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Research Article

Cardioprotective and Hypolipidemic Effect of Cardamom Oil-Loaded Lipid Carrier Nanoparticles in a Rat Model of Streptozotocin-Induced Diabetes

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Abstract

Background: Diabetic cardiomyopathy (DCM) is a serious complication of poorly managed diabetes. Inflammation, hyperglycemia, oxidative stress, hyperlipidemia, and other factors all play a role in DCM pathogenesis. ***Objective***: To investigate the cardioprotective effects of cardamom oil-loaded lipid carrier nanoparticles (CEO-LC NPs) on streptozotocin (STZ)-induced diabetes in rats. ***Methods***: Twenty-four male rats were randomly divided into four groups of six each. STZ (50 mg/kg) caused diabetes in all groups but the negative control. The diabetic control group (G1) received a normal saline solution. For 28 days, group G2 received CEO-LC NPs (600 mg/kg), group G3 received empagliflozin (10 mg/kg), and group G4 (no diabetes) received normal saline as a negative control. On day 29, blood samples were taken to determine blood glucose, cholesterol, LDL, HDL, and triglyceride levels, as well as oxidative stress markers. Additionally, atherogenic indices were calculated. Heart tissue was sent for histopathological examination. ***Results***: In diabetic rats treated with CEO-LC NPs, serum glucose, cholesterol, LDL, and triglyceride levels were significantly reduced, while HDL levels increased. The CEO-LC NP treatment also reduced oxidative stress by increasing total antioxidant capacity while decreasing malondialdehyde (MDA). Furthermore, diabetic rats treated with CEO-LC NP had significantly lower AIP, CRI-I, and CRI-II ratios. ***Conclusions***: CEO-LC NPs improve cardioprotection in STZ-induced diabetic rats by lowering plasma lipid levels and oxidative stress.

Keywords: Cardamom oil, Cardioprotective effect, Diabetes, Lipid nanoparticles, Oxidative stress, Rats.

التأثير الوقائي للقلب وخفض شحوم الدم للجسيمات النانوية الحاملة المحملة بزيت الهيل في نموذج السكري المستحث بالستبتوزوسين في الجرذان

الخلاصة

الخلفية: اعتلال عضلة القلب السكري هو أحد المضاعفات الخطيرة لمرض السكري الذي تتم إدارته بشكل سيئ. يلعب الالتهاب وارتفاع السكر في الدم والإجهاد التأكسدي وفرط شحميات الدم وعوامل أخرى دورا فيه. **الهدف**: التحقيق في التأثيرات الوقائية للقلب للجسيمات النانوية الحاملة للدهون المحملة بالهيل (CEO-LC NPs) على مرض السكري الناجم عن الستربتوزوتوسين (STZ) في الجرذان. **الطريقة**: استخدم 24 جرذا ذكرا قسموا عشوائيا إلى أربع مجموعات من ستة لكل منها. أعطيت المجموعة الضابطة (G1) محلول ملحي فقط. لمدة 28 يوما، تلقت المجموعة G2 600 مجم/كجم من CEO-LC NPs، وتلقت المجموعة G3 إمباغليفلوزين (10 مجم/كجم)، وتلقت المجموعة G4 (G4محلول ملحي طبيعي كعنصر تحكم سلبي. في اليوم 29، تم جمع عينات الدم لتقييم مستويات الجلوكوز في الدم والكوليسترول و LDL و HDL والدهون الثلاثية، بالإضافة إلى علامات الإجهاد التأكسدي. بالإضافة إلى ذلك، تم حساب مؤشرات تصلب الشرايين. تم إرسال أنسجة القلب للتحليل النسيجي المرضي. **النتائج**: في الجرذان المصابة بالسكري التي عولجت ب CEO-LC NPs، انخفضت مستويات الجلوكوز في الدم والكوليسترول و LDL والدهون الثلاثية بشكل كبير، بينما زادت مستويات HDL كما قلل علاج CEO-LC NP من الإجهاد التأكسدي عن طريق زيادة إجمالي القدرة المضادة للأكسدة مع تقليل MDA. علاوة على ذلك، كان لدى الجرذان المصابة بالسكري التي عولجت ب CEO-LC NP نسب AIP و CRI-I و CRI-II أقل بكثير. **الاستنتاجات**: تعمل CEO-LC NPs على تحسين حماية القلب في الجرذان المصابة بالسكري التي يسببها STZ عن طريق خفض مستويات الدهون في البلازما والإجهاد التأكسدي.

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INTRODUCTION

Hyperglycemia, a hallmark of diabetes mellitus (DM), is a metabolic syndrome with numerous complications that increases the risk of microvascular and macrovascular problems [1]. Cardiovascular complications are commonly acknowledged as the primary cause of morbidity and mortality in individuals with diabetes, stemming from uncontrolled, prolonged hyperglycemic states [2]. Diabetic cardiomyopathy (DCM), a cardiac condition associated with diabetes mellitus (DM), is one issue. It is brought on by alterations in the structure and function of the myocardium, which persist even after accounting for other conditions such as high blood pressure, atherosclerosis, and coronary artery disease (CAD) [3]. In addition to a number of other contributing variables, inflammation, oxidative stress, hyperglycemia, and hyperlipidemia may be considered primary DCM pathogenic mechanisms [4]. An imbalance between the body's production of free radicals and its antioxidant reserves leads to oxidative stress [5]. It has been observed that the heart of an animal model of diabetes produces too many reactive oxygen species (ROS) and has fewer antioxidant enzymes [6]. According to studies, plants with hypoglycemic, anti-inflammatory, and antioxidant properties may lessen the effects of oxidative stress and thereby prevent DCM [7]. Indian medicinal plants and spices have long been known for their antioxidant qualities and have demonstrated great promise in the treatment of a wide range of human illnesses, including cardiovascular conditions [8]. An oral hypoglycemic drug called empagliflozin was approved for clinical usage in 2014 [9]. This drug is classified as an inhibitor of the sodium-glucose co-transporter 2 (SGLT2) [10]. A novel target for the treatment of type 2 diabetes is SGLT2, the major transporter for glucose reabsorption from the glomerular filtrate [11]. The Zingiberaceae family of spices includes the sweet spice cardamom (Elettaria cardamomum), which has dual uses as a flavoring and medicinal herb. Because of its unique aroma, it is widely used in Eastern, Arab, Scandinavian, and Western cuisines [12]. Numerous experimental investigations have demonstrated the many health benefits of cardamom, including its anticancer [13], gastroprotective [14], antihypertensive [15], anti-inflammatory [16], and immunomodulatory effects. It has a strong inhibitory effect on lipid peroxidation and is a powerful scavenger of hydroxyl radicals and superoxide anions [17]. As a result, cardamom exhibits properties as an antioxidant and a free radical scavenger [18]. Apart from its antioxidant qualities, it has also been shown to have fibrinolytic, hypotensive, and antiplatelet activities [19], all of which greatly enhance its cardioprotective effects. The beneficial effects of cardamom essential oils are limited by their low permeability, low solubility, low bioavailability, uncontrollably volatile nature, and poor stability during storage. These problems can be resolved by incorporating essential oils into nanoemulsions. The stability, enhanced surface area, and nanometric size of nanoemulsions, among other special properties, enhance the efficacy of drug dosage [20]. The goal of the current study was to investigate the hypolipidemic and cardioprotective effects of lipid carrier nanoparticles loaded with cardamom essential oil in rats with STZ-induced diabetes.

METHODS

Materials

Cardamom essential oil was purchased from mountainroseherbs.com (Sri Lanka). Cocoa butter was purchased from Deluxe Shea Butter Co. (Australia), and extra virgin olive oil was purchased from Kasho Factory Co. (Sulaimani, Iraq). Germany-based Merck provided the analytical-grade DPPH powder and tween 80.

Preparation of CEO loaded NLC

The CEO-loaded NLC was synthesized by the low-energy nano-emulsification approach in conjunction with high-shear homogenization and sonication. This required the dissolution of CEO in olive oil and mixing it with melted solid lipid (cocoa butter). Then, a surfactant called Tween 80 was added drop by drop to the lipid phase while it was being mixed at 20,000 rpm for 45 minutes using high shear. Consequently, sonication was implemented ten times for one minute each, separated by a one-minute interval. Throughout these procedures, the suspension's temperature was maintained at 50±5 ^{o}C. For recrystallization of the lipid phase, the hot oil-water nanoemulsion mixture was frozen to 4 ^{o}C in a refrigerator. Eventually, the NLC was formed.

Characterization of CEO-Loaded NLC

The particle size (z-average size), zeta potential, and polydispersity index (PDI) of CEO-loaded NLCs were all determined by using dynamic light scattering (DLS) and the zetasizer. The surface morphology of the obtained NLC was investigated by scanning electron microscopy. Differential scanning calorimetry (DSC), utilizing a DSC thermal analyzer, and X-ray diffraction (XRD) analysis were both used to perform thermal analysis [21].

Animal model of diabetes

Twenty-four male Wistar rats, weighing (230±30) grams and aged 10–12 weeks, were obtained from the animal center of the College of Pharmacy, University of Sulaimani. The animals were housed in polypropylene cages under standard conditions (maintained at a temperature of 22±2.0 degrees Celsius

with 50% humidity) and subjected to a 12-hour dark-light cycle. They were provided with a conventional pellet diet and had unrestricted access to water. The entire experiment was conducted in compliance with the Institutional Animal Ethical Committee guidelines. A single intraperitoneal injection of 50 mg/kg streptozotocin (STZ) (Nanjing Duly Biotechnology, Nanjing, China) was utilized to induce diabetes mellitus. STZ was dispersed in freshly prepared citrate buffer (pH 4.5). The blood glucose level in the tail blood samples of the 12-hourly fasting rats was measured one week after the induction. Animals were defined as diabetic when their blood glucose levels were ≥ 250 mg/dL [22].

Treatment protocol

Twenty-four animals were randomly allocated into four groups, each of six, as follows: G1, the negative control group, received normal saline orally. G2, the positive control, received normal saline along with an STZ (50 mg/kg) injection to induce diabetes. G3 (CEO-LC NPs) received CEO-LC NPs (600 mg/kg) along with the STZ protocol. G4, the empagliflozin group, received empagliflozin (10 mg/kg) along with the STZ protocol. All the treatment groups received the treatments for 28 days. At the end of the experiment, the rats were fasted overnight, anesthetized with ketamine and xylazine, and blood samples were collected for various biochemical tests. Heart tissues were sent for histopathological examination.

Outcome measurements

Fasting blood glucose concentrations were assessed at three time intervals: prior to STZ injection (day zero), on the seventh day, and on the twenty-eighth day of the experiment. Glucose levels were measured using a One Touch On-Call Plus glucometer (ACON Lab, India), obtaining a two-milliliter blood sample from a tail prick after mild ether anesthesia. Prior to blood collection, the animals underwent a fasting period of 12 to 15 hours. The serum levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were determined at the end of the experiment by the enzymatic assay method using analytical kits (Biolab SA Maizy, France). Lipid peroxidation degree was evaluated by measuring malondialdehyde (MDA) level, and antioxidant capacity was estimated using an ELISA kit (bioassay technology laboratory, Korea). For histopathological evaluation, the isolated heart tissue was cut into small fragments and immersed in 10% formalin for a day. Subsequently, these fragments were sliced into sections measuring 3–5 µmm in thickness using a microtome and stained with hematoxylin-eosin After staining, the sections were mounted using disterene phthalate xylene (DPX). Then, Olympus Soft Imaging Solution (GmbH, Munster, Germany) developed Cell Imaging software specifically designed for Life Science microscopy to examine the images of each tissue section.

Atherogenic index and lipid ratio evaluation

Atherogenic index of plasma (AIP), Castelli risk index-I (CRI-I), and the Castelli risk index-II (CRI-II) ratio are diagnostic substitutions for predicting cardiovascular risks [23]. The results obtained from the AIP calculation can be classified as follows: −0.3 to 0.1 indicates low risk, 0.1 to 0.24 indicates medium, and more than 0.24 indicates a high risk of cardiovascular disease [24]. The Castelli risk index-I (CRI-I), also known as the cardiac risk ratio (CRR), is a diagnostic measure assessing total cholesterol levels, indicative of coronary plaque formation progression [25]. The following formulas were used for calculating AIP, CRI-I, and CRI-II [26, 27].

Atherogenic index (AIP) = log (TG/HDL-c)

Castelli risk index-I (CRI-I) = TC/HDL-c

Castelli risk index-II (CRI-II) = LDL-c/HDL-c

Statistical analyses

This analysis was conducted using GraphPad Prism software version 8. Data were presented as mean ± standard deviation. A one-way analysis (ANOVA) was employed for comparisons among all groups, followed by Dunnett's post hoc test. Additionally, the Student's t-test was utilized for comparisons between groups. Pearson's correlation coefficient (r) was utilized to assess the association between and across the means of the variables. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

After the seventh day of STZ injections, all groups exhibited a marked increase in blood glucose levels compared to the negative control group ($p<0.0001$). Following the oral administration of CEO-LC NPs for 28 days, there was a significant reduction in blood glucose levels in the CEO-LC NPs-treated group ($p= 0.0005$) when compared to the diabetic control group. Additionally, empagliflozin (10 mg/kg) exhibited a decrease in blood glucose levels compared to the diabetic control group ($p= 0.028$) (Figure 1). The oral administration of CEO-LC NPs for 28 days resulted in a significant decrease in serum total cholesterol (*p-value* = 0.0019), triglycerides ($p= 0.0008$), and LDL levels ($p= 0.0004$), alongside an increase in HDL levels ($p= 0.0145$) compared to the diabetic control group. Moreover, the group treated with empagliflozin showed a significant reduction in total cholesterol and LDL levels compared to the diabetic control group (*p-value* = 0.0017 and 0.0010, respectively).

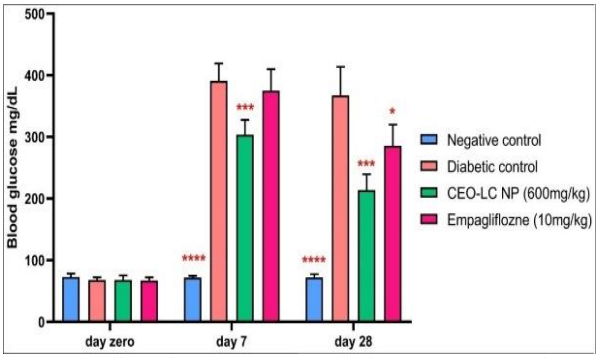

Figure 1: Effect of oral administration of CEO-LC NPs on fasting blood glucose concentrations on days zero, 7 and 28 of the experiment. Values are expressed as mean±SD (*n*= 6). Statistical differences from the diabetic control group are denoted as: * p<0.05, *** p< 0.001, and **** p< 0.0001.

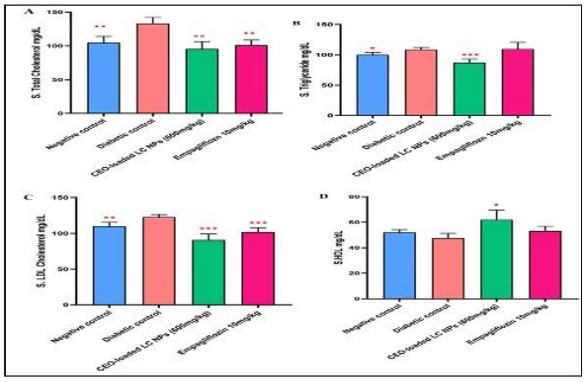

Figure 2: The effect of CEO-LC NPs 600mg/kg on lipid profile parameter. Values are expressed as mean ±SD (n = 6). Statistical differences from diabetic control group are indicated as: * p<0.05), ** p<0.01, *** p<0.001.

Additionally, the CEO-LC NPs-treated group exhibited lower serum triglyceride and higher serum HDL levels than those observed in the empagliflozin-treated group (Figures 2A, 2B, 2C, and 2D). The Atherogenic Index of Plasma (AIP) demonstrated a noteworthy reduction in both the CEO-LC NPs-treated group (p= 0.0007) and the negative control group (p= 0.0078) compared to the diabetic control group (Figure 3A).

Both Castelli Risk Index-I (CRI-I) and Castelli Risk Index-II (CRI-II) displayed a significant decrease in the CEO-LC NPs treated group (p= 0.0005 and < 0.001), the Empagliflozin treated group (p= 0.0043 and 0.0055), and the negative control group (p= 0.0086 and 0.0055) in comparison to the diabetic control group (Figure 3B and 3C).

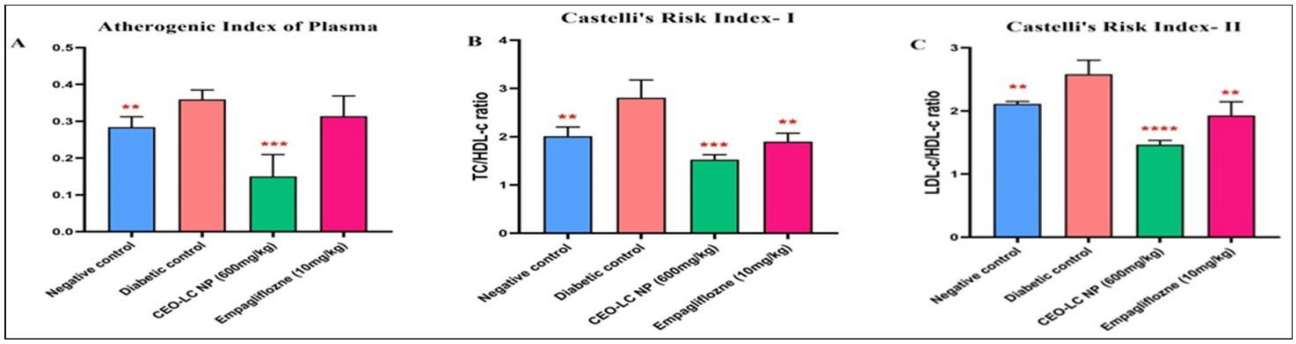

Figure 3: The effect of CEO-LC NPs on lipid ratio and Atherogenic index of plasma. Values are expressed as mean ±SD (n = 6). Statistical variances from the diabetic control group are denoted as: ** p<0.01, *** p< 0.001, and **** p< 0.0001.

Table 1 depicts the correlation between traditional lipid parameters and both the AIP and emerging lipid ratios. Total cholesterol (TC), LDL-C, and TG exhibited a positive correlation with AIP and the atherogenic indices (p<0.05). Conversely, HDL-C displayed a negative correlation with all the atherogenic indices (p<0.05). The CEO-LC NPs-treated group exhibited a significant decrease in MDA levels (p= 0.0023) compared to the diabetic control group. Moreover, the CEO-LC NPs-treated group displayed a remarkable reduction in MDA levels (p= 0.0082) compared to the empagliflozin-treated group (Figure 4A).

Table 1: Relationship between AIP, Lipid Ratios and the Traditional Lipid Profile in the experiment

Variable	AIP		CRI-I		CRI-II	
	r-value	*p*-value	*r*-value	*p*-value	*r*-value	*p*-value
TC (mmol/l)	0.5664	0.0222	0.9140	<0.0001	0.7970	0.0002
HDL-c (mmol/l)	-0.8101	0.0001	-0.7691	0.0005	-0.8458	<0.0001
LDL-c (mmol/l)	0.6902	0.0031	0.8331	<0.0001	0.9011	<0.0001
TG (mmol/l)	0.8823	<0.0001	0.4757	0.0625	0.5629	0.0232

AIP: Atherogenic index of plasma; CRI: Castelli's risk index, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, TC: Total cholesterol, TG: Triglyceride.

Furthermore, a substantial increase in total antioxidant capacity levels in the CEO-LC NPs-treated group (p<0.0001) compared to the diabetic control group was observed. This elevation was statistically higher than that produced by the empagliflozin-treated group (Figure 4B).

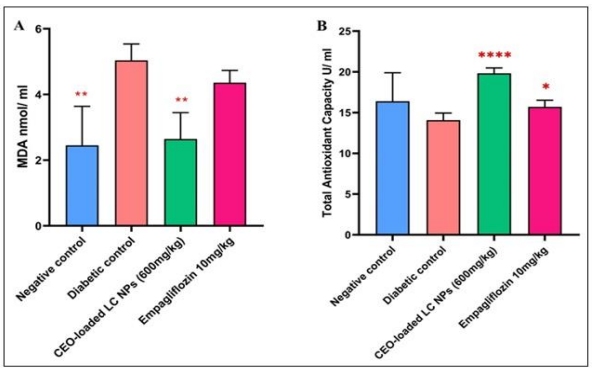

Figure 4: The effect of CEO-LC NPs on lipid peroxidation (A), and antioxidant status (B). Values are expressed as mean±SD (n = 6). Statistical differences from the diabetic control group are denoted as: * p<0.05, ** p< 0.01, and **** p< 0.0001.

The histopathological examination of the rat hearts from all groups is shown in Figure 5. The hearts of the normal control group displayed an intact and homogeneous histoarchitecture without necrosis, edema, or inflammation. They were composed of cardiomyocytes with eosinophilic cytoplasm (C) and nuclei (N), predominantly oriented longitudinally (Figure 5A). The hearts of the diabetic rats in the control group grew bigger in some places (hypertrophy) (H), and there were also small areas of damage and death in the hypertrophied myocardium (N). Additionally, small areas of fibrosis surrounded by active fibroblasts were observed, along with blood vessel congestion (F). It's important to note that necrotic changes may arise due to relative hypoxia in the hypertrophied muscle, as the blood supply might not adequately meet the demands of increased fiber size (Figure 5B). In contrast, rats given CEO-LC NPs (Figure 5C) showed protection from myocardial injury in the form of mild myocardial hypertrophy without significant other pathological changes. Conversely, the empagliflozin-treated group exhibited hypertrophy of cardiomyocytes (H), blood vessel congestion (C), and a fibrotic area with active fibroblasts (Figure 5D).

DISCUSSION

Many factors, such as oxidative stress, hyperglycemia, and hyperlipidemia, are linked to the start and development of DCM [28]. To the best of our knowledge, this is the first study to look into how oral CEO-LC NP treatment affects the plasma glucose levels and lipid profile in male diabetic rats. In the current study, the groups treated with CEO-LC NP and empagliflozin showed a notable improvement in hyperlipidemia and hyperglycemia. Comparing the streptozotocin-treated animals to the normal control group, there was a noticeable increase in the animals' blood glucose and cholesterol levels. After receiving CEO-LC NPs for 28 days, diabetic rats' levels of HDL-c were considerably higher and their levels of glucose, triglycerides, cholesterol, and LDL-c were significantly lower.

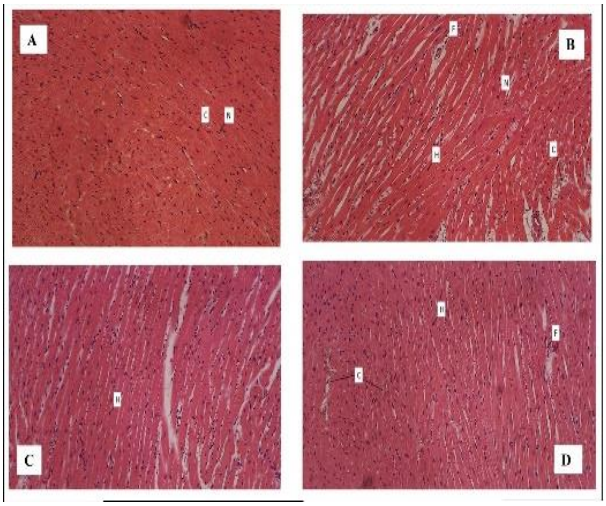

Figure 5: effect of CEO-LC NPs on histopathology of heart in the streptozotocin-induced diabetic in rats. (A) Representative photomicrographs of the heart showing normal-looking myocardium in negative control group. (B) diabetic control group reveal increased in size of individual muscle fibers (hypertrophy) (H), with minute foci of degenerative changes and necrosis in hypertrophied myocardium (N), minute area of fibrosis surrounded by active fibroblast, Blood vessels congestion is seen (F). (C) CEO-LC NPs 600mg/kg treated group shows mild myocardial hypertrophy, without other significant pathological changes. (D) empagliflozin 10mg/kg treated group exhibit hypertrophy of cardiomyocytes (H), congestion of blood vessels (C), active fibroblasts are seen within fibrotic area (F) H&E staining 10X.

The CEO-LC NPs group produced a reduction that was higher than the reduction resulting from empagliflozin. These results align with previous studies [29–35]. Cardamom has been used in traditional medicine since ancient times to treat a wide range of ailments, including asthma, kidney and digestive issues, dental and gum infections, cataracts, nausea, diarrhea, and heart problems [36,37]. It's possible that cardamom lowers cholesterol because it increases lipoprotein lipase activity, increases metabolism of cholesterol, and decreases intestinal lipid absorption [39,40]. It has been demonstrated that cardamom enhances glycemic status, insulin resistance, and glucose metabolism. By boosting the activities of Sirtuin 1 (SIRT1), Peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator-1 alpha (PGC-1α) [41], and reducing the activity of nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB), the bioactive components found in *Elettaria cardamomum* have the ability to control both insulin secretion and insulin resistance. Furthermore, it controls the metabolism of glucose by blocking the activities of α-glucosidase and α-amylase [39]. The atherogenic index of plasma (AIP) was considerably reduced in the CEO-LC NPs-treated group when compared to the diabetic control, negative control, and empagliflozin-treated groups, according to the estimation of lipid ratios. Acute myocardial infarction is well predicted by the inverse relationship between triglycerides and high-density lipoprotein cholesterol [42]. An increased risk

of cardiovascular complications is associated with an AIP value greater than 0.24 [24]. It is also thought to be helpful for tracking the efficacy of treatments and forecasting cardiovascular risk [26]. Additionally, the CEO-LC NPs-treated group showed significantly lower values for both Castelli's Risk Ratio I (CRI-I) and Castelli's Risk Ratio II (CRI-II) when compared to the diabetic control group. The development of coronary plaques is linked to CRI-I, which is measured by the ratio of total cholesterol (TC) to HDL-C [43]. Research has highlighted the usefulness of these metrics as indicators of cardiovascular disease (CVD), particularly when lipid profile parameters seem normal [26]. Diabetes and its related cardiovascular complications, including stroke, atherosclerotic disease, and myocardial infarction, are significantly influenced by oxidative stress [44,45]. In diabetic rats, lipid peroxidation—which is characterized by elevated MDA levels—is used as a marker of oxidative stress. Comparing the CEO-LC NPs group to the diabetic control group and the empagliflozin-treated group, there was a notable decrease in MDA levels and an increase in total antioxidant capacity. CEO-LC NPs may have an antioxidant effect by increasing antioxidant enzymes and reducing lipid peroxidation [37,46–48]. The natural enzymatic antioxidant defense mechanisms of the body are crucial for preventing tissue damage caused by free radicals. Lipid peroxidation and the production of free radicals can both rise in response to modifications in the activities of antioxidant enzymes [49]. Prior to receiving CEO-LC NPs, heart tissue's antioxidant enzyme activity was higher. This was probably due to the endogenous antioxidants' superior capacity to eliminate free radicals.

Conclusion

This study demonstrates that by reducing metabolic abnormalities and cell damage, CEO-LC NP administered to rats with streptozotocin-induced diabetes helped protect their hearts. Due to their increased risk of cardiovascular problems, diabetics may benefit from the early detection provided by atherogenic indices.

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Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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