

RESEARCH PAPER

Enhanced Anti-Obesity Efficacy of Orlistat via Co-Administration with Iron Oxide Nanoparticles (SPIONs) in a High-Fat Diet-Induced Obese Mice Model

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ARTICLE INFO

Article History:

Received 05 September 2025

Accepted 27 October 2025

Published 01 January 2026

Keywords:

Iron Oxide Nanoparticles Fe_3O_4

Lipid Profile

Obesity

Orlistat

ABSTRACT

Obesity is a chronic, progressive condition associated with dyslipidemia, insulin resistance, and a proinflammatory state. Orlistat, a lipase inhibitor, is approved for weight management but has limited efficacy and side effects. Superparamagnetic iron oxide nanoparticles (SPIONs) have been reported to modulate lipid metabolism-related gene expression. This study aimed to evaluate whether co-administration of iron oxide nanoparticles enhances the lipid-lowering effects of orlistat in an obese mouse model. Seventy adult male mice (20–25 g) were divided into five groups (n = 14 each): (A) control (standard diet + saline), (B) obese (high-fat diet, no treatment), (C) obese + orlistat (120 mg/kg/d), (D) obese + SPIONs (dose proportional to body weight), and (E) obese + orlistat + SPIONs. Treatments were administered orally (0.2 mL/d) for 21 days. Seven mice per group were sacrificed on days 7 and 21. Serum triglycerides (TG), total cholesterol (CH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were measured using commercial kits. One-way ANOVA with LSD post hoc test was performed; $P < 0.05$ was considered significant. Compared to controls, obese mice exhibited significantly elevated TG, CH, and LDL ($P < 0.05$) and decreased HDL ($P < 0.05$). Orlistat alone (group C) reduced TG marginally (136.2 ± 32.6 mg/dL) compared to obese (151.6 ± 27.3 mg/dL) but did not significantly alter CH or LDL levels. SPIONs alone (group D) produced a slightly greater TG reduction (125.9 ± 31.8 mg/dL) than orlistat ($P > 0.05$) and significantly decreased HDL relative to orlistat ($P < 0.05$). The combination (group E) decreased TG (133.7 ± 30.3 mg/dL) and VLDL more than orlistat alone ($P > 0.05$) but had no additive effect on CH, LDL, or HDL. VLDL levels remained statistically similar across all groups ($P > 0.05$). Co-administration of SPIONs with orlistat modestly improved TG and VLDL levels compared to orlistat alone but did not significantly affect CH, LDL, or HDL within 21 days. These findings suggest that SPIONs may potentiate certain lipid-lowering effects of orlistat. Further studies optimizing dose, treatment duration, and nanoparticle functionalization are warranted to clarify mechanistic pathways and long-term metabolic benefits.

How to cite this article

Ramadan S. Al-Ali Z. Al-Abboodi A. Enhanced Anti-Obesity Efficacy of Orlistat via Co-Administration with Iron Oxide Nanoparticles (SPIONs) in a High-Fat Diet-Induced Obese Mice Model. J Nanostruct, 2026; 16(1):995-1003. DOI: 10.22052/JNS.2026.01.089

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INTRODUCTION

Obesity is defined as the excessive accumulation of fat, known as extracorporeal fat, in various parts of the body, organs, or throughout the body. It is a chronic, progressive, and recurrent condition with multiple factors leading to negative health consequences for metabolic, psychological, and social well-being [1]. A body mass index (BMI) greater than 30 kg/m^2 , defined as weight in kilograms divided by the square of height in meters, is used to diagnose obesity. For adults, a BMI between 25.0 and 29.9 kg/m^2 is defined as overweight, and a BMI of 30 kg/m^2 or greater is defined as obese [2]. The BMI scale is not used for children and adolescents aged 2–18 years; instead, a centile scale based on the child's sex and age is recommended [3, 4]. Anthropometric methods are the most appropriate and common methods for estimating the extent of obesity. In addition to BMI, these methods include waist and hip circumferences, waist-to-hip ratio (WHR), skinfold thickness, and waist-to-height ratio (WSR). Since shorter individuals are typically lighter, weight alone cannot be relied upon to determine the amount of fat stored in an individual. Therefore, both WSR and waist circumference are relatively easy and accurate techniques for estimating visceral fat [5].

The lipid abnormalities seen in patients who are obese which include elevated triglycerides (TG), very low density lipoprotein (VLDL), apolipoprotein B (Apo B), and non-high density lipoprotein cholesterol (non-HDL-c) levels, which are all commonly observed, HDL-C and apolipoprotein A-I (Apo A-I) levels are typically low, low density lipoprotein cholesterol (LDL-C) levels are frequently in the normal to slightly elevated range, but an increase in small dense LDL is often seen resulting in an increased number of LDL particles [6, 7]. Orlistat induces weight reduction via the inhibition of lipases in the mucous membranes of the stomach, small intestine, and pancreas, thereby preventing the breakdown of triglycerides into fatty acids and their absorption in the intestines [8]. Orlistat and other treatments used to combat obesity are associated with unpleasant side effects, and the failure rate of weight loss treatment is extremely high (90%), which reduces its chances of reducing the prevalence of obesity and its comorbidities [9]. Regarding bariatric surgery, it is considered the most effective treatment to date [10, 11]. However,

despite its high effectiveness, it is associated with a number of adverse intraoperative outcomes and postoperative complications such as anemia, depression, fractures, malabsorption, etc. Therefore, due to the lack of desired or confirmed weight loss, it raises a lot of controversy and concern among obese patients [12]. The issue of funding for this type of treatment is also not without importance [13]. All of these reasons have led to the search for new strategies to safely reduce the prevalence of obesity. Anti-obesity nanomedicine could be developed and improved, reducing the risk of obesity [14].

Nanotechnology is a branch of science that involves the design and manufacture of nanoscale materials (1 to 100 nanometers) for applications in various fields, such as medicine. Recent meta-analyses show that artificial-intelligence workflows now accelerate every stage of nanomaterial discovery and clinical translation, from in-silico molecular screening to predictive toxicology [15]. Metal- and polysaccharide-based nanomaterials have already proved their biomedical versatility ranging from broad-spectrum antibacterial coatings [16-18] to magnetically guided vaccine carriers built from protein-conjugated superparamagnetic iron oxide nanoparticles [19]. Alarming, recent surveys of some food products in southern Iraq have revealed multiple enteric parasites on raw vegetables [20], underscoring the urgent need for low-cost nanoscale decontamination platforms that can be deployed along the food-supply chain. Machine-learning hydrological models further underline how data-driven tools can guide nanoparticle-based clean-up strategies in Iraq's food sector [21]; this can shape responsible nanomaterial deployment [22, 23]. These exemplars underline why SPIONs are an attractive co-therapeutic for metabolic disorders, where both drug solubility and tissue-specific delivery are paramount.

Superparamagnetic Iron Oxide nanoparticles (SPIONs) have been shown to be involved in regulating genes involved in lipid and glucose metabolism, suggesting they could be used as treatments for diabetes and obesity [24, 25]. Beyond lipid regulation, iron-oxide nanostructures modulate innate-immune signalling inside macrophages and dendritic cells, a property harnessed to suppress biofilms and hard-to-treat infections [26]. Such immunometabolic cross-talk may further potentiate the metabolic

benefits observed in obesity models. Therefore, our study aims to increase the efficacy of orlistat in treating obesity by adding PEG-coated SPIONs. This study aims to evaluate lipid profile changes following treatment with orlistat, SPIONs, and their combination.

MATERIALS AND METHODS

Animal Ethics and Housing

Seventy adult male Swiss albino mice (20–25 g) were obtained from the Animal Husbandry Unit, Biology Department, College of Science, University of Misan. All procedures adhered to institutional guidelines for animal care and use. Mice were housed in standard plastic cages at 20–25 °C with a 12 h light/12 h dark cycle and had ad libitum access to food and water. Animals were acclimatized for one week before experimental interventions.

Induction of Obesity

All mice, except the control group (A), were fed a high-fat diet (HFD; 60 % kcal from fat) for four weeks to induce obesity. Body weight and food intake were measured weekly. Obesity was confirmed when mice gained ≥ 20 % of baseline body weight.

Experimental Design

Once obesity was established, mice were randomly assigned ($n = 14$ per group) into five groups: Group A (Control): Standard diet + saline (0.2 mL/d, oral), Group B (Obese): HFD + saline (0.2 mL/d, oral), Group C (Orlistat): HFD + orlistat (120 mg/kg/d in 0.2 mL saline, oral), Group D (SPIONs): HFD + iron oxide nanoparticles (Fe₃O₄; dose proportional to body weight, in 0.2 mL saline, oral) and Group E (Orlistat + SPIONs): HFD + orlistat (120 mg/kg/d) + Fe₃O₄ SPIONs (same dose as Group D). Treatments were administered

once daily for 21 days. Seven mice per group were sacrificed on day 7, and the remaining seven on day 21 for biochemical analyses.

Nanoparticle Preparation

Iron oxide nanoparticles (Fe₃O₄; Superparamagnetic iron oxide nanoparticles, SPIONs) were purchased from US Research Nanomaterials, Inc. The nanoparticles were dispersed in sterile saline by sonication for 15 min immediately prior to administration to ensure homogeneity.

Blood Collection and Serum Preparation

At each time point (days 7 and 21), mice were euthanized by chloroform inhalation. Cardiac puncture was performed using a 3 mL syringe to collect blood into gel-coated tubes. Samples were allowed to clot at room temperature for 30 min, then centrifuged at 3,000 rpm for 15 min. Serum was aliquoted into pre-labeled Eppendorf tubes and stored at –80 °C until analysis [27].

Biochemical Analyses

Serum levels of TG, total cholesterol (CH), HDL, LDL, and VLDL were quantified using the BioSystem A15 analyzer (BioSystem, Spain) with manufacturer-provided kits, following the supplied protocols. The TG measurement has been conducted using the enzymatic glycerol-3-phosphate oxidase method, while the CH measurement using the enzymatic cholesterol oxidase–peroxidase method. Precipitation method and enzymatic assay have been exploited to measure the HDL measurement, direct enzymatic assay to measure the LDL measurement and calculated as TG/5 (mg/dL) to measure the VLDL estimation.

Statistical Analysis

Data were presented as Mean \pm standard error

Table 1. Mean serum lipid parameters for groups. Data was pooled from days 7 and 21.

Group	TG (mg/dL)	CH (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
A (Control)	78.93 \pm 15.66 a	91.14 \pm 15.14 a	49.57 \pm 14.11 a	47.86 \pm 16.69 a	27.29 \pm 10.48 a
B (Obese)	151.57 \pm 27.34 b	128.29 \pm 20.26 b	35.93 \pm 6.01 bc	73.79 \pm 11.51 b	29.86 \pm 5.63 a
C (Orlistat)	136.21 \pm 32.58 bc	142.00 \pm 29.57 b	43.36 \pm 12.06 ab	87.86 \pm 19.73 bc	26.93 \pm 7.07 a
D (SPIONs)	125.86 \pm 31.77 c	135.00 \pm 31.19 b	34.71 \pm 7.16 c	89.93 \pm 21.69 c	24.93 \pm 6.60 a
E (Orlistat+SPIONs)	133.71 \pm 30.25 bc	144.57 \pm 35.49 b	39.14 \pm 8.59 bc	86.21 \pm 23.38 bc	28.64 \pm 9.44 a

a, b, c: Means within a column sharing different superscripts differ significantly ($P < 0.05$).

(SE). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Least Significant Difference (LSD) post hoc test for multiple comparisons. A P-value < 0.05 was considered statistically significant. All analyses were conducted using SPSS version 24.0 (IBM Corp., USA).

RESULTS AND DISCUSSION

All groups started with comparable mean body weights (20–25 g). After four weeks of HFD, groups B–E exhibited a significant weight gain (> 20 % of baseline; P < 0.05 vs. group A), confirming obesity induction. Throughout the 21-day treatment, groups C, D, and E showed modest weight reductions compared to group B, although these changes did not reach statistical significance (data not shown). Table 1 presents mean serum lipid parameters for all groups (pooled data from days 7 and 21, since no significant time-dependent differences were observed).

Triglycerides (TG): Obese mice (group B) exhibited a significant TG increase (151.57 ± 27.34 mg/dL) compared to controls (78.93 ± 15.66 mg/dL; P < 0.05). Orlistat (group C) and SPIONs (group D) reduced TG compared to group B, but only

SPIONs produced a significant decrease (125.86 ± 31.77 mg/dL; P < 0.05 vs. group B) as shown in Fig. 1. The combination (group E) yielded TG of 133.71 ± 30.25 mg/dL—lower than group B (P < 0.05) but not significantly different from orlistat alone (P > 0.05).

Total Cholesterol (CH): All obese groups (B–E) had significantly higher CH (128.29–144.57 mg/dL) than controls (91.14 ± 15.14 mg/dL; P < 0.05). Neither orlistat, SPIONs, nor the combination reduced CH significantly compared to group B (P > 0.05 for all comparisons) as presented in Fig. 2.

High-Density Lipoprotein (HDL): Obese mice (group B) exhibited lower HDL (35.93 ± 6.01 mg/dL) than controls (49.57 ± 14.11 mg/dL; P < 0.05). SPIONs (group D) further decreased HDL (34.71 ± 7.16 mg/dL) compared to orlistat (43.36 ± 12.06 mg/dL; P < 0.05). Orlistat alone (group C) preserved HDL (43.36 ± 12.06 mg/dL) at a level not significantly different from controls (P > 0.05). The combination (group E) produced intermediate HDL (39.14 ± 8.59 mg/dL), which was lower than orlistat alone (P > 0.05) but not significantly different from group B (P > 0.05) as shown in Fig. 3.

Low-Density Lipoprotein (LDL): Obese mice (group B) showed a significant increase in LDL

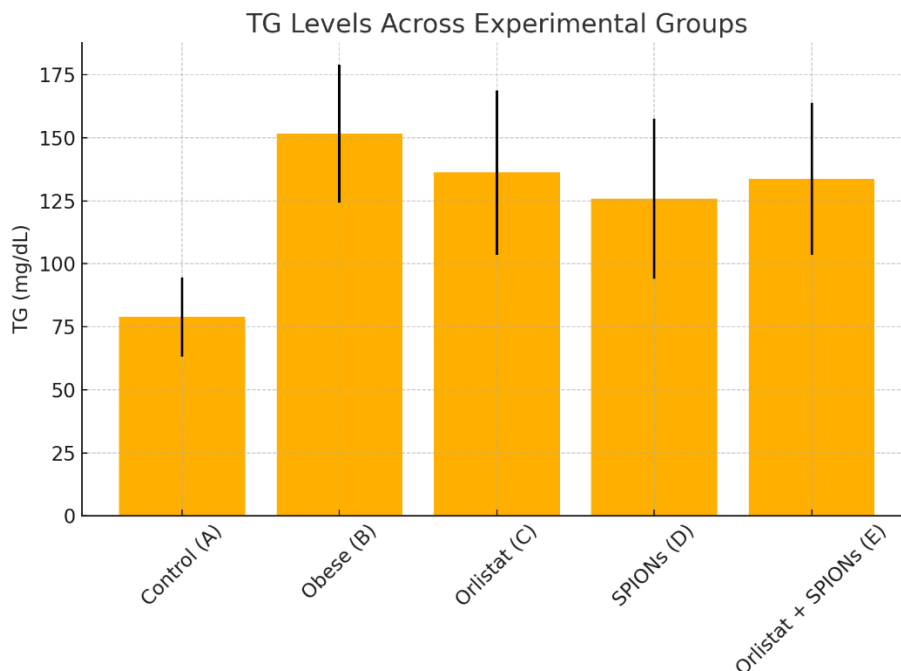


Fig. 1. TG levels across the study groups.

(73.79 ± 11.51 mg/dL) versus controls (47.86 ± 16.69 mg/dL; P < 0.05). Orlistat alone (87.86 ± 19.73 mg/dL) and SPIONs alone (89.93 ± 21.69 mg/dL) both led to significantly higher LDL

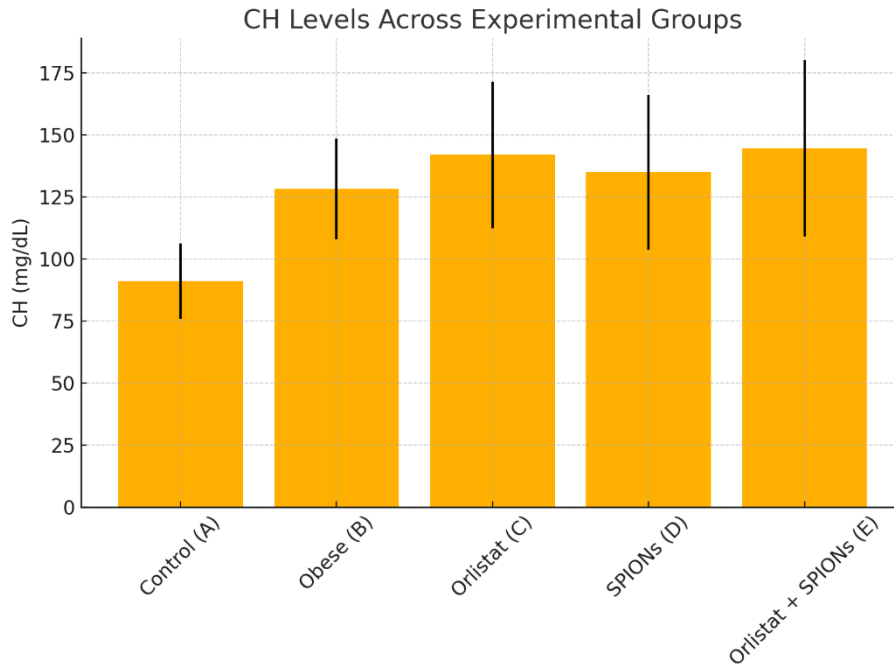


Fig. 2. CH levels across the study groups.

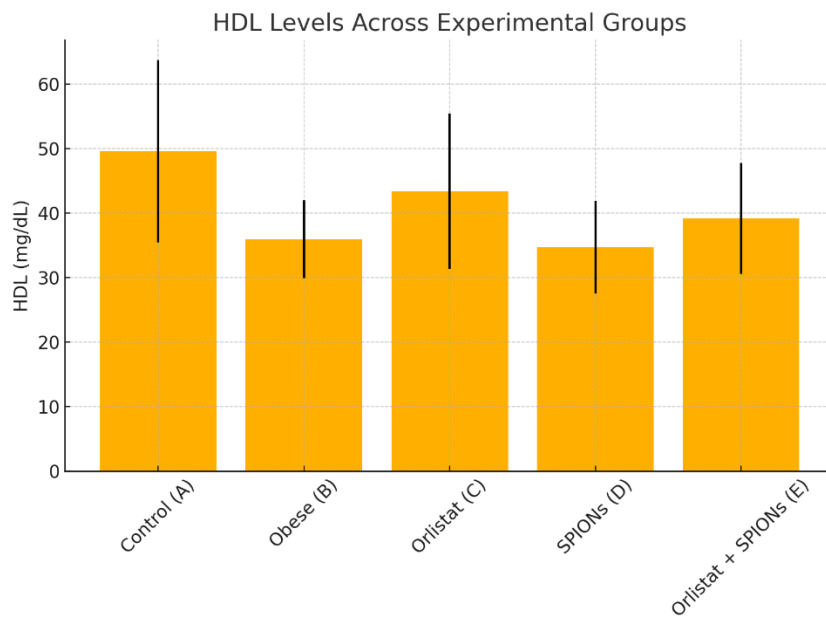


Fig. 3. HDL levels across the study groups.

than controls ($P < 0.05$). Neither treatment nor combination reduced LDL compared to group B ($P > 0.05$ across comparisons) as shown in Fig. 4. Very Low-Density Lipoprotein (VLDL): VLDL remained statistically similar across all groups (24.93–29.86 mg/dL; $P > 0.05$) as presented in Fig. 5.

Obesity Induction and Dyslipidemia: High-fat diet–induced obesity significantly elevated TG, CH, and LDL, while reducing HDL consistent with prior rodent studies demonstrating obesity-associated dyslipidemia [28]. Excess FFA flux and insulin resistance impede lipoprotein lipase (LPL) activity, leading to delayed clearance of TG-rich lipoproteins [29]. Elevated TG promotes CETP-mediated lipid exchanges that reduce HDL cholesterol [30]. Parallel findings in murine chemotaxis-on-a-chip studies show that nanoparticle exposure can rapidly re-programme stromal cells and their metabolic output [31–33], supporting the notion that SPIONs act through both systemic and micro-tissue remodelling.

In line with previous reports [34], orlistat attenuated TG levels compared to obese controls, although the decrease was not statistically robust in our short-term (21 days) protocol. Orlistat’s lipase inhibition prevents dietary TG absorption, partially explaining the observed TG reduction.

However, CH and LDL remained unchanged or increased slightly possibly due to compensatory hepatic VLDL production and limited treatment duration. Orlistat preserved HDL, albeit not significantly higher than obese controls, suggesting partial protection against HDL depletion [35].

SPIONs Monotherapy: SPIONs alone yielded a more pronounced TG reduction than orlistat ($P < 0.05$ vs. group B), corroborating reports that iron oxide nanoparticles promote mitochondrial biogenesis and white adipose tissue browning, thereby enhancing FFA oxidation (Li et al., 2019). Surprisingly, SPIONs also reduced HDL significantly compared to orlistat ($P < 0.05$). This may reflect altered HDL remodeling via increased hepatic lipase activity or shifts in apolipoprotein expression [25]. A comparable HDL dip has been reported when some plant seedlings were exposed to salinity stress an abiotic insult that likewise perturbs membrane lipid turnover [36]. This cross-kingdom parallel suggests that SPION-driven lipid remodelling may engage conserved oxidative-stress pathways. LDL was elevated in SPION-treated obese mice relative to controls; prolonged treatment or different dosing may be required to achieve normalization [37].

Combined Therapy (Orlistat + SPIONs): The

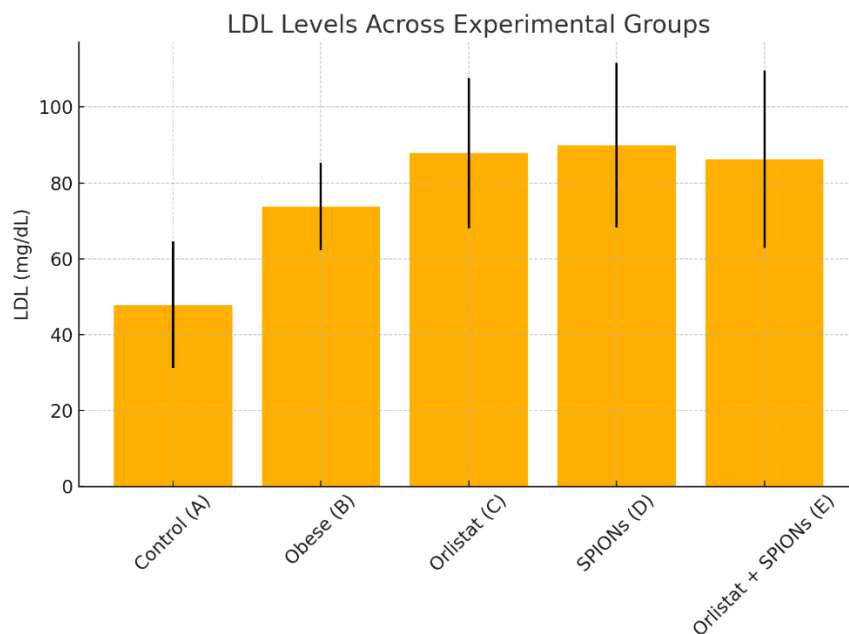


Fig. 4. LDL levels across the study groups.

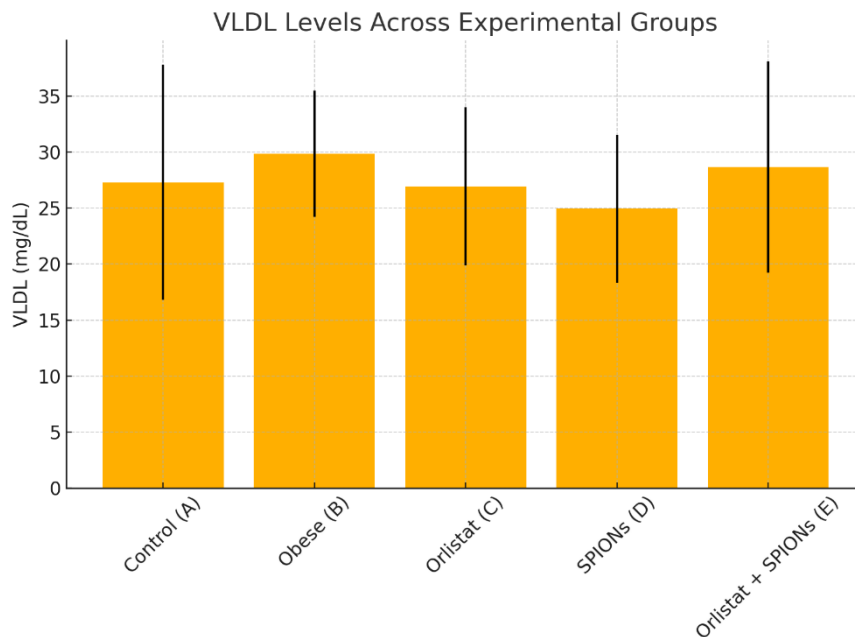


Fig. 5. VLDL levels across the study groups.

combination modestly improved TG (133.71 ± 30.25 mg/dL) relative to orlistat alone (136.21 ± 32.58 mg/dL), but this difference was not statistically significant. VLDL followed a similar trend. CH and LDL remained elevated, suggesting that short-term co-administration may be insufficient to reverse lipid perturbations fully. Notably, combined therapy-maintained HDL at intermediate levels (39.14 ± 8.59 mg/dL), indicating partial mitigation of SPION-induced HDL reduction. Prior rodent studies reported that SPIONs plus orlistat synergistically improved lipid profiles better than either agent alone [14, 37, 38]. Discrepancies between our findings and previous studies may stem from differences in nanoparticle size, surface coating such as PEGylation, dosing regimens, and administration routes.

Mechanistic Considerations: SPIONs may enhance adipocyte mitochondrial biogenesis via activation of PGC-1 α and upregulation of UCP1, leading to increased FFA oxidation [39]. Additionally, iron oxide internalization by adipose-resident macrophages could modulate local inflammation, improving insulin sensitivity [25, 41]. Orlistat's inhibition of gastrointestinal lipases reduces dietary fat absorption, reducing substrate availability for hepatic VLDL production [34, 40].

Co-administration aims to combine peripheral (adipose) and intestinal mechanisms to achieve additive lipid-lowering effects. Translational roll-out of nanomedicine must also anticipate cyber-risks: a recent healthcare study showed that ensemble machine-learning filters can block phishing URLs targeting electronic patient records, an essential safeguard for data-rich nano-therapeutic trials [42].

CONCLUSION

Co-administration of iron oxide nanoparticles with orlistat in HFD-induced obese mice yielded modest improvements in serum TG and VLDL beyond orlistat monotherapy but failed to significantly impact CH, LDL, or HDL within a 21-day period. SPIONs alone exhibited a notable TG-lowering effect but also reduced HDL, suggesting complex lipid remodeling. These findings indicate that SPIONs may partially potentiate orlistat's metabolic effects; however, optimization of nanoparticle coating, dosing, administration route, and treatment duration is essential to achieve clinically meaningful dyslipidemia improvement. Further mechanistic investigations are warranted to elucidate SPION-mediated modulation of adipose and hepatic pathways, thereby guiding

development of effective nanomedicine-based anti-obesity strategies.

ACKNOWLEDGMENTS

The authors thank the Animal Husbandry Unit of the Biology Department, College of Science, University of Maysan, for technical support. We also acknowledge US Research Nanomaterials, Inc. for providing the SPIONs used in this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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