DOI: https://doi.org/10.21323/2618-9771-2025-8-3-362-369

Received 06.01.2025
Accepted in revised 16.08.2025
Accepted for publication 21.08.2025

Available online at https://www.fsjour.com/jour
Original scientific article
Open access

INFLUENCE OF ZINC-WHEY PROTEIN NANOPARTICLES IN FERMENTED MILK ON OXIDATIVE STRESS AND LIVER FUNCTION IN OBESE RATS

Mona A. Hassan[1], Mahmoud Salama[2], Ahmed A. Abd El-Maksoud[3*]

[1] Food Evaluation and Food Science Department, National Organization for Drug Control and Research, Giza, Egypt
[2] Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt
[3] Dairy Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt

KEY WORDS:
hepatic enzymes, fermented milk, oxidative stress, zinc-whey protein nanoparticles, malonaldehyde

ABSTRACT

Obesity refers to fat accumulation in patients' adipose tissue and is widespread globally. It is necessary to develop anti-obesity foods and promote a healthy lifestyle before it reaches epidemic proportions. This study aimed to evaluate the effects of zinc-whey protein nanoparticles (Zn-WPNPs), incorporated into fermented milk (FM), on oxidative stress, inflammation, and liver fibrosis in obese rats. Chemical analysis included the determination of total solids, protein, fat, carbohydrates, ash contents, and pH. Zn efficacy was within 91.22–97.12 %, with particle size within 41.4–228 nm. Zn-WPNPs demonstrated stability after encapsulation, as confirmed by transmission electron microscopy photographs. Moreover, forty female albino rats were divided into five groups and were orally treated for 30 consecutive days: a control group, high-fat diet (HFD; Ch; 1 %), FM alone, FM-Zn, and FM-Zn-WPNPS. Blood and liver samples were then collected for analysis. Highly significant increases in body weight, lipid profile (TG and LDL), and malonaldehyde, along with decreases in the levels of hepatic Ch, HDL, and enzyme activity (AST and ALT) were found in the HFD group. On the other hand, the animals treated with FM-Zn-WPNPs showed reduced levels of all the oxidative stress markers (MDA, CAT, and GSH) to 1.19, 1.89, and 43.25, compared to 2.29, 1.20, and 27.40 in the HFD group, respectively, along with improvements in all the liver measurements. Thus, FM-Zn-WPNPs may serve as an appropriate food for nonalcoholic fatty liver disease (NAFLD) patients to improve their health.

ACKNOWLEDGEMENTS: The authors are grateful to the National Organization for Drug Control and Research for providing research facilities. Authors are thankful to Arla Foods Ingredients Group for providing whey protein isolate..

Поступила 06.01.2025
Поступила после рецензирования 16.08.2025
Принята в печать 21.08.2025

https://www.fsjour.com/jour
Научная статья
Open access

ВЛИЯНИЕ НАНОЧАСТИЦ ЦИНКА-СЫВОРОТОЧНОГО БЕЛКА В КИСЛОМОЛОЧНОМ ПРОДУКТЕ НА ОКИСЛИТЕЛЬНЫЙ СТРЕСС И ФУНКЦИИ ПЕЧЕНИ У КРЫС С ОЖИРЕНИЕМ

Мона А. Хасан [1], Махмуд Салама [2], Ахмед А. Абд Эль-Максуд [3*]

[1] Отдел оценки пищевых продуктов и науки о продуктах питания,
Национальная организация по контролю за наркотиками и научным исследованиям, Гиза, Египет
[2] Кафедра пищевых наук, сельскохозяйственный факультет, Каирский университет, Гиза, Египет
[3] Кафедра молочных наук, сельскохозяйственный факультет, Каирский университет, Гиза, Египет

КЛЮЧЕВЫЕ СЛОВА:
ферменты печени, кисломолочный продукт, окислительный стресс, наночастицы цинка -сывороточного белка, малоновый альдегид

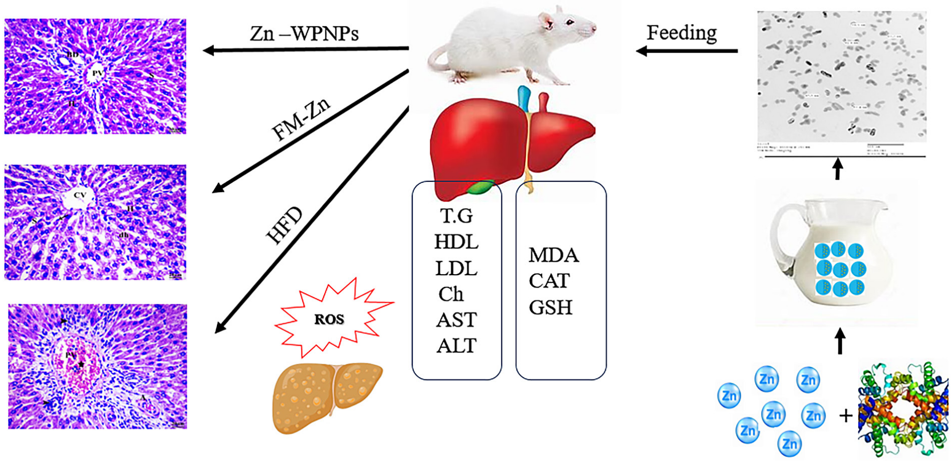
АННОТАЦИЯ

Ожирение характеризуется накоплением жировой ткани в организме человека и широко распространено во всём мире. Необходимо разрабатывать продукты, направленные на борьбу с проблемой ожирения и продвигать здоровый образ жизни пока проблема не достигнет масштабов эпидемии. Целью данного исследования было оценить влияние наночастиц цинка и сывороточного белка (Zn-WPNPs), включённых в состав кисломолочного продукта (FM), на окислительный стресс, воспаление и фиброз печени у крыс с ожирением. Химический анализ включал определение содержания сухих веществ, белка, жира, углеводов, золы и измерение pH. Эффективность связывания цинка составила 91,22–97,12 %, при среднем размере частиц 41,4–228 нм. Zn-WPNPs сохраняли стабильность после инкапсуляции, что было подтверждено фотографиями, полученными с помощью просвечивающей электронной микроскопии. В исследовании использовали 40 самок белых крыс, разделённых на пять групп и получавших пероральное лечение в течение 30 последовательных дней: контрольная группа, группа на высокожировой диете (HFD; холестерин 1 %), FM без добавок, FM-Zn и FM-Zn-WPNPs. После эксперимента у животных отбирали кровь и печень для анализа. В группе HFD наблюдались значительные увеличения массы тела, показателей липидного профиля (триглицеридов и ЛПНП) и малонового диальдегида, а также снижение уровня печёночного холестерина, ЛПВП и активности ферментов (АСТ и АЛТ). В то же время у животных, получавших FM-Zn-WPNPs, уровень всех маркеров окислительного стресса (MDA, CAT и GSH) снизился до 1,19; 1,89 и 43,25 соответственно, по сравнению с 2,29; 1,20 и 27,40 в группе HFD, а также отмечалось улучшение всех печёночных показателей. Таким образом, FM-Zn-WPNPs могут рассматриваться как перспективный продукт питания для пациентов с неалкогольной жировой болезнью печени (НАЖБП), способный улучшать их состояние здоровья.

БЛАГОДАРНОСТИ: Авторы выражают благодарность Национальной организации по контролю и исследованию лекарственных средств (The National Organization for Drug Control and Research) за предоставленные условия и оборудование для проведения исследований. Также авторы благодарят компанию Arla Foods Ingredients Group за предоставленный изолят сывороточного белка.

FOR CITATION: **Hassan, M. A., Salama, M., Abd El-Maksoud, A. A.** (2025). Influence of zinc-whey protein nanoparticles in fermented milk on oxidative stress and liver function in obese rats. *Food Systems,* 8(3), 362–369. https://doi.org/10.21323/2618-9771-2025-8-3-362-369

ДЛЯ ЦИТИРОВАНИЯ: **Хасан, М. А., Салама, М., Абдель-Максуд, А. А.** (2025). Влияние наночастиц цинка-сывороточного белка в кисломолочном продукте на окислительный стресс и функции печени у крыс с ожирением. *Пищевые системы*, 8(3), 362–369. https://doi.org/10.21323/2618-9771-2025-8-3-362-369



Graphical abstract

1. Introduction

Obesity-related health problems are increasing daily, reaching epidemic levels. It is estimated that over 4 million deaths occur annually due to obesity. In 2022, global statistics of the WHO showed that 2.5 billion adults are overweight, with 890 million of them classified as obese, and by 2024, more than 35 million children were overweight [1]. This concerning trend requires prevention measures; lifestyle and genetic susceptibility play a crucial role in causing obesity, and estimating the cost of obesity-related diseases on healthcare systems suggests it will reach 3 trillion US$ annually by 2030 [2]. Obesity results from energy imbalance and excessive fat accumulation, leading to complications such as nonalcoholic fatty liver disease (NAFLD), inflammation, hyperinsulinemia, hypertension, and cardiovascular disorders [3–5].

NAFLD encompasses a wide range of liver disorders from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis [6,7]. Excessive calorie intake and high-fat diets significantly contribute to NAFLD, atherosclerosis, and metabolic syndrome [1,5,8]. The chemical composition of dietary fat affects fat oxidation rates and alters low-density/high-density lipoprotein, cholesterol, body weight, and adipocyte morphology [9]. The treatment of NAFLD starts with lifestyle changes, weight loss through diet and exercise, and reducing blood glucose/cholesterol [10]. In addition, oxidative stress (an imbalance between antioxidants and free radicals) plays a key role in NAFLD progression and inflammatory cytokine expression [11]. The recent treatment of obese individuals with type-2 diabetes mellitus is metformin, which contributes to reducing oxidative stress [12]. It offers comparable benefits to polyphenols and antioxidants against oxidative stress, insulin resistance, and liver inflammation [13,14]. Furthermore, prebiotics and probiotics are also used as therapeutic options for NAFLD [15,16].

Milk has an excellent nutritional profile, including protein, carbs, fatty acids, and many vitamins and minerals. It also contains bioactive (linoleic acid, stearic acid, butyric, caprylic, caproic, and capric acids) and functional (β-lactoglobulin, α-lactalbumins, immunoglobulins, lactoferrin, and transferrin) compounds, which are reported to improve human health [17,18]. Rayeb (fermented milk) is one of the popular fermented dairy products (FDPs) in many countries, including Russia, China, Finland, Australia, and the Middle East [19,20]. FDPs are also beneficial for patients who suffer from milk hypersensitivities, lactose intolerance, viral diarrhea, hay fever, stomach flu, and asthma. It has also been used for the treatment of digestive problems, high blood pressure, reducing the risk of diabetes, and helping to prevent cancer [16,21–23].

Moreover, zinc (Zn) is an important signalling molecule involved in human growth and development. It is one of the most common trace elements in the human body [24] and regulates inflammation by reducing inflammatory cytokines [25]. Hence, it reduces oxidative stress and participates in antioxidant enzyme formation [26]. These enzymes serve as biocatalysts in metabolic processes. Furthermore, Zn regulates cell expressions to avoid inflammation. Some studies have shown that Zn lowers blood pressure, reduces harmful cholesterol, and helps to prevent stroke and angina pectoris [25–27]. In addition, zinc is involved in lipid metabolism, which may explain its ability to decrease low-density lipoprotein (LDL) cholesterol and increase high-density lipoprotein (HDL) cholesterol levels [28]. All these effects emphasize the importance of zinc as a protective element against cardiovascular diseases and hypertension [25–27]. Therefore, maintaining a proper diet or using zinc supplements may be beneficial in the prevention and treatment of health issues.

Numerous studies suggest that the bioavailability and efficacy of bioactive compounds could be enhanced through complexation with carrier molecules such as whey proteins (WPs) [29–31]. The combination of whey protein and zinc can be synergistic, as both help to reduce oxidative stress and regulate liver-expressed genes [32]. This dual approach can provide a dietary option for the prevention of diseases. Therefore, this study aims to encapsulate Zn using whey protein nanoparticles through noncovalent interactions and improve its delivery via fermented milk (rich in probiotics) to study the effect of the mixture (FM-Zn-WPNPs) on oxidative stress and liver functions in rats.

2. Materials and methods

2.1. Materials, chemicals, and kits

Raw buffalo milk was obtained from the Agriculture Research Station, Cairo University, Egypt. DVS of *Lactobacillus delbrueckii* ssp., *bulgaricus,* and *Streptococcus salivarius* ssp., *thermophilus* were obtained from Chr. Hansen's Lab, Copenhagen, Denmark. Whey protein isolate was purchased from Davisco Foods International Inc., Eden Prairie, Minnesota, USA. Zinc citrate was provided by El Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. 1% Cholesterol (Ch) was purchased from Sigma Chemical Co., St. Louis, Missouri, USA. The tests for Ch, TG, HDL, and LDL were supplied by FAR Diagnostics Co., Via Fermi, Italy, while MDA, CAT, and GSH assays were from Oxis Research™ Co., USA.

2.2. Preparation of zinc loaded whey protein nanoparticles (Zn-WPNPs)

Whey protein nanoparticles loaded with 7 mg of zinc citrate were prepared and characterized following the method previously reported by Hassan et al. [32].

2.3. Skimmed milk preparation

Raw buffalo milk was obtained and processed using an Alfa Laval separator, Sweden, to obtain skimmed milk. Skimmed milk powder was used for standardization. The prepared skimmed milk was divided into three parts: skimmed milk only, skimmed milk fortified with Zn (10 mg/250 g), and skimmed milk fortified with Zn-WPNPS (10 mg/250 g) according to Kanoni et al. [33]. It was then heat treated for 5 min in a water bath at 85 °C, followed by cooling to 42 °C according to the method described by Hamed et al. [34].

2.4. Fermented milk (FM) processing

Fermented milk was prepared using Tamime and Robinson's method [35]. Skimmed milk was heat treated at 80 °C for 5 minutes and then inoculated with 2% of the culture. After that, the inoculated milk was divided into three parts: fermented milk only (FM), fermented milk fortified with zinc (FM-Zn), and fermented milk fortified with Zn-WPNPs (FM-Zn-WPNPs). All the samples were incubated at 42 °C until they reached a pH of 4.6. Then, the samples were mixed using an electric stirrer, poured into 100 ml sterile cups, and stored in a refrigerator at 4 °C for subsequent use.

2.5. Chemical analysis of FM, FM-Zn, and FM-Zn -WPNPs

Total solids, ash, pH values, and protein content were determined according to AOAC [36]; total carbohydrate was estimated using the method of DuBois et al. [37].

2.6. Determination of Zn content

Zinc content was determined using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) at wavelengths 213.857 nm and 206.200 nm, following the method described by Soliman and Hassan [38].

The analysis was performed using an Agilent 5110 ICP-OES system (Agilent Technologies, USA) equipped with a SeaSpray nebulizer and a radial view configuration. Samples were first digested with a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) before analysis.

The total zinc efficiency after fermentation with yogurt culture was calculated using the following equation:

$$\text{Zn Efficiency}\,(\%) = \frac{\text{Zn content after fermentation}}{\text{Total added Zn}} \times 100.$$

2.7. Transmission electron microscopy

Transmission electron microscopy (TEM) was performed following the procedure described by Soliman and Hassan [38], with minor modifications. Samples were initially fixed by adding glutaraldehyde in a 1:7 (v/v) ratio, then diluted with deionized water at a 1:100 (v/v) ratio. One drop of the diluted sample was placed on a formvar-coated copper grid and allowed to adsorb for 1 minute. Excess fluid was removed, and a 2% phosphotungstic acid solution (pH 7.2) was added for negative staining. After drying, the samples were examined using a JEOL JEM-1400Plus transmission electron microscope, operated at an accelerating voltage of 80 kV. The mean particle size of the prepared Zn-WPNPs was detected using TEM according to the method of Zhang and Wang [39].

2.8. Experimental animals

Animals: 40 female Sprague-Dawley rats, five weeks old and weighing approximately 120 ± 15 g, were obtained from the Animal House Lab, National Organization of Drug Control and Research. The animals were given a standard laboratory diet obtained from Meladco Feed Co., Aubor City, Cairo, and tap water. The animals were kept in cages within suitable rooms free from any source of chemical contamination, thermally controlled (25 ± 10 °C), with a 12-hour light/dark cycle, and humidity (50 ± 5%) at the Animal House Lab. The rats were treated according to the National Institutes of Health (NIH publication 86–23, revised 1985) and the Animal Care and Use Committee guidelines of the National Research Centre (Committee of general division for Biological Control and Research, No 253, 2021).

2.9. Experimental design

After 7 days of acclimatization, 40 Sprague-Dawley albino rats were randomly divided equally into five groups (8 rats per group): Group 1 served as the control and received a basal diet; Group 2 received a high-fat diet (HFD) with 1% cholesterol; Group 3 was treated with fermented milk (FM) at a dose of 0.5 ml/100 g b. w.; Group 4 was treated with FM fortified with zinc citrate (FM-Zn) at a dose of 50 mg/kg b. w. and Group 5 received FM fortified with zinc-loaded whey protein nanoparticles (FM-Zn-WPNPs) at a dose of 50 mg/kg b. w. All treatments were administered orally for 30 days. Except for the control group, which received only the standard diet, all other groups were fed a high-fat diet (HFD). Body weight was recorded on the first and final days using a digital scale with a precision of two decimal digits (Sartorius, Germany) with an accuracy of ±0.1 grams. All rats were fasted for 12 hours before blood was collected from the retro-orbital venous plexus under diethyl ether anesthesia to analyze lipid profile, liver function parameters, catalase, malonaldehyde, and reduced glutathione [40–42]. The rats were sacrificed by cervical dislocation, and liver samples were obtained. These samples were fixed in formalin (10%) and then embedded in paraffin. Sections (5 µm) were stained with hematoxylin and eosin for histological evaluation using an Olympus microscope (100X) with a camera [41,43].

2.10. Statistical analysis

All data obtained were expressed as mean ± SD. Statistical analysis was performed with SPSS11.5 (SPSS, Inc. © Chicago, IL, USA) by using one-way analysis of variance ANOVA. The difference between the samples was considered significant. All the statements of significance were based on a P-value of < 0.05.

3. Results

3.1. Chemical analysis of fermented milk (FM), FM-Zn, and FM-Zn-WPNPs

Table 1 shows no significant difference in total solids or carbohydrate content between the FM-Zn and Zn-WPNPs treatments and the control sample. The protein content of the FM-Zn-WPNPs samples significantly increased compared to the FM-Zn sample. Furthermore, FM-Zn has the highest acidity values compared to FM (control) and FM-Zn-WPNPs. Figure 1 shows that FM (control) contains 3.22 mg/kg of zinc. FM-Zn contains 39.32 mg of zinc/kg of FM, while FM-Zn-WPNPs have a total zinc content of 41.26 mg/kg. Our results showed that the efficiency of Zn ranged from 91.22% to 97.12%. Besides, significant changes in Zn content after fermentation were observed among all samples (Figure 1). Notably, Zn's efficiency in FM-Zn-WPNPs was the highest (97.12%) compared to FM and FM-Zn samples.

The results are presented as means, and lowercase letters above the bars indicate significant differences ($p < 0.05$).

3.2. Transmission electron microscopy (TEM)

TEM studies were performed on nanoparticles of Zn-WPNPs. As shown in Figure 2, the FM-Zn-WPNPs were nearly spherical, with the dark region representing Zn and the bright region indicating WPNPs [32]. Furthermore, the FM-Zn-WPNPs sample was well dispersed, and no aggregation was observed. However, the particle size showed the diameters of WP particles and Zn elements in nm (Figure 2). In this study, the mean particle size of FM (A), FM-Zn (B), and FM-Zn-WPNPs (C) ranged from 41.4 to 228 nm, with the mean particle size of Zn being was 55.5 nm.

Table 1. **Chemical composition of FM, FM-Zn, and FM-Zn-WPNPs**

Таблица 1. **Химический состав образцов FM, FM-Zn и FM-Zn-WPNPs**

Samples	Total solids	Protein	Fat	Carbohydrate	Ash	pH
FM	13.41 ± 0.2^{a}	5.33 ± 0.18^{ab}	0.21 ± 0.02^{ab}	6.7 ± 0.28^{a}	0.86 ± 0.02^{b}	4.52 ± 0.11^{b}
FM-Zn	13.11 ± 0.20^{a}	5.21 ± 0.16^{b}	$0.17 \pm 0.0\ 3^{b}$	6.4 ± 0.35^{a}	0.97 ± 0.03^{a}	4.68 ± 0.04^{a}
FM-Zn-WPNPs	13.34 ± 0.18^{a}	5.53 ± 0.12^{a}	0.24 ± 0.02^{a}	6.5 ± 0.18^{a}	0.97 ± 0.01^{a}	4.55 ± 0.08^{ab}

FM: fermented milk; FM-Zn: fermented milk with zinc; FM-Zn-WPNPs: fermented milk with zinc-whey protein nanoparticles; data are presented as mean ± SE. Within each column, values with different lowercase letters differ significantly ($P \leqslant 0.05$).

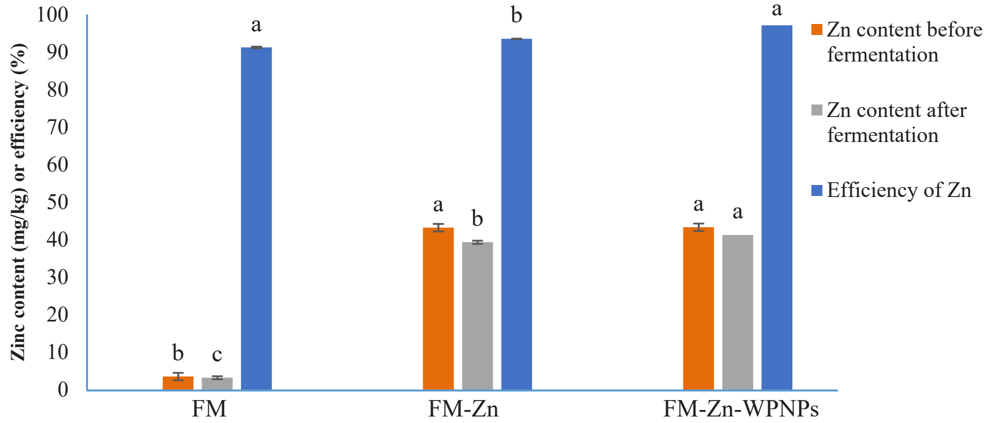


Figure 1. **Zinc content (mg/kg) and efficiency (%) before and after fermentation of milk**

Рисунок 1. **Содержание цинка (мг/кг) и эффективность сохранения цинка (%) после сквашивания молока**

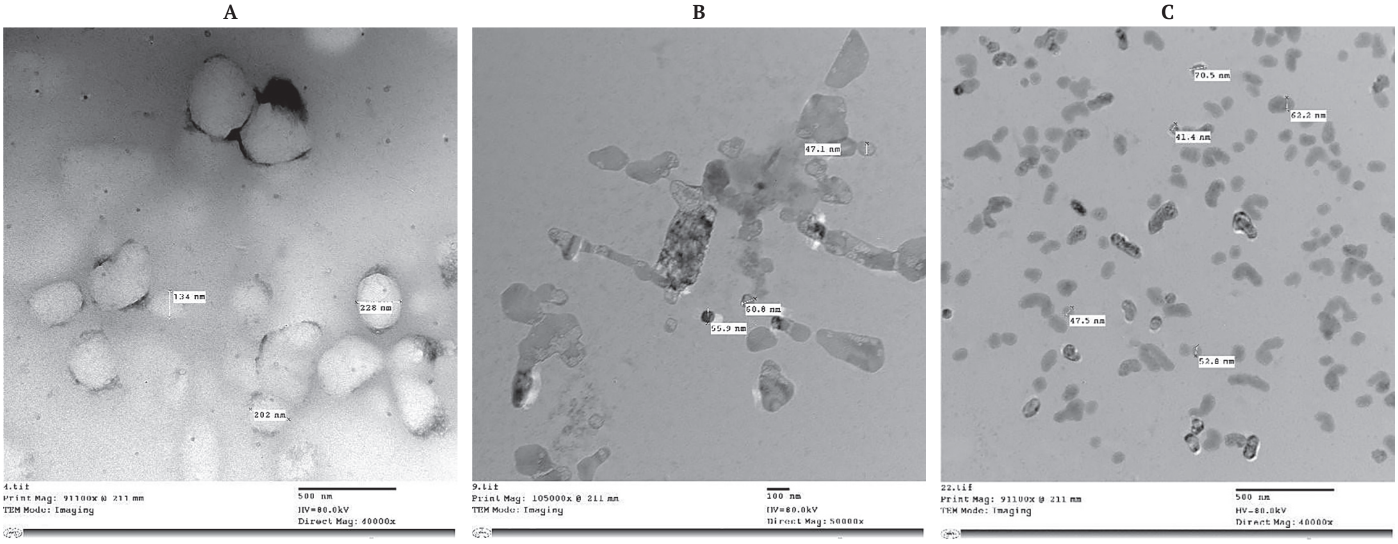


Figure 2. **Transmission electron microscopy (TEM) of FM (A), FM-Zn (B), and FM-Zn-WPNPs (C) after fermentation**

Рисунок 2. **Снимки, полученные методом просвечивающей электронной микроскопии (ПЭМ) образцов FM (A), FM-Zn (B) и FM-Zn-WPNPs (C) после сквашивания**

3.3. Effects of FM, FM-Zn, and FM-WPNPs on weight gain

As indicated in Table 2, the HFD-treated group had remarkably higher body weight after 30 days than the control and other treatment groups. The FM and FM-Zn-WPNPs treated groups significantly reduced the body weight gain induced by HFD and undid the HFD-induced obesity alone; the FM-Zn group showed a non-significant effect with HFD. There was no significant difference between the body weight of the FM-Zn-WPNPs administered groups and the control group in this study.

3.4. Biochemical assays

As shown in the HFD group (Table 3), there were significantly higher levels of cholesterol, triglycerides, and LDL, while HDL levels significantly decreased. Although HDL showed a significant decline compared to the control and treatment groups, the diets of FM, FM-Zn, and FM-Zn-WPNPs enhanced the lipid profile compared to the HFD group. Furthermore, the group treated with FM-Zn-WPNPs showed the best values for most lipid profile parameters compared to FM or FM-Zn (Table 3).

Hepatic injury was assessed by measuring the activities of liver enzyme markers, which increase in response to hepatic injury. Table 4 shows that AST and ALT levels significantly increased in HFD-fed animals (67.80 and 36.00, respectively) compared to their control and other treatment groups. Treatments with FM, FM-Zn, and FM-Zn-WPNPs showed improvements in all biochemical markers of liver function. It can be observed from the current results (Table 4) that HFD-fed rats experienced a significant reduction in catalase (CAT) and glutathione (GSH) levels (1.20 and 27.40, respectively) compared to the control (1.92 and 47.00, respectively) and other treated groups. In addition, CAT and GSH levels were higher ($p < 0.05$) in the control, FM-Zn-WPNPs, FM-Zn, and FM groups. Moreover, the FM-Zn-WPNPs group showed a significantly greater increase in GSH levels compared to the FM-Zn and FM groups. GSH levels in the FM-Zn-WPNPs, FM-Zn, and FM groups were 57.8 %, 47.8 %, and 49.6 % higher, respectively, than in the HFD group.

Malonaldehyde is used as an indicator of oxidative stress. Current results (Table 4) revealed that HFD-treated rats have significantly higher levels of MDA compared to the control and treated groups (FM, FM-Zn, and FM-Zn-WPNPs). FM, FM-Zn, and FM-Zn-WPNPs showed significant reduction ($p < 0.05$) in MDA, with decreases of 45.4, 44.1, and 48.0 %,

Table 2. **Body weight of albino rats fed with FM, FM-Zn, and FM-Zn-WPNPs**

Таблица 2. **Масса тела крыс-альбиносов, вскармливаемых FM, FM-Zn и FM-Zn-WPNPs**

Weight (g)	Control	HFD	FM	FM-Zn	FM-Zn-WPNPs
Initial weight	131.2 ± 1.35^{a}	130.4 ± 1.22^{a}	132.41 ± 1.04^{a}	120 ± 0.82^{d}	122.5 ± 1.16^{bc}
Final weight	165.75 ± 6.07^{bc}	182.5 ± 5.40^{a}	168.5 ± 3.81^{bc}	162.67 ± 6.65^{c}	160 ± 5.02^{cd}
Weight gain	34.54 ± 5.27^{bc}	52.1 ± 4.15^{a}	36.09 ± 3.22^{bc}	42.64 ± 6.28^{ab}	37.48 ± 4.52^{bc}

Control: basal diet; HFD: high-fat diet; FM: fermented milk; FM-Zn: fermented milk with zinc; FM-Zn-WPNPs: fermented milk with zinc-whey protein nanoparticles; data are presented as mean ± SE. Within each row, values with different lowercase letters differ significantly ($P \leqslant 0.05$).

Table 3. **Serum lipid profiles of albino rat groups fed on FM, FM-Zn, and FM-Zn-WPNPs**

Таблица 3. **Липидный профиль сыворотки крови крыс-альбиносов, вскармливаемых FM, FM-Zn и FM-Zn-WPNPs**

Parameters	Control	HFD	FM	FM-Zn	FM-Zn-WPNPs
Ch	93.25 ± 0.96^{e}	151.2 ± 3.11^{a}	117.40 ± 2.19^{b}	113 ± 1.22^{c}	108.8 ± 3.56^{d}
TG	78 ± 3.56^{d}	139.6 ± 3.36^{a}	95.16 ± 4.24^{c}	102 ± 2.45^{b}	96.75 ± 2.63^{c}
HDL	49.6 ± 2.70^{a}	33.2 ± 2.39^{c}	41.57 ± 2.70^{b}	50.2 ± 1.48^{a}	50.43 ± 1.72^{a}
LDL	27.55 ± 2.60^{d}	90.28 ± 3.28^{a}	45.01 ± 3.24^{b}	43.77 ± 1.59^{bc}	40.48 ± 1.29^{c}

Control: basal diet; HFD: high-fat diet; FM: fermented milk; FM-Zn: fermented milk with zinc; FM-Zn-WPNPs: fermented milk with zinc-whey protein nanoparticles; data are presented as mean ± SE. Within each row, values with different lowercase letters differ significantly ($P \leqslant 0.05$).

Table 4. **Effects of FM, FM-Zn, and FM-Zn-WPNPs on liver function, antioxidant enzyme activity, and lipid peroxidation (MDA) in albino rats**

Таблица 4. **Влияние FM, FM-Zn и FM-Zn-WPNPs на функции печени, активность антиоксидантных ферментов и перекисное окисление липидов (по малоновому диальдегиду) у крыс-альбиносов**

Parameters	Control	HFD	FM	FM-Zn	FM-Zn-WPNPs
AST	40.40 ± 1.03^{c}	67.80 ± 4.02^{a}	43.25 ± 1.71^{b}	42.25 ± 2.21^{bc}	41.75 ± 1.50^{bc}
ALT	19.80 ± 1.24^{c}	36.00 ± 1.22^{a}	22.50 ± 1.29^{b}	21.00 ± 1.58^{bc}	20.25 ± 2.22^{bc}
MDA	1.12 ± 0.02^{d}	2.29 ± 0.02^{a}	1.25 ± 0.04^{bc}	1.28 ± 0.03^{b}	1.19 ± 0.03^{c}
CAT	1.92 ± 0.01^{a}	1.20 ± 0.02^{c}	1.85 ± 0.04^{b}	1.87 ± 0.03^{b}	1.89 ± 0.03^{ab}
GSH	47.00 ± 0.82^{a}	27.40 ± 1.52^{d}	41.00 ± 2.94^{bc}	40.50 ± 1.29^{c}	43.25 ± 1.71^{b}

Control: basal diet; HFD: high-fat diet; FM: fermented milk; FM-Zn: fermented milk with zinc; FM-Zn-WPNPs: fermented milk with zinc-whey protein nanoparticles; data are presented as mean ± SE. Within each row, values with different lowercase letters differ significantly ($P \leqslant 0.05$).

respectively, compared to HFD, indicating suppression of oxidative stress markers caused by high cholesterol in HFD.

3.5. Histological examination

Figure 3 and Table 5 represent photomicrographs and parameters of liver sections. Figures 3A and 3B show the control group with standard structure, no histological change, and a clear appearance of the central vein and hepatocyte structure. Hepatocyte nuclei could be identified as dark red bodies within the cells, and the cytoplasm is stained red. The sinusoids, regular portal areas, and parallel hepatic cords that extend from the lobule's periphery to the central vein were visible. In contrast, in the HFD-treated group, shown in Figures 3C, 3D, and 3E, the lobular form was changed, with nuclear disintegration appeared in some places, degeneration of normal hepatic cells, necrosis of liver tissue, and fatty degeneration. The hepatic central vein showed hypertrophy and congestion. Lymphocytes infiltrated the portal area.

The group treated with FM alone is shown in Figures 3F and 3G; mild degeneration was present in some hepatocytes, with some thin, fibrous filaments interposed. There was moderate fatty degeneration, significant infiltration of mononuclear cells, and pyknosis/necrotic changes;

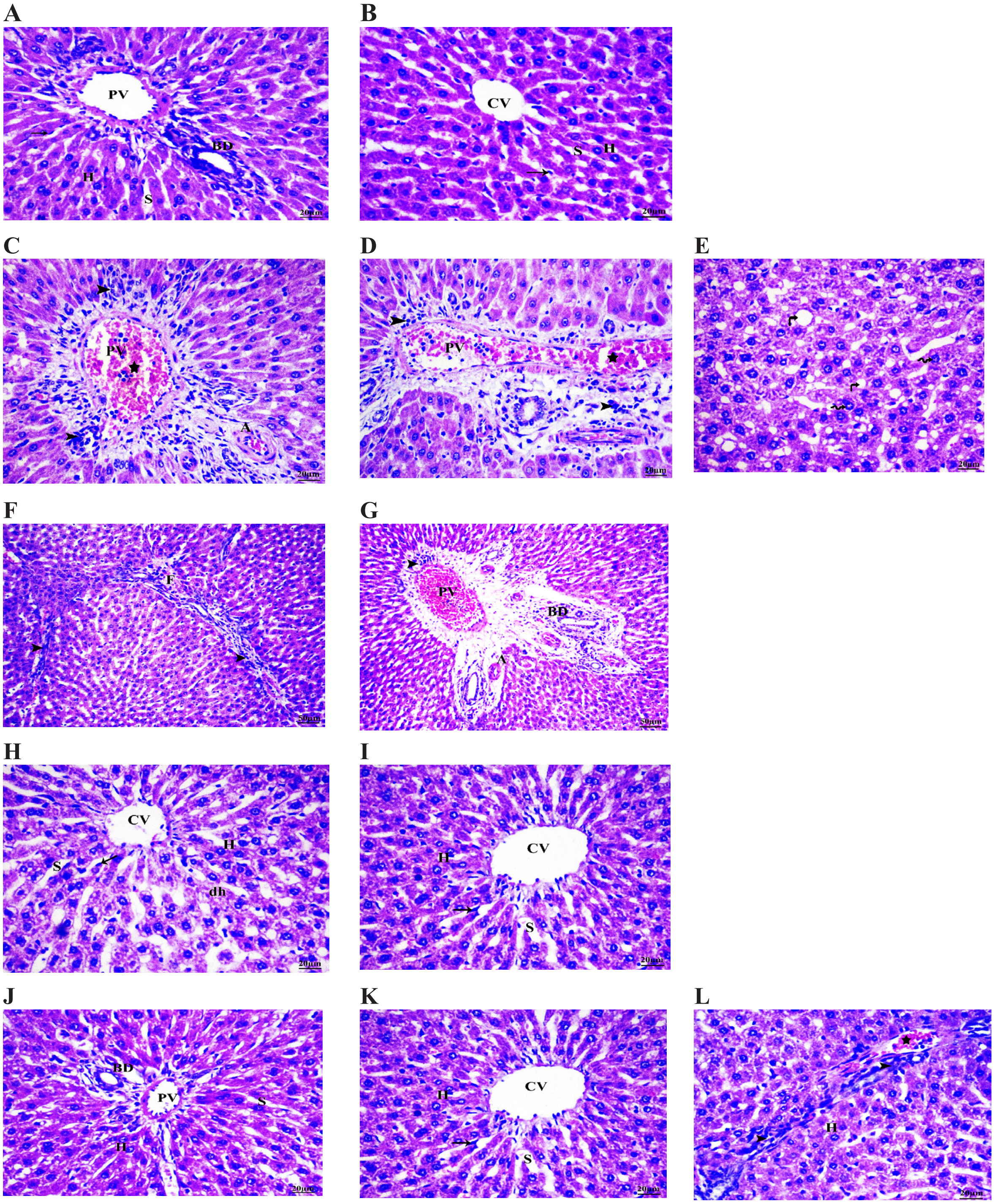


Figure 3. **Photomicrographs of liver sections from rat groups: control (A and B), high-fat diet (C, D, and E), fermented milk (F and G), fermented milk-zinc (H and I), and zinc-whey protein nanoparticles (J, K, and L). Abbreviations, PV, portal vein; BD, bile duct; CV, central vein; H, hepatocyte; A, artery, dh, degenerated hepatocyte; N, necrosis; F, fibrosis; curved arrow, fatty degeneration; Arrow, Kupffer cell; Arrowhead, lymphatic infiltration; Bold arrow, collagen fibers; zigzag arrow, normal hepatocytes; star, congested blood vessel; wavy arrow, karyolysis**

Рисунок 3. **Микрофотографии срезов печени крыс из следующих групп: контрольная (A и B), высокожирная диета (C, D и E), употреблявшие FM (F и G), употреблявшие FM-Zn (H и I) и FM-Zn-WPNPs (J, K и L). Сокращения: PV — портальная вена; BD — желчный проток; CV — центральная вена; H — гепатоцит; A — артерия; dh — гепатоцит с признаками дегенерации; N — некроз; F — фиброз; изогнутая стрелка — жировая дегенерация; стрелка — клетка Купфера; наконечник стрелки — лимфатическая инфильтрация; жирная стрелка — коллагеновые волокна; зигзагообразная стрелка — нормальные гепатоциты; звездочка — застойный кровеносный сосуд; волнистая стрелка — кариолиз**

Table 5. **Effect of fermented milk (FM), FM fortified with Zn, and n-WPNPs on the liver of obese rats**

Таблица 5. **Влияние FM, FM-Zn и Zn-WPNPs на печень крыс с ожирением**

Groups	Congestion	Lymphatic infiltration	Necrosis	Fatty degeneration	Fibrosis
Control	-	-	-	-	-
HFD	++	+++	+++	+++	+
FM	+	++	+	+++	+
FM-Zn	+	+	-	+	-
FM-Zn-WPNPs	+	+	+	+	-

Control: basal diet; HFD: high-fat diet; FM: fermented milk; FM-Zn: fermented milk with zinc; FM-Zn-WPNPs: fermented milk with zinc-Whey protein nanoparticles; (–) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates high.

congestion was mild. Hepatocytes in the FM-Zn group (Figures 3H and 3I) had a uniform look. Only a few mononuclear cells invade the area. There was no fibrosis present. There were no signs of necrosis, and blood vessels showed only mild constriction. Mild fatty degenerative changes were seen. The hepatic cords were properly aligned around the sinusoids, which looked normal. The FM-Zn-WPNPs group (Figures 3J, 3K, and 3L) showed that hepatocytes and portal components were in a focal state. Mild infiltration of mononuclear cells was present. There was no fibrosis present. Bile ducts were diffusely present. Some hepatocytes exhibit mild necrotic changes. Blood vessels showed mild congestion and fatty degeneration.

4. Discussion

Health complications are increasing worldwide [44]. A high-energy diet and a high-fat intake are critical factors in the problem's development. Changes in body weight are considered an essential parameter when evaluating the effects of a high-fat diet in causing obesity and monitoring the treatment of obesity [1,45]. Our study hypotheses that FM, FM-Zn, and FM-Zn-WPNPs impact hepatotoxicity and oxidative stress in HFD-induced rats. Previous studies provided scientific evidence on how milk and dairy products influence body weight and composition, especially FM [20,46]. FM, as a type of dairy product, is considered a favorable and satiating food because of the presence of high-quality protein and essential amino acids. After the entire study period, changes in body weight indicated obesity caused by the HFD. Rats fed with HFD showed an increase in body weight compared to control rats. The addition of FM, FM-Zn, or FM-Zn-WPNPs to the diet of HFD-fed rats led to a significant reduction in final body weight compared to rats that received only HFD. FM, FM-Zn, and FM-Zn-WPNPs showed no significant effect on body weight compared to control rats (basal diet). These results align with studies by Park et al. [46] and ALSuhaymi et al. [47], who found changes in metabolism genes, reduced weight gain, and adipose tissues.

On the other hand, feeding a diet containing short-chain fatty acids (butter) promoted obesity. However, in today's environment, high accessibility to foods with saturated fatty acids may promote the development and maintenance of obesity [48]. Modulation of the gut microbiome and its composition, involving *Lactobacillus plantarum, Lactobacillus curvatus, Lacticaseibacillus rhamnosus, Lactobacillus delbrueckii*, and *Lactobacillus bulgaricus* could be a significant factor in promoting weight loss and mitigating the deleterious impact of obesity [46,47,49].

Intake of dietary fatty acids regulates the content of lipids in muscle through fat metabolites. Moreover, a diet rich in unsaturated fatty acids impairs the accumulation of lipids in muscle cells, while high-fat diets usually contain more saturated fats than other types of diets [50]. In this study, the HFD-fed rats led to an increase in Ch, TG, LDL, and a decrease in HDL parameters in blood serum in a dose-dependent manner compared to the control, FM, FM-Zn, and FM-Zn-WPNPs. Feeding FM in the rats' diet improved the lipid profile (Ch, TG, HDL, and LDL) in FM, FM-Zn, and FM-Zn-WPNPs-fed rats. The results align with Chu et al. [51], who suggested that consuming *L. bulgaricus* powder (10^8 CFU) for 12 weeks significantly decreased TG and LDL by modulating the accumulation of LDL/HDL in obese individuals. In addition, zinc supplements affect the lipid profile, as stated in the study by Thoen et al. [52]. They found a reduction in blood TG and insulin levels in obese rats fed zinc supplements (6mg/kg bw).

Moreover, in the present study, HFD-fed rats showed increases in ALT, AST, and MDA, and a significant decrease in CAT and GSH. The results have shown that rats treated with FM-Zn-WPNPs had increased CAT and GSH levels, and significantly decreased levels of ALT, AST, and MDA compared to HFD-rats. Previous studies indicated that the FM-diet (*Streptococcus thermophilus, Lactobacillus acidophilus,* and *Bifidobacterium bifidum*) may prevent obesity-related hepatotoxicity due to bacteria's efficiency in the treatment of liver disorders (ALT and AST), which aligns with our results [53]. In another study by Chu et al. [51]. They found that *Lactobacillus bulgaricus* led to a decrease in AST and no significant change in ALT levels.

On the other hand, the oxidative stress markers (MDA, CAT, and GSH) showed the best values within the FM-Zn-WPNPs group, indicating the positive and synergistic effect of probiotics and zinc matrix against HFD-fed rats. Consumption of FM or multispecies probiotic capsules (*Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus casei, L. acidophilus, Lactobacillus rhamnoses, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, and Streptococcus thermophilus*) for 6 weeks showed enhanced biomarkers of oxidative stress (GSH) in petrochemical workers [54]. Besides, whey protein (immunoglobulins and albumin) protects the liver from obesity-induced damage by acting as an antioxidant and enhancing levels of GSH in tissues [55]. Zinc serves as an enzyme catalyst in fat and carbohydrate metabolism and has a significant role in treating type 2 diabetes, metabolic syndrome, and atherosclerosis [25,56].

Furthermore, dietary supplementation with zinc nanoparticles showed significant increases, along with improvements in the treatment of genotoxicity (3 mg ZnNPs) [57], antidiabetic effects (10 mg ZnNPs), blood glucose levels [58], and TG [59] in rats. Dietary zinc intake in chlorpyrifos-exposed rats demonstrated general improvement in the structural integrity of the liver tissues, supporting the potential of zinc for protection, as the study reports on the role of zinc in reducing chlorpyrifos-induced hepatotoxicity [60].

In our study, HFD-fed animals showed histological changes in the liver, such as necrosis, fatty degeneration, congestion, lymphocyte infiltration, and fibrosis. Moreover, among all treated groups, the best result was observed with FM-Zn-WPNPs (7mg/g), showing a synergistic effect of zinc and whey-nanoparticles. Our results indicated that FM combined with Zn-WPNPs significantly reduced oxidative stress parameters in the liver and was more effective compared to either fermented milk or Zn supplementation alone.

5. Conclusion

This study investigated the potential therapeutic effects of FM, FM-Zn, and FM-Zn-WPNPs on obesity, hepatotoxicity, and oxidative stress in HFD-induced obese rats. The findings demonstrated that FM-Zn-WPNPs significantly improved lipid profiles (Ch, TG, HDL, and LDL), liver function biomarkers (AST and ALT), and oxidative stress markers (MDA, CAT, and GSH) compared to the HFD group. FM-Zn-WPNPs showed the therapeutic and protective effects of the synergistic interaction between zinc and the nano-whey protein carrier.

FM-contained probiotics positively affected weight management, confirming their value as a functional food. However, its combination with bioavailable zinc delivered via whey protein nanoparticles reduced hepatic damage and NAFLD by combating reactive oxygen species and LDL. The findings suggest that FM-Zn-WPNPs may be a promising dietary strategy for preventing or managing obesity-associated liver dysfunction and oxidative stress. Further clinical research is required to confirm these results in humans and study the mechanisms of action.

REFERENCES

1. World Health Organization. (2025). Obesity and overweight. Retrieved from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight Accessed July 14, 2025.
2. World Obesity Federation. (2022). The Economic Impact of Overweight Obesity. Retrieved from https://data.worldobesity.org/publications/WOF-Economic-Impacts-2-V2.pdf Accessed July 14, 2025.
3. Öner-İyidoğan, Y., Koçak, H., Seyidhanoğlu, M., Gürdöl, F., Gülçubuk, A., Yildirim, F. et al. (2013). Curcumin prevents liver fat accumulation and serum fetuin — A increase in rats fed a high-fat diet. *Journal of Physiology and Biochemistry*, 69(4), 677–686. https://doi.org/10.1007/s13105-013-0244-9
4. Ragab, S. M. M., Abd Elghaffar, S. K., El-Metwally, T. H., Badr, G., Mahmoud, M. H., Omar, H. M. (2015). Effect of a high fat, high sucrose diet on the promotion of

non-alcoholic fatty liver disease in male rats: The ameliorative role of three natural compounds. *Lipids in Health and Disease*, 14(1), Article 83. https://doi.org/10.1186/s12944-015-0087-1
5. Petrie, J. R., Guzik, T. J., Touyz, R. M. (2018). Diabetes, hypertension, and cardiovascular disease: Clinical insights and vascular mechanisms. *Canadian Journal of Cardiology*, 34(5), 575–584. https://doi.org/10.1016/j.cjca.2017.12.005
6. Grundy, S. M. (2006). Atherogenic dyslipidemia associated with metabolic syndrome and insulin resistance. *Clinical Cornerstone*, 8(Suppl 1), S21-S27. https://doi.org/10.1016/S1098-3597(06)80005-0
7. Castro-Barquero, S., Ruiz-León, A. M., Sierra-Pérez, M., Estruch, R., Casas, R. (2020). Dietary strategies for metabolic syndrome: A comprehensive review. *Nutrients*, 12(10), Article 2983. https://doi.org/10.3390/nu12102983
8. Ulla, A., Alam, M. A., Sikder, B., Sumi, F. A., Rahman, M. M., Habib, Z. F. et al. (2017). Supplementation of *Syzygium cumini* seed powder prevented obesity, glucose intolerance, hyperlipidemia and oxidative stress in high carbohydrate high fat diet induced obese rats. *BMC Complementary and Alternative Medicine*, 17(1), Article 289. https://doi.org/10.1186/s12906-017-1799-8
9. Anders, L. C., Yeo, H., Kaelin, B. R., Lang, A. L., Bushau, A. M., Douglas, A. N. et al. (2016). Role of dietary fatty acids in liver injury caused by vinyl chloride metabolites in mice. *Toxicology and Applied Pharmacology*, 311, 34–41. https://doi.org/10.1016/j.taap.2016.09.026
10. Zelber-Sagi, S., Moore, J. B. (2024). Practical lifestyle management of nonalcoholic fatty liver disease for busy clinicians. *Diabetes Spectrum*, 37(1), 39–47. https://doi.org/10.2337/dsi23-0009
11. Coelho, D. F., Pereira-Lancha, L. O., Chaves, D. S., Diwan, D., Ferraz, R., Campos-Ferraz, P. L. et al. (2011). Effect of high-fat diets on body composition, lipid metabolism and insulin sensitivity, and the role of exercise on these parameters. *Brazilian Journal of Medical and Biological Research*, 44(10), 966–972. https://doi.org/10.1590/S0100-879X2011007500107
12. Hoey, H., Roche, E. (2024). Obesity a triple pandemic, the trillion dollar disease: Prevention is imperative. *Global Pediatrics*, 7, Article 100141. https://doi.org/10.1016/j.gpeds.2024.100141
13. Naghdi, A., Goodarzi, M. T., Karimi, J., Hashemnia, M., Khodadadi, I. (2022). Effects of curcumin and metformin on oxidative stress and apoptosis in heart tissue of type 1 diabetic rats. *Journal of Cardiovascular and Thoracic Research*, 14(2), 128–137. https://doi.org/10.34172/jcvtr.2022.23
14. Yan, Y., Jun, C., Lu, Y., Jiangmei, S. (2019). Combination of metformin and luteolin synergistically protects carbon tetrachloride-induced hepatotoxicity: Mechanism involves antioxidant, anti-inflammatory, antiapoptotic, and Nrf2/HO-1 signaling pathway. *BioFactors*, 45(4), 598–606. https://doi.org/10.1002/biof.1521
15. Bovi, A.P.D., Marciano, F., Mandato, C., Siano, M. A., Savoia, M., Vajro, P. (2021). Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. *Frontiers in Medicine*, 8, Article 595371. https://doi.org/10.3389/fmed.2021.595371
16. Naeem, H., Hassan, H. U., Shahbaz, M., Imran, M., Memon, A. G., Hasnain, A. et al. (2024). Role of probiotics against human cancers, inflammatory diseases, and other complex malignancies. *Journal of Food Biochemistry*, 2024, Article 6632209. https://doi.org/10.1155/2024/6632209
17. Miller, G. D., Jarvis, J. K., McBean, L. D. (2006). Handbook of Dairy Foods and Nutrition. CRC Press, 2006. https://doi.org/10.1201/9781420004311
18. Fox, P. F., McSweeney, P. L. (1998). Dairy chemistry and biochemistry. Blackie Academic and Professional, 1998.
19. Kolokolova, A. Y., Kishilova, S. A., Rozhkova, I. V., Mitrova, V. A. (2024). The Role of postbiotic composition in the growth stimulating of bifidobacteria. *FOOD METAENGINEERING*, 2(2), 12–21. https://doi.org/10.37442/fme.2024.2.56 (In Russian)
20. Tamang, J. P., Cotter, P. D., Endo, A., Han, N. S., Kort, R., Liu, S. Q. et al. (2020). Fermented foods in a global age: East meets West. *Comprehensive Reviews in Food Science and Food Safety*, 19(1), 184–217. https://doi.org/10.1111/1541-4337.12520
21. Patel, P., Butani, K., Kumar, A., Singh, S., Prajapati, B. G. (2023). Effects of fermented food consumption on non-communicable diseases. *Foods*, 12(4), Article 687. https://doi.org/10.3390/foods12040687
22. Kaur, H., Kaur, G., Ali, S. A. (2022). Dairy-based probiotic-fermented functional foods: An update on their health-promoting properties. *Fermentation*, 8(9), Article 425. https://doi.org/10.3390/fermentation8090425
23. Stachelska, M. A., Karpiński, P., Kruszewski, B. (2025). Health-promoting and functional properties of fermented milk beverages with probiotic bacteria in the prevention of civilization diseases. *Nutrients,* 17(1), Article 9. https://doi.org/10.3390/nu17010009
24. Hara, T., Yoshigai, E., Ohashi, T., Fukada, T. (2022). Zinc transporters as potential therapeutic targets: An updated review. *Journal of Pharmacological Sciences*, 148(2), 221–228. https://doi.org/10.1016/j.jphs.2021.11.007
25. Patil, R., Sontakke, T., Biradar, A., Nalage, D. (2023). Zinc: An essential trace element for human health and beyond. *Food and Health*, 5(3), Article 13. https://doi.org/10.53388/fh2023013
26. Luan, R., Ding, D., Xue, Q., Li, H., Wang, Y., Yang, J. (2023). Protective role of zinc in the pathogenesis of respiratory diseases. *European Journal of Clinical Nutrition*, 77(4), 427–435. https://doi.org/10.1038/s41430-022-01191-6
27. Hara, T., Yoshigai, E., Ohashi, T., Fukada, T. (2023). Zinc in cardiovascular functions and diseases: epidemiology and molecular mechanisms for therapeutic development. *International Journal of Molecular Sciences*, 24(8), Article 7152. https://doi.org/10.3390/ijms24087152
28. Long, Q., Feng, Y., Chen, F., Wang, W., Ma, M., Mao, S. (2022). Association between serum zinc level and lipid profiles in children with spinal muscular atrophy. *Frontiers in Nutrition*, 9, Article 960006. https://doi.org/10.3389/fnut.2022.960006
29. Luparelli, A., Trisciuzzi, D., Schirinzi, W. M., Caputo, L., Smiriglia, L., Quintieri, L. et al. (2025). Whey proteins and bioactive peptides: Advances in production, selection and bioactivity profiling. *Biomedicines*, 13(6), Article 1311. https://doi.org/10.3390/biomedicines13061311
30. Jiang, L., Zhang, Z., Qiu, C., Wen, J. (2024). A review of whey protein-based bioactive delivery systems: design, fabrication, and application. *Foods*, 13(15), Article 2453. https://doi.org/10.3390/foods13152453
31. El-Maksoud, A. A. A., Korany, R. M. S., El-Ghany, I. H. A., El-Beltagi, H. S., de Gouveia, G. M. A. F. (2020). Dietary solutions to dyslipidemia: Milk protein–polysaccharide conjugates as liver biochemical enhancers. *Journal of Food Biochemistry*, 44(3), Article e13142. https://doi.org/10.1111/jfbc.13142
32. Hassan, M. A., El-Nekeety, A. A., Abdel-Aziem, S. H., Hassan, N. S., Abdel-Wahhab, M. A. (2019). Zinc citrate incorporation with whey protein nanoparticles alleviate the oxidative stress complication and modulate gene expression in the liver of rats. *Food and Chemical Toxicology*, 125, 439–451. https://doi.org/10.1016/j.fct.2019.01.026
33. Kanoni, S., Dedoussis, G. V., Herbein, G., Fulop, T., Varin, A., Jajte, J. et al. (2010). Assessment of gene–nutrient interactions on inflammatory status of the elderly with the use of a zinc diet score — ZINCAGE study. *The Journal of Nutritional Biochemistry*, 21(6), 526–531. https://doi.org/10.1016/j.jnutbio.2009.02.011
34. Hamed, A. M., Taha, S. H., Darwish, A. A., Aly, E. (2021). Antioxidant activity and some quality characteristics of buffalo yoghurt fortified with peanut skin extract powder. *Journal of Food Science and Technology*, 58(6), 2431–2440. https://doi.org/10.1007/s13197-020-04835-2
35. Tamime, A. Y., Robinson, R. K. (2007). Tamime and Robinson's yoghurt: Science and technology. Woodhead Publishing-Elsevier, 2007.
36. AOAC. (2005). Association of Official Analytical Chemists, Official Methods of Analysis of AOAC international, 18th Edition, Published by AOAC international Maryland, USA. 2005.
37. DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350–356. https://doi.org/10.1021/ac60111a017
38. Soliman, T. N., Hassan, M. A. E. F. (2018). Preparation and characterization of sustained released zinc citrate encapsulated in whey protein nanoparticles. *Pakistan Journal of Biological Sciences*, 21(9), 448–453. https://doi.org/10.3923/pjbs.2018.448.453
39. Zhang, S., Wang, C. (2023). Precise analysis of nanoparticle size distribution in TEM image. *Methods and Protocols*, 6(4), Article 63. https://doi.org/10.3390/mps6040063
40. Konecny, F. (2021). Rodent general anesthesia suitable for measurement of experimental invasive hemodynamics. *European Journal of Biology and Biotechnology*, 2(4), 33–43. https://doi.org/10.24018/ejbio.2021.2.4.259
41. McClure, D. E. (1999). Clinical pathology and sample collection in the laboratory rodent. *Veterinary Clinics of North America: Exotic Animal Practice*, 2(3), 565–590. https://doi.org/10.1016/S1094-9194(17)30111-1
42. Plate, A. Y. A., Crankshaw, D. L., Gallaher, D. D. (2005). The effect of anesthesia by diethyl ether or isoflurane on activity of cytochrome P450 2E1 and P450 reductases in rat liver. *Anesthesia and Analgesia*, 101(4), 1063–1064. https://doi.org/10.1213/01.ane.0000166791.30963.ef
43. Suvarna, S.K., Layton, C., John D. Bancroft, J.D. (2019). Bancroft's Theory and Practice of Histological Techniques. Elsevier Ltd, 2019. https://doi.org/10.1016/C2015-0-00143-5
44. World Health Organization. (2024). Noncommunicable diseases. Retrieved from https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases Accessed July 14, 2025.
45. Farhana, A., Rehman, A. (2023). Metabolic consequences of weight reduction. In StatPearls [Internet]. Treasure Island (FL). StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK572145/ Accessed July 14, 2025.
46. Park, D. -Y., Ahn, Y. -T., Park, S. -H., Huh, C. -S., Yoo, S. -R., Yu, R. et al. (2013). Supplementation of *Lactobacillus curvatus* KY1032 in diet-induced obese mice is associated with gut microbial changes and reduction in obesity. *PLoS ONE*, 8(3), Article e59470. https://doi.org/10.1371/journal.pone.0059470
47. ALSuhaymi, N., Darwish, A. M., Khattab, A. E.-N. (2023). Assessment of two potential probiotic strains as anti-obesity supplements under high-fat feeding conditions. *Probiotics and Antimicrobial Proteins*, 15(4), 856–867. https://doi.org/10.1007/s12602-022-09912-w
48. Younossi, Z. M., Zelber-Sagi, S., Henry, L., Gerber, L. H. (2023). Lifestyle interventions in nonalcoholic fatty liver disease. *Nature Reviews Gastroenterology and Hepatology*, 20(11), 708–722. https://doi.org/10.1038/s41575-023-00800-4
49. Koutnikova, H., Genser, B., Monteiro-Sepulveda, M., Faurie, J. -M., Rizkalla, S., Schrezenmeir, J. et al. (2019). Impact of bacterial probiotics on obesity, diabetes and non-alcoholic fatty liver disease related variables: A systematic review and meta-analysis of randomised controlled trials. *BMJ Open*, 9(3), Article e017995. https://doi.org/10.1136/bmjopen-2017-017995
50. Timmers, S., den Bosch, J. de V-v., de Wit, N., Schaart, G., van Beurden, D., Hesselink, M. et al. (2011). Differential effects of saturated versus unsaturated dietary fatty acids on weight gain and myocellular lipid profiles in mice. *Nutrition and Diabetes*, 1(7), Article e11. https://doi.org/10.1038/nutd.2011.7
51. Chu, P.-Y., Yu, Y.-C., Pan, Y.-C., Dai, Y.-H., Yang, J.-C., Huang, K.-C. et al. (2024). The efficacy of lactobacillus delbrueckii ssp. bulgaricus supplementation in managing body weight and blood lipids of people with overweight: A randomized pilot trial. *Metabolites*, 14(2), Article 129. https://doi.org/10.3390/metabo14020129
52. Thoen, R. U., Barther, N. N., Schemitt, E., Bona, S., Fernandes, S., Coral, G. et al. (2019). Zinc supplementation reduces diet-induced obesity and improves insulin sensitivity in rats. *Applied Physiology, Nutrition, and Metabolism*, 44(6), 580–586. https://doi.org/10.1139/apnm-2018-0519
53. Refaat, O. G., Arafa, M. A., Rabeh, N. M., Sabra, R. S. (2020). Biological evaluation of probiotic fermented milk (RAYEB) on obese rats. *Egyptian Journal of Applied Science*, 35(9), 85–102. https://doi.org/10.21608/ejas.2020.128871
54. Mohammadi, A.A., Jazayeri, S., Khosravi-Darani, K., Solati, Z., Mohammadpour, N., Asemi, Z. et al. (2015). Effects of probiotics on biomarkers of oxidative stress and inflammatory factors in petrochemical workers: A randomized, double-blind, placebo-controlled trial. *International Journal of Preventive Medicine*, 6(1), Article 82. https://doi.org/10.4103/2008-7802.164146
55. Bayram, T., Pekmez, M., Arda, N., Yalçın, A. S. (2008). Antioxidant activity of whey protein fractions isolated by gel exclusion chromatography and protease treatment. *Talanta*, 75(3), 705–709. https://doi.org/10.1016/j.talanta.2007.12.007

56. Jin, D., Wei, X., He, Y., Zhong, L., Lu, H., Lan, J. et al. (2024). The nutritional roles of zinc for immune system and COVID-19 patients. *Frontiers in Nutrition*, 11, Articl 1385591. https://doi.org/10.3389/fnut.2024.1385591
57. El-Maddawy, Z. K., Abd El Naby, W. S. H. (2019). Protective effects of zinc oxide nanoparticles against doxorubicin induced testicular toxicity and DNA damage in male rats. *Toxicology Research*, 8(5), 654–662. https://doi.org/10.1039/c9tx00052f
58. Alkaladi, A., Abdelazim, A. M., Afifi, M. (2014). Antidiabetic activity of zinc oxide and silver nanoparticles on streptozotocin-induced diabetic rats. *International Journal of Molecular Sciences*, 15(2), 2015–2023. https://doi.org/10.3390/ijms15022015
59. Rahman, H. S., Othman, H. H., Abdullah, R., Edin, H. Y. A. S., AL-Haj, N. A. (2022). Beneficial and toxicological aspects of zinc oxide nanoparticles in animals. *Veterinary Medicine and Science*, 8(4), 1769–1779. https://doi.org/10.1002/vms3.814
60. Goel, A., Dani, V., Dhawan, D. K. (2005). Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. *Chemico-Biological Interactions*, 156(2–3), 131–140. https://doi.org/10.1016/j.cbi.2005.08.004

AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Mona A. Hassan, Assistant Professor, Food Evaluation and Food Science Department, National Organization for Drug Control and Research 51 Wezaret El-Zeraa Street, Agouza, 12654, Giza, Egypt E-mail: monasaa2016@gmail.com ORCID: https://orcid.org/0000-0002-8423-6066	**Мона А. Хассан** — ассистент-профессор, Отдел оценки пищевых продуктов и науки о продуктах питания, Национальная организация по контролю за наркотиками и научным исследованиям 12654, Египет, Гиза, Агоуза, ул. Везарет Эль-Зераа, 51 E-mail: monasaa2016@gmail.com ORCID: https://orcid.org/0000-0002-8423-6066
Mahmoud Salama, Assistant Professor, Food Science Department, Faculty of Agriculture, Cairo University 1 Gamaa Street, 12613, Giza, Egypt E-mail: mahmoudsalama2100@agr.cu.edu.eg ORCID: https://orcid.org/0000–0002–8486–0360	**Салама Махмуд** — ассистент-профессор, кафедра пищевых наук, сельскохозяйственный факультет, Каирский университет 12613, Египет, Гиза, ул. Гамаа, 1 E-mail: mahmoudsalama2100@agr.cu.edu.eg ORCID: https://orcid.org/0000–0002–8486–0360
Ahmed A. Abd El-Maksoud, Associate Professor, Department of Dairy Science, Faculty of Agriculture, Cairo University 1 Gamaa Street, 12613, Giza, Egypt E-mail: ahmed_ali@cu.edu.eg ORCID: https://orcid.org/0000-0001-6631-5900 * corresponding author	**Ахмед А. Абдель-Максуд** — Адъюнкт-Профессор, кафедра молочных наук, сельскохозяйственный факультет, Каирский университет, Египет 12613, Египет, Гиза, ул. Гамаа, 1 E-mail: ahmed_ali@cu.edu.eg ORCID: https://orcid.org/0000-0001-6631-5900 * автор для контактов
Contribution	**Критерии авторства**
Authors are equally relevant to the writing of the manuscript, and equally responsible for plagiarism.	Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат.
Conflict of interest	**Конфликт интересов**
The authors declare no conflict of interest.	Авторы заявляют об отсутствии конфликта интересов.