

Dose-dependent effects of transdermal fentanyl on hepatic structural integrity, biochemical function and markers of intrinsic mitochondrial apoptosis in rats

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Abstract: Background: Transdermal fentanyl is widely used for pain management; however, whether high systemic doses exert direct hepatotoxic effects or trigger intrinsic liver apoptotic pathways independently of systemic hypoxia remains unclear. **Objectives:** This study aimed to investigate the dose-dependent effects of transdermal fentanyl on hepatic structural integrity, biochemical function and markers of intrinsic mitochondrial apoptosis (Bcl-2 and caspase-3) in rats. **Methods:** Thirty-two Wistar albino rats were randomly assigned to four groups (n = 8): a control group and three experimental groups treated with transdermal fentanyl at doses of 3.125 µg/kg/h (Group I), 6.25 µg/kg/h (Group II) and 12.5 µg/kg/h (Group III). Patches were applied to the abdominal region and replaced every 72 hours for nine days. At the end of the experiment, liver tissues were collected for biochemical and histopathological and molecular analyses of apoptotic markers. Apoptotic changes were assessed by measuring caspase-3 and Bcl-2 levels. **Results:** Serum alanine aminotransferase (ALT) and total cholesterol levels were significantly higher in Group II and III than in the control group ($p < 0.05$). Glucose levels were significantly increased in all fentanyl-treated groups ($p < 0.05$). Total protein levels were significantly elevated in Group III compared to the control group ($p < 0.05$). Lactate dehydrogenase (LDH) levels were significantly lower in Groups II and III ($p < 0.05$). The anti-apoptotic marker Bcl-2 was significantly decreased in Group III compared with the other groups ($p < 0.05$), whereas no statistically significant differences were observed in caspase-3 levels among any of the groups. No evident histopathological alterations were detected in liver tissues. **Conclusion:** High-dose transdermal fentanyl induces significant biochemical alterations and reduces Bcl-2 levels in rat liver tissue, suggesting a potential shift toward apoptotic signaling despite the absence of significant caspase-3 changes. However, short-term administration does not appear to cause evident histopathological damage.

Keywords: Apoptosis; Bcl-2; Caspase-3; Histopathology; Liver function tests; Transdermal fentanyl

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INTRODUCTION

Pain is a complex response of the organism to internal and external stimuli and is defined as a subjective experience involving both physiological and psychological components. From a physiological perspective, pain typically arises in response to tissue injury and serves as a defense mechanism aimed at protecting the organism from harmful stimuli. Acute pain, which occurs as a result of tissue damage, is often associated with inflammatory processes and usually subsides once healing is complete. However, in some cases, pain may persist despite tissue recovery, a condition referred to as chronic pain. Chronic pain is more than a physical symptom; it represents a significant health problem that impairs quality of life, may lead to mood disorders and poses a complex therapeutic challenge (Moussa and Ogle, 2022; Benzon *et al.*, 2022; Cohen *et al.*, 2021).

Effective pain management is essential for ensuring patient comfort, hemodynamic stability and overall well-being.

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Accordingly, a variety of analgesic agents are used in clinical practice. Among these, opioid analgesics are the most frequently employed, particularly for the treatment of severe pain. Fentanyl, a synthetic phenylpiperidine derivative, is distinguished by its potent and rapid onset of action. However, because of its short duration of effect, continuous systemic administration is often required. This limitation has been largely overcome by the development of transdermal fentanyl formulations. Consequently, transdermal fentanyl is widely used in the management of cancer-related and other forms of severe chronic pain (O'Neill and Lirk, 2022; Mestdagh *et al.*, 2023; Niculae *et al.*, 2024).

Fentanyl is highly lipophilic, enabling rapid penetration into the central nervous system and a prompt analgesic effect. It is primarily metabolized in the liver by the cytochrome P450 3A4 (CYP3A4) enzyme. Because CYP3A4 is also involved in the metabolism of numerous other drugs, the liver is particularly vulnerable to the potential toxic effects of pharmacological agents (Taylor *et al.*, 2023; Skrzypczak *et al.*, 2022).

Beyond its metabolic role, the liver is central to vital processes such as protein synthesis, biochemical storage and detoxification. Therefore, any structural or functional impairment of the liver may lead to serious systemic consequences. Although many drugs are known to exert hepatotoxic effects, studies specifically examining the impact of fentanyl on hepatic tissue remain limited. This gap highlights the need for further investigation into whether fentanyl induces histopathological or cellular alterations in the liver (Ono *et al.*, 2023).

Chronic pain associated with malignancy is common among oncology patients and profoundly affects quality of life. In this population, transdermal fentanyl is frequently preferred because of its efficacy and ease of administration. At the cellular level, however, malignancies are often characterized by disruptions in apoptotic mechanisms, which may contribute to disease progression. Caspase-3, a key executioner enzyme in apoptosis, plays a critical role in programmed cell death, whereas Bcl-2 functions as an anti-apoptotic regulator that protects cells from apoptotic destruction (Qian *et al.*, 2023; Pisani *et al.*, 2020). However, despite the extensive clinical use of transdermal fentanyl, its specific dose-dependent impact on organ-level toxicity and apoptotic signaling pathways, particularly within hepatic tissue, remains insufficiently characterized. Therefore, the combined assessment of caspase-3 and Bcl-2 levels represents a valuable approach for evaluating the potential apoptotic effects of transdermal fentanyl. Such data may clarify the biological impact of fentanyl beyond its analgesic properties.

In this context, the primary aim of the present study is to evaluate the dose-dependent biochemical and histopathological effects of transdermal fentanyl on liver tissue in an experimental animal model. The secondary objective is to investigate potential cellular damage by assessing apoptotic markers, particularly caspase-3 and Bcl-2. The findings are expected to contribute valuable evidence to the literature regarding the safe use of transdermal fentanyl.

MATERIALS AND METHODS

This study was conducted following the approval of the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University (Approval date: 29.09.2022; Decision No: 2022/09-10). Additionally, this research was supported by the Van Yuzuncu Yil University Scientific Research Projects Coordination Unit (Project No: TTU-2022-10403).

Animals and housing conditions

Thirty-two healthy male Wistar albino rats, aged 3 months and weighing approximately 250 g, were used in this study. All rats were housed under controlled environmental conditions (temperature: 21 ± 1 °C; relative humidity: 40–

70%; 12 hours light/dark cycle) and were provided with standard laboratory chow and tap water *ad libitum* throughout the experiment.

Inclusion and exclusion criteria

Inclusion criteria: Healthy male Wistar albino rats with no history of previous experimental procedures or exposure to pharmacological agents before enrollment.

Exclusion criteria: Animals showing signs of illness, injury, or abnormal behavior before randomization were to be excluded. During the experiment, animals would have been excluded if severe treatment-related complications or inadequate blood or liver tissue samples had prevented biochemical or histopathological evaluation. However, no animals met these criteria and no exclusions occurred during the study.

Experimental Design and Animal Grouping

The animals were randomly assigned to four groups ($n = 8$ per group) and identified by tail marking. Transdermal fentanyl patches (Duragesic®, Janssen, Belgium; Licence No: 10.03.2000-107/66) used in the experimental groups were supplied by the pharmacy of Van Yuzuncu Yil University Hospital within the scope of the institutional research project (Project No: TTU-2022-10403), in accordance with national regulations for Schedule II controlled substances.

- **Group C (Control):** Rats received no intervention and were observed under the same conditions as the experimental groups.
- **Group I:** Rats received transdermal fentanyl at a dose of 3.125 µg/kg/h.
- **Group II:** Rats received transdermal fentanyl at a dose of 6.25 µg/kg/h.
- **Group III:** Rats received transdermal fentanyl at a dose of 12.5 µg/kg/h.

Based on a preliminary pilot study, higher doses of transdermal fentanyl (100 and 200 µg/kg/h) resulted in rapid mortality within minutes, whereas 12.5 µg/kg/h was identified as the highest dose compatible with survival. Therefore, doses of 3.125, 6.25 and 12.5 µg/kg/h were selected for the present study.

Before patch application, the abdominal area of each rat was shaved and cleaned. The patches were then affixed directly to this region. To prevent removal, animals were housed individually in separate cages. Patches were replaced every 72 hours in accordance with the manufacturer's instructions and treatment was continued for a total of 9 days. At the end of the experimental period, all animals were anesthetized by intraperitoneal injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg) and subsequently euthanized by exsanguination. Blood samples were collected by intracardiac puncture and serum and plasma were separated and stored at -80 °C until analysis.

Apoptotic and biochemical analyses

Serum caspase-3 and Bcl-2 levels were determined using rat-specific commercial ELISA kits (Sunred, Catalog No: SRB-T-81677-96T; supplied by Farmasina Tibbi ve Kimyevi Urunler San. ve Dis Tic., Turkey) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (BioTek ELx800, USA).

Serum biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total cholesterol, triglycerides, total protein, albumin, glucose and total bilirubin, were analyzed using commercial assay kits (Vacusera, Turkey) with an automated biochemical analyzer (Abbott ARCHITECT ci16200, USA) in accordance with the manufacturer's protocols.

Histopathological analyses

After euthanasia, liver tissue specimens were promptly harvested and fixed in 10% neutral-buffered formalin for at least 24 hours. Following fixation, tissues underwent routine histopathological processing, including dehydration through graded ethanol, clearing in xylene and paraffin embedding. Sections of 4 µm thickness were cut using a rotary microtome (Leica RM2135, Germany) and mounted on glass slides. The sections were then deparaffinized, rehydrated and stained with hematoxylin and eosin (H&E) according to standard procedures to evaluate general hepatic architecture. Stained sections were examined under a light microscope (Nikon 80i-DSRI2, Japan) at various magnifications. All histopathological assessments were performed by an experienced pathologist blinded to the experimental groups.

Statistical analysis

Descriptive statistics were expressed as mean, standard deviation (SD) or median (Q1-Q3), as appropriate. The distribution of variables was assessed using the Kolmogorov-Smirnov test. Given the relatively small sample size ($n = 8$ per group), the limited power of the Kolmogorov-Smirnov test to detect deviations from normality was taken into consideration. The Kruskal-Wallis test was used for overall comparisons among groups. When a statistically significant difference was observed, pairwise comparisons were performed using the Mann-Whitney U test as a post hoc analysis. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Biochemical results

Baseline liver function biomarkers

Table 1 summarizes the biochemical results. Comparative evaluation of liver function biomarkers, including albumin,

ALP, AST, bilirubin and triglycerides, showed no statistically significant differences among the groups ($p > 0.05$)

Alanine aminotransferase (ALT)

ALT levels were significantly higher in Groups II and III than in the Control Group ($p < 0.05$). However, no significant differences were observed between Group I and the control group or among Groups I, II and III ($p > 0.05$)

Total cholesterol

Total cholesterol levels were significantly increased in Groups II and III compared with the Control Group ($p < 0.05$). In contrast, no statistically significant differences were found between Group I and the Control Group or between Groups II and III ($p > 0.05$)

Blood glucose

Glucose levels in Groups I, II and III were significantly higher than those in the Control Group ($p < 0.05$). However, no significant differences were detected among the fentanyl-treated groups ($p > 0.05$)

Lactate dehydrogenase (LDH)

LDH levels were significantly lower in Groups II and III than in both Group I and the Control Group ($p < 0.05$). No significant differences were observed between Group I and the Control Group or between Groups II and III ($p > 0.05$)

Total protein

Total protein levels were significantly increased in Group III compared with the Control Group ($p < 0.05$). However, no statistically significant differences were observed among Groups I, II and III or between Group I or II and the control group ($p > 0.05$)

Bcl-2 protein expression

Table 2 presents the Bcl-2 results. The anti-apoptotic marker Bcl-2 was significantly decreased in Group III compared with Groups I and II and the Control Group ($p < 0.05$). In contrast, no significant differences were observed among the Control Group, Group I and Group II ($p > 0.05$)

Caspase-3 activity

Table 3 summarizes the caspase-3 results. Intergroup comparisons revealed no statistically significant differences among the groups ($p > 0.05$)

Histopathological evaluation of hepatic structure

Microscopic examination of liver tissues from the Control Group revealed normal histological architecture. Hepatocytes and portal areas appeared normal, with hepatocytes arranged in regular hepatocyte plates around the central vein and normal-appearing sinusoids between the plates (Fig. 1A). Histopathological evaluation of liver sections from Groups I–III (Figs. 1B–D) demonstrated no morphological alterations. All sections exhibited histological features similar to those observed in the Control Group.

Table 1: Comparison of liver function tests between groups

Biochemical parameters	Group C Mean $\pm$ SD	Group I Mean $\pm$ SD	Group II Mean $\pm$ SD	Group III Mean $\pm$ SD	<i>p</i> -value
Albumin (g/L)	39.3 $\pm$ 5.8	43.2 $\pm$ 1.52	44.0 $\pm$ 1.49	42.3 $\pm$ 1.38	0.119*
ALP (IU/L)	148 $\pm$ 40.9	147 $\pm$ 19.5	143 $\pm$ 27.3	150 $\pm$ 35.8	0.955*
ALT (IU/L)	32.9 $\pm$ 6.8	37.0 $\pm$ 4.64	39.0 $\pm$ 3.53	54.0 $\pm$ 37.5	0.024*
AST (IU/L)	115 $\pm$ 24.9	113 $\pm$ 16.2	103 $\pm$ 15.5	117 $\pm$ 35.3	0.453*
Bilirubin (mg/dL)	0.06 $\pm$ 0.02	0.06 $\pm$ 0.01	0.07 $\pm$ 0.05	0.06 $\pm$ 0.01	0.166*
Total cholesterol (mg/dL)	51.6 $\pm$ 8.7	61.2 $\pm$ 8.9	60.5 $\pm$ 5.8	64.5 $\pm$ 6.6	0.039*
Glucose (mg/dL)	139 $\pm$ 23.8	180 $\pm$ 38.1	357 $\pm$ 504	176 $\pm$ 25.1	0.024*
LDH (IU/L)	1450 $\pm$ 336	1560 $\pm$ 261	1073 $\pm$ 363	902 $\pm$ 436	0.007*
Total protein (g/L)	55.6 $\pm$ 8.7	62.0 $\pm$ 2.47	61.8 $\pm$ 2.19	64.0 $\pm$ 2.20	0.026*
Triglycerides (mg/dL)	82.7 $\pm$ 27.4	75.1 $\pm$ 23.3	55.8 $\pm$ 16.5	70.6 $\pm$ 28.7	0.135*

*Kruskal-Wallis, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase

Table 2: Evaluation of Bcl-2 results between groups

Group Name	Bcl-2 (ng/mL) Median (Q1-Q3)	<i>p</i> -value
Group C ^a	1.17 (1.02-1.34)	0.024
Group I ^a	1.14 (1.10-1.26)	
Group II ^a	1.22 (1.10-1.28)	
Group III ^b	0.55 (0.24-1.12)	

Kruskal–Wallis test; Mann–Whitney U test for pairwise comparisons. ^aGroups not significantly different from each other ($p > 0.05$);

^bSignificantly different from groups marked with superscript ^a ($p < 0.05$).

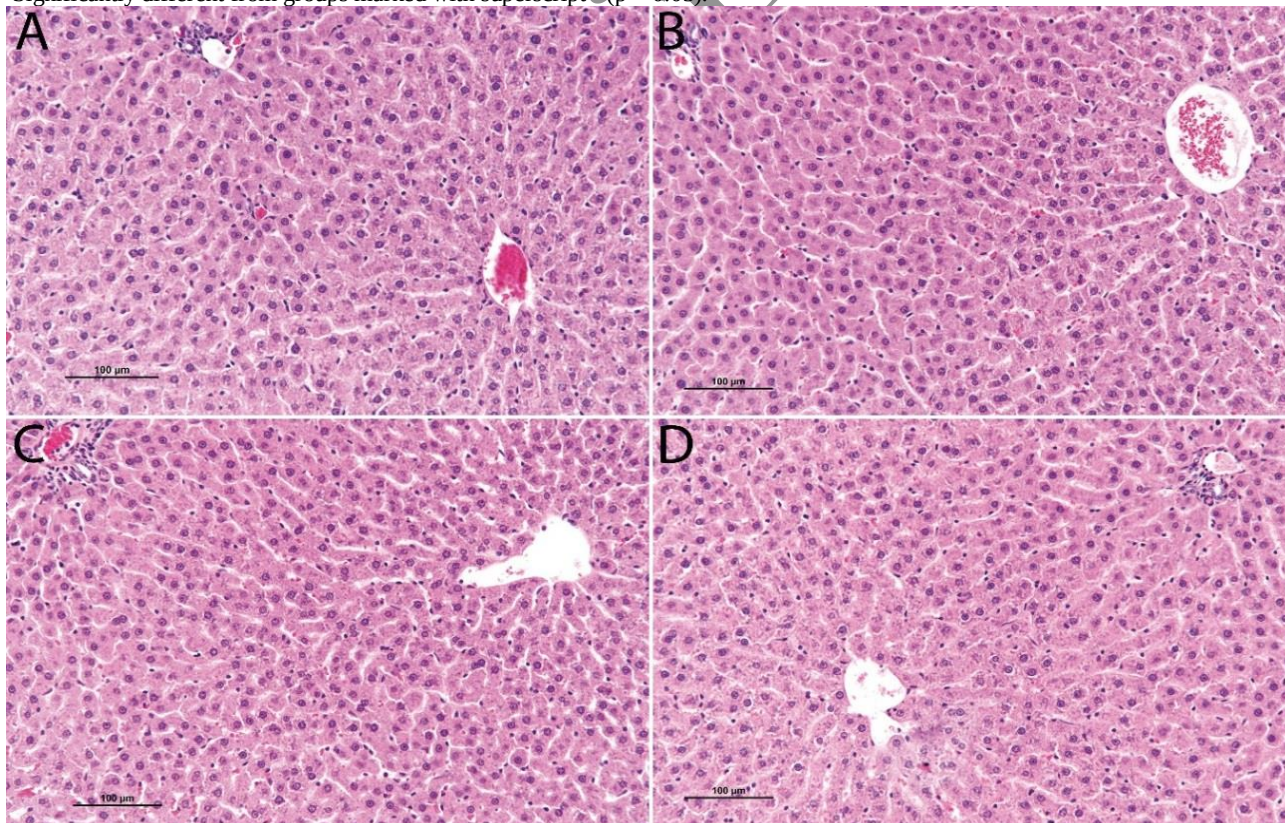


Fig. 1: Representative histological images of liver sections across experimental groups. (A) Control Group; (B) Group I; (C) Group II; (D) Group III, All groups exhibit normal hepatic architecture with regular hepatocyte plates radiating from the central vein and intact sinusoids (H&E stain; scale bar = 100 μ m).

Table 3: Evaluation of caspase-3 results between groups

Group Name	Caspase-3 (ng/mL) Median (Q1-Q3)	p-value
Group C	1635 (1405-1795)	0.233
Group I	1494 (1349-1692)	
Group II	1487 (1270-1565)	
Group III	1338 (1160-1550)	

Kruskal–Wallis test.

DISCUSSION

The aim of the present study was to investigate the apoptotic, biochemical and histopathological effects of transdermal fentanyl, which is widely used in the management of chronic pain, particularly malignancy-related pain. Given that malignancies are associated with dysregulation of apoptotic balance at the cellular level, understanding the effects of fentanyl on apoptosis-related markers may provide insight into its broader biological impact beyond analgesia. Accordingly, caspase-3, a key executioner enzyme in apoptosis and Bcl-2, a major anti-apoptotic protein, were evaluated, together with routine biochemical and histopathological parameters in healthy rat liver tissue.

One of the main findings of this study was that administration of high-dose transdermal fentanyl (12.5 µg/kg/h) significantly reduced Bcl-2 levels in liver tissue, whereas no significant changes were observed at lower doses. These results suggest that fentanyl at higher doses may shift the apoptotic balance toward a pro-apoptotic state by downregulating anti-apoptotic defense mechanisms.

Similar reductions in Bcl-2 expression have been reported in tumor models treated with fentanyl, in which decreased Bcl-2 and increased Bax expression were associated with inhibition of tumor growth (He *et al.*, 2016). In the healthy rat model, a comparable decrease in Bcl-2 was observed; however, this finding should be interpreted with caution, as tumor microenvironments and signaling pathways differ substantially from those in normal hepatic tissue.

In contrast, no statistically significant differences in caspase-3 levels were detected among the groups. This finding differs from previous studies reporting increased caspase-3 activity following fentanyl administration in tumor models (Zhang *et al.*, 2014) or in other tissues, such as the cerebellum of neonatal pigs (Sabir *et al.*, 2018). Moreover, transdermal fentanyl was recently shown to increase caspase-3, -8 and -9 activity in the reproductive tissues of male rats (Kosal *et al.*, 2025), indicating that fentanyl can activate classical apoptotic pathways in a tissue-dependent manner. The discrepancy between these findings and the present results may be explained by differences in experimental design, including disease status, tissue examined, route of administration, dosage and

duration of exposure. Importantly, the reduction in Bcl-2 without a concomitant increase in caspase-3 suggests that fentanyl-induced apoptotic signaling in healthy liver tissue may involve caspase-3-independent pathways, potentially mediated by mitochondrial dysfunction, altered Bax/Bcl-2 balance, oxidative stress, or alternative downstream effectors.

The biochemical findings demonstrated significant increases in ALT, total cholesterol, glucose and total protein levels in fentanyl-treated groups, together with decreased LDH levels at higher doses. These alterations may reflect early metabolic and hepatocellular responses to fentanyl exposure rather than overt liver injury, as histopathological examination revealed preserved hepatic architecture in all groups. Consistent with the findings of the present study, Yadav *et al.* (2018) reported elevations in ALT following fentanyl exposure in rodents, supporting the view that fentanyl may induce mild biochemical disturbances depending on dose, route and duration of administration.

The variability observed in glucose levels, particularly in Group II, may reflect individual metabolic responses and stress-related neuroendocrine activation induced by opioid exposure. Although no inappropriate outliers were identified, this variability represents a limitation of the present study and may have influenced statistical outcomes. The decrease in LDH levels in Groups II and III is noteworthy, as LDH is commonly regarded as a marker of tissue injury; however, reduced LDH activity may also indicate altered hepatic metabolic activity or suppressed cellular turnover under opioid exposure, as suggested by previous experimental observations (Yadav *et al.*, 2018).

In-vitro studies have reported that fentanyl may protect certain tumor-associated endothelial cells from apoptosis and promote angiogenesis (Liu *et al.*, 2020), highlighting the complex and context-dependent effects of fentanyl on apoptotic signaling. In contrast, these *in-vivo* findings in healthy liver tissue indicate a potential pro-apoptotic tendency at high doses, reflected by decreased Bcl-2 levels. These divergent results further emphasize that fentanyl's biological effects depend strongly on tissue type, disease state and experimental conditions.

Dose selection represents another important aspect of this study. In pilot observations, higher transdermal doses (100

and 200 µg/kg/h) resulted in rapid mortality, whereas 12.5 µg/kg/h was identified as the highest dose compatible with survival. This finding is consistent with the report by Dutriez-Casteloot *et al.* (2022), who observed adverse systemic effects at very high transdermal fentanyl doses in neonatal rats, underscoring the narrow safety margin of fentanyl in experimental settings.

From a clinical perspective, transdermal fentanyl is widely prescribed for chronic and cancer-related pain because of its efficacy and ease of use (Lakshmi and Chandrasekhar, 2022; Hall *et al.*, 2023; Niculae *et al.*, 2024). Although the present findings were obtained in an experimental animal model, the observed biochemical alterations and modulation of apoptotic markers at higher doses suggest that even transdermal administration may exert early hepatic effects. These results highlight the potential importance of monitoring liver function, particularly in patients receiving high-dose or long-term fentanyl therapy.

Several limitations of the present study should be acknowledged. First, the control group comprised naive (untreated) rats without the application of a drug-free vehicle or sham patch. Consequently, potential confounding stress responses associated with skin shaving, adhesive patch placement and physical wraps (such as elevated systemic corticosterone levels that can independently influence blood glucose, liver metabolism and apoptotic pathways) could not be fully isolated from fentanyl-specific effects. Second, abdominal patch placement presents a potential risk of animal grooming, licking, or chewing. Oral ingestion of patch contents alters drug pharmacokinetics by introducing direct first-pass hepatic metabolism, which differs from true continuous transdermal absorption. Third, transdermal fentanyl patches are engineered for 72-hour zero-order delivery through human skin; however, rodent skin possesses higher permeability and drug flux rates, which may lead to accelerated drug depletion and variable plasma concentration dynamics over 72 hours. Fourth, the administration duration was relatively brief (9 days) and longer chronic exposure may yield different results.

Future studies should evaluate the long-term hepatic effects of transdermal fentanyl using extended treatment periods, different dosing regimens and additional molecular markers involved in apoptosis and oxidative stress. Inclusion of vehicle control groups and pharmacokinetic assessments would further improve understanding of the hepatic safety profile of transdermal fentanyl.

CONCLUSION

Administration of transdermal fentanyl for 9 days did not result in observable histopathological damage in rat liver tissue. However, exposure to the highest dose (12.5

µg/kg/h) was associated with a significant reduction in serum Bcl-2 levels alongside alterations in specific biochemical parameters, including elevated ALT, blood glucose, total cholesterol and total protein levels, alongside reduced serum LDH activity, suggesting early hepatic and apoptotic responses. At the molecular level, high-dose fentanyl significantly downregulated the anti-apoptotic marker Bcl-2, indicating early mitochondrial apoptotic priming. However, the absence of concomitant changes in serum caspase-3 levels, combined with preserved tissue architecture, indicates that short-term transdermal fentanyl administration at these doses does not induce overt hepatic necrosis or executioner caspase activation.

Overall, while high-dose transdermal fentanyl induces select serum biochemical and protein shifts, these findings should be interpreted cautiously due to the 9-day exposure duration and small sample size ($n = 8$). Further studies incorporating tissue-specific molecular assays (such as Western blotting or immunohistochemistry), extended treatment periods and varied dosing regimens are required to fully define the hepatic safety profile of transdermal fentanyl.

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Author's contributions

Conceptualization: Ozdemir Z., Kurt N., Keles O.F. and Tekeli A.E.; Methodology: Kurt N., Ozdemir Z. and Soyalp C.; Resources: Ozdemir Z., Kurt N., Keles O.F., Soyalp C. and Kilic M.; Investigation: Ozdemir Z., Kurt N., Keles O.F., Tekeli A.E., Kilic M. and Soyalp C.; Formal analysis: Ozdemir Z., Kurt N., Kilic M. and Soyalp C.; Writing – original draft: Ozdemir Z., Kurt N. and Soyalp C.; Writing – review & editing: Ozdemir Z., Kurt N., Keles O.F., Tekeli A.E. and Kilic M.; Visualization: Kurt N. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The datasets generated and/or analysed during the current study are available from the corresponding author (N.Kurt) on reasonable request.

Ethical approval

This study was conducted following the approval of the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University (Approval date: 29.09.2022; Decision No: 2022/09-10). All animal handling, experimental procedures and manuscript reporting were

carried out in compliance with the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines. This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agreed with the contents of the manuscript and there is no financial interest to report. The authors certify that the submission is original work and is not under review at any other publication.

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