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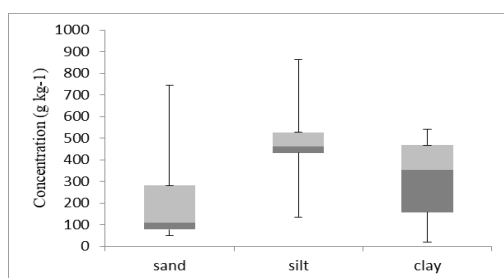
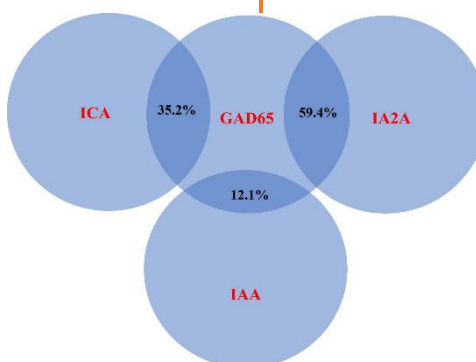
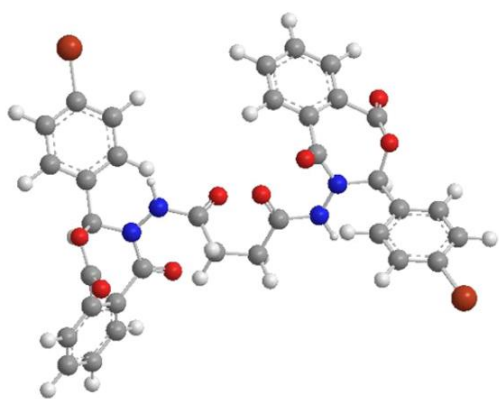
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Anti-lipogenic, anti-inflammatory, hepatic, and renal effects of Cardamom Essential Oil Loaded Nanostructured Lipid on Rats Fed High-Fat Diet

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Article info	Abstract
<p>Original: 10/06/2023 Revised: 09/07/2023 Accepted: 10/07/2023 Published online: 20/12/2023</p> <p>Keywords: <i>Nanoparticles, animal study, plant products, metabolic diseases</i></p>	<p>Background: Cardamom has a variety of pharmacological properties. Objectives: This study examined the potential anti-lipogenic, anti-inflammatory, hepatic, and renal effects of Cardamom Essential Oil-Loaded Nanostructured Lipid Carrier (CEO-NLC) in rats fed high-lipid diets. Methodology: Male Sprague Dawley rats (No.= 42) were divided into 7 groups. The negative control group was fed standard normal rat chaw; the positive control group was fed a high-fat diet (HFD); the LCEO-NLC group was fed HFD and low doses of CEO-NLC, HCEO-NLC group fed HFD and high amount of CEO-NLC, atorvastatin group fed HFD with atorvastatin, atorvastatin/LCEO-NLC group fed HFD with atorvastatin in combination with LCEO-NLC, and CEO group fed HFD with CEO. All drenching processes were done for 14 consecutive weeks. The body weights were measured, and blood samples were taken to determine lipid profile, renal/hepatic enzymes, IL-1, IL-6, and TNF-α. Results: The LCEO-NLC, HCEO-NLC, atorvastatin, and atorvastatin/LCEO-NLC groups showed a lower body weight than the PC group ($p < 0.05$). Compared with the PC group, the LCEO-NLC and other HFD groups significantly reduced serum cholesterol and triglyceride. Supplementation with LCEO-NLC decreases the ALP to 193.3 U/L vs 388.5 U/L in PC, while HCEO-NLC, atorvastatin and CEO groups recorded less improvement in ALP. The ALT and AST were significantly high ($p < 0.05$) in the LCEO-NLC group when compared to NC or PC groups, and this increase was still high in the atorvastatin/LCEO-NLC group or in HCEO-NLC. The mean TSB was 0.125 mg/dl in the PC group, the mean was lower (0.117 mg/dl) in the LCEO-NLC group, and more decline was recorded (0.099 mg/dl) in the HCEO-NLC group. All study groups which received CEO-NLC did not show a significant change in urea/creatinine levels. The IL-1 and TNF-α were lowered in the LCEO-NLC group, while the HCEO-NLC group did not show a cytokine decline. Conclusions: CEO-NLC could control the rise in serum cholesterol and triglyceride but did not cause significant changes in renal function. Different doses of CEO-NLC had effects on liver enzymes and bilirubin. There were mild non-significant effects of atorvastatin on coadministration with CEO-NLC. Low concentrations of CEO-NLC cause reduce in the IL-1 and TNF-α but not IL-6.</p>

Introduction

Nanoparticles (NPs) are tiny materials with dimensions under 100 nm. The use of NPs in medicine is increasing, and they are newly emerging players in the medical field with diverse clinical applications ranging from diagnostic to therapeutic agents; for example, they are used as contrast substances in imaging techniques, carriers for drugs, and genetic manipulation of tumours (1).

The use of NPs as drug delivery is an advanced application for their use in the medical field; this is partly due to the success of forming NPs made up of liposomes or polymers which can enclose a drug. This improves the delivery of many medicinal compounds, particularly chemotherapeutic drugs, and offers creative, alternative ways to get around many problems with their effectiveness and safety (2). NPs have unique features that make them suitable for drug delivery, such as the sizeable surface-to-mass ratio. They have high adsorbing properties, which make them able to carry other compounds like drugs (3).

The Nanostructured lipid carriers (NLC) encompass a mixture of solid lipids enclosing a liquid phase. This results in the incompletely crystallized molecule and conveys many benefits over solid phase NPs, like enhancing the drug loading capacity and improving drug release and stability. NLCs have many applications in pharmaceutical and decorative substances due to their easy preparation, scale-up feasibility, biological compatibility, nontoxic effect, efficient targeting and the possibility of their administration by various routes using site-specific delivery (6).

Cardamom is a perennial herb commonly used in food to improve flavour. Cardamom belongs to the *Zingiberaceae* family and is frequently found in India, Indonesia, Bangladesh, Pakistan, and Burma. It is widely used as a phytotherapeutic agent in traditional medicine due to its anti-inflammatory activity (8). People may benefit from cardamom in improving metabolic syndrome and fatty liver, decreasing obesity, controlling hypertension and diabetes mellitus, and many other gastrointestinal diseases and complaints, such as respiratory and urinary diseases. Moreover, in traditional medicine, cardamom is an antibacterial, antiviral, and antifungal agent (9). It is also used in treating cardiovascular diseases and epilepsy. It also has anticancer properties. So, evaluating the potential benefits of cardamom is one of the researchers' priorities in pharmacology.

Cardamom was shown to contain many compounds like geraniol, terpinene, stigmasterol, β -pinene, citronellol, borneol, bisabolene, limonene, geranyl acetate, eugenol acetate, β -sitosterol, cineol, nerolidol, linalool, heptane, phytol, α -pinene, myrcene, menthone, sabinene, and α -terpineol (10).

Recent studies have concentrated on natural substances' anti-inflammatory properties as prospective therapeutic agents against infections and inflammatory illnesses. As "natural goods," cardamom extracts and their essential oils (EOs) have been used to cure various ailments, such as asthma, indigestion, and congestive jaundice. Cardamom has a variety of pharmacological qualities, including anti-inflammatory, anticancer, antioxidant, and antibacterial effects (11), which may be attributed to the plant's abundance in physiologically active phenolic chemicals, terpenoids, alkaloids, anthocyanins, and flavonoids (12). Thus, we planned to examine the potential anti-lipogenic, anti-inflammatory, hepatic, and renal effects of Cardamom Essential Oil-Loaded Nanostructured Lipid Carrier (CEO-NLC) in male rats fed with a high-lipid diet.

Materials and Methods

Male Sprague Dawley rats (No.= 42), aged 6 – 8 weeks, weighed 150 – 200 g, were acclimatized on tap water and a standard rat chow for seven days and kept in a well-ventilated room with a 12 hours' dark/light cycle at 27 ± 3 °C. Then, they were randomly divided into seven groups (Table 1).

Table 1: The nutritional and treatment characteristics of study groups.

Group	Diet	Treatment
Negative Control (NC)	Standard normal rat chow	No
Positive control (PC)	High-fat diet	No
LCEO-NLC	High-fat diet	LCEO-NLC (Low dose, 300 mg/Kg body weight)
HCEO-NLC	High-fat diet	HCEO-NLC(High dose, 600 mg/Kg body weight)
Atorvastatin	High-fat diet	Atorvastatin 40 mg/Kg body weight
Atorvastatin and LCEO-NLC	High-fat diet	Atorvastatin 20 mg/kg body weight in combination with LCEO-NLC 300 mg/Kg body weight
Cardamom essential oil (CEO)	High-fat diet	Cardamom essential oil (CEO) alone

All drenching processes were done using force-feeding, ball-tipped needles; for exactly fourteen consecutive weeks. The body weights were measured weekly using a scientific measuring scale, and the animals were observed twice daily for clinical and behavioural abnormalities, toxicological symptoms, food consumption, and gross appearance. The blood samples were collected and centrifuged at 5000 rpm for 5 minutes, and the separated serum was divided into two tubes (one for biochemical tests and the other for ELISA tests). Then both sera were stored at -20 °C.

Biochemical analysis

Serum-measured lipid parameters, including triglycerides (TG), cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Liver enzymes, including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), were also measured as an indicator of liver cell damage. The blood urea and serum creatinine were also investigated. The laboratory investigations were done using the standard diagnostic kits (Roche/Germany) in an automated biochemistry analyzer (HITACHI, Roche COBAS C 311, Germany, and Japan). The inflammatory biomarkers IL-1, IL-6, and TNF- α , were determined by commercial ELISA kits (BT Lab, China). All the inflammatory biomarkers depended on the sandwich ELISA technique and had similar basic procedures.

Statistical analysis

The results were expressed as mean \pm standard deviation. If normally distributed, the data were analyzed using one-way ANOVA, followed by post hoc Bonferroni tests (Statistica 10. StatSoft Inc. OK, USA). If the data was not normally distributed, they were log-transformed to achieve distribution normality before being analyzed. $P < 0.05$ was considered significant.

Ethical approval

The Ethical Committee of the College of Pharmacy, University of Sulaimani, approved this experimental animal research.

Results

The changes in body weight in different groups after 14 weeks of treatment are shown in Figure 1. In contrast to the CP group, the LCEO-NLC, HCEO-NLC, atorvastatin, and LCEO-NLC groups showed a lower body weight than the PC group ($p < 0.05$). The weight loss in LCEO-NLC and HCEO-NLC groups was dose-related. Furthermore, compared with the PC group, the CEO group also displayed a better effect in terms of weight loss.

Compared with the NC group, the mean serum cholesterol level in PC was significantly elevated (136 mg/dl in PC vs 68.7 mg/dl in NC), implying that the hyperlipidemia model was successful. Similar findings were obtained for serum triglyceride (131.9 mg/dl in PC vs 93.5 mg/dl in NC). Compared with the PC group, serum cholesterol and triglyceride were significantly reduced in the LCEO-NLC group ($p < 0.05$). The effect was more pronounced when the dosage of NPs increased in the HCEO-NLC group. In the atorvastatin, atorvastatin & LCEO-NLC, and CEO groups, the serum cholesterol and triglyceride were also distinctly decreased compared to the PC group. Noticeably, triglyceride markedly declined in the CEO group compared with the PC group. In addition, only the HCEO-NLC group and, to less extent CEO group showed a decline in the LDL level, while LDL was not declined in other groups (Table 2). The HDL concentrations significantly increased in the LCEO-NLC group but decreased in HCEO-NLC.

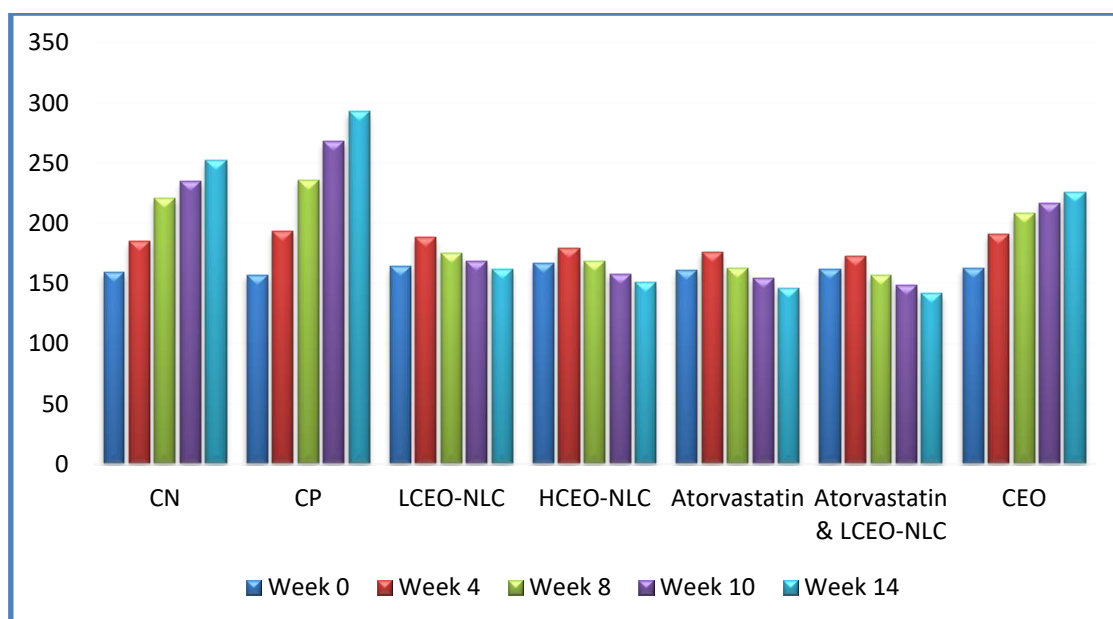


Figure 1: Body weight of rats in different study groups at different time intervals. CN: Control negative, CP: Control Positive; CEO: Cardamom essential oil; NLC: Nanostructured lipid carrier; LCEO: Low dose cardamom essential oil; HCEO: High dose cardamom essential oil

Moreover, the present study showed that the PC group with a high-fat diet did not have an increase in blood urea and serum creatinine; actually, the blood urea in PC was 76.62 mg/dl, which is less than blood urea (124.47 mg/dl) in NC group, while creatinine was 0.45 mg/dl in PC group vs 0.8 mg/dl in NC group. The administration of CEO-NLC did not cause a significant change in blood urea and serum creatinine, and the same was found in other study groups ($p>0.05$) (Table 2).

Table 2: Effects of cardamom, cardamom nanoparticles, and atorvastatin on some parameters in high-fat rats.

Parameter	NC	PC	LCEO-NLC	HCEO-NLC	Atorvastatin	Atorvastatin & LCEO-NLC	CEO
Cholesterol (mg/dl)	68.7±24.1	136.0±27.1	89.0±8.19	72.2±10.5	75.0±3.8	85.9±6.7	82.0±12.4
TG (mg/dl)	93.5±8.6	131.9±48.2	99.0±50.9	77.5±19.6	95.4±27.4	72.8±9.1	57.9±11.2
LDL (mg/dl)	11.8±6.2	16.3±3.8	18.0±7.3	11.7±0.45	16.3±1.8	17.7±4.1	15.3±5.7
HDL (mg/dl)	58.4±8.2	61.3±12.4	97.0±15.6	58.9±9.2	56.3±16.2	78.4±8.3	66.6±10.5
ALP (U/L)	177.0±22.1	388.5±133.2	193.3±44.3	209.8±54.9	259.2±86.1	195.5±8.9	208.5±29.4
ALT (U/L)	57.7±24.7	90.3±35.9	134.6±83.3	86.4±43.9	78.1±24.2	116.2±3.4	110.5±83.6
AST (U/L)	98.9±30.5	125.4±29.7	197.1±129.9	141.1±60.9	165.3±42.4	170.8±8.1	130.8±56.1
Bilirubin (mg/dl)	0.096±0.041	0.125±0.033	0.117±0.035	0.099±0.014	0.092±0.018	0.121±0.012	0.132±0.030
Urea (mg/dl)	124.47±93.4	76.62±29.9	115.77±58.6	73.78±8.8	53.92±21.8	95.93±57.7	102.87±49.4
Creatinine (mg/dl)	0.80±0.39	0.45±0.14	0.54±0.10	0.52±0.04	0.42±0.11	0.64±0.14	0.56±0.11
IL-1 (ng/L)	46.9±8.7	49.3±12.0	44.2±7.12	52±15.1	40.35±6.2	48.61±2.7	59.27±15.1
IL-6 (ng/L)	3.5±0.30	3.4±0.42	4.3±1.27	4.1±0.41	3.1±0.31	3.2±0.21	3.8±0.82
TNF- α (pg/ml)	146.5±32.84	197.5±141.9	108.5±27.9	233±142.7	146.5±13.9	169.5±8.4	184.8±23.5
MDA (nmol/ml)	0.503±0.181	0.667±0.145	0.570±0.97	0.986±0.324	0.529±0.044	0.663±0.191	0.558±0.137

ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transferase; CN: Control negative; CEO: Cardamom essential oil; CP: Control Positive; HCEO: High dose cardamom essential oil; IL: Interleukin; LCEO: Low dose cardamom essential oil; MDA: Malonaldehyde; NLC: Nanostructured lipid carrier; TNF: Tumor necrosis factor; TG: Triglyceride

In the PC group, rats fed a high-fat diet had significantly increased liver enzymes (ALP, ALT and AST) compared to the NC control rats. Supplementation with LCEO-NLC decreases the ALP activity to 193.3 U/L vs 388.5 U/L in PC. The results of ALP in the atorvastatin & LCEO-NLC group (195.5 U/L) were similar to

that of the LCEO-NLC group. While HCEO-NLC, atorvastatin and CEO groups recorded less improvement in ALP activity. The ALT and AST activities were significantly high ($p < 0.05$) in the LCEO-NLC group compared to NC or PC groups, and this increase was still high even when atorvastatin was added in the atorvastatin & LCEO-NLC group. The HCEO-NLC group recorded ALT and AST activities equal to 86.4 U/L and 141.1 U/L, respectively (Table 2). The mean total serum bilirubin was 0.125 mg/dl in the PC group and was lower (0.117 mg/dl) in the LCEO-NLC group, and more decline was recorded (0.099 mg/dl) in the HCEO-NLC group. The atorvastatin & LCEO-NLC group recorded a TSB of 0.112 mg/dl.

Serum levels of IL-1 and TNF- α , but not IL-6, were elevated in the PC group compared to the NC group; this elevation was mild for IL-1 (49.3 ng/L in the PC group vs 46.9 NG/l in the NC group) and significant rise for TNF- α (197.5 pg/ml in PC group vs 151.5 pg/ml in NC group). The IL-1 and TNF- α were lowered in the LCEO-NLC group, while the HCEO-NLC group did not show a decline in these two cytokines. The atorvastatin & LCEO-NLC group did not show significant differences in IL-1, IL-6, and TNF- α compared to the PC group.

Discussion

In the current study, the HFD for 12 weeks resulted in hypercholesteremia and hypertriglyceridemia, and increased body weight, which is similar to that of Jia et al. (13). Dyslipidemia and increased body weight are predisposing factors for many systemic conditions such as ischemic heart diseases, metabolic syndrome, and inflammation (14). We also revealed that CEO-NLC could reduce cholesterol and triglyceride, which HFD increased. Previous reports suggested that cardamom can prevent rising cholesterol levels in the plasma due to HFD feeding in rats (15, 16). In the current study, the higher dose of CEO-NLC was associated with more decline in hypercholesterolemia and hypertriglyceridemia. Moreover, the CEO without NPs were also able to reduce these lipids. These results suggest that HCEO-NLC, LCEO-NLC, and CEO can control hyperlipidemia.

In a meta-analysis of randomized clinical trials, Omid et al. (17) found that cardamom supplementation did not significantly affect LDL-cholesterol or HDL-cholesterol. At the same time, the current study revealed the decrease of LDL only by HCEO-NLC and CEO, while the use of LCEO-NLC or atorvastatin did not cause a decline in LDL. Moreover, LCEO-NLC in the current study caused an increase in HDL concentrations which is not agreed with the results of Omid et al.; however, our results revealed a decrease in HDL upon using HCEO-NLC; thus, CEO-NLC has a dose-related effect on HDL.

In this study, rats fed HFD displayed significant elevations in ALP, AST, and ALT levels compared to those who received a regular diet; the results of Lasker et al. (18) were in line with ours. The HFD mediates oxidative stress on the liver tissue and causes leakage of liver enzymes in the blood circulation. The HFD + LCEO-NLC showed notable control on ALP but the unexpected rise in ALT and AST; these findings are against the results of Rahman et al. (19), who found improvement in liver function and a decrease in ALT and AST activity after administration of cardamom supplement to experimental rats. Adding atorvastatin to LCEO-NLC did not make a significant difference, and it did not have a synergistic effect when combined to control liver injury. The use of HCEO-NLC was associated with less rise in ALT and AST when compared to LCEO-NLC; on the other hand, both concentrations of NPs were associated with improvement in bilirubin concentration. Different concentrations of CEO-NLC have different effects on liver hepatocytes with consequent different results of ALP, ALT, AST, and TSB.

Blood urea and serum creatinine are markers of renal dysfunction, and there is a lack of difference between these renal markers in HFD and NC rats. These outcomes are similar to that of another study indicating that the 12-week feeding protocol was not long enough to induce functional renal damage (20). The LCEO-NLC, HCEO-NLC, or CEO showed no significant change in blood urea and serum creatine compared to the NC,

which may reflect their nontoxic effect on the kidney. Moreover, Elkomy et al. (21) recorded the renal protective effect of cardamom on rats with induced gentamicin toxicity.

Rats fed HFD showed significantly higher levels of TNF- α and mild elevation in IL-1, while IL-6 was nearly similar to rats fed a regular diet. The differences in the secretion of these cytokines may be affected by rat ages, cytokine stimulation pathways, and HFD feeding duration. These results may reflect different ways for cytokines stimulation and secretion, which is affected by the inflammatory process induced by HFD. Moreover, the secretion of these cytokines may be related duration of feeding. Thus, the faster response is achieved by TNF- α than IL-1, while serum IL-6 may require a longer duration HFD to be elevated. The elevated proinflammatory cytokines induce a chronic inflammatory process with long-term HFD administration and consequent weight gain and obesity; this chronic inflammation affects insulin sensitivity of the tissues and induces tumorigenesis such as hepatocellular carcinoma and colorectal carcinoma (22). Cortez et al. (23) recorded a rise in all these three proinflammatory cytokines among rats fed HFD.

Furthermore, LCEO-NLC caused a decrease in IL-1 and TNF- α , while HCEO-NLC or CEO couldn't improve the rise in these biomarkers. These results clarify the beneficial effect of LCEO-NLC in improving the proinflammatory cytokines among rats fed HFD. Daneshi-Maskooni et al. (24) found a decline in IL-6 and TNF- α serum levels among obese adult humans provided oral cardamom supplements, while Kazemi et al. (25) saw improvement in IL-6. Still, no TNF- α in obese prediabetic women received cardamom supplementation.

Conclusions

CEO-NLC can control the rise in serum cholesterol and triglyceride, but only high doses of CEO-NLC and CEO can cause a decline in LDL levels in hyperlipidemic status. Only low doses of CEO-NLC can increase HDL. The liver enzymes were unequally affected by CEO-NLC, as different doses of CEO-NLC have other positive or negative effects on liver enzymes. At the same time, the TSB was improved by high doses of CEO-NLC in high-fat-fed rats. There were mild non-significant effects of atorvastatin on CEO-NLC. CEO-NLC do not cause significant changes in kidney function among rats fed HFD. Low concentrations of CEO-NLC cause reduce in the inflammatory biomarkers IL-1 and TNF- α but not IL-6 among rats fed HFD. CEO-NLC can lower body weight in hyperlipidemic rats, and the weight loss is dose-related; the higher the dose, the more weight loss.

Conflict of interest

The authors confirm that they are not affiliated with or involved in any organization or entity with financial interests.

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