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INVESTIGATING THE OBESITY EFFECT OF CHITOSAN-COATED ELLETTARIA CARDAMOMUM OIL NANOEMULSION THROUGH FTO AND LEPTIN GENE EXPRESSION IN AN ANIMAL MODEL

[1]*Azadeh Mehraban, [2]Ali Nemati

[1]Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran, [2]Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran

Corresponding Author: Azadeh Mehraban

ABSTRACT

Obesity is a serious concern for people's public health as it is a factor involved in causing various diseases. Using herbal compounds has emerged as a promising potential to fight obesity and its associated diseases. Their nano-formulation can be an effective solution due to some limitations of their clinical application. Thus, the present study investigated the biological effects of chitosan-coated Ellettaria cardamomum nanoemulsions on obesity. In this study, the above nanoemulsions were synthesized using the ultrasonic method and the minimum amount of surfactant (Tween 80), cosurfactant (ethylene glycol), and chitosan. DLS, TEM, and Real-Time PCR techniques were used to characterize the synthesized nanoemulsions and evaluate their biological effects. The results indicated that the synthesized nanoemulsions had a diameter of 178.92 nm, a dispersion index 0.18, and a zeta potential of 25.50 mV. Thus, they have a homogeneous dispersion in addition to the appropriate size. The results of the evaluation of FTO gene expression also revealed that the level of FTO gene expression decreased significantly in the groups receiving nanoemulsion, compared to the negative control group ($P \leq 0.05$). Additionally, comparing the mean weights of the samples in the treated groups with the control groups in the fourth and fifth weeks of treatment revealed that the weight of the experimental groups decreased significantly compared to the control groups ($P \leq 0.05$). Therefore, chitosan-coated Ellettaria cardamomum nanoemulsions can inhibit obesity by reducing FTO gene expression. However, more studies are needed to further evaluate and investigate the potential of their clinical use.

Keywords: Nanoemulsion, Ellettaria cardamomum oil, Obesity, FTO gene, Leptin gene

INTRODUCTION

Nowadays, bioactive substances are considered safer treatment methods to replace synthetic substances. This change in replacing natural bioactive molecules requires more studies and the creation of more stable and useful formulations. The oily phase of nanoemulsions mainly includes triglycerides, alkanes, fatty esters, or acids. Issues such as non-toxicity, physical stability, and affinity of the oily core of these materials should be considered for the better efficiency of nanoemulsions in treatment (McClements 2012). Only a few therapeutic agents including Lorcaserin, phentermine-topiramate, orlistat, and sibutramine are used to treat obesity. The mentioned drugs can reduce body weight by reducing food intake or absorption or by increasing energy distribution. Nanotechnology has opened new perspectives in medicine, especially in the field of drug delivery in recent years. This technology can transfer specific anti-obesity drugs to white fat tissue and help to prevent the side effects of drugs from reaching other parts of the body (Natarajan et al. 2021). Several studies published between 2000 and 2022 have been based on nanoformulations based on herbal compounds for the treatment of obesity. Thus, nanotechnology plays a promising role in the delivery of phytochemicals and nutrients in obesity management (Sharma et al. 2022).

Gmnitasha et al. (2015) compared the effects of cardamom with pioglitazone. The reduction in blood sugar level after glucose measurement with pioglitazone was significant compared to cardamom. Cardamom has an effect comparable to pioglitazone in preventing dexamethasone-induced hepatomegaly, dyslipidemia, and hyperglycemia (Bhat et al. 2015). In a study in 2016, hepatocellular carcinoma (HCC) was induced by dimethylnitrosamine (DENA) in mice. Treatment with cardamom prevented all the changes caused by DENA (Elguindy et al. 2016). Yaqublu et al. conducted a clinical trial on obese women with a history of diabetes. They showed that consuming 3 g of cardamom for 2 months reduced the level of triglycerides in the blood from 192.6 to 183.7 mg/dL, LDL-C level from 118.1 to 110.5 mg/dL, and HDL-C level from 44.1 to 42.7 mg/dL (Fatemeh et al. 2017). Rahman et al. (2017) evaluated the potential benefits of cardamom powder supplementation in high-carbohydrate food (HCHF)-induced obese mice. Gomaa et al. (2019) investigated the effect of cardamom on Alzheimer's patients with a history of diabetes. In this study, the administration of cardamom extract reversed the symptoms of AD and significantly reduced the level of ACHE and caspase 3. Delgadillo-Puga et al. (2023) investigated the effect of cardamom in modulating neural circuits and increasing mitochondrial oxidative metabolism in the liver and muscle. The results revealed that the consumption of cardamom seeds could lead to the creation of a regulated hormonal environment, an adjustment in nerve circuits, and an increase in metabolism.

Recently, extensive studies have evaluated the relationship between a gene called FTO, obesity, and type 2 diabetes. This gene, whose locus is located on the long arm of chromosome 16, was first discovered in mice (Peters et al. 1999). This gene is expressed in the pituitary, adrenal, and hypothalamus glands, which regulate energy homeostasis in the body. This gene establishes an energy balance in the body. Obesity increases the rate of type 2 diabetes up to 10 times (Gerken et al. 2007). Studies on this gene have shown that the FTO protein is less effective on Asian people than on Europeans (Tan 2004; Thorleifsson et al. 2009). This is due to the difference in their body structure because abdominal fat is more than muscle fat in the Asian race. Given what was stated, Ellettaria cardamomum vegetable oil was used to prepare the oil core of nanoemulsion and its effects on obesity through the expression of the FTO gene in an animal model.

MATERIALS AND METHODS

In this study, 30 c57bl/6 male mice were purchased from the Ferdowsi Faculty of Medical Sciences in Mashhad. They were transferred to the animal room of the Islamic Azad University

of Mashhad. The intraperitoneal injection was used for the treatment of the samples and the mice were randomly divided into 5 groups including positive control with a food ration 2 times the negative control group and as in the injection group, negative control including half the food ration of the other groups, the third group was treated with 200 nanomg/kg, the fourth treatment group was treated with 400 nanomg/kg, and the fifth treatment group was treated with 800 nanomg/kg. The samples received 100 µl for 4 weeks and one hour a day. After the treatment period, the samples were killed by chloroform, and their liver tissue was removed and transferred to microtubes containing LATAR RNA manufactured by Dena Zist Company.

In this study, the ultrasonic method was used to synthesize cardamom oil nanoemulsion. First, we dissolved chitosan in 1% acetic acid and incubated the resulting solution for 24 hours under a magnetic stirrer. To prepare the nanoemulsion, we added a specific amount of Tween 80 and 20 to 500 µl of polyethylene glycol (PEG). Then, we added an equal amount of PEG, essential oil/cardamom oil, and brought the resulting solution to a volume of 50 ml with deionized distilled water. Then, the chitosan-containing solution was added drop by drop to the nanoemulsion solution, and the sample was homogenized after 2 hours. This nanoemulsion consisted of an oil phase (cardamom oil), aqueous phase (distilled water), quinine surfactant (20% and 80%), and cosurfactant (ethylene glycol). Nanoemulsion is investigated using a transmission electron microscope (TEM). To verify PSD, we used the dynamic light dispersion technique. In this technique, a hydrodynamic diameter or radius is obtained through the Stokes-Einstein equation and provides us with an overall size of the particle perpendicular to the light source at that moment. To achieve this goal, we use a NANO-ZS zeta sizer to achieve the zeta potential of particles.

RNA extraction

1 -First, we poured some of the mouse liver tissues into a mortar and poured liquid nitrogen on the tissue so we could turn the tissues into smaller components at a low temperature.

2 -We transferred some of the liver from the previous step to the microtube and added 1ml or 750 µl of lysis buffer to the contents of the microtube. Then, we performed pipetting several times and incubated for 5 minutes at room temperature.

3-We added 200 µl of chloroform to it and repeated it several times with up and down intensity.

4 -We incubated the contents of the previous step for 3 minutes at room temperature and centrifuged for 12 minutes at 4°C and 13000rpm.

5-We transferred the supernatant to a new 1.5 ml microtube, added 600 to 800 µl of isopropanol to it, and gently shook it until they were mixed.

6 -We transferred the solution to the purification column and centrifuged at 13000 RPM for one minute.

7 -After this step, we discard the solution in the collecting tube.

8 -Using a sampler, we added 500 µl of ethanol with a concentration of 70% to the microtube of the previous step and centrifuged it at 13000 RPM for one minute.

9 -We discarded the solution in the collecting tube.

10-We centrifuged it at 13000 RPM for two minutes, removed the remaining buffer, and transferred the column to a new microtube.

11-We added 30 to 40 µl of DEPC-treated water to the column and incubated for 3 minutes at room temperature.

12 -We centrifuged it at 13000 RPM for 2 minutes, collected the RNA, and kept it at -70 C.

Real-time PCR technique was used to investigate the expression changes of apoptotic genes. The GAPDH housekeeping gene was used as a control gene. In this study, the BioRad apparatus and the Cybergreen method were used to quantitatively determine the expression of the mentioned gene (Table 1).

Table 1. Sequence of the primers used

Gene	Forward	Reverse
GAPDH	ACCATCTTCCAGGAGCGAGA	GCAAATGAGCCCCAGCCTTC
FTO	GGTGGAACAAAGGAGTGAGAT	GGGTCATCCTTCTCCCAATATG

In this study, Prism software, a one-way ANOVA test, and the Dunnett and LSD test were used to analyze the data. A confidence level of 5% was considered for calculations.

RESULTS

The results of the dynamic light dispersion technique

The synthesized nanoparticles have a dynamic diameter of 178.92 nm, which is the sum of the diameter of the nanoparticles and the charge around them. Another important index in this table is the Pdi or particle dispersion index, which is about 0.29. If this number is in the range of 0.2, it indicates that the nanoemulsion synthesized in this study has a homogeneous dispersion. The mean size of nano-droplets is 54.15 nm.

Zeta potential

To examine the stability of nanoemulsion, we use zeta potential, which is an index to measure the surface charge of droplets. The acceptable level for this index is in the range of more than 30 MV or less than -30 MV. The results show the zeta potential of 25.50 MV and this number indicates that the synthesized nanoemulsion has good stability.

Results of transmission electron microscopy TEM

Analyzing the results indicated that the synthesized nano-droplets are spherical and uniformly dispersed (Fig. 1). Analyzing the results indicate that the synthesized nano-droplets are spherical and uniformly dispersed (Fig. 1).

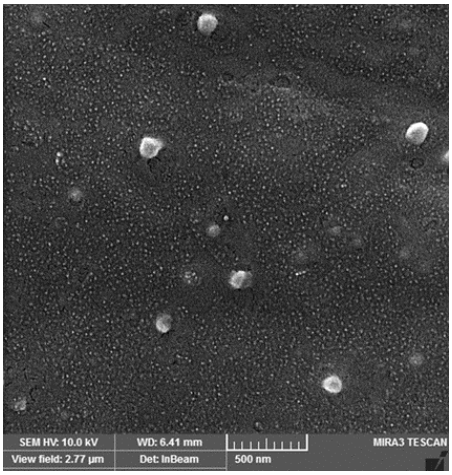
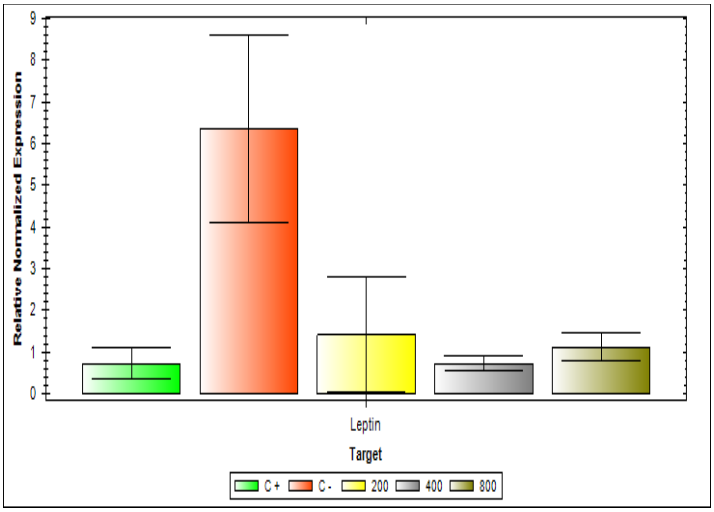
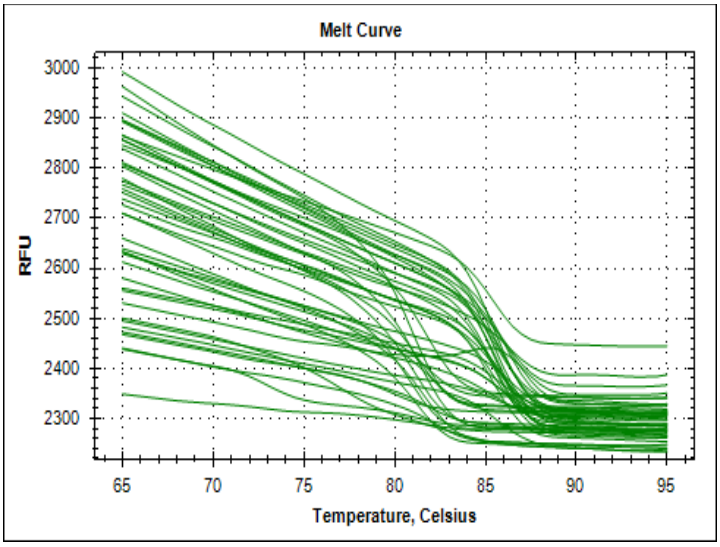


Fig. 1 The results of transmission electron microscopy TEM

Leptin gene expression

Leptin gene expression results are presented in Fig. 2.





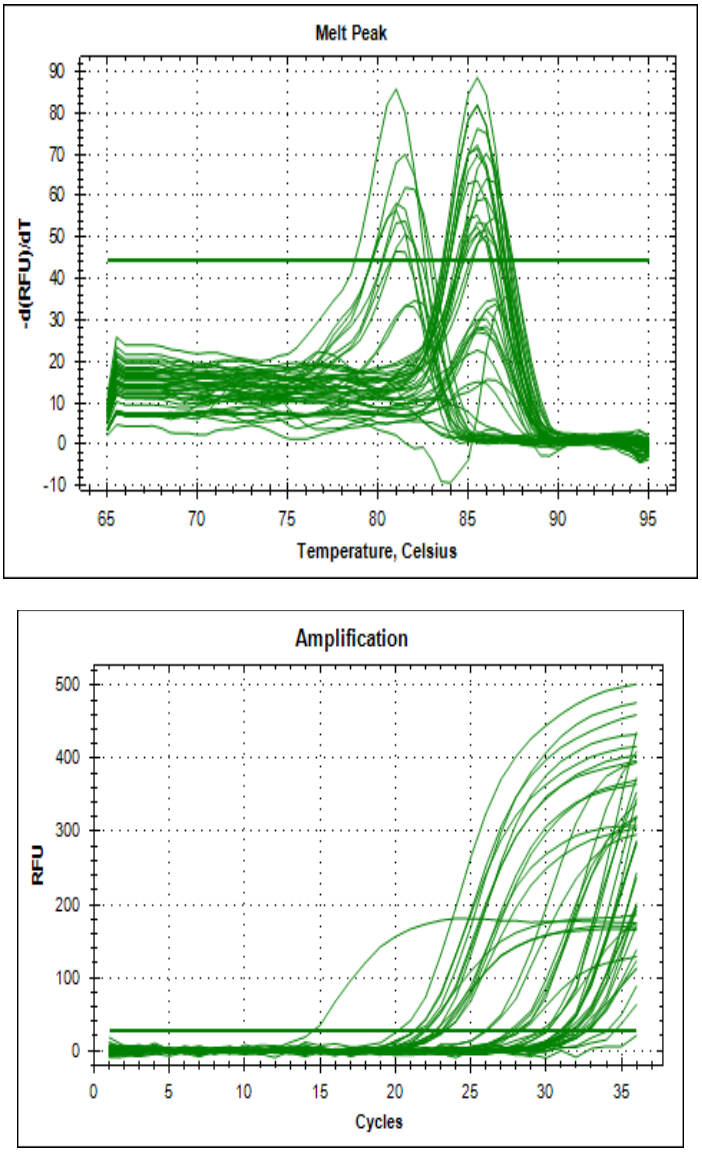


Fig. 2 Leptin gene expression results

Evaluation of FTO gene expression rate

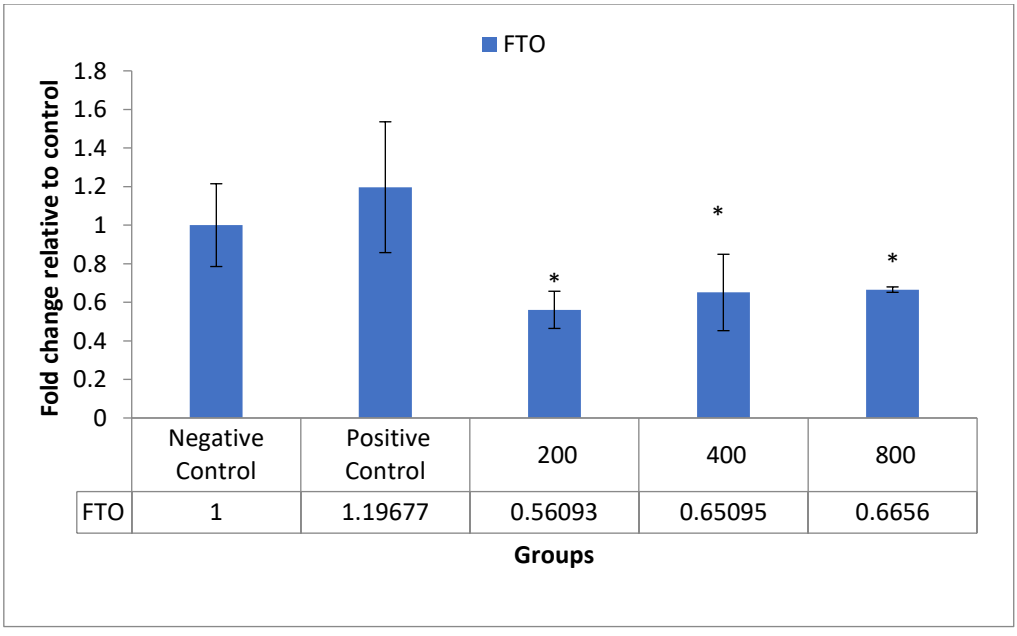


Fig. 3 Evaluation of FTO gene expression rate

FTO is one of the obesity genes and its increased expression causes overweight. Investigating the expression level of this gene in mice treated with different concentrations of nanoemulsion showed that the expression of this gene in mice treated with 800, 400, and 200 nanomg/kg decreased by half compared to other groups, and it was statistically significant ($p<0.01$). The results showed that the treatment with nanoemulsion reduces obesity by reducing the expression rate of the FTO gene (Fig. 3).

Investigating the effect of nanoemulsion doses on weight loss of treated groups of C57BL/6 mice

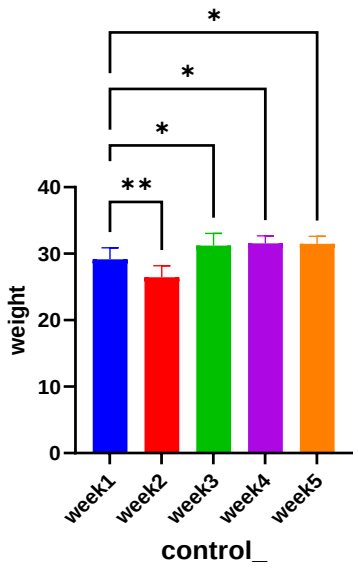


Fig. 4 The mean weight of the samples in the negative control group during the treatment

The weight of the samples of the negative control group increased slightly over time and reached 31 g in the last week, which is almost 2 g more than the first week. This means that the samples grew with the passage of time and aging (Fig. 4).

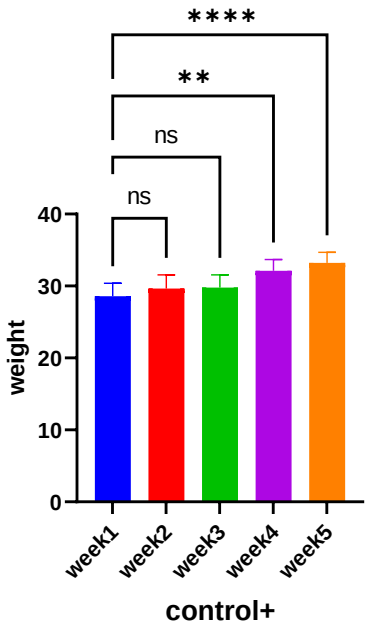


Fig. 5 The mean weight of the samples in the positive control group during the treatment

In the last week, the weight of the positive control samples, which received double the food

ration of the negative control, reached 32 g from 28.58 g in the first week, which was 2 times more overweight than the negative control group (Fig. 5).

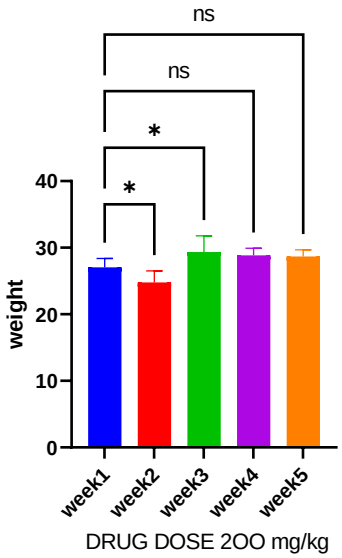


Fig. 6 The mean weight of the samples in the 200 mg/kg nanoemulsion group during the treatment

In the treatment group with a concentration of 200 mg/kg, the mean weight of the samples increased by about 1 g in the last week compared to the first week. This means that the nano was not very effective, but compared to the negative and positive control groups, less weight gain is observed (Fig. 6).

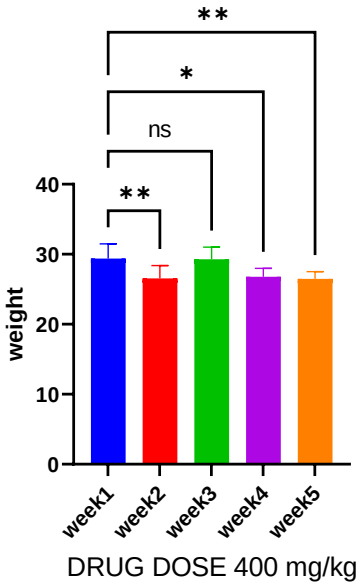


Fig. 7 The mean weight of the samples in the group received 400 mg/kg of the drug during the treatment

In the group treated with 400 nanomg/kg in the last week, the mean weight of the samples decreased by 3 g, indicating that nano was effective in reducing weight compared to the negative and positive control groups. In addition, the comparison with the group treated with 200 mg/kg concentration shows that increasing nano concentration affects weight loss (Fig. 7).

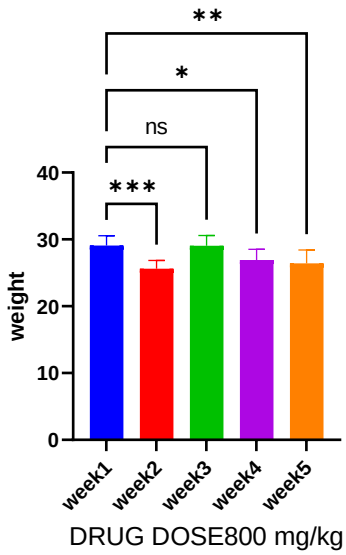


Fig. 8 The mean sample weight in the group received 800 mg/kg cardamom nanoemulsion during the treatment

This nano concentration (800 mg/kg) is similar to the group treated with a concentration of 400 mg/kg, and in both groups, the mean weight of mice decreased by 3 g (Fig. 8).

Investigating the weight of mice in different groups over time with a negative control group

Comparing the mean weight of the groups, before treatment with cardamom nanoemulsion in the first week with the negative control group, shows that the mean weight of the samples is not significantly different. In the second week, there is a significant difference between the negative and positive control groups, which is due to the double food of the positive control group. In the third week, there is no significant difference between any of the groups compared to the negative control. In the fourth week, the mean weight of the samples in the three groups treated with different nano concentrations decreased significantly compared to the negative control group. In the fifth week, it is similar to the fourth week and it shows that in the samples treated with nanoemulsion, they maintained their mean weight loss trend.

Comparison of the weight of mice in the nanoemulsion treatment groups with the positive control group

In the first week of treatment, no significant difference was observed between the experimental groups and the positive control group. In the second week, significant changes were observed between samples of the experimental groups and the positive control group regarding weight loss.

The highest weight loss was related to the group treated with 200 mg/kg of nanoparticles. Similar to the results from the first week of treatment, in the third week of treatment, no significant difference was found between the experimental groups and the positive control group. In the fourth week of treatment, a significant difference was found between the experimental groups and the positive control group regarding the weight loss of the samples. The weight loss was related to the groups treated with f 400, 800, and 200 mg/kg of nanoparticles. The weight loss of the samples of the experimental groups compared to the positive control group in the fifth week of treatment is also significant. The highest weight loss was related to the experimental group treated with 400 mg/kg of nanoparticles.

Investigating the cholesterol and triglyceride levels of mice in different groups over time compared to the control groups

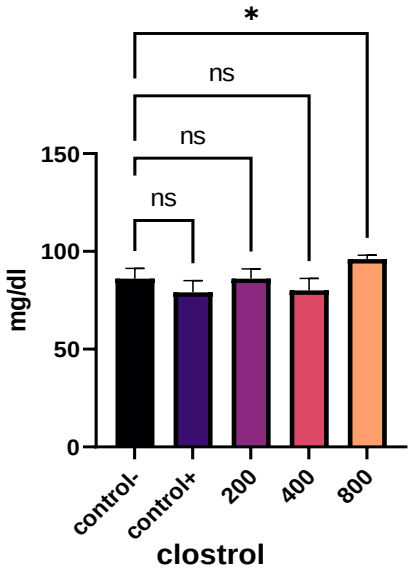


Fig. 9 Comparison of cholesterol levels of different groups with negative control

Based on the Fig. 9, significant changes were observed only in the experimental group with a dose of 800 mg/kg of nanoparticles compared to the negative control group, and no significant changes were observed in the other groups.

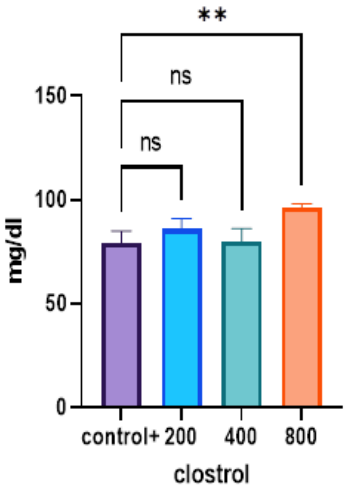


Fig. 10 Comparison of the cholesterol levels of the group treated with nanoemulsion with the positive control group

Similar to the results of Figure 3-19, significant changes were observed only in the experimental group with a dose of 800 mg/kg of nanoparticles compared to the positive control group, and no significant changes were observed in the other groups (Fig. 10).

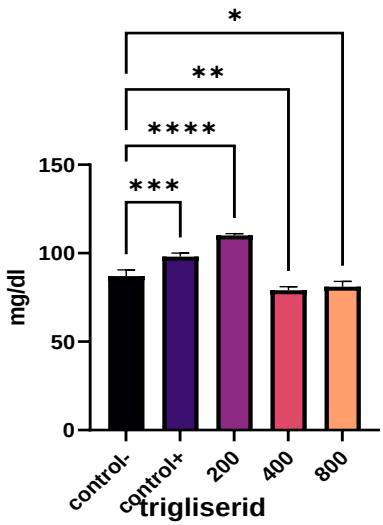


Fig. 11 Comparison of triglyceride levels of different treated groups with negative control

Based on the above Figure, a significant difference is observed between the triglyceride levels of experimental mice treated with nanoparticles in all concentrations compared to the negative control group. The highest significant increase in triglycerides is seen in the experimental group treated with a concentration of 200 mg/kg, and the highest significant decrease in triglycerides is seen in the group treated with a concentration of 400 mg/kg compared to other groups (Fig. 11).

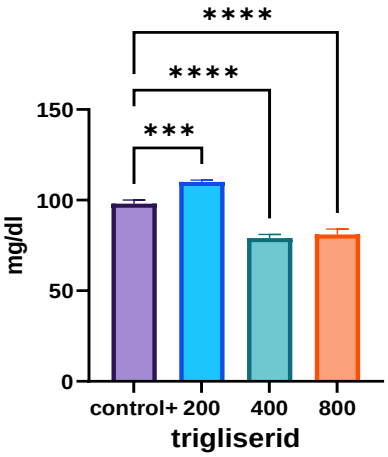
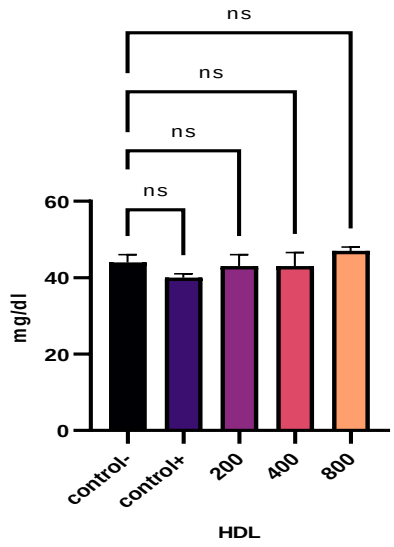


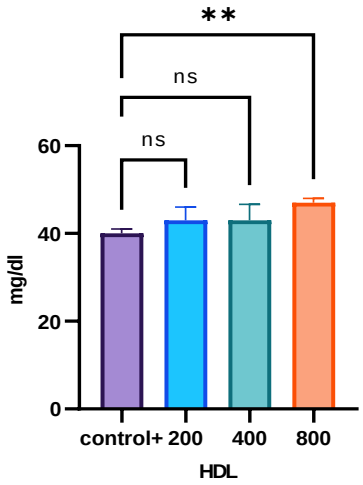
Fig. 12 Comparison of triglyceride levels of groups treated with nanoemulsion with positive control

As shown in Fig. 12, a significant difference is observed between all the groups treated with different concentrations of nanoparticles compared to the positive control group (Fig. 12).

Investigating HDL and LDL levels of mice in different groups over time compared to control groups



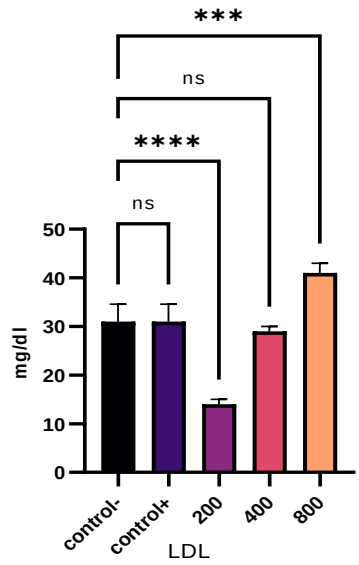
(a)



(b)

Fig. 13 Comparison of HDL levels of groups treated with nanoemulsion with positive and negative control groups

Based on Fig. 13-a, no significant changes in HDL levels were observed in any of the experimental groups treated with nanoparticles compared to the negative control group. Based on Fig. 13-b, there was no significant difference between the HDL levels of the experimental groups treated with nanoparticles, except for the group treated with a concentration of 800 mg/kg, and the control group.



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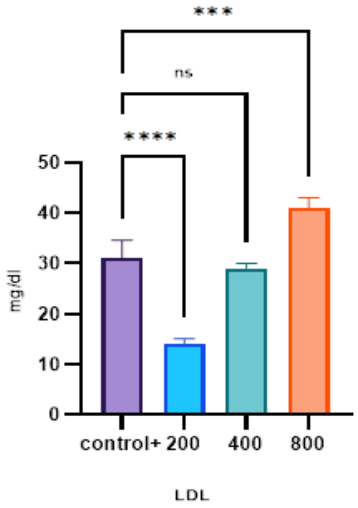
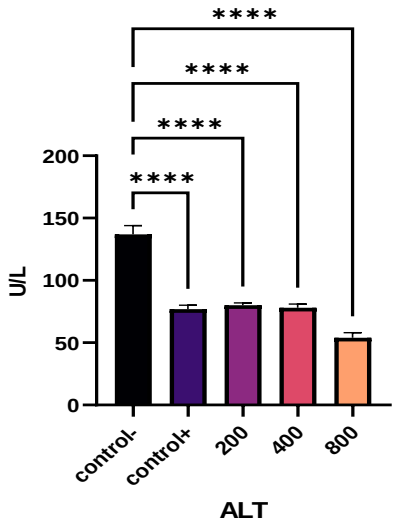


(b)

Fig. 14 Comparison of LDL levels in different groups treated with positive and negative control

According to Fig. 14-a, a significant difference was observed between the LDL levels of experimental groups treated with concentrations of 800 and 200 mg/kg of nanoparticles and the negative control group. These significant changes were not observed in other groups. Based on Fig. 14-b, a significant difference was found between the LDL levels of all the experimental groups treated with different concentrations of nanoparticles, except the experimental group treated with a concentration of 400 mg/kg, and the positive control group,

Investigating the ALT and AST levels of mice in different groups over time compared to the control groups



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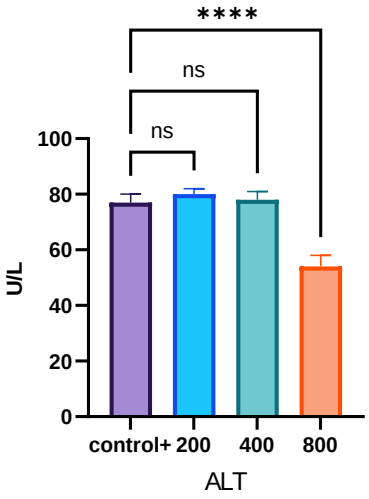
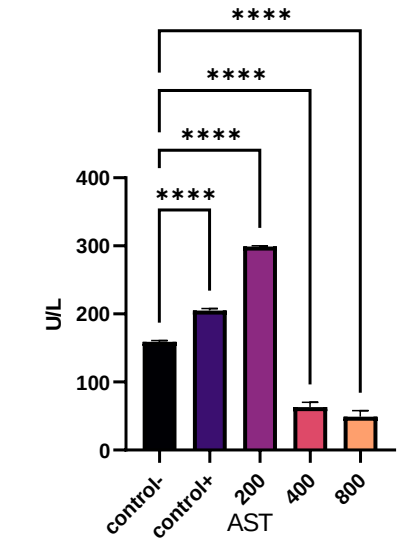


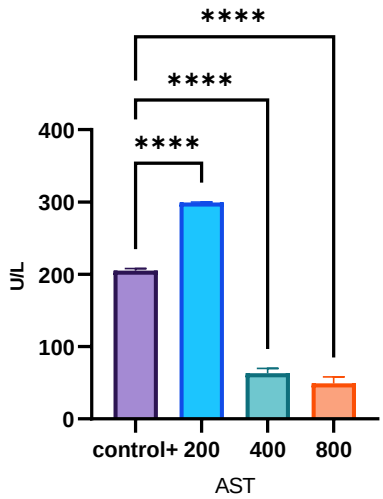
(b)

Fig. 15 Comparison of ALT levels in different groups treated with positive and negative control

Based on Fig. 15-a, in all groups treated with nanoparticles in different concentrations, a significant difference between ALT levels was observed compared to the negative control group. Based on Fig. 15-b, significant changes compared to the positive control group were seen only in the experimental group treated with 800 mg/kg of nanoparticles, but these changes were not significant in other groups.



(a)



(b)

Fig. 16 Comparison of AST levels in different groups treated with positive and negative control

Based on Fig. 16-a, in all the experimental groups treated with nanoparticles in different concentrations, a significant difference between the AST levels of the samples was observed compared to the negative control group.

In the groups treated with 400 and 800 mg/kg of nanoparticles, it caused a significant decrease in AST levels, and in the group treated with 200 mg/kg of nanoparticles, it caused a significant increase in the above enzyme. Based on Fig. 16-b, there is a significant difference was observed between AST levels in experimental groups treated with nanoparticles in different concentrations and the positive control group.

DISCUSSION

In this study, the resulting nanoemulsion was prepared using cardamom oil, Tween 20 and 80 in equal ratio, ethylene glycol, and water by ultrasound method. Then, it was analyzed. The

results of DLS showed that the resulting particles have an average size of 178.92 nm with a dispersion index of 0.2940, indicating the formation of particles with nanometer size and uniform dispersion. The surface charge of nano-droplets was estimated to be 25.50 mV, indicating the stability of the resulting nanoemulsion. Various studies have used ultrasound methods to prepare nanoemulsion, which is similar and comparable to the present study. For example, Kentish et al. (2008) used linseed oil and non-ionic surfactant (Tween 40) for nanoemulsion synthesis. Their results confirmed the formation of nanoemulsion with a mean size of 70 nm (Kentish et al. 2008).

Comparing the results with the data from the present study indicates that using Tween 40 is probably a more appropriate option for the formation of nano-droplets since the same method was used for synthesis in both studies. However, the diameter of the nano-droplets in the present study is almost double the size of nanoparticles obtained from linseed oil. The comparison of these results indicates the higher efficiency of the formulation in the study by Kentish et al. Similarly, de Moraes et al. (2010) used the high-intensity ultrasound method to prepare nanoemulsions with a droplet size of 20 nm. Given the similarity of the nanoemulsion synthesis methods, comparing the results of the mentioned study with the present study shows that the formulation of Span 80 and Tween 80 is probably more effective in reducing droplet size.

The results of SDS-PAGE before and after treatment with eugenol oil nanoemulsion indicate that the content of extracellular soluble small molecular proteins in mushrooms treated with eugenol oil nanoemulsion is significantly reduced. This nanoemulsion is smaller compared to the cardamom nanoemulsion, but its micrograph shows spherical and dispersed particles, similar to the cardamom micrograph, and the eugenol oil nanoemulsion shows more stability to temperature. However, cardamom nanoemulsion is more stable at 4 °C, the refrigerator temperature (Abd-Elsalam and Khokhlov 2015).

The present study aimed to obtain a colloidal system with andiroba oil using a low-energy method without organic solvents. Additionally, the larvicidal activity of the initial nanoemulsion residue versus Aedes aegypti was evaluated. Oleic and palmitic acids, in addition to phytosterol β-sitosterol and limonoids (tetranorterpenoids), were the primary fatty acids. The required hydrophilic-lipophilic ratio was about 0.11 and the optimal nanoemulsion was obtained using polysorbate 85. The distribution of particle size suggested the presence of small droplets (mean diameter of about 150 nm) and a low dispersion index (about 0.150). The temperature impact on particle size distribution showed no significant increase in droplet size. The present study provided the possibility of obtaining a potential bioactive oil in water nanoemulsion, which may be a promising controlled release system. The results of this investigation showed a smaller size, a lower dispersion index, and a more homogeneous mixture than the cardamom nanoemulsion. The difference in variables such as the nano size and dispersion index can be related to the differences in the synthesis method, i.e. the low energy method and the type of surfactant in Andiroba nanoemulsion (Jesus et al. 2017).

This study revealed that multilayered nanoemulsions could be used to increase satiety by delaying lipid digestion, which can be crucial for the development of functional foods to fight obesity (Silva et al. 2018). This study confirms a lower bioavailability for multilayer nanoemulsions of curcumin, which contradicts the purpose of this study, which is to coat the nanoemulsion with cardamom to improve bioavailability.

Oil-in-water nanoemulsion loaded with quercetin was prepared by the aqueous phase titration method. The optimized formula had a mean particle size of 19.3 ± 0.17 nm with a zeta potential of -0.34 ± 0.13 mV. The in vitro permeability of quercetin nanoemulsion through artificial intestinal membrane and Caco-2 cell monolayer was 188 and 3.37 times higher than an aqueous dispersion of quercetin, respectively, and the resulting in vivo oral bioavailability was 33.51 times higher. High-fat diet (HFD)-treated mice that received daily oral nanoemulsion containing quercetin reduced maximum weight gain by 23.5% compared to the HFD control

group. These results suggest that a nanoemulsion containing quercetin may be a promising oral therapy for the treatment of obesity. This review aimed to design an effective oral delivery system for quercetin to increase its solubility and bioavailability and thus improve its anti-obesity effects. The results of the mentioned study are in line with those of the present study (Pangeni et al. 2017). In a study by Kim et al. (2014), the results confirmed the effect of nanoemulsions derived from red pepper plants on obesity.

Daneshi et al. concluded that cardamom supplementation reduces total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c), and increases high-density lipoprotein cholesterol (HDL-c) (Daneshi-Maskooni et al. 2019). It is consistent with the results of our study. However, the total cholesterol level is contradictory to our results. This contradiction may be related to the different conditions of the two experiments. In addition, there was no significant difference between HDL-C levels in our experiment and the mean level of HDL-C was maintained. Another study in 2017 revealed that the consumption of 3 g of cardamom within 2 months reduced the level of triglycerides in the blood from 192.6 to 183.7 mg/dL and the LDL-c level from 118 to 5.1 110 mg/dL, and HDL-c level from 44.1 to 42.7 mg/dL. The results of this study are consistent with those of our study. This study is one of the few randomized clinical trials evaluating the effects of Ellettaria cardamomum supplementation on serum lipids, glycemic indices, and blood pressure, in overweight and obese pre-diabetic women. The results indicate that compared to the placebo, eight weeks of cardamom supplements are not effective on blood biomarkers and blood pressure. However, TC and LDL-C significantly decreased and insulin sensitivity increased in the cardamom group. In addition, in the cardamom group, the mean level of HDL-C was maintained, while in the control group, a significant decrease was observed after the study, indicating the protective effect of cardamom on the level of HDL-C.

The study by Verma reported that consumption of 3 g of cardamom powder for 12 weeks by people with hypertension (stage 1) significantly reduced systolic and diastolic blood pressure. The mentioned study did not show any change in the level of blood lipids in the intervention group (Verma et al. 2009). The results of some studies on patients with metabolic syndrome have indicated that foods with high flavonoid content decrease serum TG, TC, and LDL-C and increase HDL-C. Additionally, flavonoids affect transcription factors such as proteins, sterol regulatory element binding protein 1 (SREBP-1), and sterol regulatory element binding protein 2 (SREBP-2). These regulatory factors and proteins increase cholesterol and TG synthesis. The role of flavonoids in the prevention of insulin resistance has also been examined. The increase in fat storage in fat cells is one of the major factors that cause insulin resistance in adipose tissue, leading to inflammation. Flavonoids may improve insulin function in the body by reducing fat storage (Galleano et al. 2012).

CONCLUSION

The obesity effect of prepared nanoemulsion was investigated through the expression level of the FTO gene. It was observed that the expression level of FTO decreased significantly in the groups receiving nanoemulsion compared to the negative control group ($P \leq 0.05$). Also, comparing the mean weights in the treated groups with the positive control and negative control groups in the fourth and fifth weeks of treatment showed that the weight of the samples receiving the drug dose decreased significantly ($P \leq 0.05$) compared to the control groups. Thus, chitosan-coated Ellettaria cardamomum oil nanoemulsion can inhibit obesity by reducing FTO gene expression. Thus, this formulation can be further studied for the treatment of obesity. Based on the results, it is recommended to investigate the expression level of other obesity-related genes using cardamom nanoemulsion, the effects of cardamom nanoemulsion on insulin resistance, and its effect on beta cells in the islets of Langerhans.

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