase-1, which activates Gasdermin-D (GSDMD), producing a membrane hole and killing the cell (62). Pyroptosis increases DCM incidence, when diabetic hearts upregulated caspase-1, NLRP3, and GSDMD. Circular RNA activation of caspase-1 exacerbated DCM pyroptosis and cardiomyocyte death. Thus, reducing cardiomyocyte pyroptosis may prevent DCM (63). Serum CTSB levels were strongly related to cardiovascular events in stable coronary heart disease patients (64) So when the treatments have been given to the diabetic rats the release of cathepsin B declined significantly (as it's clear from the highlighted brown area) after four weeks in comparison with the N groups and restored to near normal which means that they decreased the cardiomyopathic change effectively. Moreover, treatment groups in which ALA has been added provided a better reduction on the level of cathepsin B because ALA inhibited intraliposomal Cathepsin-mediated apoptosis by chelating excess iron in endothelial lysosomes after intimal damage (65).

In the context of STZ-induced diabetic rats, the presence of diabetes and glucotoxicity has resulted in a notable increase in oxidative stress biomarkers, such as MDA and TXNIP, alongside inflammatory biomarkers like NF Kb105 and LPPLA2 besides myopathy biomarkers such as NEP and Hs-cTnT. Promising therapeutic interventions, including trelagliptin or remogliflozin, either alone or in conjunction with the novel antioxidant ALA, have demonstrated significant enhancements in cardiac biomarkers. The supplementation of these treatments with ALA holds the potential for clinical benefits in ameliorating the aforementioned cardiac biomarkers, which directly or indirectly contribute to the pathophysiology of diabetic DCM. The efficacy of this combination was supported by immunohistochemical examination, which revealed a reduction in the percentage area of CTSB, a marker secreted following cardiac cell damage when ALA was included as part of the treatment. However, further investigation is warranted to assess the potential adverse reactions and impact on other organs implicated in diabetes complications

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Interest conflict
Conflict of interest not discovered

Author's contribution
Each author contributed equally to the study's conception, execution, statistical analysis, and paper writing.

Availability of data and material
This paper has been published with all the data produced during this investigation.

Ethical approval
Permission number 2282022-844 HMU-EC-PH was

granted by the ethical committee of Hawler Medical University's College of Pharmacy for this study.

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