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Assessment of nanoliposomes loaded with daidzein for ameliorating diabetes in alloxan-induced mice: A promising nutraceutical approach

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ABSTRACT

The present study synthesized and evaluated the effects of nanoliposomes loaded daidzein (NLD) in diabetic mice induced by alloxan. Five groups of Balb-c mice were treated for 5 weeks (Group 1 and 2 referred to as normal and diabetic control receiving normal feed). Group 3 and 4 were diabetic mice receiving 50 and 100 mg/kg/BW NLD respectively. Group 5, diabetic mice, received the standard drug, Metformin, 250 mg/kg/BW per day orally. Results indicated that NLD significantly ($p < 0.05$) improved the final body weight, triglyceride (TG), total cholesterol (TC) concentrations, low-density lipoprotein cholesterol and malondialdehyde (MDA) levels and enhanced the high-density lipoprotein. Furthermore, NLD enhanced the expression of CAT and suppressed iNOS gene in the pancreas. The mRNA expression of GLUT2 and GLUT4 also showed increase in the diabetic mice. Findings demonstrated the potential of NLD as a nutraceutical for the prevention and/or treatment of diabetes without toxic side effects.

1. Introduction

Fat deposition in humans or animals depends on the balance between dietary caloric intake and whole-body energy expenditure. Complex genetic and/or environmental factors can induce chronic imbalance in energy metabolism (more energy input than energy output) and leads to excess fat accretion or obesity in humans (Hall et al., 2022). Obesity is a major risk factor to multiple diseases conditions such as high blood pressure, diabetes, impaired vascular function, sleep disorders, colon and breast cancers which is ultimately incurs tremendous socio-economic burdens (Hecker, Freijer, Hiligsmann, & Evers, 2022).

There are multiple side effects to commercially available anti-obesity drugs. A modest (5–7 %) weight loss in obese subjects can significantly improve risk factors of cardiovascular disease and diabetes, and new techniques can help reduce body fat and offer beneficial effects to health development and treatment options (Haase et al., 2021; White et al., 2023). Global strategies are mostly focused on dietary and lifestyle modifications, i.e., caloric intake restriction and increasing physical activity to slow obesity development. Dietary phytochemicals have recently attracted much attention, thanks to antioxidant effects and modifying epigenetic mechanisms that could potentially counteract obesity (Dincer & Yuksel, 2021; Wang, Xiang, Qi, Du, & Nutrition, 2022). The anti-obesity effects are also mediated by regulation of pathways such as lipid absorption, intake and expenditure of energy, increasing lipolysis, and decrease lipogenesis, and differentiation and proliferation of pre-adipocytes (Cho, Lu, Kim, Jeon, & Lee, 2022).

Daidzein is a phytoestrogen isoflavone present in soybeans and other legumes with various therapeutic properties (Alshehri et al., 2021; Sanatkar, Rahimi Kalateh Shah Mohammad, Karimi, Oskoueian, & Hendra, 2021). In many pathological conditions, daidzein have shown protective actions particularly those triggered by oxidative stress including cardiovascular disease and metabolic disorders (Alshehri et al., 2021). Dietary phytochemicals can suppress adipose tissue growth by anti-angiogenic activity and modulating adipocyte metabolism (Petrine and Del Bianco-Borges, 2021).

Drug delivery systems can improve the bioavailability of a drug, decrease drug degradation, improve therapeutic effects and minimize side effects (Etter, Mei, & Nguyen, 2021). Liposomes with peculiar features i.e., stability, biocompatibility, target specificity, further compliance, constant encapsulation, and controlled release scheme can

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help reduce the dosage frequency and toxicity (Guimarães, Cavaco-Paulo, & Nogueira, 2021).

It is therefore hypothesized that the administration of nano-liposome loaded daidzein would exhibit beneficial effects on various physiological parameters in diabetic mice induced by alloxan.

The overall objective of the present study was to comprehensively investigate the potential therapeutic benefits of nano-liposome loaded daidzein in diabetic mice, providing insights into its mechanism of action. As such, the specific objectives were defined as follows:

(I)To investigate the impact of nano-liposome loaded daidzein on antioxidant status and assess changes in the expression of fundamental antioxidant genes and oxidative stress markers to determine the influence of nano-liposome loaded daidzein on antioxidant defenses. II) To evaluate the anti-inflammatory potential of nano-liposome loaded daidzein. III) To assess the impact of nano-liposome loaded daidzein on body weight. IV) To determine the potential of nano-liposome loaded daidzein as a therapeutic agent as a viable therapeutic option for diabetes management without significant side effects. To the best of our knowledge, the present study is the first report on using nanoliposomes loaded with daidzein for ameliorating diabetes in alloxan-induced mice.

2. Materials and methods

2.1. Chemicals

Daidzein, alloxan monohydrate and lecithin were obtained from Sigma Aldrich (Steinheim, Germany). Other regents and chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Nano-liposome preparation and characterization

For the synthesis of nano-liposome loaded daidzein (NLD), 245 mL distilled water (80 °C) and five gram lecithin were mixed and stirrer (300 rpm, 50 min), after which, daidzein was added and mixed for 2 h. The mixture was sonicated for 7 min as described previously (Beyrami, Karimi, Oskoueian, & Research, 2020). The NLD was freeze-dried and maintained at −20 °C. The size, morphology, ascertain and stability (zeta potential) of prepared nanoliposome loaded daidzein was evaluated by particle size analyser (ZEN3600, Malvern, UK), field emission scanning electron microscopy (FESEM) and Malvern Zetasizer Nano ZS (Malvern, UK), respectively (Beyrami et al., 2020). The daidzein content of the NLD was determined by HPLC previously as describer (Sanatkar, Rahimi Kalateh Shah Mohammad, Karimi, Oskoueian, & Hendra, 2021). In Brief, the solvents used consisted of deionized water (labeled as A) and acetonitrile (labeled as B). Deionized water's pH was modified to 2.5 by incorporating trifluoroacetic acid. An analysis was performed using a C-18 column (dimensions: 10 cm × 4.6 mm) with a gradient method, transitioning from 15 % to 50 % solvent B over a 50-minute period, employing a flow rate of 0.6 mL/min. The chromatogram was recorded at 254 nm. It is worth noting that the selection of the NLD doses for *in-vivo* assay was based upon preliminary test earlier performed by our research team (data not shown hereto).

2.3. In vivo assay

Forty male Balb-c mice (4-week-old, body weight 30–32 g) were caged individually. Following acclimatization, all mice received a standard chow diet for 1 week. Mice were provided with clean water ad libitum and maintained under a controlled room environment (23 ± 1 °C, 85 % humidity and a 12-h dark and light cycle). Prior to diabetes induction, animals were fasted overnight for 13 h. Data such as weight and fasting blood glucose levels were collected. The diabetic conditions were induced by a single intraperitoneal injection (1 mL/kg) of freshly prepared alloxan monohydrate solution (20 mg per kg body weight). After 48 h of alloxan injection, the glucose level in plasma blood was tested by taking the blood from the tail and animals showing > 200 mg/dL for glucose level were thereby selected for the trials. The animals were divided in 5 groups (n = 8), with the following treatment details: Group 1 and 2 served as normal and diabetic control and received normal food. Group 3 and 4 (diabetic mice), received 50 and 100 mg/kg/BW NLD respectively. Group 5 were diabetic mice which received standard drug, Metformin, 250 mg/kg/BW per day orally. At the end of the experiment, after a period of 5 weeks, the mice were anesthetized by 300 (mg per kg body weight) ketamine and 30 (mg per kg body weight) xylazine that were given intraperitoneally following 12 h fasting. All experimental protocols were conducted in accordance with the guidelines of the Institute of Animal Care and Use Committee (IAU/UP-R008/2019).

2.4. Blood biochemical analysis

Blood collection was conducted following rigorous aseptic techniques to ensure the integrity of the samples. Prior to blood collection, all equipment, including syringes and needles, was sterilized to prevent contamination. A volume of one milliliter of blood sample was aseptically obtained via cardiac puncture. Cardiac puncture, considered a highly precise method, involves the direct withdrawal of blood from the heart, providing a representative and uncontaminated sample. The collected blood samples were then meticulously processed to obtain serum for subsequent analysis. To accomplish this, the blood was transferred to sterile, labeled tubes specifically designed for serum collection. These tubes were carefully handled to prevent hemolysis or clot formation. Following the sample transfer, the tubes were subjected to centrifugation at a consistent speed of 2000 revolutions per minute (rpm) for precisely 10 min. Centrifugation is a critical step for the separation of serum from other blood components. The resultant clear, yellowish serum was cautiously extracted, ensuring that no cellular or fibrin components were included.

The analysis of a wide array of important biochemical parameters in the serum was carried out to gain a comprehensive understanding of the physiological status of the study subjects. Serum levels of triglycerides, total cholesterol, high-density lipoprotein (HDL) low-density lipoprotein (LDL), insulin, GPX, SOD and catalase were analysed by auto-analyzer (Hitachi 902, Japan) using commercial kits (Asan Pharm, Seoul, Korea).

2.5. Histopathological assessment

To initiate the histopathological analysis, tissue samples from the liver and pancreas were carefully excised with precision. These samples were subsequently rinsed with physiological serum, a step taken to remove any external contaminants or residues that may have been present on the tissue surfaces. After the rinsing process, the tissue samples were placed in a 10 % buffered formalin solution, as previously described (Beyrami et al., 2020). This formalin solution serves as a fixative, preserving the tissue's cellular structure and preventing degradation. The preserved tissue samples were then subjected to processing for histological examination. This involved embedding the tissues in paraffin, a step that facilitates the creation of thin tissue sections for microscopic analysis. The tissues were embedded in paraffin to maintain their structural integrity and allow for precise sectioning. Sections of 4 μm (μm) thickness were carefully cut from the paraffin-embedded tissues. These thin sections are ideal for microscopic examination and offer a detailed view of the tissue's internal structures.

To visualize the tissue structures and cellular components, the tissue sections were stained using a classic histological staining method. Hematoxylin and Eosin staining, a widely employed technique in histopathology, was utilized. Hematoxylin stains cell nuclei blue, highlighting the cellular arrangement and density, while Eosin stains the cytoplasm and extracellular matrix pink. This dual staining method provides excellent contrast and clarity, enabling the examination of cellular morphology and tissue architecture. All stained tissue specimens were meticulously evaluated under a high-quality Eclipse light

microscope (Nikon, Tokyo, Japan).

2.6. Lipid peroxidation assay

The assay was carried out as previously described (Shafaei et al., 2020a). Initially, 1 g of pancreas tissue was meticulously collected for analysis. These tissue samples serve as a representative source of lipids for the assay. To ensure the maintenance of physiological conditions and prevent tissue degradation, the collected pancreas tissue was mixed with 9 mL of phosphate-buffered saline (PBS).

Initially, 1 g of pancreas tissue was meticulously collected for analysis. These tissue samples serve as a representative source of lipids for the assay. To ensure the maintenance of physiological conditions and prevent tissue degradation, the collected pancreas tissue was mixed with 9 mL of PBS. This buffered solution creates an environment that closely resembles the tissue's native state. To separate the tissue components and isolate the supernatant for analysis, the tissue and PBS mixture underwent centrifugation. This centrifugation step was performed at a speed of 9800 rpm for 15 min. The pancreas tissue was subsequently homogenized to ensure a uniform mixture. This homogenization process aids in the extraction of lipids and other relevant compounds. From the homogenized tissue mixture, a 100 μL sample was extracted for further analysis. The extracted sample underwent a series of treatments to facilitate the lipid peroxidation assay. It was vortexed with 150 μL of deionized water, which serves as a solvent and aids in the dissolution of lipid compounds. To prevent oxidation during the analysis, 17.7 μL of butylated hydroxytoluene, an antioxidant, was added. For effective solubilization of lipids, 85 μL of sodium dodecyl sulfate, a detergent, was included. To initiate the specific chemical reactions necessary for lipid peroxidation measurement, 1 mL of thiobarbituric acid was introduced. After the sample treatments, the mixture was incubated for a precisely timed duration of 60 min. This incubation was conducted in a water bath, creating the ideal conditions for the chemical reactions to occur. Following incubation, the mixture was allowed to cool to ambient temperature. To extract the compounds of interest, the cooled mixture was mixed with 2 mL of n-butanol. N-butanol acts as a solvent, aiding in the separation of the targeted compounds from the rest of the mixture. Once the n-butanol phase was separated from the mixture, the absorbance of the resulting solution was measured at a specific wavelength of 532 nm.

2.7. Gene expression protocol

Immediately after sacrificing the mice, the pancreas tissues were quickly excised and snap-frozen in liquid nitrogen and stored at − 80 °C until RNA extraction. Total RNA was extracted using the RNeasy lipid tissue mini kit (Qiagen, Hilden, Germany). Single-stranded cDNA was reverse transcribed from total RNA by denaturing 1 μg RNA in DNase/RNase-free water kit (Zymo, CA, USA). Finally, SYBR Green Master Mix (BioRad, CA, USA) was used in a Real-time PCR System (BioRad, CA, USA) as previously described (Rahmani et al., 2022). The expressions of genes such as Catalase (CAT), inducible nitric oxide synthase (iNOS), Glucose transporter 2 and 4 (GLUT-2, GLUT-4) were normalized to β-actin and to the expression of corresponding genes in the control group. Details of primers are presented in Table 1.

2.8. Statistical analyses

Data were analyzed by Analysis of Variance (ANOVA) using IBM statistical packages (SPSS, Version 21.0, Chicago, IL, USA). Data were checked for normality using the Univariate function and α at 95 % was defined as significant difference level.

3. Results and discussion

3.1. Physicochemical assessment of nanoliposome

The fundamental characterization of nano-liposomes, including measurements of size, polydispersity index (PDI), and ζ-potential, serves as an essential indicator of their stability and structural properties. In our study, the hydrodynamic size of the nanoliposome-encapsulated daidzein was determined to be 293.0 ± 46.5 nm (as shown in Table 2). This measurement is crucial as it reflects the overall dimensions of the nanoliposomes, providing insights into their physical characteristics.

Moreover, the PDI, which stood at 0.27, is an important parameter that quantifies the size distribution within the nanoliposome population. A low PDI value, as observed in our study, suggests a relatively narrow size distribution, indicating uniformity in the nanoliposome sizes. This uniformity is desirable as it contributes to the overall stability and consistency of the nanoliposome formulation.

The ζ-potential, a measure of the surface charge of nanoparticles, was determined to be −27.22 mV (as presented in Table 2). This negative ζ-potential indicates the presence of negatively charged groups on the surface of the nanoliposomes, which can contribute to their stability by preventing aggregation or coalescence due to electrostatic repulsion.

Interestingly, the findings from the characterization techniques were consistent with the observations from field emission scanning electron microscopy (FESEM), as depicted in Fig. 1. The FESEM image clearly illustrates the spherical shape and uniform distribution of the synthesized nanoliposomes, further confirming their structural integrity and stability. Lastly, the daidzein content within the nano-liposomes was quantified to be 389 mg/g dry weight (DW). This measurement is of prime importance as it validates the successful encapsulation of daidzein within the nanoliposomes, indicating their potential as effective delivery carriers for this bioactive compound.

3.2 Weight. alteration and dietary intake analyses

The weight changes observed in the various experimental groups over the course of the 35-day study period provide intriguing insights into the potential impact of dietary inclusion of nano-liposome loaded daidzein (NLD) on body weight dynamics. In our study, the control group of mice experienced a weight gain pattern that closely paralleled that of the alloxan-induced diabetic mice group, which exhibited the most substantial weight loss, as illustrated in Table 3. This alignment in

Table 2
Physical characteristics of synthesized daidzein-loaded nanoliposomes.

Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
293.0 ± 46.5	0.27	−27.22

Table 1
Names and sequences of the primers.

Gene	Forward (5′ →3′)	Reverse (5′ →3′)	References
CAT	ACATGGTCTGGGACTTCTGG	CAAGTTTTTGATGCCCTGGT	(Beyrami et al., 2020)
iNOS	CACCTTGGAGTTCACCCAGT	ACCACTCGTACTTGGGATGC	(Kou, Qi, Dai, Luo, & Yin, 2011)
GLU2	ATGTCAGAAGACAAGATCACCGG	CCTACTGGACGGATTTTGGCTC	(Samadder et al., 2011)
GLU4	AAGATGGCCACGGAGA	GTGGGTTGTGGCAGTGAGTC	(Samadder et al., 2011)
β-actin	CCTGAACCCTAAGGCCAACC	CAGCTGTGGTGGTGAAGCTG	(Shafaei et al., 2020b)

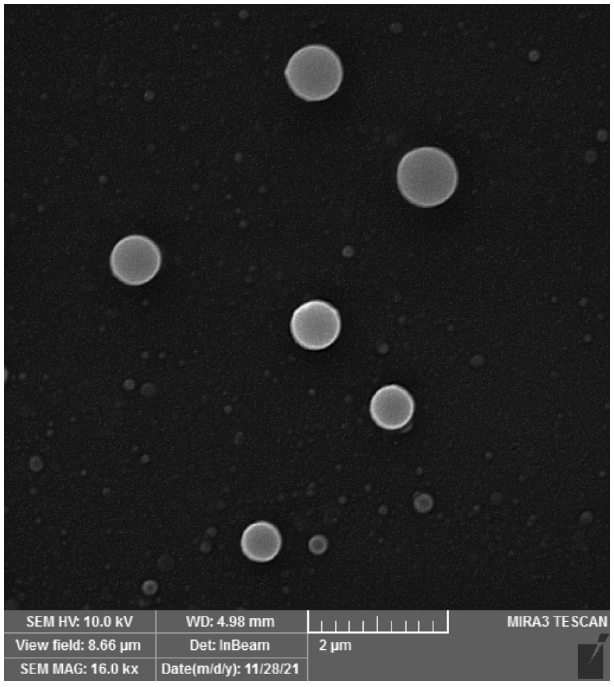

Fig. 1. The FESEM image of synthesized daidzein-loaded nanoliposomes.

Table 3
The averages of mice body weight changes and feed intake during experiment receiving different treatments.

Average	T1	T2	T3	T4	T5	SEM
Average daily weight gain (mg/day)	81.42^{a}	2.09^{e}	10.25^{d}	32.85^{c}	77.23^{b}	0.59
Average daily feed intake (g/day)	3.65^{a}	2.14^{d}	2.76^{c}	3.15^{b}	3.27^{b}	0.21

T1: Control; T2: Diabetic mice; T3: Diabetic mice + 50 mg NL/kg BW; T4: Diabetic mice + 100 mg NL/kg BW; T5: Diabetic mice + 250 mg Metformin/kg BW.
Different letters in the same raw indicated significant difference at $p < 0.05$ level. Analysis were performed in triplicates.
SEM: standard Error Mean.

weight trends underscores the severity of weight loss commonly associated with uncontrolled diabetes, as it often leads to decreased appetite, muscle wasting, and metabolic disruptions.

However, the introduction of nano-liposome loaded daidzein into the diets of the experimental groups marked a notable turning point. Specifically, the groups receiving NLD at concentrations of 50 mg NL/kg BW (T3) and 100 mg NL/kg BW (T4) exhibited a significant improvement in body weight compared to their diabetic counterparts. This outcome is particularly remarkable because it suggests that NLD could potentially counteract the weight loss typically observed in diabetic mice, which can be attributed to factors such as hyperglycemia, insulin resistance, and altered metabolism.

The improvement in body weight in response to NLD supplementation underscores the potential of this compound in ameliorating the adverse effects of diabetes, which often include unintentional weight loss. It suggests that NLD may contribute to better overall health and nutritional status in diabetic individuals by mitigating the weight loss associated with the condition. However, it is important to note that the specific mechanisms underlying such weight improvement require further investigation. Potential factors could include improved glycemic control, enhanced metabolic function, or alterations in appetite regulation (Lima, Moreira, & Sakamoto-Hojo, 2022).

Similar findings were also observed for the food intake. The dietary supplementation of NLD showed an improvement in the food intake. Natural products i.e., crude extracts and plant-derived compounds were previously reported to improve pancreas function, such as apigenin, genistein, and catechins (Ahmad et al., 2020). Phenolic compounds can substantially attenuate the hepatic steatosis, inflammation and oxidative stress in pancreas tissues in the diabetic condition (Zhao & Xu, 2021).

3.3. Blood analysis

We observed notable alterations in various biochemical parameters in diabetic mice induced by alloxan, which were consistent with findings reported in previous scientific research. Specifically, the diabetic-induced alloxan group (T2) exhibited a significant increase in cholesterol, LDL (Low-Density Lipoprotein), and glucose levels when compared to the normal control group, as indicated in Table 4. This elevation in cholesterol, LDL, and glucose levels is a common hallmark of diabetes mellitus and aligns with the existing literature.

Remarkably, our study demonstrated the potential of nano-liposome loaded daidzein (NL) supplementation in ameliorating these diabetic-associated changes. Groups 3 and 4 (T3 and T4), which were supplemented with 50 and 100 mg of NL per kilogram of body weight, respectively, exhibited a significant reduction in cholesterol, LDL, and glucose levels compared to the control group. This reduction aligns with the findings of prior research that explored the hypolipidemic and hypoglycemic properties of daidzein, especially when delivered through nano-liposomes.

Furthermore, our investigation revealed a significant decrease in triglyceride and LDL levels in the diabetic mice induced by alloxan when supplemented with nano-liposome loaded daidzein, as compared to the normal control group (Table 4). This outcome is in line with previous studies that have suggested the potential of daidzein to modulate lipid profiles and reduce cardiovascular risk factors.

Beyond lipid and glucose parameters, our study also assessed the antioxidant defense system in the serum of diabetic mice. We found that diabetic mice treated with 50 and 100 mg of NL per kilogram of body weight exhibited an enhanced antioxidant defense system. This observation corresponds with previous research that has highlighted the antioxidant properties of daidzein and the potential of nano-liposomes to improve its bioavailability.

Furthermore, serum biomarkers are important criteria for the evaluation of toxicity in the body. The amounts of enzymes leaking into the blood stream indicate the severity of hepatic damage (Aman et al., 2021). Obese individuals tend to have a decreased antioxidant defences in which antioxidant enzymes e.g., SOD and catalase are lowered (Li et al., 2021). Evidence suggests a cluster of sources which induce oxidative stress in obesity such as hyperglycemia, increased tissue lipid levels, inadequate antioxidant defences, increased rates of free radical formation, and chronic inflammation (Nono Nankam, Nguelefack, Goedecke, & Blüher, 2021).

3.4. Histopathological characteristics and lipid peroxidation

The evaluation of liver and pancreas cells, as depicted in Fig. 2, did not reveal any statistically significant differences within the groups. This suggests that the administration of nano-liposome loaded daidzein at both 50 and 100 mg NL/kg BW to the diabetic mice induced by alloxan did not result in significant changes in the condition of these vital organs, at least at the cellular level. These findings provide valuable insights into the safety profile of nano-liposome loaded daidzein as a potential therapeutic agent for diabetes, indicating that it does not cause discernible harm to the liver and pancreas.

Fig. 3 depicts an increase in malondialdehyde (MDA) levels in the pancreas tissue of diabetic mice induced by alloxan compared to the control group. Elevated MDA levels are indicative of oxidative stress and lipid peroxidation, which are often associated with diabetes. However,

Table 4
The blood parameters analysis during experiment receiving different treatments.

Treatment	TG (mg/dl)	Chol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Glu (mg/dl)	Insulin (μlU/ml)	GPX (U/L)	SOD (U/ml)	Catalase (U/ml)
T1	197.5^{b}	112.5^{b}	47^{a}	31.5^{d}	159^{c}	8.17^{a}	180^{a}	300^{d}	141.5^{b}
T2	191^{c}	159^{a}	36.5^{bc}	52.5^{a}	281^{a}	3.93^{d}	148.5^{c}	254.5^{e}	100.5^{e}
T3	160.5^{d}	108^{c}	39.5^{b}	40.5^{b}	180^{b}	5.46^{c}	161.5^{b}	310^{b}	118.5^{d}
T4	159^{d}	93^{d}	41.5^{b}	37.5^{c}	139^{d}	6.56^{c}	184.5^{a}	337.5^{a}	186.9^{a}
T5	387^{a}	99^{d}	35^{bc}	35.5 ^{cd}	113^{e}	7.32^{b}	163.5^{b}	306.5^{c}	130.2^{c}
SEM	3.68	5.73	3.19	4.96	7.58	1.62	4.39	6.48	8.14

T1: control; T2: Diabetic mice; T3: Diabetic mice + 50 mg NL/kg BW; T4: Diabetic mice + 100 mg NL/kg BW; T5: Diabetic mice + 250 mg Metformin/kg BW. Different letters in the same raw indicated significant difference at $p < 0.05$ level. Analyses were performed in triplicates.

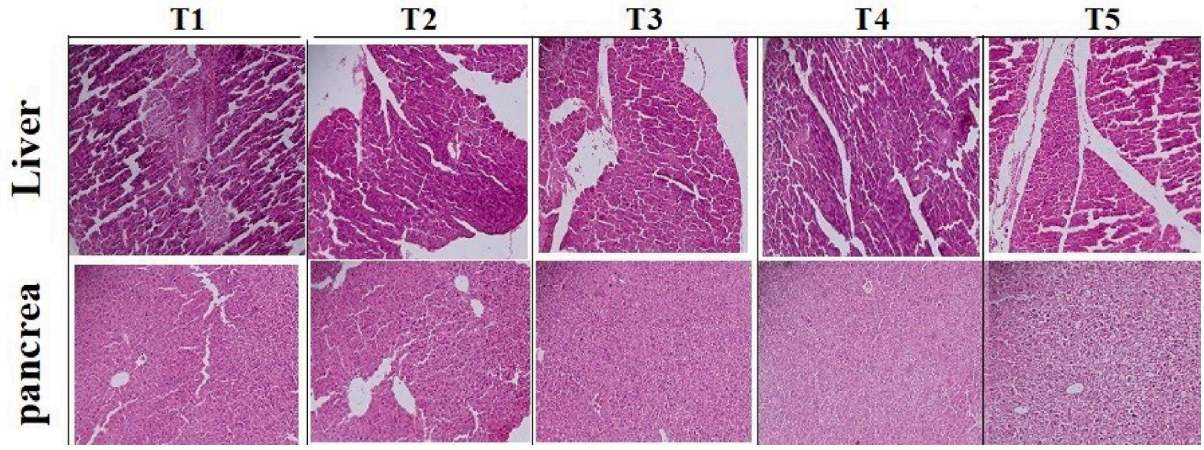

Fig. 2. The histopathological changes in the mice liver and pancreas under different treatments. T1: Control; T2: Diabetic mice; T3: Diabetic mice + 50 mg NL/kg BW; T4: Diabetic mice + 100 mg NL/kg BW; T5: Diabetic mice + 250 mg Metformin/kg BW.

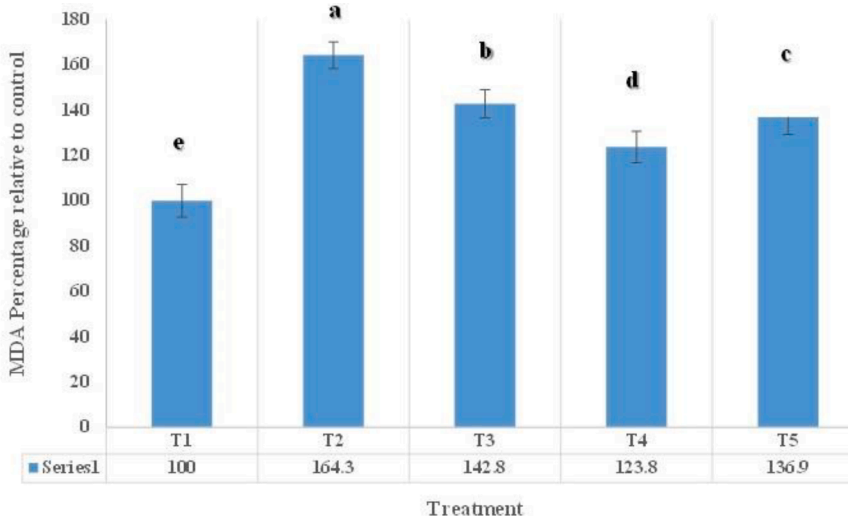

Fig. 3. The lipid peroxidation in the pancreas of mice under different treatments. T1: control; T2: Diabetic mice; T3: Diabetic mice + 50 mg NL/kg BW; T4: Diabetic mice + 100 mg NL/kg BW; T5: Diabetic mice + 250 mg Metformin/kg BW. Different letters indicate significant difference at $p < 0.05$ level. Analysis was performed in triplicates.

our study revealed a significant ($p < 0.05$) prevention of this rise in MDA levels, particularly when nano-liposome loaded daidzein was administered at a concentration of 100 mg NL/kg BW. Moreover, this effect displayed a dose-dependent pattern, suggesting that higher doses of nano-liposome loaded daidzein may offer enhanced protection against oxidative stress in the pancreas. These results are encouraging as they highlight the potential of nano-liposome loaded daidzein to mitigate oxidative damage in the pancreas, a key component in the pathogenesis of diabetes. The observed dose-dependent effect underscores the importance of dosage optimization in future therapeutic applications of this formulation.

Oxidative stress, characterized by an excess of reactive oxygen species (ROS) or a deficiency in antioxidant defenses, has been implicated in the pathogenesis and progression of numerous health conditions. Aman et al. (2021) further solidify this notion by underlining the prevalence of MDA measurement in oxidative stress research across

diverse disease states. Their findings reflect the growing awareness of the pivotal role oxidative stress plays in the molecular and cellular mechanisms underlying diseases such as cardiovascular disorders, neurodegenerative diseases, cancer, and metabolic disorders. The concentration of such oxidative stress biomarkers can be lowered by changing life style and nutritional intake of antioxidants (Pisoschi et al., 2021).

3.5. Gene profiling

Obesity is associated with a chronic inflammatory response, characterized by abnormal adipokine production, and the activation of pro-inflammatory signaling pathways, leading to the induction of several biological markers of antioxidant and inflammation (Ahmed, Sultana, & Greene, 2021). The investigation into the molecular mechanisms underlying the effects of nano-liposome loaded daidzein in diabetic mice provided intriguing insights into the modulation of critical biomarker genes related to antioxidant defense and inflammation within pancreatic tissues, as outlined in Table 5. One of the pivotal findings was the discernible impact on the expression of key antioxidant genes, particularly catalase (CAT). This enzyme is a cornerstone of the cellular defense against oxidative stress, as it catalyzes the breakdown of harmful hydrogen peroxide into water and oxygen, thus safeguarding cells from oxidative damage. Strikingly, our study unveiled a significant ($p < 0.05$) down-regulation of CAT expression in diabetic mice, reflecting the compromised antioxidant defense mechanisms often observed in diabetes. However, with the introduction of nano-liposome loaded daidzein into the mice's diet, a noteworthy reversal was observed. CAT expression was significantly enhanced, indicating a bolstered capacity to combat oxidative stress within the pancreatic tissues.

Conversely, our investigation also delved into the expression of the inducible nitric oxide synthase (iNOS) gene, a marker associated with inflammation. An up-regulation of iNOS gene expression was detected in the diabetic mice, signifying an inflammatory response. Intriguingly, when nano-liposome loaded daidzein was administered, it induced a significant suppression of iNOS gene expression in the pancreas. This finding underscores the potential anti-inflammatory properties of daidzein-loaded nanoliposomes in mitigating the inflammatory milieu often associated with diabetes.

In addition to these observations, our study delved into the mRNA expression of glucose transporter genes, GLUT2 and GLUT4, in both pancreatic and muscle tissues. In the diabetic mice, there was an elevation in the mRNA expression of both GLUT2 and GLUT4 compared to the negative control (T1), which might be an adaptive response to increased glucose levels in diabetes. However, the positive control group (T2) exhibited a significant ($p < 0.05$) decrease in the mRNA expression of both GLUT2 and GLUT4 compared to the control group, indicative of impaired glucose transport.

Remarkably, the administration of nano-liposome loaded daidzein displayed a dose-dependent effect, leading to a significant decrease in the expression of both GLUT2 and GLUT4 genes in muscle tissue. This suggests that nano-liposome loaded daidzein could potentially improve glucose homeostasis by modulating the expression of these crucial glucose transporters.

Table 5
Gene expression profiling in mice received different treatment.

Gene expression (Fold changes)						SEM
Genes	T1	T2	T3	T4	T5	
Gene expression in pancreas tissue						
CAT	1.0^{a}	-3.8^{c}	-1.4^{b}	1.1^{a}	1.3^{a}	0.08
iNOS	1.0^{c}	5.8^{a}	3.1^{b}	1.2^{c}	1.4^{c}	0.09
Gene expression in muscle tissue						
GLUT-2	1.0^{b}	-3.7^{d}	-1.5^{c}	1.3^{ab}	1.6^{a}	0.05
GLUT-4	1.0^{c}	-2.3^{e}	-1.1^{d}	1.7^{b}	2.3^{a}	0.07

T1: Control; T2: Diabetic mice; T3: Diabetic mice + 50 mg NL/kg BW; T4: Diabetic mice + 100 mg NL/kg BW; T5: Diabetic mice + 250 mg Metformin/kg BW; Different letters in the same raw indicated significant difference ($p < 0.05$). The analyses were performed in triplicates. SEM: standard Error Mean.

4. Conclusion

The current study investigated the impact of nano-liposome loaded daidzein on diabetic mice, shedding light on its potential therapeutic mechanisms. While this research offers valuable insights, it is important to acknowledge limitations, as well as the potential benefits it could bring to the industry.

For instance, I) the study primarily utilized a diabetic mouse model induced by alloxan. While this model provides valuable information, it's important to recognize that the pathophysiology of diabetes in humans is complex and may not be entirely recapitulated in mice. II) The study focused on the effects of nano-liposome loaded daidzein. Diabetes is a multifaceted disease, and its management often involves a combination of interventions. Future research might explore the synergistic effects of daidzein with other bioactive compounds or pharmaceutical agents. III) While our findings are promising, clinical translation to human subjects requires further investigation. Human trials and safety assessments are necessary steps to validate the potential therapeutic applications of nano-liposome loaded daidzein.

The findings of the present study can offer benefits to the industry as follows:

I) Should the effects observed in this study can be replicated in human trials, nano-liposome loaded daidzein may offer a complementary or alternative option for managing diabetes alongside existing therapies. This diversification of treatment options can benefit the pharmaceutical and healthcare industries by providing more tailored solutions to patients. II) The utilization of nanoliposomes as a delivery system is a burgeoning field within pharmaceutical and biomedical research. This study showcases the potential of nanotechnology in enhancing the bioavailability and effectiveness of bioactive compounds, paving the way for future developments in drug delivery systems. The dose-dependent effects observed in the study suggest that dosages of nano-liposome loaded daidzein can be tailored to individual patient needs. This aligns with the growing trend in personalized medicine, where treatments are customized based on patients' unique characteristics and requirements.

In summary, while this study has limitations, it represents a significant step forward in understanding the potential therapeutic applications of nano-liposome loaded daidzein in diabetes management. By addressing these limitations and conducting further research, the industry can explore new avenues for developing innovative treatments and delivery systems, ultimately benefitting both patients and healthcare providers.

CRediT authorship contribution statement

Negar Chalaki Rad: Methodology, Formal analysis, Conceptualization. **Ehsan Karimi:** . **Homa Mahmoodzadeh Akherat:** Writing – original draft, Validation, Software, Data curation, Conceptualization. **Ehsan Oskoueian:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Mohsen Zareian:** Writing – review & editing, Writing – original draft, Software, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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