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RESEARCH ARTICLE

Cardiometabolic Risks of Tramadol in the Setting of Obesity: Evidence from a High-Fat Diet Mouse Model

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SUMMARY

Globally, obesity has become a significant problem and contributes to the development of metabolic syndrome. Metabolic syndrome affects the pharmacokinetics of various opioids, raising drug toxicity. Tramadol is an opioid analgesic used to treat moderate to severe pain. Chronic tramadol misuse can cause oxidative stress, inflammation, and alteration of the γ-aminobutyric acid (GABA) neurotransmitter system. Although some studies pointed out the risk of increased lipid peroxidation and hepatic and renal damage, very little research has been done on how tramadol affects the cardiovascular system in obese conditions. Therefore, this study investigated the effects of tramadol in high-fat diet-induced obese mice. In this study, the obese mice received tramadol at 5 mg/kg while continuing the high-fat diet. Following six weeks of treatment, we measured body weight, Lee index, food efficiency ratio, organ weight, serum lipid profile (triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and Very Low-Density Lipoprotein cholesterol), hepatic biochemical markers (Aspartate aminotransferase and Alkaline Phosphatase), creatinine level, adipocyte cell size, and coronary risk indices (Atherogenic Index of Plasma, Atherogenic coefficient, and Coronary Risk Ratio). Our data indicate that tramadol reduces food intake and the weight of the organs. Body weight reduction is attributed to the reduced food intake in obese mice treated with tramadol. Tramadol treatment was associated with increased lipid profiles, liver-kidney function markers, and lipid-derived atherogenic indices. These cardiometabolic alterations suggest that tramadol exposure may contribute to an adverse cardiovascular risk profile in obese individuals, increasing their susceptibility to cardiac complications.

Keywords: *Obesity, tramadol, lipid profile, liver functions, cardiac complications*

Obezite Bağlamında Tramadolün Kardiyometabolik Riskleri: Yüksek Yağlı Diyetli Fare Modelinden Elde Edilen Kanıtlar

ÖZ

Küresel olarak obezite önemli bir sorun haline gelmiş ve metabolik sendromun gelişimine katkıda bulunmaktadır. Metabolik sendrom, çeşitli opioidlerin farmakokinetiğini etkileyerek ilaç toksisitesini artırmaktadır. Tramadol, orta ila şiddetli ağrıyı tedavi etmek için kullanılan bir opioid analjeziktir. Kronik tramadol kötüye kullanımı oksidatif strese, inflamasyona ve γ-aminobütirik asit (GABA) nörotransmitter sisteminin bozulmasına neden olabilir. Bazı çalışmalar artmış lipid peroksidasyonu ve karaciğer ve böbrek hasarı riskine işaret etse de, tramadolün obez koşullarda kardiyovasküler sistemi nasıl etkilediği konusunda çok az araştırma yapılmıştır. Bu nedenle, yüksek yağlı diyetle indüklenen obez farelerde tramadolün etkilerini araştırmayı amaçladık. Bu çalışmada, obez bir fare grubuna yüksek yağlı diyet (HFD) ile birlikte 5 mg/kg tramadol uygulandı. Altı haftalık tedavinin ardından vücut ağırlığı, Lee indeksi, besin verimlilik oranı, organ ağırlığı, serum lipid profili (trigliseritler, toplam kolesterol, yüksek yoğunluklu lipoprotein kolesterolü, düşük yoğunluklu lipoprotein kolesterolü ve çok düşük yoğunluklu lipoprotein kolesterolü), karaciğer biyokimyasal belirteçleri (Aspartat aminotransferaz ve Alkalin Fosfataz), kreatinin düzeyi, adiposit hücre boyutu ve koroner risk indeksleri (Plazmanın Aterojenik İndeksi, Aterojenik katsayı ve Koroner Risk Oranı) ölçüldü. Verilerimiz tramadolün besin alımını ve organ ağırlığını azalttığını göstermektedir. Vücut ağırlığındaki azalma, tramadol ile tedavi edilen obez farelerde azalan besin alımına bağlanmaktadır. Tramadol tedavisi, artmış lipid profilleri, karaciğer-böbrek fonksiyon belirteçleri ve lipid kaynaklı aterojenik indekslerle ilişkilendirilmiştir. Bu kardiyometabolik değişiklikler, tramadol maruziyetinin obez bireylerde olumsuz bir kardiyovasküler risk profiline katkıda bulunabileceğini ve kalp komplikasyonlarına yatkınlıklarını artırabileceğini düşündürmektedir.

Anahtar Kelimeler: *Obezite, tramadol, lipid profili, karaciğer fonksiyonları, kardiyak komplikasyonlar*

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INTRODUCTION

Obesity is a major global health problem and is closely associated with metabolic syndrome, which includes insulin resistance, dyslipidemia, and hypertension (Takahashi et al., 2015). This syndrome not only predisposes individuals to cardiovascular and hepatic complications but also influences the pharmacokinetics and pharmacodynamics of various drugs, including opioids (Armani et al., 2024; Morales Castro, Dresser, Granton, & Fan, 2023). In obese individuals, physiological changes such as reduced blood flow due to adipose tissue accumulation and increased risk of thrombosis can significantly alter drug distribution, metabolism, and excretion, leading to enhanced drug toxicity (Cheymol, 2000; Gouju & Legeay, 2023).

Tramadol, a synthetic opioid analgesic, is frequently administered to relieve moderate to severe pain because it inhibits serotonin and norepinephrine reuptake and exerts μ-opioid receptor agonist activity (Minami, Ogata, & Uezono, 2015; Subedi, Bajaj, Kumar, & YC, 2019). Although generally considered to have a favorable safety profile compared to other opioids, prolonged or excessive use of tramadol has been linked to a range of adverse effects. These include oxidative stress, systemic inflammation, and γ-aminobutyric acid (GABA) neurotransmission disturbance (Mohamed & Mahmoud, 2019; Xia et al., 2020). Tramadol may also stimulate leptin production through inflammatory stress, sympathetic activation, and opioid receptor activation (Gromov & Filonenko, 1999; Minami et al., 2015; Zhang, Fan, Zhang, & Cao, 2023). Tramadol exposure may potentially influence leptin signaling (Berthoud, 2007; Engin, 2024). Prior research has also demonstrated tramadol-induced lipid peroxidation, along with hepatic and renal toxicity, under various pathological conditions (Ali et al., 2020).

In particular, it remains unclear whether tramadol administration in the obese state is associated with variations in circulating lipid profiles, hepatic and renal biochemical markers, and lipid-derived indices of atherogenic risk. Alterations in triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), and High-density lipoprotein cholesterol (HDL-C) may provide a more comprehensive assessment of metabolic status than body weight alone. In addition, lipid-derived indices such as the atherogenic index of plasma (AIP), coronary risk ratio (CRR), and atherogenic coefficient (AC) are commonly used as surrogate indicators of an unfavorable lipid-associated cardiovascular risk profile (Lioy, Webb, & Amirabdollahian, 2023; Lumu et al., 2023). However, most available experimental investigations have been conducted in healthy animals. In contrast, the metabolic consequences of tramadol exposure in an established obese state remain comparatively less well characterized. Nevertheless, the available evidence remains insufficient to determine whether tramadol produces a distinct pattern of metabolic alterations under conditions of obesity.

Because of this research gap, the present study investigated the effects of tramadol on the cardiovascular risk profile in high-fat diet-induced obese female mice. We hypothesized that tramadol exposure in HFD-induced obese mice would alter metabolic and biochemical parameters. To address this gap, we assessed key physiological and biochemical endpoints, including changes in body weight and relative organ weights, serum lipid profiles, liver and kidney function tests, and lipid-derived atherogenic indices. By elucidating the cardiometabolic consequences of tramadol exposure in an obesity model, this study seeks to provide critical insights into the safe therapeutic use of tramadol in obese experimental models.

MATERIALS AND METHODS

Animals and Materials

We used diet-induced obese mouse models in this research because they reproduce several key physiological and metabolic features of human obesity (Della Vedova et al., 2016). Fifteen female Swiss al-

bino mice weighing 22–25 g were used in the study. Obesity was induced by feeding the mice a high-fat diet. The International Centre for Diarrheal Disease Research in Bangladesh (ICDDR, B) provided those mice. The mice were housed in an animal facility maintained at 22 ± 1 °C, with a 12-h light/dark cycle. Food and water were provided ad libitum. Tramadol was supplied as a generous gift from Incepta Pharmaceuticals Limited. All experimental procedures were conducted at the Molecular Pharmacology Laboratory of the Department of Pharmacy in Noakhali Science and Technology University.

Study Design

Initially, 15 female mice (5 weeks old) were divided into two groups at random: high-fat diet (HFD) group (n=10) given a high-fat diet (normal diet with 20% cow fat through melting and mixing; approximate caloric content: 4.65 kcal/g) for six consecutive weeks, and the Normal diet (ND) group (n=5) given a normal diet also for six consecutive weeks. Figure 1 summarizes and illustrates the study design. Body weight was measured daily using a weighing balance during the experiment, and the average weight of each group of mice was calculated. Additionally, their food intake tendencies were also monitored and recorded.

After the 6-week obesity-induction period, the HFD-induced obese mice were randomly allocated to either the untreated HFD group or the tramadol-treated group (n = 5/group). Mice in the tramadol group received tramadol at 5 mg/kg (equivalent to 300 mg/day/65-kg human) by oral gavage for 6 weeks. Regular high-fat diets were continued for both of these groups, while normal diets were followed for the preceding ND group. After the experimental period ended, all of the animals were dissected. Serum, adipose tissue, kidneys, liver, and heart were collected for subsequent analyses. The study was reviewed and approved by the Noakhali Science and Technology University Ethical Committee (NSTUEC) (Ref: NSTU/SCI/EC/2023/161, August 13, 2023). The experiments were conducted in accordance with the guidelines of the committee.

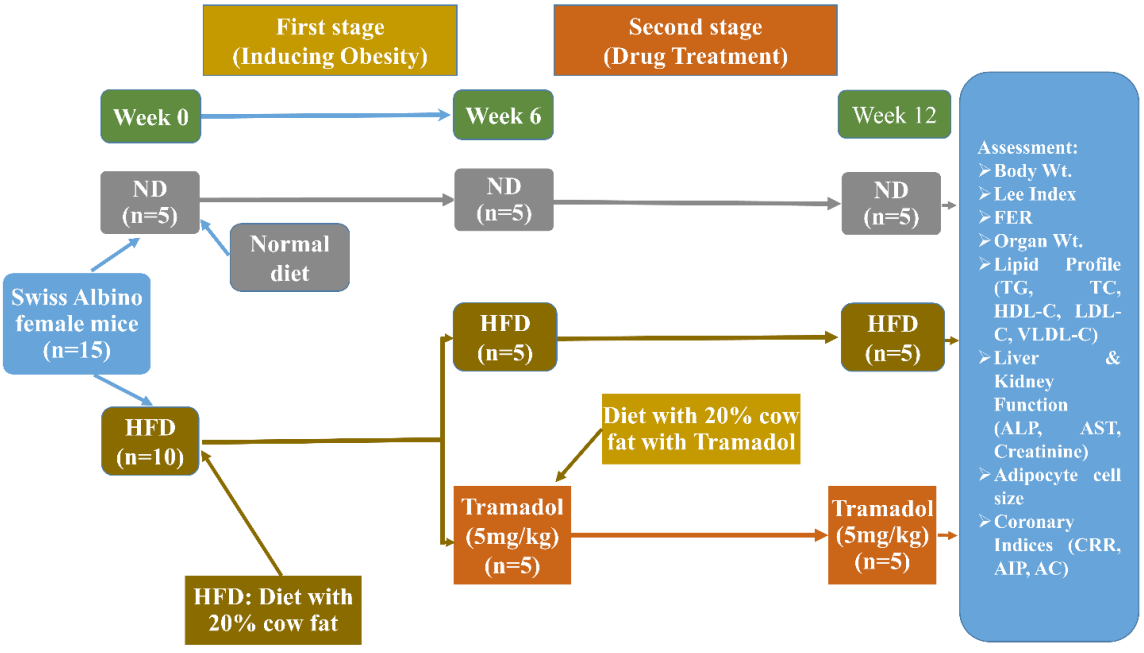

Figure 1. Flowchart of the study design of this research

Food Intake Measurement

Dietary consumption was tracked throughout the experimental period by providing a pre-weighed quantity of the respective diet to each cage and weighing the remaining food after 24 h. Daily dietary intake was assessed as the difference between the amount of food provided and the amount remaining at the end of each 24-h period. Visible food spillage was collected and accounted for whenever possible. Food consumption was documented according to individual cages and expressed as grams of food consumed per cage per day.

Randomization and Blinding Procedures

Following the acclimatization period and the confirmation of the HFD-induced obese model, the animals were individually identified and randomly allocated to the experimental groups using a simple lottery-based randomization procedure. Each animal was assigned an identification number, and the numbers were randomly drawn and distributed among the predefined experimental groups. Baseline body weights were noted before distribution to determine early uniformity across groups. To minimize observer bias, the animals from specific groups were identified by coded numbers. Then, the researchers doing outcome evaluation and data analysis were kept uninformed of the treatment distribution. Group codes were disclosed only after completion of the relevant measurements and data recording.

Method of Serum Collection for Biochemical Tests and Analysis

Blood specimens were collected by cardiac puncture and allowed to clot at room temperature for 30 min. The samples were subsequently centrifuged at 3500 rpm for 10 min to separate the serum. The separated serum samples were transferred into Eppendorf tubes and stored at −80 °C until biochemical analysis. Hepatic biochemical parameters were measured using Human Diagnostics kits (Germany), particularly ALP (Ref# 12127) and AST (Ref# 12031). Lipid parameters were measured using Cromatest kits (Linear Chemical S.L.), specifically TG (Ref# 1155017), TC (Ref# 1118017), HDL-C (Ref# 1133010), LDL-C (Ref# 1133105), and creatinine (Ref# 1123005). VLDL-C was estimated using the Friedewald equation (Friedewald, Levy, & Fredrickson, 1972). We performed all assays according to the manufacturers' protocols using a semi-automated chemistry analyzer (Mindray BA-88A, China). These tests were validated in our lab environment, and the same procedure was followed as described in our previous study (Begum, Roy, Ripon, Amin, & Hossain, 2025).

Finally, the lipid-derived coronary risk indices, including the atherogenic index of plasma (AIP), atherogenic coefficient (AC), and coronary risk ratio (CRR), were calculated from the measured lipid profile parameters using the following formulas (Akici, Onal, Gürbüz, Sağ, & Kilinç, 2020; Dobiášová & Frohlich, 2001):

$$\text{CRR} = \frac{\text{TC}}{\text{HDLC}}$$

$$\text{AIP} = \text{Log}_{10}\frac{\text{TG}}{\text{HDLC}}$$

$$\text{AC} = \frac{\text{TC}-\text{HDLC}}{\text{HDLC}}$$

Collection and Measurement of Adipose Tissues and Body Organs

Following a 6-week treatment, the mice were euthanized with chloroform and subsequently dissected using a standard dissection box. At that time, the adipose tissue in the abdomen was carefully separated from the adjacent tissues, collected, and weighed. A representative portion was subsequently processed for histological examination and adipocyte-area measurement. The liver, the heart, and the kidneys were then separated and weighed.

Histological Staining of Adipose Tissue

Adipose tissue samples from mice were fixed in Bouin's solution, processed, and then embedded in paraffin for histological examination. The tissues fixed in paraffin were sectioned at 4 μm thickness using a

microtome (Leica RM2235). The sectioned tissue was stained with hematoxylin and eosin (H&E) and examined under a light microscope at 10× magnification. The pictures of the stained sections were taken, and the adipocyte area was measured using ImageJ software. Histological procedures were performed as previously described by Roy et al. (2022).

Statistical Analysis

The GraphPad Prism software (version 8.00 for Windows) was used for all statistical analyzes. At first, the normality test was assessed before parametric analyses. Then, the statistical significance of the data was determined using the Student's t-test for only two groups. For comparisons involving the three independent groups at a single time point, one-way analysis of variance (ANOVA) with Tukey's post hoc multiple comparison test was applied to control for type I error originating from multiple comparisons. For longitudinal variables such as body weight and food intake measured repeatedly during the treatment period, a two-way repeated-measures ANOVA with Tukey's post hoc multiple comparison test was also used. A priori sample size was determined using power analysis (effect size = 0.5, $\alpha = 0.05$, and statistical power = 80%). In addition, the number of animals was reduced in each experimental group in accordance with the principles of Replacement, Reduction, and Refinement (the 3Rs). The power analysis indicated that a sample size of n = 5 animals per group provided sufficient power to detect biologically meaningful differences with moderate-to-large effect sizes for the primary outcome measures. Results are presented as mean ± SEM (n=5). Statistical significance was defined as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

RESULTS

Obese Mice Development and Obesity Confirmation

After six weeks of high-fat diet (HFD) administration, the body weight of the HFD group increased significantly compared with the normal diet (ND) group (42 g vs. 30 g). Obesity development was further evaluated using the Lee index, for which a value above 310 has been used as an indicator of obesity in mice (Lee, 1929). The HFD group exhibited a higher Lee index (339) than the ND group (306), exceeding the reported threshold and supporting the development of an obese phenotype. In addition, the food efficiency ratio (FER) was higher in the HFD group (5) than in the ND group (1.9). The average body weight also increased by 47% in the HFD group compared with 20% in the ND group. Collectively, these findings confirmed the establishment of the HFD-induced obese model. Figure 2 presents the data.

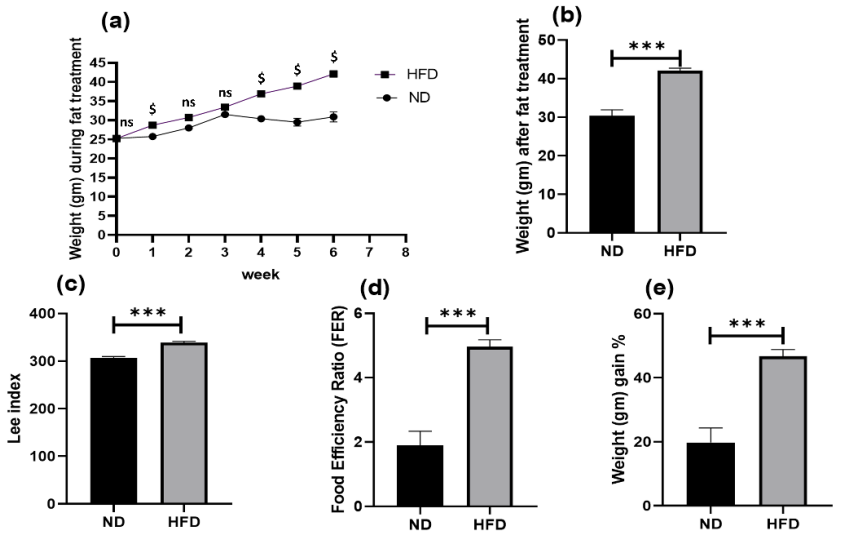

Figure 2. Assessment of HFD-induced obesity in mice. (a) Body-weight changes during the HFD induction period of 6 weeks, (b) Final body weight, (c) Lee index, (d) Food efficiency ratio, and (e) % of body-weight gain. Values are presented as mean ± SEM; Statistical significance was calculated using both two-way ANOVA with a post hoc multiple comparison test (for Fig. 2 (a)) and Student's t-test (for Fig. 2 (b) to 2 (e)). The symbol $ in Fig. 2 (a) indicates statistically significant differences in the HFD group compared with the ND group at the same time point. In the case of Fig. 2 (b)-2 (e), *p < 0.05, **p < 0.01, and ***p < 0.001

Impact of Tramadol on Food Consumption and Body Weight

During the 6 weeks of treatment with tramadol, the body weight and the food intake of the mice were measured. During treatment, food intake and body weight were lower in the tramadol-treated group than in the HFD group. Following the medication, the tramadol group's body weight significantly decreased compared to the obese (HFD) group. Figure 3 presents the data.

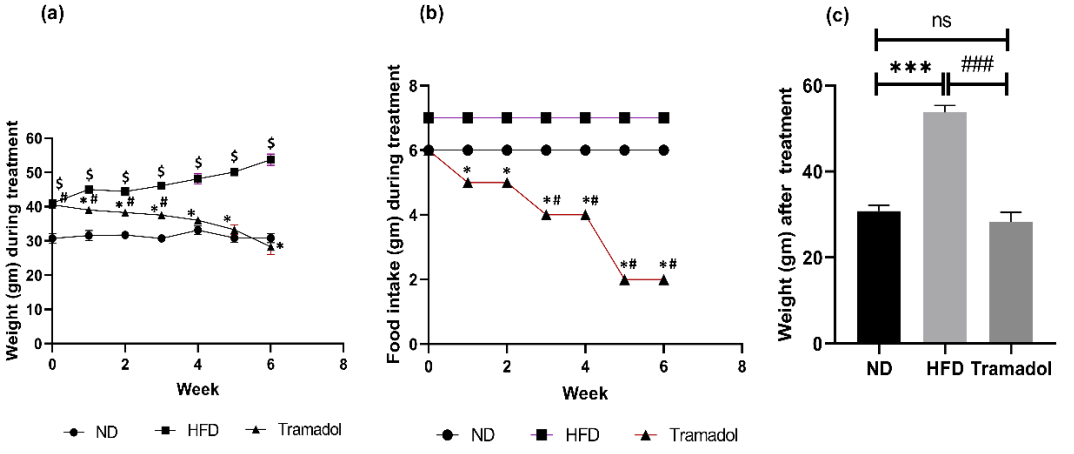

Figure 3. The effect of tramadol on the body weight and food intake of obese mice. (a) Changes in mean body weight during the 6 weeks of drug administration, (b) Food intake pattern during treatment, and (c) Final body weight after treatment. Values are presented as Mean ± SEM. Statistical significance was calculated using both two-way ANOVA (for Fig. 3 (a) and 3 (b)) and one-way ANOVA (for Fig. 3 (c)) with Tukey's post hoc multiple comparison. Different symbols ($ for ND vs HFD, # for ND vs Tramadol, and * for HFD vs Tramadol) in Fig. 3 (a) and 3 (b) indicate statistically significant differences among the other specified groups at the same time point. In Fig. 3 (c), ***p < 0.001 in comparison with the ND group, and ###p < 0.001 in comparison with the HFD group

Effect of Tramadol on Adipose Tissue

Following 6 weeks of treatment, the mice were dissected, and adipose tissue was collected. The area of fat cells was histologically observed and measured. The area of adipocytes in the tramadol-treated group increased non-significantly compared with the HFD group. Data are presented in Figure 4.

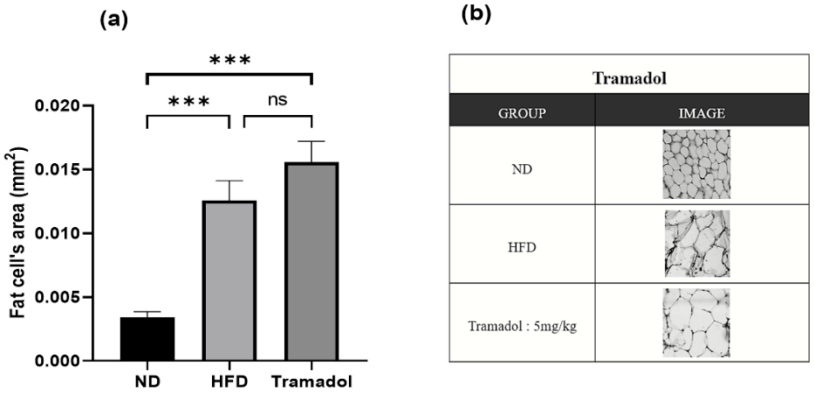

Figure 4. The effect of tramadol on adipose tissue of obese mice. (a) Tramadol treatment for 6 weeks increased the area of fat cells in obese mice (ND vs HFD: p = 0.0004), and (b) Comparison of fat cells from the histology study. Values are presented as Mean ± SEM. One-way ANOVA with Tukey's post hoc multiple comparison test; ***p < 0.001 in comparison with the ND group

Effect of Tramadol on Body Organ Weight

The average weight of the kidneys, liver, and heart in the three mouse groups was determined. Following tramadol treatment, organ weights were significantly lower in the tramadol-treated group than in the HFD group. Figure 5 shows the data.

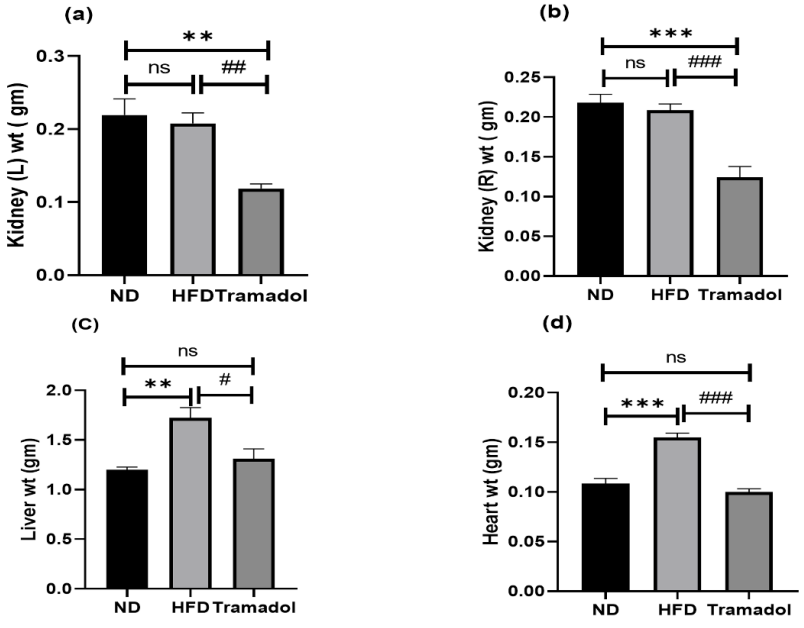

Figure 5. The effect of tramadol on the body organ weights of obese mice. (a) Left Kidney (HFD vs Tramadol: p = 0.0089 and ND vs Tramadol: p = 0.0039), (b) Right Kidney (HFD vs Tramadol: p = 0.0005 and ND vs Tramadol: p = 0.0002), (c) Liver (ND vs HFD: p = 0.0019 and HFD vs Tramadol: p = 0.0140), and (d) Heart. Values are presented as Mean ± SEM. Statistical significance was calculated using a one-way ANOVA test with Tukey's post hoc multiple comparison; **p < 0.01, ***p < 0.001 when compared with the ND group, and #p <0.05, ##p < 0.01, ###p < 0.001 when compared with the HFD group

Effect of Tramadol on Lipid Profiles

In this study, the levels of TG, TC, LDL-C, and VLDL-C were significantly higher in the HFD group compared to the ND group, and the HDL-C levels there were significantly lower compared to the controls. However, after 6 weeks of medication treatment, the tramadol group showed a significant increase in all lipid profiles compared to the HFD group. The data are presented in Figure 6.

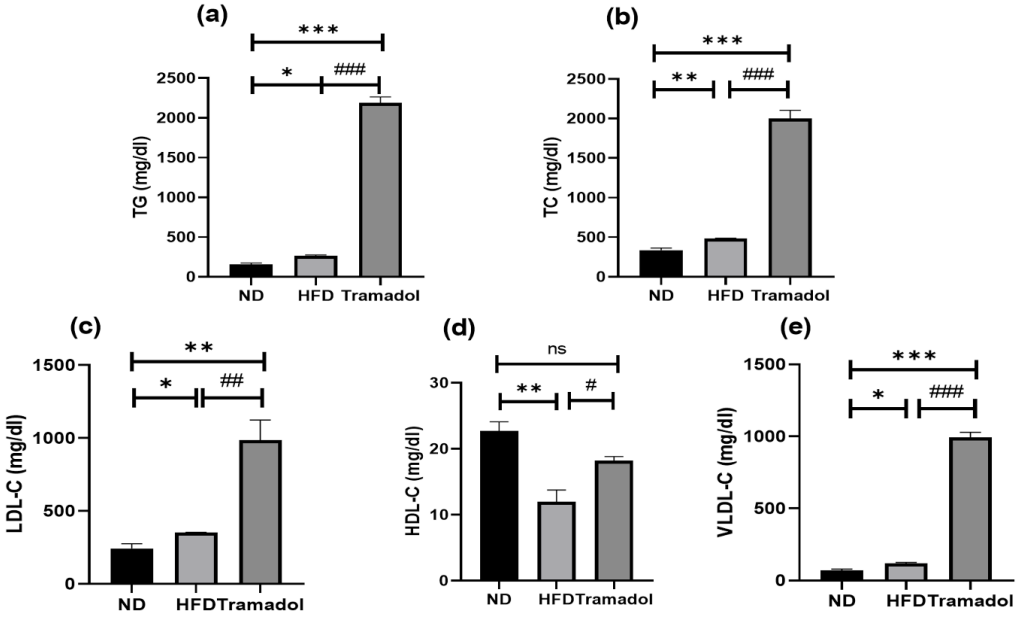

Figure 6. The effect of tramadol on lipid profiles of HFD-induced obese mice. (a) Triglyceride (TG), (b) Total cholesterol (TC), (c) Low-density lipoprotein cholesterol (LDL-C) (ND vs Tramadol: p = 0.0016 and HFD vs Tramadol: p = 0.0037), (d) High-density lipoprotein cholesterol (HDL-C) (ND vs HFD: p = 0.0035 and HFD vs Tramadol: p = 0.0410), and (e) Very low-density lipoprotein cholesterol (VLDL-C). Values are presented as Mean ± SEM. Statistical significance was assessed using a one-way ANOVA with Tukey's post hoc multiple comparison test; *p < 0.05, **p < 0.01, ***p < 0.001 when compared with the ND group, and #p <0.05, ##p < 0.01, ###p < 0.001 when compared with the HFD group

Impact of Tramadol on Hepatic & Renal Function

Here, the biochemical tests of AST and ALP were performed to assess liver function effectiveness. Additionally, creatinine levels were also measured as a kidney function test. Compared to the ND group, AST, ALP, and creatinine levels in the HFD group were significantly higher. The tramadol group showed a non-significant increase in ALP values but a significant increase in AST and creatinine values compared with the HFD group. The data are shown in Figure 7.

Effect of Tramadol on the Coronary Indices

We calculated CRR, AIP, and AC levels from the lipid profile data collected in this study using specific mathematical formulas. CRR, AIP, and AC levels were significantly higher in the HFD group than in the ND group. In addition, CRR, AIP, and AC levels in the tramadol group were significantly higher than in the HFD group. Figure 8 presents the data.

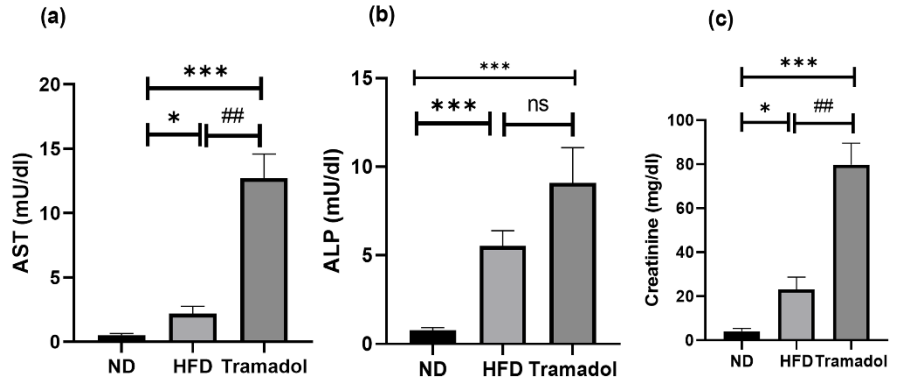

Figure 7. Effects of tramadol on hepatic and renal biochemical parameters in HFD-induced obese mice. (a) Aspartate aminotransferase (AST) Level (HFD vs Tramadol: p = 0.0001), (b) Alkaline phosphatase (ALP) Level, and (c) Creatinine level (ND vs Tramadol: p = 0.0004 and HFD vs Tramadol: p = 0.0021). Values are presented as Mean ± SEM. Statistical significance was determined using a one-way ANOVA with Tukey's post hoc multiple comparison test; *p < 0.05, ***p < 0.001 when compared with the ND group, and ##p < 0.01 when compared with the HFD group

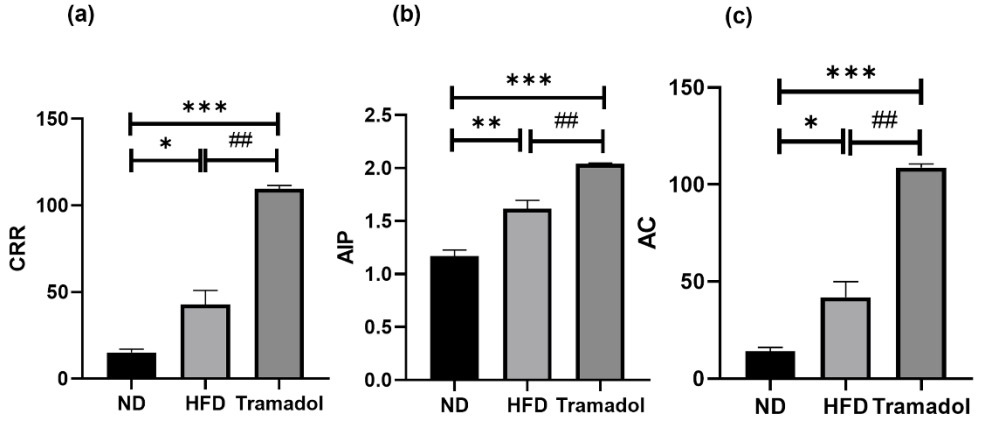

Figure 8. Effects of tramadol on the coronary indices of obese mice. (a) Coronary risk ratio (CRR) (ND vs HFD: p = 0.0161 and HFD vs Tramadol: p = 0.0002), (b) Atherogenic index of plasma (AIP) (ND vs HFD: p = 0.0028 and HFD vs Tramadol: p = 0.0039), and (c) Atherogenic coefficient (AC) (ND vs HFD: p = 0.0161 and HFD vs Tramadol: p = 0.0002). Values are presented as Mean ± SEM. Statistical significance was assessed using a one-way ANOVA with Tukey's post hoc multiple comparison test; *p < 0.05, **p < 0.01, ***p < 0.001 in comparison with the ND group, and ##p < 0.01 in comparison with the HFD group

DISCUSSION

The present study investigated the effects of tramadol administration in high-fat diet (HFD)-induced obese mice. The study evaluated food intake, body weight, adipose morphology, organ weight, lipid profiles, hepatic and renal biochemical markers, and lipid-derived cardiovascular risk indices. The principal finding was a discordant metabolic response to tramadol exposure. Tramadol treatment was associated with reduced food intake and body weight. However, these alterations coincided with a rise in adipocyte area, an unfavorable serum lipid profile, variations in hepatic and renal biochemical markers, and elevated atherogenic indices. Thus, the reduction in body weight should not be interpreted independently as evidence of a beneficial metabolic effect of tramadol in obese mice.

A particularly important finding was the alteration of the serum lipid profile following tramadol treatment. Compared with the HFD group, tramadol ad-

ministration significantly increased TG, TC, LDL-C, and VLDL-C. This pattern indicates an adverse change in lipid homeostasis and differs from the decrease in body weight. Consistent with the present findings, Barbosa et al. (2020) also reported alterations in serum lipid parameters following repeated administration of therapeutic doses of tramadol, together with biochemical evidence of hepatobiliary involvement in rats (Barbosa et al., 2020). The increased CRR, AIP, and AC observed in the present study further support the presence of an adverse lipid-associated phenotype following tramadol exposure. Since these indices are calculated from circulating lipid contents, they should be interpreted as surrogate indicators of atherogenic risk. This suggests that tramadol in the context of obesity may shift the lipid balance toward an atherogenic state. This observation aligns with prior reports that chronic tramadol exposure may elevate oxidative stress and disrupt lipid homeostasis in animal models and humans (Ali et al., 2020; Barbosa et al., 2020; Owoade, Adetutu, & Olorunnisola, 2019).

The observed changes in hepatic and renal biochemical parameters provide additional evidence of altered biochemical status following tramadol administration. In the present study, tramadol treatment was associated with increased AST, ALP, and creatinine levels. A previous study also reported dose-dependent increases in hepatic and renal function markers, including AST and creatinine, following tramadol administration in rats, together with tissue oxidative and molecular alterations (Ali et al., 2020). Similarly, repeated administration of clinical doses of tramadol in Wistar rats results in increased hepatic enzymes, altered lipid metabolism, and consistent biochemical and histological findings with hepatic and renal injury (Barbosa et al., 2020). These previous observations provide experimental context for the hepatic and renal biochemical changes observed in the present study. However, the available data cannot determine the underlying mechanisms. Changes in hepatic biochemical markers may indicate oxidative damage to liver tissues and impaired liver function, as previously reported in models of tramadol-induced hepatotoxicity (Arafa & Atteia, 2018). These findings are consistent with the understanding that obesity itself predisposes to organ vulnerability, and tramadol exposure can amplify this susceptibility.

The worsening of lipid and coronary risk indices, despite the reduction in body weight, may imply the involvement of nutrient-independent mechanisms. This could be due to alterations in pharmacokinetics and increased exposure to O-desmethyltramadol (M1), a metabolite with greater μ-opioid receptor affinity and analgesic potency than the parent drug (Xu et al., 2021). In addition, it has also been reported that chronic or high-dose tramadol administration leads to a significant rise in plasma leptin concentrations in both lean and obese individuals (Fathy Ahmad, Attalla, Attalla, & Nader, 2022). Despite elevated leptin levels, tramadol abuse can lead to leptin resistance (Cui, López, & Rahmouni, 2017; Engin, 2024). It is unclear if changes in leptin signaling contribute to the observed metabolic phenotype, which should be studied further in future investigations. Thus, the interplay between CYP2D6-mediated metabolism, oxidative stress, and lipid dysregulation could explain the observed metabolic changes.

Taken together, these findings suggest that tramadol use in obese individuals may carry unrecognized cardiometabolic risks, particularly with prolonged administration. A recent systematic review and meta-analysis has indicated that higher AIP is associated with increased TG, lower HDL-C, obesity-related metabolic abnormalities, and cardiovascular risk (Lioy et al., 2023). Elevated CRR, AIP, and AC levels have been determined to be established predictors of atherogenic risk and atherosclerotic cardiovascular disease (Akici et al., 2020; Nimmanapalli, Kasi, Devapatla, & Nuttakki, 2016). Therefore, the increased AIP, CRR, and AC observed in the present study strengthen the interpretation that tramadol exposure may be associated with unfavorable cardiometabolic risks in HFD-induced obese mice. Clinically, these data

highlight the importance of cautious tramadol use in obese patients, with consideration for regular lipid monitoring and cardiovascular risk assessment.

Several limitations in this research should also be acknowledged, including the relatively small sample size, short treatment duration, and use of a mouse model. These may limit generalizability to humans. Direct cardiovascular, molecular, and pharmacokinetic assessments were not performed. Furthermore, the use of chloroform for euthanasia represents a potential methodological limitation, although the animal experiment was conducted following approval by the institutional ethics committee. The potential toxicological effects of chloroform euthanasia and the possibility of its influence on biochemical or tissue-related measurements cannot be completely excluded.

CONCLUSION

In this study, tramadol treatment in high-fat diet-induced obese mice was associated with a complex metabolic profile. While tramadol treatment was associated with reduced food intake and decreased body weight, these changes were accompanied by significant adverse alterations in multiple cardiometabolic parameters. Specifically, tramadol exposure led to unfavorable lipid profiles characterized by elevated TG, TC, LDL-C, and VLDL-C, along with increased hepatic and renal biochemical markers (AST, ALP, and creatinine). Furthermore, the significant elevations in atherogenic indices, including the AIP, AC, and CRR, indicate an unfavorable atherogenic lipid profile and potentially increased cardiometabolic risk.

Collectively, these findings suggest that the reduction in body weight observed with tramadol treatment does not translate into metabolic benefit in the context of obesity. Instead, tramadol may contribute to an unfavorable cardiometabolic profile through mechanisms that cannot be explained solely by the reduction in food intake and body weight. The coexistence of reduced body weight with unfavorable alterations in lipid profiles and atherogenic indices warrants further investigation. These findings may support consideration of metabolic monitoring in future clinical studies of tramadol use in individuals with obesity. Further studies incorporating detailed metabolic, pharmacokinetic, molecular, histopathological, and cardiovascular assessments are warranted to clarify the physiological significance.

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AUTHOR CONTRIBUTION STATEMENT

ME: Study design, Experiment, Data analysis. MARR and IA: Data analysis, Manuscript drafting, and finalization. KNJ and MAR: Data analysis, Manuscript correction. MSH: Concept, Study design, Supervision, Manuscript finalization. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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