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ORIGINAL ARTICLE

# Synthesized nanoliposome-encapsulated kaempferol attenuates liver health parameters and gene expression in mice challenged by cadmium-induced toxicity

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**Abstract**

In the present research, we encapsulated a flavonoid called kaempferol into nanoliposomal structures and the health-promoting effects of synthesized nanoliposome-loaded kaempferol (NLK) were evaluated in mice challenged by cadmium-induced . The NLK characteristics, such as size, zeta potential, and polydispersity index, were 218.4 nm, −28.55 mV, and 0.29, respectively. The in vivo experiment revealed that the mice receiving water containing cadmium (2 mg/kg body weight/day) showed significant ($p < 0.05$) weight loss, an increase in liver enzyme activities, and hepatic oxidative stress. Dietary supplementation with NLK at concentrations of 2.5 and 5 mg/kg mice body weight notably ($p < 0.05$) improved the body weight, liver enzyme activities, hepatic oxidative stress, and antioxidant potential of the liver. Our findings elucidated that NLK could alleviate the toxicity of cadmium in mice challenged by cadmium-induced toxicity.

**KEYWORDS**
encapsulation, drug delivery, nanoliposomes, kaempferol, heavy metals

## 1 | INTRODUCTION

Metal toxicity is associated with their catalytic nature, which induces cellular oxidative stress and thus leads to massive tissue damage. Cadmium ($Cd^{2+}$) is an inorganic heavy metal that influences human health through polluted air, soil, water, and dietary regimens. Cellular oxidative stress is considered the main result of excessive reactive oxygen species (ROS) and antioxidant defense inability. In other words, cadmium-induced ROS generation leads to lipid peroxidation and DNA and protein damage, which can alter cell function and lead to various degenerative diseases.[1] To defend against such conditions, the antioxidant defense system, which comprises superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) enzymes, is activated to alleviate the effects of oxidative stress and counteract ROS.[2,3]

Flavonoids protect cells by neutralizing the generated ROS and preventing oxidative stress.[4] Kaempferol is a major flavonoid mainly found in fruits, vegetables, and herbs and is reported to possess anti-inflammatory, antioxidant, neuroprotective, and hepatoprotective activities. It is also known to be effective in cancer chemotherapy.[5–10] Dong et al. reported the hepatoprotective effect of kaempferol, which is a flavonoid of *E. mollis*, on D-galactosamine (GalN) and lipopolysaccharide (LPS)-

**Abbreviations:** ALP, Alkaline phosphatase; AST, Aspartate transaminase; ALT, Alanine transaminase; BW, Body weight; CAT, Catalase; DLS, Dynamic light scattering; FESEM, Field emission scanning electron microscope; GPx, Glutathione peroxidase; iNOS, Nitric oxide synthase; LPS, Llipopolysaccharide; NLK, Nano-liposome-loaded kaempferol; PDI, Polydispersity index; SOD, Super-oxide dismutase; TBA, Thiobarbituric acid.

induced acute liver injury in mice, who administered them through the water. Kaempferol exerted anti-inflammatory, antiapoptotic, and antioxidative effects on GalN/LPS-induced acute liver injury, and their results suggested that these activities are attributed to the downregulation of TLR4 and NF-κB signaling pathway activity.[11] In another study by Wang et al., the hepatoprotective effects of kaempferol 3-O-glucoside (K-3-G) and kaempferol 3-O-rutinoside (K-3-R) were isolated from *C. tinctorius*, which were injected intraperitoneally, on $CCL_4$-induced oxidative liver damage in mice.[12] A significant step in defining the potential mechanism of action of flavonols is bioactive compound bioavailability. Bioavailability studies in humans suggest that some plant conjugated forms of kaempferol are more bioavailable than free forms of the compound since flavonols are rapidly metabolized in the human body.[13] In addition, free flavonoids are more susceptible to oxidation and degradation, which decreases their health benefits.[14] Hence, the encapsulation or conjugation of flavonoids with other biomolecules not only increases their intestinal bioavailability but also enhances their stability against degradation and oxidation.[15,16]

Nanoliposomes consist of phospholipid bilayers that are microscopic carriers that can control the release of natural phytochemicals to the target place and enhance the effectiveness and cellular uptake of the encapsulated natural constituent.[17] Liposomes can also provide a slow release of an encapsulated natural constituent, resulting in sustained exposure to the site of action and increased efficacy.[18] Thus, encapsulation using liposomal technology as carriers is aimed at enhancing the bioavailability of pharmaceutical and nutraceutical potent bioactive compounds.[19] Flavonoids such as kaempferol have low solubility in polar solutions. Therefore, it is not an efficient natural antioxidant compound due to its low solubility and intestinal absorption.[20] To solve this problem, loading kaempferol into an appropriate carrier, such as liposomes, could improve its solubility and intestinal absorption. Liposomal-based drugs can benefit from the increased solubility of the cargo, the longer half-life, and the ability to overcome the resistance of cancer cells to chemotherapy. As a result of the pharmacokinetic changes that follow, adverse effects could become less prevalent and the therapeutic index of encapsulated drugs could improve.[21] In this regard, it has been shown that by utilizing nanotechnological strategies such as liposomes, scientists could increase the bioavailability of silymarin, a flavonoid. For instance, Ochio et al. indicated that encapsulation into PEGylated nanoliposomes of silymarin and gallic acid led to a synergistic influence on the liver cancer cell line HepG2.[22] Analyses of scanning electron microscopy revealed that the nanoliposome capsules are spherical in shape with no signs of aggregation.[23,24]

**Highlights**

- Nanoliposome-loaded kaempferol, a natural flavonoid, was synthesized.
- Cadmium in the drinking water induced weight loss and increased liver enzyme activities and hepatic oxidative stress in mice.
- Nanoliposome-loaded kaempferol alleviated the toxicity of cadmium in mice challenged by cadmium.
- The nanoliposome-loaded kaempferol could be considered as a dietary supplement against cadmium toxicity.

Liposomes are tiny synthetic sphered-shaped vesicles consisting of one or more phospholipid bilayers that can separate into hydrous solutions and can be made from cholesterol and natural nontoxic phospholipids.[25] These bipolar structures can trap polar and nonpolar compounds. Accordingly, the loaded flavonoids could be protected against external factors such as pH, oxygen, and enzymatic degradation in the gastrointestinal tract, and thus bioavailability and absorption could be increased.[17] Lecithin is used for liposomal encapsulation due to its safety and accessibility. Hence, in this experiment, we encapsulated a flavonoid called kaempferol into lecithin-based nanoliposomal structures, and the health-promoting effects of synthesized nanoliposome-loaded kaempferol (NLK) were evaluated in mice challenged by cadmium-induced toxicity.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

Kaempferol (Cat: 14590) and soybean lecithin (Cat: 429415) were purchased from Sigma Aldrich (Germany). Male Balb/c mice were purchased from Razi Vaccine and Serum Research Institute (Mashhad, Iran). The other materials and reagents not mentioned here obtained were from Merck (Germany).

### 2.2 | Nanoliposome-loaded kaempferol

The NLK preparation was carried out according to the study conducted by Oskoueian et al.[27] Precisely, 196 g of hot distilled water (80°C) and 4 g of lecithin were mixed for 60 min on a stirrer (300 rpm). Kaempferol was dissolved in

ethanol and added to the mixture to reach a concentration of 2000 ppm. The blending was continued for an additional 2 h at 60°C. To sonicate the mixture at 80% power for 5 min, an ultrasonic disrupter was utilized. The synthesized NLK was then freeze-dried and stored at −20°C. Freeze-dried NLK rehydration was executed by suspending NLK pastes in deionized water at room temperature under continuous stirring for 60 min.

#### 2.2.1 | NLK characterization

The NLK mixture was diluted (1:20) with distilled water to decrease aggregation and inhibit noise scattering.[28] Employing the dynamic light scattering (DLS) method, the average size of particles and their stability (zeta potential) and polydispersity index (PDI) were determined. After all, field emission scanning electron microscopy (FESEM) was utilized to ascertain the sizes and morphology of NLK particles. The PDI and zeta potential parameters were measured by a Zetasizer Nano ZS-90 (Malvern, UK).

### 2.3 | Mice trial

Thirty-two male Balb/c mice with a mean body weight (BW) of 20–25 g (by the age of 8 weeks) were randomly separated into four groups that consisted of eight mice in each treatment. The mice were kept in cages at 58 ± 10% humidity and 23 ± 1°C with alternating 12-h light/dark periods for 7 days to adapt to lab conditions. The treatments included a control group that received a normal diet without obtaining NLK particles and cadmium-containing water. Three other groups received an NLK-supplemented diet in various concentrations of NLK (0, 2.5, and 5 mg/kg mice BW) and cadmium-containing water (2 mg/kg mouse BW). The mice were weighed three times at the beginning, middle, and end of the treatment time. The experiment was performed for 30 days. All animal experiments were carried out according to the ethical principles approved by the Islamic Azad University, Mashhad, Iran, by the ethics code IR.IAU.MSHD.REC.1398.094.

### 2.4 | Histological evaluation

At the end of the trial, the mice were sacrificed, and their tissues, such as the liver, brain, kidney, and jejunum, were carefully biopsied, washed with physiological serum for histopathological study, and then maintained in buffered formalin (10% formalin in 0.1 M sodium phosphate buffer, pH 7). Tissues were paraffinized, sliced, and stained (using the hematoxylin/eosin protocol) after washing samples with 0.9% NaCl solution based on the reference protocols.[29] The liver, brain, and kidney tissues were then evaluated for histopathological examination, while the jejunum was used for histomorphological analysis. The liver samples were kept at −80°C for mineral analysis and gene expression study.

### 2.5 | Liver parameter evaluation

#### 2.5.1 | Liver enzyme analysis

The liver enzymes of mice containing alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) were characterized by an autoanalyzer (Hitachi 902, Japan).

#### 2.5.2 | Metal deposition in the liver metal

To define the concentrations of various metals comprising copper (Cu), cadmium (Cd), selenium (Se), manganese (Mn), and zinc (Zn) in the liver tissue of mice, inductively coupled plasma–mass spectrometry (ICP–MS) analysis was applied. In this way, the liver tissues were freeze-dried at −20°C for 12 h and placed in an electric oven (70°C) for 3 h. The sample powder was hydrolyzed through a mixed acid solution (nitric acid (68%): hydrofluoric acid 38% with a ratio of 7.5:1) and warmed for 3 h at 80°C. The samples were chilled and dried in a Teflon beaker at 150°C. Then, the acid solution (0.5 mL $HCIO_4$ (70%) and 1.0 mL $HNO_3$) was made and mixed with the sample powder, and this heating and cooling were repeated (20). The ultimate powder was blended in nitric acid and analyzed by an ICP–MS device (Agilent 7500, USA) as previously explained by Yoshida et al.[30]

#### 2.5.3 | Lipid peroxidation assay

The lipid peroxidation of liver tissues was determined with slight modifications. The definitive product of lipid peroxidation is malondialdehyde (MDA). It can form a colored complex by reacting with thiobarbituric acid (TBA). Hence, the absorption index demonstrates the rate of lipid oxidation. Compactly, liver tissues were incorporated in phosphate-buffered saline (PBS) at a ratio of 1 g: 9 mL (0.01 M) at pH 7.4, and the lysate was centrifuged at 10,000 rpm for 10 min. The 200-$\mu$L of homogenized tissues was blended with 35 $\mu$L of BHT, 300 $\mu$L of distilled water, 2 mL of TBA, and 165 $\mu$L of sodium dodecyl sulfate. The composition was heated for 60 min in a water bath at 90°C. The suspension was refrigerated, and 2 mL of *n*-butanol was added and vortexed

**TABLE 1** The primer sets of targeted genes

| Gene | Forward (5→3) | Reverse (3→5) |
|---|---|---|
| GAPDH | GCAGGGGGGAGCCAAAACGGT | GGGTGGCAGTGATGGCATGG |
| iNOS | CACCTTGGAGTTCACCCAGT | ACCACTCGTACTTGGGATGC |
| SOD | GAGACCTGGGCAATGTGACT | GTTTACTGCGCAATCCCAAT |
| CAT | ACATGGTCTGGGACTTCTGG | CAAGTTTTTGATGCCCTGGT |
| GPx | CAAGTTTTTGATGCCCTGGT | TCGGACGTACTTGAGGGAAT |

Abbreviations: CAT: catalase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; GPx: glutathione peroxidase; iNOS: inducible nitric oxide synthase; SOD: superoxide dismutase.

for 60 s. It was then centrifuged at 2000 *g* for 5 min. The *n*-butanol was segregated, and the absorbance was distinguished at 532 nm with a visible spectrophotometer (UNICO, China). The results were standardized to the control group and stated as a percentage relative to the control.[31]

#### 2.5.4 | Gene expression analysis

The gene expression profile of the mice's liver tissues was measured for four different genes, including inducible nitric oxide synthase (iNOS), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase SOD, under disparate NLK concentrations and cadmium cytotoxic effects. The mice's liver tissues of all four treated groups of mice were used for the RNA extraction phase with an RNeasy Mini kit (Qiagen, Hilden, Germany). Then, cDNA libraries were produced by a Quantitect Reverse Transcription kit (Qiagen, Hilden, Germany). Next, the forward and reverse primer sequences for the intended genes (CAT, iNOS, GPx, and SOD) and housekeeping gene (GAPDH) were designed (Table 1). SYBR Green PCR Master Mix (Qiagen, Hilden, Germany) was employed for comparative real-time PCR (Roche Diagnostics). The intended genes were reinforced as follows: 95°C for 5 min (1×), 95°C for 20 s, then 55°C for 20 s, and 72°C for 25 s (35×). Gene expression was normalized to that of GAPDH as a reference gene and then normalized to the expression of related genes in the control group.[31]

### 2.6 | Statistics

One-way analysis of variance (ANOVA) using the GLM procedure of SPSS (Version 21) was applied to conduct the statistical analysis. The averages were compared by Duncan's multiple range test. The means are supposed to be significantly distinct when $p \leq 0.05$. All the analyses were accomplished three times, and the results are represented as the mean values ± standard error of the mean or standard deviation.

## 3 | RESULTS

### 3.1 | NLK characterization

Figure 1 indicates the NLK physical features, including the zeta potential, *Z*-average particle size, and FESEM image. The zeta potential, *Z*-average particle size, and PDI were −28.5 mV, 218.4 nm, and 0.26, respectively. A PDI less than 0.3 is considered a sufficient index for homogenous dispersions of NLK particles. The zeta potential of −28.55 mV indicates that the NLK surface electric potential showed acceptable particles with repulsion and stability. The FESEM analysis confirmed that the nanosizes of NLK, which were morphologically spherical and exhibited almost the same size, confirmed the particle size results.

#### 3.1.1 | Mice trial

The control group mice displayed an increase in weight of approximately 7.34 ± 0.27 g/30 days. Instead, the mice receiving cadmium showed a weight loss of 9.1 ± 0.46 (g/30 days) during treatment. Dietary supplementation with NLK at concentrations of 2.5 and 5 mg/kg mice body weight significantly ameliorated weight loss ($p \leq 0.05$) (Table 2). The results of the relationship between food consumption and changes in body weight are given. These results showed an increase in food intake in the control group, while food intake in the group of cadmium recipients decreased due to appetite loss. However, in the groups receiving dietary supplements NLK, we observed an increment in appetite and, accordingly, a rise in food intake (Table 2). Liver enzymes such as AST, ALT, and ALP are biomarkers that show the function and health of the liver. The rates of these enzymes were increased in the serum of the mice receiving cadmium (Cd-treated group); however, dietary supplementation with NLK modulated the levels of these enzymes (Table 3).

Mineral deposition alterations in the liver of mice, including copper, cadmium, selenium, manganese, and zinc, are shown in Table 4. The control group had the

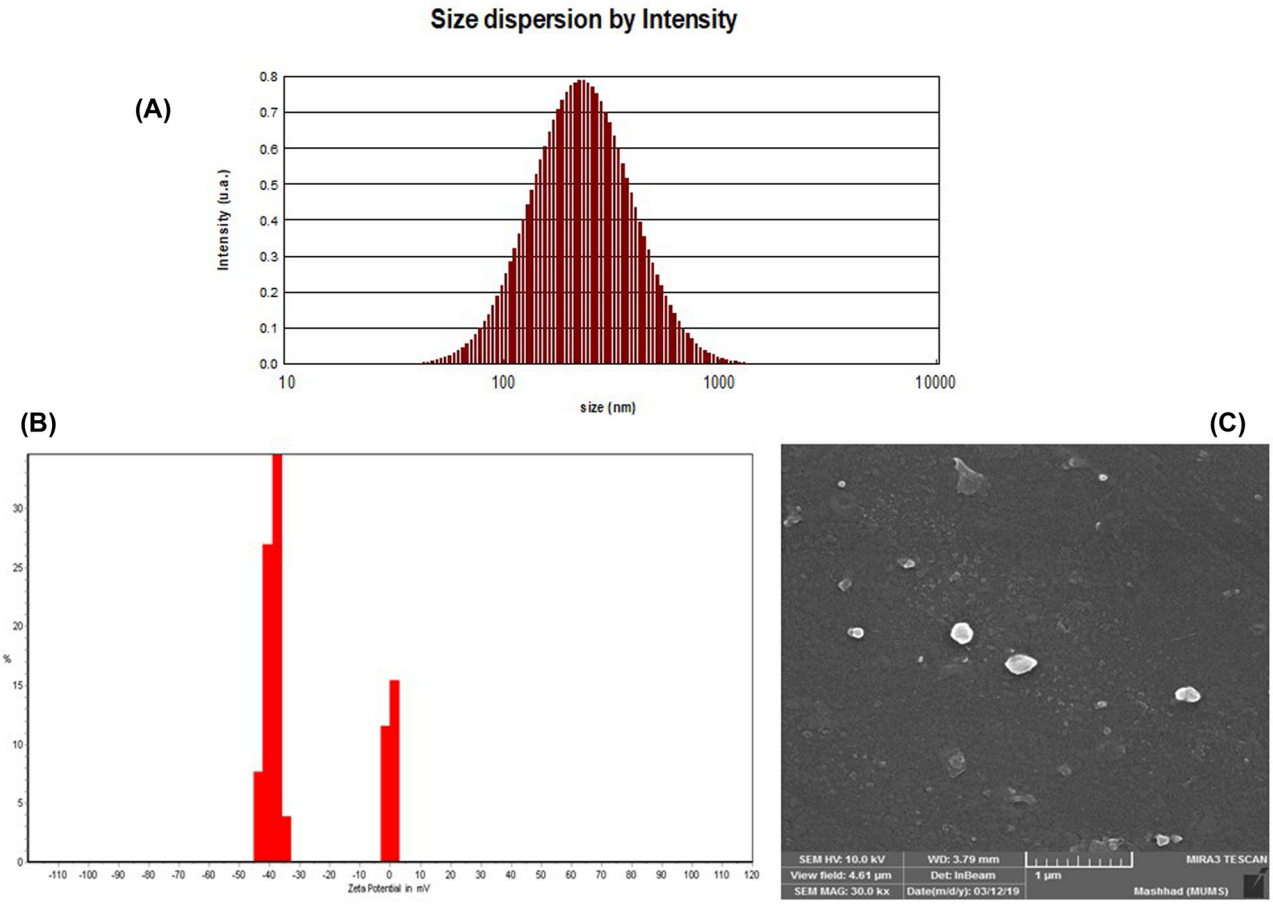


**FIG 1** The features of nanoliposome-loaded kaempferol

**TABLE 2** Changes in the mice's body weight and food consumption within the experiment after receiving various treatments

| Average | Control group | Cd-treated group | Group 1 (Cd + 2.5 mg/kg BW NLK) | Group 2 (Cd + 5 mg/kg BW NLK) |
|---|---|---|---|---|
| Bodyweight changes (g/30 days) | +7.34 ± 0.27[a] | −9.1 ± 0.46[d] | −6.2 ± 0.81↓[c] | −4.4 ± 0.79↓[b] |
| Food consumption (g/30 days) | 58.8 ± 4.26[a] | 34.2 ± 3.60[d] | 38.7 ± 3.12[c] | 44.6 ± 2.12[b] |

*Note*: Values with various superscript letters in the same line are significantly different ($p \leq 0.05$). All analyses were performed in triplicate.
Abbreviations: −, weight lost; +, weight gain; Cd, cadmium; NLK, nanoliposome loaded kaempferol.

**TABLE 3** Aspartate AminotransferaseAST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP) serum concentrations in the mice that received various treatments

| Liver enzymes (IU/L) | Control group | Cd-treated group | Group 1 (Cd + 2.5 mg/kg NLK) | Group 2 (Cd + 5 mg/kg NLK) |
|---|---|---|---|---|
| AST | 318 ± 13.68[d] | 787.5 ± 24.91[a] | 682 ± 14.72[b] | 505 ± 11.94[c] |
| ALT | 47.5 ± 9.67[d] | 193 ± 8.91[a] | 151.5 ± 7.92[b] | 110.5 ± 8.01[c] |
| ALP | 121.5 ± 6.16[d] | 278 ± 7.52[a] | 224.5 ± 6.31[b] | 167 ± 5.81[c] |

*Note*: Values with various superscript letters in the same line are significantly different ($p \leq 0.05$). All analyses were performed in triplicate.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase.

**TABLE 4** The alterations in the liver mineral compound of mice receiving distinct treatments

| Liver Elements (ppm) | Control group | Cd-treated group | Group 1 (Cd + 2.5 mg/kg BW NLK) | Group 2 (Cd + 5 mg/kg BW NLK) |
|---|---|---|---|---|
| Cadmium | 0.09 ± 0.04$^{d}$ | 6.5 ± 0.06$^{a}$ | 4.2 ± 0.08$^{b}$ | 3.3 ± 0.06$^{c}$ |
| Copper | 13.5 ± 0.21$^{a}$ | 10.1 ± 0.11$^{d}$ | 11.3 ± 0.18$^{c}$ | 12.3 ± 0.15$^{b}$ |
| Manganese | 4.3 ± 0.09$^{a}$ | 2.36 ± 0.07$^{d}$ | 2.9 ± 0.08$^{c}$ | 3.8 ± 0.06$^{b}$ |
| Selenium | 1.42 ± 0.07$^{a}$ | 0.82 ± 0.05$^{d}$ | 1.2 ± 0.06$^{c}$ | 1.3 ± 0.09$^{b}$ |
| Zinc | 51.8 ± 0.22$^{a}$ | 45.8 ± 0.28$^{d}$ | 47.3 ± 0.16$^{c}$ | 49.5 ± 0.16$^{b}$ |

*Note*: Values with various superscript letters in the same line are significantly different ($p \leq 0.05$). All analyses were performed in triplicate.

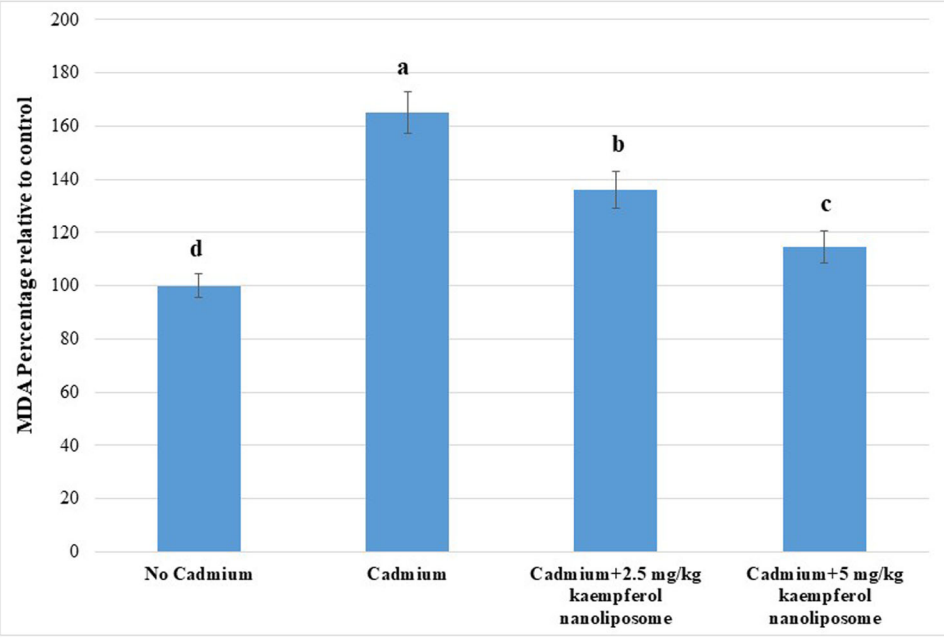


**FIG 2** Lipid peroxidation in the liver tissue of mice that received different treatments

lowest amount of cadmium, and the group that received cadmium through drinking water had the highest amount of cadmium. A diet containing NLK inhibited cadmium deposition in the liver. The liver deposition of other minerals, including zinc, manganese, copper, and selenium, in the cadmium-containing water-treated mice decreased ($p \leq 0.05$). Instead, the embodiment of NLK as a dietary supplement resulted in these depositions.

## 3.2 | Lipid peroxidation

The results showed that lipid peroxidation increased in the liver in the presence of cadmium. Consumption of NLK significantly ($p \leq 0.05$) reduced lipid peroxidation. In this way, NLK supplementation had a high antioxidant capacity and cytoprotective activity that inhibited lipid peroxidation (Figure 2).

## 3.3 | Gene expression analysis

The results of the inflammatory gene, inducible iNOS expression, and antioxidant regulatory genes such as CAT, SOD, and GPx are reported in Table 5. Cadmium resulted in inflammation in the mice's liver, and the increase in the expression of the iNOS gene confirmed the presence of inflammation. Dietary supplementation with NLK significantly ($p \leq 0.05$) alleviated the expression of the iNOS gene and thus reduced liver inflammation. Antioxidant-related gene expression comprising SOD, CAT, and GPx was significantly ($p \leq 0.05$) downregulated in the cadmium-treated mice. Cadmium increases free radicals and induces oxidative stress, thereby suppressing antioxidant-related gene expression (SOD, CAT, and GPx). Treatment of mice with NLK removes free radicals and enhances the cellular redox potential, thus promoting antioxidant-related gene expression.

**TABLE 5** Gene expression analysis of the livers of mice that received different treatments (fold changes)

| Liver Genes | Control group | Cd-treated group | Group 1 (Cd + 2.5 mg/kg BW NLK) | Group 2 (Cd + 5 mg/kg BW NLK) |
|---|---|---|---|---|
| Upregulated gene | | | | |
| iNOS | $1.0 \pm 0.13^{d}$ | $+7.1 \pm 0.26^{a}$ | $+4.7 \pm 0.13^{b}$ | $+3.4 \pm 0.37^{c}$ |
| Downregulated genes | | | | |
| CAT | $1.0 \pm 0.28^{d}$ | $-4.4 \pm 0.63^{a}$ | $-3.5 \pm 0.39^{b}$ | $-1.9 \pm 0.17^{c}$ |
| SOD | $1.0 \pm 0.14^{d}$ | $-5.4 \pm 0.62^{a}$ | $-4.1 \pm 0.38^{b}$ | $-3.2 \pm 0.18^{c}$ |
| GPx | $1.0 \pm 0.21^{d}$ | $-3.5 \pm 0.37^{a}$ | $-2.7 \pm 0.26^{b}$ | $-2.1 \pm 0.16^{c}$ |

*Note*: Values with various superscript letters in the same line are significantly different ($p \leq 0.05$). All analyses were performed in triplicate. +, upregulated; −, downregulated.

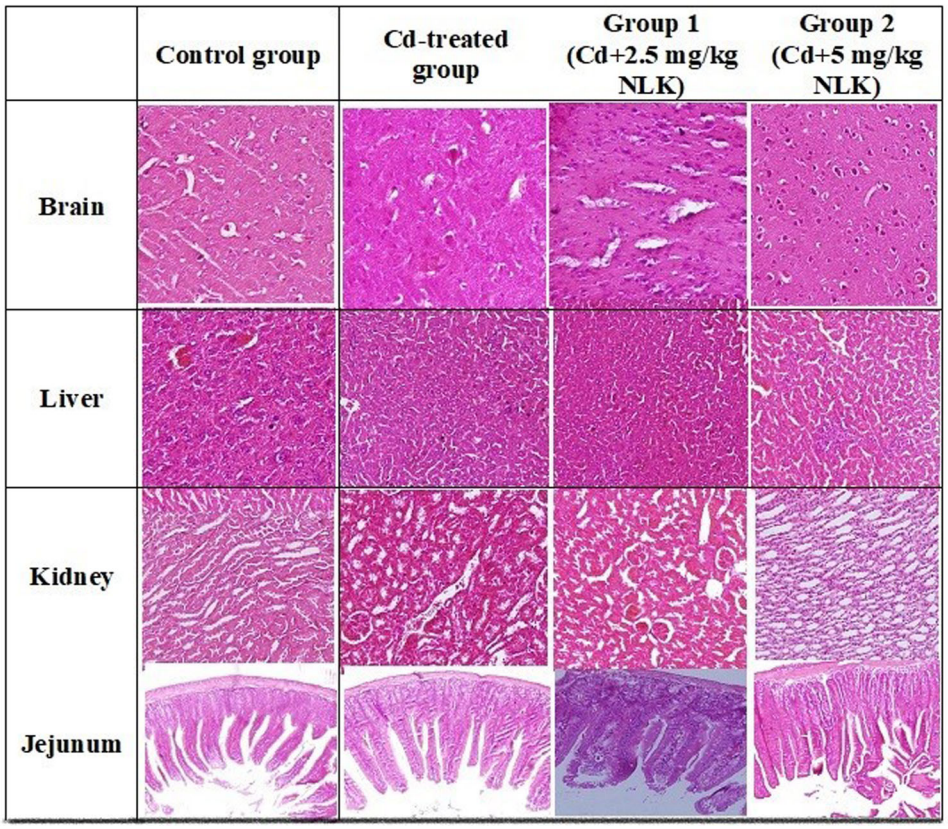


**FIG 3** The histopathology of the brain, kidney, liver, and jejunum tissues of the mice that received various treatments

### 3.3.1 | Histopathology results

Figure 3 shows the histopathological characteristics of the mice's liver, kidney, brain, and jejunum. The mice challenged by cadmium-induced oxidative stress revealed the normal architecture of tissues in the liver, kidney, brain, and jejunum in comparison to the control. The administration of cadmium for 30 days did not induce any prominent histopathological changes. The treatment of mice with NLK did not affect the histopathology of the liver, kidney, and brain.

## 3.4 | Jejunum morphological characteristics

The villus height, crypt depth, and villus width in the mice jejunum that received various treatments are depicted in Table 6. The presence of cadmium significantly ($p \leq 0.05$) impaired the villus width, crypt depth, and villus height. Dietary supplementation with NLK improved the villus width and height and crypt depth.

**TABLE 6** The morphometric features of mice jejunum receiving diverse treatments

| Description | Control group | Cd-treated group | Group 1 (Cd + 2.5 mg/kg NLK) | Group 2 (Cd +5 mg/kg NLK) |
|---|---|---|---|---|
| Villus height ($\mu$m) | $408.2 \pm 29.24^{a}$ | $357.4 \pm 22.7^{d}$ | $369.1 \pm 17.8^{c}$ | $384.9 \pm 26.64^{b}$ |
| Villus width ($\mu$m) | $156.9 \pm 16.9^{a}$ | $88.6 \pm 13.16^{cd}$ | $93.1 \pm 5.89^{c}$ | $110.5 \pm 6.9^{b}$ |
| Crypt depth ($\mu$m) | $164.8 \pm 13.64^{d}$ | $212.8 \pm 14.8^{a}$ | $194.4 \pm 17.15^{b}$ | $183.8 \pm 18.4^{c}$ |

*Note*: Values with various superscript letters in the same line are significantly different ($p \leq 0.05$). All analyses were performed in triplicate.

## 4 | DISCUSSION

Nanotechnology and nanoscience are the study and usage of tiny things and can be utilized in several other science fields, such as biology, chemistry, materials science, physics, and engineering. The advantages of nanoliposomal drug delivery systems are biocompatibility and bioaccessibility, which make them an appropriate tool in the pharmaceutical industry for drug delivery. Nanoliposomes are capable of elevating the functional properties of bioactive compounds by increasing their bioavailability and solubility in addition to inhibiting their undesirable interactions with other molecules. Another positive aspect of nanoliposomes is cell-specific targeting, which is essential to deliver the required drug concentrations into the targeted location while lowering detrimental effects on healthy cells and tissues.

Environmental cadmium pollution is an important public health-threatening problem due to its potential for accumulation in tissues.[32] The liver is usually considered the primary defense system against various poisonous compounds, including heavy metals,[33,34] and ALT, AST, and ALP are important biomarkers of liver function.[35] Cadmium deposited in liver cells influences liver function and causes cellular damage. In the current study, cadmium at a concentration of 2 mg/kg body weight in drinking water led to liver hepatotoxicity and dysfunction, as revealed by the increase in liver enzymes and suppression of antioxidant-related genes. Cadmium not only reduced appetite but also impaired intestinal morphological characteristics and consequently reduced nutrient absorption, which altogether resulted in weight loss in the mice. Similar toxicity symptoms have been reported by early studies of Cd-mediated toxicity in in vivo conditions.[36–38] Previous studies have also indicated the detrimental impacts of cadmium on the cellular ion-regulatory network,[39,40] which then leads to interruption of molecular signaling pathways and gene expression regulation.[41] Cadmium in the cells increases the generation of hydroxyl radicals and superoxide radicals indirectly.[42,43] Hence, elevated rates of ROS induce oxidative stress. Cd-induced oxidative stress compels cells to activate antioxidant defense system genes, including SOD, CAT, and GPx, to neutralize the redundant contents of ROS and to prevent oxidative stress. In this study, the animals that were exposed to cadmium showed increased expression of iNOS, SOD, CAT, and GPx genes and confirmed substantial liver oxidative stress. ROS-induced lipid peroxidation is one of the reasons for cell membrane damage.[31,44] Therefore, it has been considered a useful biomarker for metal-induced oxidative damage.[45,46] In the existing study, there was a significant increase in lipid peroxidation in response to cadmium poisoning in mice liver tissue. The histopathological examinations in the mice's kidney, liver, and brain tissues did not indicate any histopathological variations in any of the treatments. Several studies reported different observations regarding the pathophysiology changes in mice and rats upon cadmium exposure. Some studies reported significant alterations in the liver and kidney tissues[47–51], while others reported no significant alterations.[31,52,53] The lack of histopathological alterations in some studies could be attributed to the short length of cadmium treatment or the low concentration of applied cadmium.

Cadmium impaired the morphological characteristics of the jejunum, such as villus width and height and crypt depth. Early studies reported that heavy metals impaired the morphological characteristics of the intestine through intoxication in epithelial cells and gut microbial dysbiosis.[54,55] In this study, NLK modulated the toxicity induced in epithelial cells and regulated microbial gut function, which improved the morphological characteristics of the jejunum.

In this study, dietary supplementation with NLK was able to increase appetite and, consequently, increase the weight of mice. It also improved the function of the liver, as indicated by the modulation of liver enzymes and inflammatory biomarkers (iNOS). NLK could regulate antioxidant gene expression in liver cells, which was interrupted by cadmium. Earlier surveys have shown that nanoliposome-encapsulated compounds such as fisetin, quercetin, resveratrol, epigallocatechin gallate, and curcumin can be used as appropriate and effective dietary supplements against liver oxidative stress.[56–58]

## 5 | CONCLUSION

The dietary supplementation of NLK synthesized in this study improved the mice's body weight, liver function, and antioxidant potential of the liver in mice challenged by cadmium-induced toxicity.

### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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### REFERENCES

1. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem. 2001;1:529–39.
2. Patlevič P, Vašková J, Švorc Jr P, Vaško L, Švorc P. Reactive oxygen species and antioxidant defense in human gastrointestinal diseases. Integr Med Res. 2016;5:250–8.
3. Kim J-H, Rhee J-S, Lee J-S, Dahms H-U, Lee J, Han K-N, et al. Effect of cadmium exposure on expression of antioxidant gene transcripts in the river pufferfish, *Takifugu obscurus* (Tetraodontiformes). Comp Biochem Physiol C: Toxicol Pharmacol. 2010;152:473–9.
4. Srivastava S, Somasagara RR, Hegde M, Nishana M, Tadi SK, Srivastava M, et al. Quercetin, a natural flavonoid interacts with DNA, arrests cell cycle and causes tumor regression by activating mitochondrial pathway of apoptosis. Sci Rep. 2016;6: 24049.
5. Li H, Ji H-S, Kang J-H, Shin D-H, Park H-Y, Choi M-S, et al. Soy leaf extract containing kaempferol glycosides and pheophorbides improves glucose homeostasis by enhancing pancreatic $\beta$-cell function and suppressing hepatic lipid accumulation in db/db mice. J Agric Food Chem. 2015;63:7198–210.
6. Arif H, Sohail A, Farhan M, Rehman AA, Ahmad A, Hadi S. Flavonoids-induced redox cycling of copper ions leads to generation of reactive oxygen species: a potential role in cancer chemoprevention. Int J Biol Macromol. 2018;106:569–78.
7. Nascimento AM, Maria-Ferreira D, Dal Lin FT, Kimura A, de Santana-Filho AP, Werner MFP, et al. Phytochemical analysis and anti-inflammatory evaluation of compounds from an aqueous extract of *Croton cajucara* Benth. J Pharm Biomed Anal. 2017;145:821–30.
8. Zhao J, Zhang S, You S, Liu T, Xu F, Ji T, et al. Hepatoprotective effects of nicotiflorin from nymphaea candida against concanavalin a-induced and d-galactosamine-induced liver injury in mice. Int J Mol Sci. 2017;18:587.
9. Wu Y, Sun J, George J, Ye H, Cui Z, Li Z, et al. Study of neuroprotective function of G inkgo biloba extract (EG b761) derived-flavonoid monomers using a three-dimensional stem cell-derived neural model. Biotechnol Progr. 2016;32:735–44.
10. M Calderon-Montano J, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. Mini Rev Med Chem. 2011;11:298–344.
11. Dong L, Yin L, Quan H, Chu Y, Lu J. Hepatoprotective Effects of kaempferol-3-O-$\alpha$-l-arabinopyranosyl-7-o-$\alpha$-l-rhamno pyranoside on D-galactosamine and lipopolysaccharide caused hepatic failure in mice. Molecules. 2017;22.
12. Wang Y, Tang C, Zhang H. Hepatoprotective effects of kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside from Carthamus tinctorius L. on CCl(4)-induced oxidative liver injury in mice. Journal of Food and Drug Analysis. 2015;23:310–7.
13. Dabeek WM, Marra MV. Dietary quercetin and kaempferol: bioavailability and Potential Cardiovascular-Related Bioactivity in humans. Nutrients. 2019;11:2288.
14. Huang M, Su E, Zheng F, Tan C. Encapsulation of flavonoids in liposomal delivery systems: the case of quercetin, kaempferol and luteolin. Food Funct. 2017;8:3198–208.
15. Williamson G, Kay CD, Crozier A. The bioavailability, transport, and bioactivity of dietary flavonoids: a review from a historical perspective. Compr Rev Food Sci Food Saf. 2018;17: 1054–112.
16. dos Santos Lima B, Shanmugam S, Quintans JSS, Quintans-Junior LJ, de Souza Araujo AA. Inclusion complex with cyclodextrins enhances the bioavailability of flavonoid compounds: a systematic review. Phytochem Rev. 2019;18:1337–59.
17. Hasan M, Belhaj N, Benachour H, Barberi-Heyob M, Kahn C, Jabbari E, et al. Liposome encapsulation of curcumin: physicochemical characterizations and effects on MCF7 cancer cell proliferation. Int J Pharm. 2014;461:519–28.
18. Figueroa-Robles A, Antunes-Ricardo M, Guajardo-Flores D. Encapsulation of phenolic compounds with liposomal improvement in the cosmetic industry. Int J Pharm. 2021;593:120125.
19. Aditya N, Espinosa YG, Norton IT. Encapsulation systems for the delivery of hydrophilic nutraceuticals: food application. Biotechnol Adv. 2017;35:450–7.
20. Erlejman A, Verstraeten S, Fraga C, Oteiza P. The interaction of flavonoids with membranes: potential determinant of flavonoid antioxidant effects. Free Radical Res. 2004;38:1311–20.
21. Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomed. 2015;10:975–99.
22. Ochi MM, Amoabediny G, Rezayat SM, Akbarzadeh A, Ebrahimi B. In vitro co-delivery evaluation of novel pegylated nano-liposomal herbal drugs of silibinin and glycyrrhizic acid (nano-phytosome) to hepatocellular carcinoma cells. Cell J. 2016;18:135–48.
23. Di Costanzo A, Angelico R. Formulation strategies for enhancing the bioavailability of silymarin: the state of the art. Molecules. 2019;24;2155.
24. Ochi MM, Amoabediny G, Rezayat SM, Akbarzadeh A, Ebrahimi B. In vitro co-delivery evaluation of novel pegylated nano-liposomal herbal drugs of silibinin and glycyrrhizic acid (nano-phytosome) to hepatocellular carcinoma cells. Cell J. 2016;18:135–48.
25. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013;8:102.
26. Oskoueian E, Karimi E, Noura R, Ebrahimi M, Shafaei N, Karimi E. Nanoliposomes encapsulation of enriched-phenolic fraction from pistachio hulls and it's antioxidant, anti-inflammatory and anti-melanogenic activities. J Microencapsul. 2020;37(1): 1-13.
27. Tehrany EA, Kahn CJ, Baravian C, Maherani B, Belhaj N, Wang X, et al. Elaboration and characterization of nanoliposome made of soya; rapeseed and salmon lecithins: application to cell culture. Colloids Surf B. 2012;95:75–81.

28. Cardiff RD, Miller CH, Munn RJ. Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harb Protoc. 2014;2014:pdbprot073411.
29. Yoshida S, Muramatsu Y. Determination of major and trace elements in mushroom, plant and soil samples collected from Japanese forests. Int J Environ Anal Chem. 1997;67:49–58.
30. Beyrami M, Karimi E, Oskoueian E. Synthesized chrysin-loaded nanoliposomes improves cadmium-induced toxicity in mice. Environ Sci Pollut Res. 2020;27:40643–51.
31. Satarug S, Baker JR, Urbenjapol S, Haswell-Elkins M, Reilly PE, Williams DJ, et al. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett. 2003;137:65–83.
32. Grant D, 1991 Detoxification Pathways in the Liver, Springer, pp. 421–30.
33. Ikemoto T, Kunito T, Tanaka H, Baba N, Miyazaki N, Tanabe S. Detoxification mechanism of heavy metals in marine mammals and seabirds: interaction of selenium with mercury, silver, copper, zinc, and cadmium in liver. Arch Environ Contam Toxicol. 2004;47:402–13.
34. Murali MR, Carey WD. Liver test Interpretation-Approach to the patient with liver disease: A Guide to Commo nly Used Liver Tests. Cleveland Clinic, Cleveland, USA; 2000.
35. Stacey NH, Klaassen CD. Interaction of metal ions with cadmium-induced cellular toxicity. J Toxicol Environ Health, Part A: Curr Iss. 1981;7:149–58.
36. Goering PL, Klaassen CD. Tolerance to cadmium-induced toxicity depends on presynthesized metallothionein in liver. J Toxicol Environm Health, Part A Curr Iss. 1984;14:803–12.
37. Klaassen CD, LlU J. Metallothionein transgenic and knock-out mouse models in the study of cadmium toxicity. J Toxicol Sci. 1998;23:97–102.
38. McGeer JC, Szebedinszky C, McDonald DG, Wood CM. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout. 1: iono-regulatory disturbance and metabolic costs. Aquat Toxicol. 2000;50:231–43.
39. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology. 2003;192:95–117.
40. Wang Y, Fang J, Leonard SS, Rao KMK. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radical Biol Med. 2004;36:1434–43.
41. Nemmiche S, Chabane-Sari D, Guiraud P. Role of $\alpha$-tocopherol in cadmium-induced oxidative stress in Wistar rat's blood, liver and brain. Chem Biol Interact. 2007;170:221–30.
42. Galán A, García-Bermejo L, Troyano A, Vilaboa NE, Fernández C, de Blas E, et al. The role of intracellular oxidation in death induction (apoptosis and necrosis) in human promonocytic cells treated with stress inducers (cadmium, heat, X-rays). Eur J Cell Biol. 2001;80:312–20.
43. Rikans LE, Hornbrook KR. Lipid peroxidation, antioxidant protection and aging. Biochim Biophys Acta (BBA)-Molecr Basis Dis. 1997;1362:116–27.
44. Viarengo A, Canesi L, Pertica M, Poli G, Moore M, Orunesu M. Heavy metal effects on lipid peroxidation in the tissues of mytilus gallopro vincialis lam. Comp Biochem Physiol Part C: Comp Pharmacol. 1990;97:37–42.
45. Arasu S, Reddy PS. Changes in lipid peroxidation in the gill and muscle of the marine bivalve (*Perna viridis*) during exposure to cadmium and copper. Chem Ecol. 1995;11:105–12.
46. Koyu A, Gokcimen A, Ozguner F, Bayram DS, Kocak A. Evaluation of the effects of cadmium on rat liver. Mol Cell Biochem. 2006;284:81–85.
47. El-Refaiy AI, Eissa FI. Histopathology and cytotoxicity as biomarkers in treated rats with cadmium and some therapeutic agents. Saudi J Biol Sci. 2013;20:265–80.
48. Renugadevi J, Prabu SM. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol. 2010;62:171–81.
49. Gattea Al-Rikabi Z, Abbas A, Kadhum Oudah H, Sajer Nassir H, Ali S. Histopathological study of liver and kidney tissues in C57 mice via chronic exposure to cadmium and zinc. Arch Razi Inst. 2021;76:1501–8.
50. Zhang Y, Liu Z, He Q, Wu F, Xiao Y, Chen W, et al. Construction of mode of action for cadmium-induced renal tubular dysfunction based on a toxicity pathway-oriented approach. Front Genet. 2021;12.
51. Seif MM, Madboli AN, Marrez DA, Aboulthana WMK. Hepatorenal protective effects of Egyptian purslane extract against experimental cadmium toxicity in rats with special emphasis on the functional and histopathological changes. Toxicol Rep. 2019;6:625–31.
52. Shafaei N, Barkhordar SMA, Rahmani F, Nabi S, Idliki RB, Alimirzaei M, et al. Protective effects of *Anethum graveolens* seed's oil nanoemulsion against cadmium-induced oxidative stress in mice. Biol Trace Elem Res. 2020;198:583–91.
53. Dane H, Şi'şman T. A morpho-histopathological study in the digestive tract of three fish species influenced with heavy metal pollution. Chemosphere. 2020;242:125212.
54. Kakade A, Salama E–S, Pengya F, Liu P, Li X Long-term exposure of high concentration heavy metals induced toxicity, fatality, and gut microbial dysbiosis in common carp, *Cyprinus carpio*. Environ Pollut. 2020;266:115293.
55. Mignet N, Seguin J, Romano MR, Brullé L, Touil YS, Scherman D, et al. Development of a liposomal formulation of the natural flavonoid fisetin. Int J Pharm. 2012;423:69–76.
56. Caddeo C, Díez-Sales O, Pons R, Carbone C, Ennas G, Puglisi G, et al. Cross-linked chitosan/liposome hybrid system for the intestinal delivery of quercetin. J Colloid Interface Sci. 2016;461:69–78.
57. Saengkrit N, Saesoo S, Srinuanchai W, Phunpee S, Ruktanonchai UR. Influence of curcumin-loaded cationic liposome on anticancer activity for cervical cancer therapy. Colloids Surf B. 2014;114:349–56.


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