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Effect of different doses of transdermal fentanyl on the reproductive system in male rats

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Abstract: Fentanyl, a potent synthetic opioid analgesic, is commonly used to manage severe pain. However, the effects of fentanyl use on male reproductive health have not been adequately studied. This study aimed to investigate the effects of different doses of transdermal fentanyl patches on various reproductive parameters in male rats. Adult male Albino Wistar rats were divided into four groups. The treatment groups received transdermal fentanyl at doses of 25 mcg/h (Group II), 50 mcg/h (Group III), and 100 mcg/h (Group IV) for 9 days, respectively. Sperm motility, sperm concentration, abnormal sperm, live/dead sperm, testicular apoptosis, testicular oxidative stress, and androgen receptor levels were evaluated. The results showed that fentanyl administration decreased the oxidative stress parameters CAT and SOD1 levels in all treatment groups ($p<0.001$). No significant changes were observed in sperm motility, abnormal sperm ratio, or live/dead sperm ratio. However, Group IV showed a significant increase in sperm concentration compared to the other groups ($p<0.001$). In addition, all fentanyl treatment groups showed a significant increase in apoptosis-related Caspase 3/8/9 enzymes ($p<0.001$). This study reveals the effects of fentanyl on male reproductive health. This is the first study to demonstrate an increase in sperm concentration associated with high fentanyl doses.

Keywords: Fentanyl, male, reproduction, sperm, testis

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INTRODUCTION

Fentanyl is a synthetic opioid analgesic drug and is used to relieve pain. Fentanyl is a synthetic phenylpiperidine that is 80 times more potent than morphine and 500 times more potent than meperidine. Fentanyl shows potent activity through opiate receptors such as mu (μ) and kappa (ƙ). These receptors are found in the central nervous system and periphery. By activating these receptors, fentanyl inhibits pain transmission and perception. The effects of fentanyl vary with the dose, duration and route of administration. Fentanyl is metabolized in the liver via the cytochrome P450 system and excreted through the kidneys. The antidote for fentanyl is naloxone (Fu *et al.,* 2024; Gauridas *et al.,* 2022; Reed *et al.,* 2024).

The Fentanyl patch consists of four layers that control the absorption of fentanyl through the skin and its passage into the bloodstream. A transdermal fentanyl patch is composed of four primary layers, each serving a specific function in drug delivery. The innermost layer consists of a silicone-based adhesive that ensures firm attachment of the patch to the skin. Above this, an ethylene-derived membrane modulates the controlled release of fentanyl over time. The third layer acts as the drug reservoir, containing fentanyl formulated with dipropylene glycol and cellulose to enhance percutaneous absorption. The outermost layer comprises a protective foil barrier that prevents drug leakage and maintains the integrity of the delivery system. (Taghizadeh *et al.,* 2010; Zecca *et al.,* 2015).

Transdermally administered fentanyl is not metabolized through the skin and has a bioavailability of 92%. Fentanyl absorption persists steadily over the 72-hour application period. Within the evaluated dosage range, serum fentanyl pharmacokinetics demonstrate linearity and remain consistent with repeated dosing (Montanari *et al.,* 2022; Barletta *et al.,* 2025).

The potential effects of powerful opioids like fentanyl, used for pain management, on the male reproductive system are not well understood. This study aims to investigate the effects of transdermal fentanyl patches, applied in different doses, on sperm motility, sperm concentration, abnormal sperm, live/dead sperm, testicular apoptosis, testicular oxidative stress, and androgen receptors. The study seeks to highlight the potential negative impacts of fentanyl use on male reproductive health.

MATERIALS AND METHODS

Animals

Healthy adult male Albino Wistar rats, aged approximately 3 to 4 months and weighing around 200 grams, were obtained from Van Yüzüncü Yıl University. The animals were housed under standardized laboratory conditions,

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including a 12-hour light/dark cycle, ambient temperature maintained between 22-24°C, and relative humidity set at 60%. Throughout the experimental period, the rats were provided with ad libitum access to standard chow and water.

Method

The abdominal areas of the rats were shaved before the experiment. No treatment was administered to Group I. Group II received a therapeutic dose of transdermal fentanyl (25 mcg/hr), Group III received a high dose (50 mcg/hr), and Group IV received a very high dose (100 mcg/hr) of transdermal fentanyl for 9 days. The transdermal fentanyl patches were replaced every 3 days. Duragesic® (Janssen, Belgium) was used as a fentanyl patch. To prevent the rats from removing the patches, each rat was housed individually in a separate cage. At the end of the application period, the rats were sacrificed under anesthesia (50 mg/kg ketamine + 10 mg/kg xylazine) using the exsanguination method (Sancak, 2023).

Spermatological examination

Motility examination, concentration analysis, abnormal sperm ratio, and live/dead sperm ratio analyses were performed immediately after sacrifice (Koşal *et al.,* 2023).

PCR

Collection of tissue samples for RNA isolation and preparation for analysis, RNA extraction and analysis, cDNA extraction, and Real-Time-qPCR analysis were performed (Livak and Schmittgen 2001). The target genes used are listed in table 1.

Histopathological examination

A complete necropsy was performed on all experimental animals, and all macroscopic changes in the testes were documented. Testicular tissue samples were collected and fixed in Bouin's solution. Following standard histologic procedures, tissues were embedded in paraffin blocks, sectioned at 4 μm thickness using a Leica RM 2135 microtome, and stained with hematoxylin and eosin (H&E). The prepared slides were then examined under a Nikon 80i light microscope equipped with a DS-Ri2 camera.

Ethical statement

The study was undertaken under agreement No. 2024/05-09 of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee, dated 30/05/2024.

STATISTICAL ANALYSIS

The statistical analysis was conducted using the SPSS v.20 software package (Chicago, IL, USA). All data were presented as mean ± standard deviation. Following PCR analysis, gene expression levels were determined using 2^(-ΔΔCt) log values derived from the Ct values. Statistical analyses of the groups were performed using one-way ANOVA, followed by post hoc multiple comparisons (Tukey's test) for comparative analysis between the groups. A p-value less than 0.05 was considered statistically significant.

RESULTS

Spermatological examination

Following the treatment of transdermal fentanyl, no statistically significant differences were observed between the groups in terms of sperm motility, abnormality rate, or live/dead ratio ($p>0.05$). However, sperm concentration analysis revealed a statistically significant increase in Group IV compared to the other groups ($p<0.001$) (table 2).

PCR analysis

The testicular PCR analysis revealed a significant decrease in antioxidant parameters, specifically in SOD1 and CAT levels, compared to Group I ($p<0.001$). In contrast, the analysis of androgen receptors and apoptosis factors (Caspase 3/8/9) showed a significant increase in all treatment groups compared to Group I ($p<0.001$) (fig. 1).

Histopathological findings

Microscopically, testicular sections from Group I (fig. 2A), Group II (fig. 2B), Group III (fig. 2C), and Group IV (fig. 2D) were found to have normal histologic appearances. However, spermatogenesis was increased in Group IV compared to the other groups. The number of spermatogenic cells increased in the basement membranes of the seminiferous tubules, and the density of spermatozoa excreted into the tubule lumen increased.

DISCUSSION

Fentanyl, a potent synthetic opioid, is widely used in clinical settings for its powerful analgesic effects, particularly in the management of chronic and severe pain (Mordeniz *et al.,* 2021). Despite its effectiveness, there is growing concern about the potential side effects of long-term opioid use, especially regarding its impact on the male reproductive system. While opioids have been extensively studied for their effects on pain perception and addiction, their influence on reproductive health remains less understood (Farzi *et al.,* 2019; Zheng, 2022). This study aims to fill that gap by investigating the effects of transdermal fentanyl patches, administered at different doses, on various reproductive parameters in males. Specifically, the study examines oxidative stress markers, sperm characteristics, testicular apoptosis, and androgen receptor levels, providing valuable insights into the broader implications of fentanyl use on male fertility.

In the present study, CAT and SOD1 levels, which are oxidative stress parameters, decreased in all Fentanyl administration groups ($p<0.05$).

Table 1: Primary sequence of target genes.

The Name of the Gene	Primary sequence sequencing	
	F. 5'-3'	R: 5'-3'
Aktin Beta (ACTB)	CTCCTCAAGGATGGCACC	GCTCATTGTAGAAAGTGTGGT
CAT	GGACGCTCAGCTTTTCATTC	TTGTCCAGAAGAGCCTGGAT
SOD1	GCTTCTGTCGTCTCCTTGCT	CATGCTCGCCTTCAGTTAATCC
AR	GTGAAATGGGACCTTGGATG	TACTGAATGACCGCCATCTG
CASPAS-3	TACCCTGAAATGGGCTTGTGT	GTTAACACGAGTGAGGATGTG
CASPAS-8	TAAGACCTTTAAGGAGCTTCATTTTGA	AGGATACTAGAACCTCATGGATTTGAC
CASPAS-9	GAGGGAAGCCCAAGCTGTTC	GCCACCTCAAAGCCATGGT

Table 2: Sperm analysis.

n=9	Motility (%)	Concentration (x10^9)	Abnormal (%)	Live/Dead (%)
Group I	82.22±4.40	1.99±0.12 ^{x}	7.55±1.42	7.88±1.61
Group II	81.25±6.40	2.01±0.12 ^{x}	7.01±1.30	9.12±1.65
Group III	80.12±5.34	2.10±0.18 ^{x}	8.12±0.64	9.87±1.72
Group IV	82.50±7.37	6.02±0.16 ^{y}	8.25±0.88	9.37±1.68

* The difference between groups with different (x. y) signs in the same column is significant (P< 0.001).

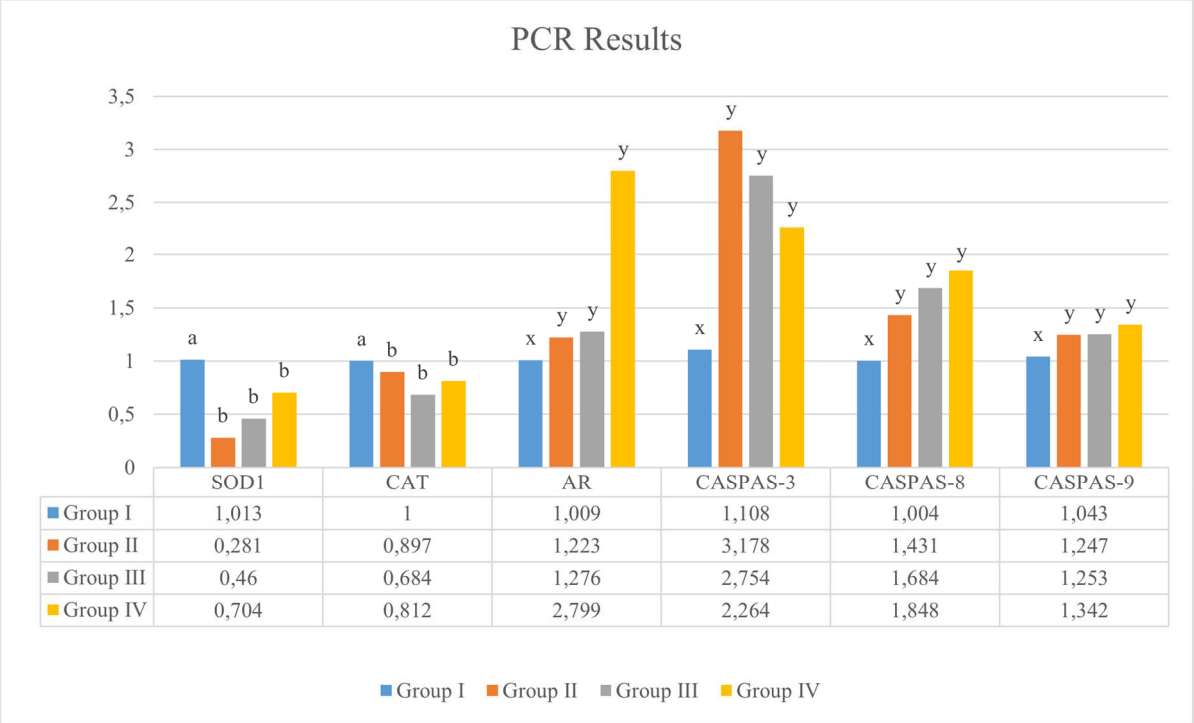

Fig. 1: PCR Results (SOD1, CAT, AR, CASPAS 3/8/9).
* The difference between groups with different (x,y; a,b) signs in the same column is significant (p< 0.001).

Fentanyl has been reported to prevent germ cell damage by suppressing ROS caused by polymorphonuclear leukocytes (PMN) and the xanthine oxidase system. Previous studies have demonstrated that opioids exert anti-inflammatory effects by suppressing endothelial and polymorphonuclear leukocyte (PMN) functions, as well as by downregulating adhesion molecules essential for PMN migration (Mordeniz *et al.,* 2021). Additionally, opioids have been reported to inhibit cell death induced by peroxynitrite, a highly reactive and toxic species formed through the interaction of superoxide radicals with nitric oxide (Hofbauer *et al.,* 2000; Yazdani *et al.,* 2022).

When sperm parameters were analyzed, a statistically significant difference was detected only in sperm concentration in Group IV. The spermatozoa concentration in mL was observed to increase approximately threefold compared to the other groups. This increase in spermatozoa count was also observed in the tubule lumens in testicular histopathology.

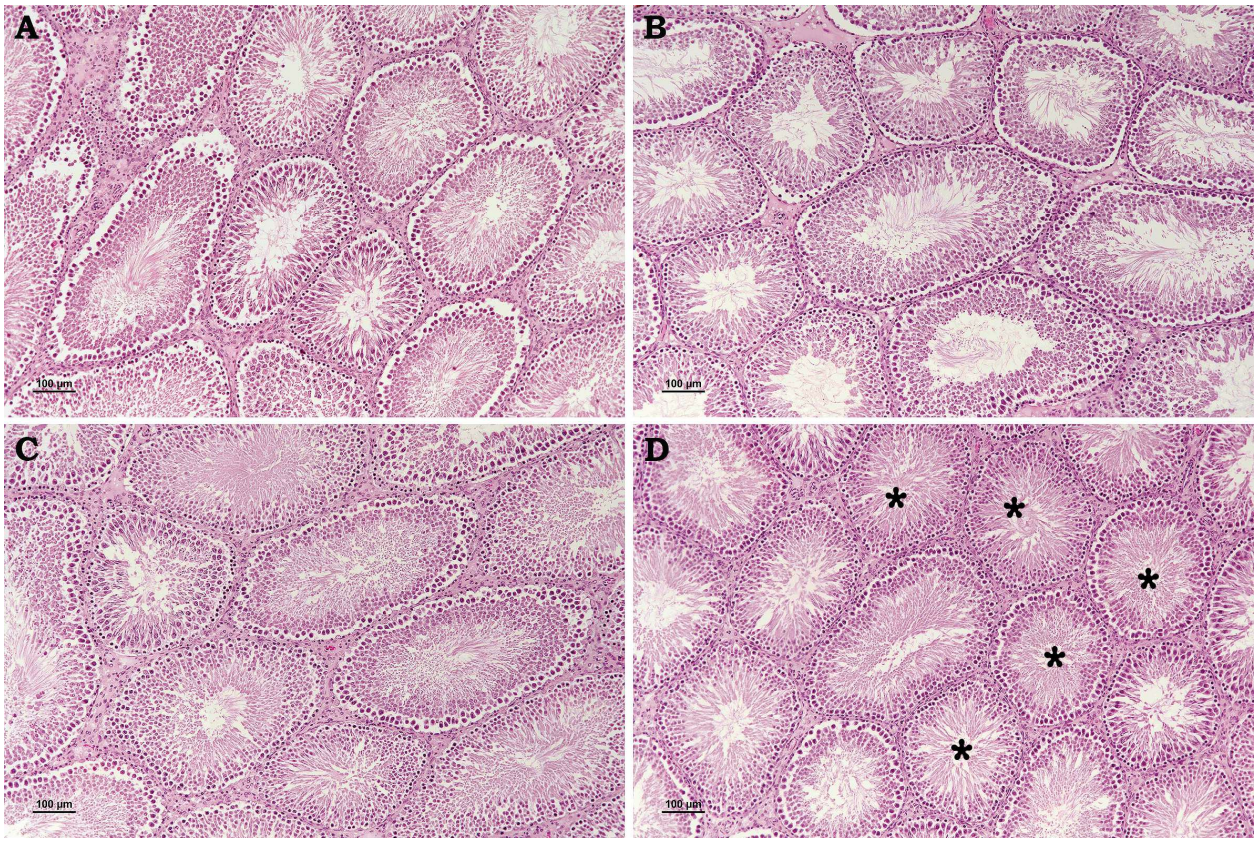

Fig. 2: Histopathologic examination of sections from rat testes revealed the following findings: (A) Group I exhibited normal seminiferous tubule architecture with well-organized spermatogenic cells at various stages of development. All germ cell types were present and showed normal morphology. (B) Group II and (C) Group III showed normal histologic appearance. histologic findings. (D) Group IV showed normal histologic appearance but an increase in spermatogenesis. Moreover, the basement membranes of the seminiferous tubules exhibited an increased number of spermatogenic cells and an increased density of spermatozoa excreted into the tubule lumens (*).

The increase in testicular AR level, histopathologic findings, and spermatozoa concentration in Group IV suggests that Fentanyl stimulates testosterone release and accelerates spermatogenesis by increasing AR levels. Fentanyl is also known to stimulate reward and pleasure centers by increasing dopamine synthesis. Consequently, it is possible that libido increases and spermatogenesis accelerates (Sayin, 2019; Zheng, 2022)

No significant difference was observed in motility, dead-to-live ratio, and abnormal spermatozoa ratio ($p>0.05$). According to the Durogesic® information published by the FDA in 2009, no statistical difference was found between pregnancy rates and litter size after 28 days of use in male and female rats (FDA, 2009). In a study using fentanyl and its derivatives, no negative effect on fertilization rates was determined (Lyseng-Williamson and Siddiqui, 2008). These studies suggest that fentanyl does not negatively impact factors such as fertilization, pregnancy, number of offspring, or sperm parameters.

In the present study, Caspase 3/8/9 enzymes were increased in all fentanyl administration groups ($p<0.001$). Numerous studies have reported that fentanyl triggers apoptosis by increasing caspase enzyme activity (Zhang *et al.,* 2014; Xu *et al.,* 2017; Carranza-Aguilar *et al.,* 2022; Wang *et al.,* 2022; Akaras, 2022). Apoptosis is primarily initiated through two major pathways: the intrinsic and extrinsic pathways. The intrinsic pathway involves disruption of mitochondrial function, release of cytochrome c, and activation of caspase-9. The extrinsic pathway is initiated by signals from outside the cell, involving the binding of death receptors to death ligands, the recruitment of an adaptor protein, and caspase-8 to the death-inducing signaling complex. These two pathways converge in the executioner phase, the final stage of apoptosis, where many cellular proteins are cleaved, leading to DNA fragmentation in the nucleus and activation of the effector caspase-3 (Mustafa *et al.,* 2024; Kartal *et al.,* 2020).

CONCLUSION

Fentanyl patches are frequently used as powerful painkillers. However, studies investigating the effects of fentanyl patch use on the male reproductive system are limited. The data from this study revealed that fentanyl

increased testicular apoptosis and oxidative stress but did not adversely affect sperm parameters. Additionally, this study is the first to determine that fentanyl administration at a dose of 100 mcg/hr increased sperm concentration. The effects of fentanyl on the male reproductive system should be examined in more detail in future studies. In this respect, our study is pioneering in exploring fentanyl's impact on the male reproductive system.

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Conflict of interest

The authors have no conflict of interest.

REFERENCES

Akaras N (2022). A histological and biochemical study of the protective role of hesperidin in testicular ischemia-reperfusion injury. *Int. J. Med. Biochem.,* **6**(1): 21-27.

Barletta C, Di Natale V, Esposito M, Chisari M, Cocimano G, Di Mauro L, Salerno M and Sessa F (2025). The rise of fentanyl: Molecular aspects and forensic investigations. *Int. J. Mol. Sci.,* **26**: 444.

Carranza-Aguilar CJ, Hernández-Mendoza A, Mejias-Aponte C, Rice KC, Morales M, González-Espinosa C and Cruz SL (2022). Morphine and Fentanyl Repeated Administration Induces Different levels of NLRP3-dependent pyroptosis in the dorsal raphe nucleus of male rats via cell-specific activation of TLR4 and opioid receptors. *Cell. Mol. Neurobiol.,* **42**: 677-694.

Farzi F, Mehrafza M, Mirmansouri A, Sorouri ZZ, Roushan ZA, Raoufi A, Shalkohi R, Samadnia S and Hosseini A (2019). Hemodynamic parameters and reproductive outcome after intracytoplasmic sperm injection and fresh embryo transfer in patients undergoing oocyte retrieval with general anesthesia using fentanyl, remifentanil, or alfentanil - A randomized clinical trial. *Taiwan J. Obstet. Gynecol.,* **58**: 536-540.

Fu Q, Han N, Li N, Gui L, Shi C, Rong P, Zeng F, Rao H, and Chen Y (2024). Guidelines for Rational Clinical Use of Fentanyl Transdermal Patch. *Drug Des. Devel. Ther.,* **18**: 233-255.

Gauridas S, Deka A and Dakua D (2022). Compliance, efficacy, and quality of life for oral morphine versus transdermal fentanyl patch in the management of cancer pain. *Int. J. Res. Med. Sci.,* **10**(3): 666-670.

Hofbauer R, Frass M, Gmeiner B, Sandor N, Schumann R, Wagner O and Kaye AD (2000). Effects of remifentanil on neutrophil adhesion, transmigration and intercellular adhesion molecule expression. *Acta Anaesthesiol. Scand.,* **44**: 1232-1237.

Kartal S, Günaydin B, Barun S and Elmas C (2020). Effects of isoflurane, ketamine, and dantrolene on apoptosis in the rat hippocampus. *Turk. J. Clin. Lab.,* **4**: 255-261.

Koşal V, Rua İ, Yüksek V and Keleş OF (2023). Investigation of the effect of Coenzyme-Q10 on Cyclophosphamide-induced testicular damage in male rats. *Rev. Científica Fac. Cienc. Vet.,* **XXXIII**: 1-7.

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT Method. *Methods,* **25**(4): 402-408.

Lyseng-Williamson KA and Siddiqui MAA (2008). Pregabalin: A review of its use in fibromyalgia. *Drugs,* **68**(15): 2205-2223.

Montanari S, Davani L, Terenzi C, Maltoni M, Andrisano V, De Simone A, and Ricci M (2022). Fentanyl pharmacokinetics in the blood of cancer patients by Gas Chromatography - Mass Spectrometry. *J. Pharm. Biomed. Anal.,* **219**: 114913.

Mordeniz C, Jafarova Demirkapu M, Akgul HM, Karabag S, Celikkol A, and Yananlı HR (2021). The effects of fentanyl on testicular ischemia-reperfusion injury *Turk. J. Anaesthesiol. Reanim.,* **49**(5): 373-378.

Mustafa M, Ahmad R, Tantry IQ, Ahmad W, Siddiqui S, Alam M, Abbas K, Moinuddin, Hassan Md I, Habib S, and Islam S (2024). Apoptosis: A comprehensive overview of signaling pathways, morphological changes, and physiological significance and therapeutic implications. *Cells,* **13**(22): 1838.

Reed RA, Berghaus LJ, Reynolds RM, Holmes BT, Krikorian AM, Sakai DM, Ishikawa Y and Knych HK (2024). The pharmacokinetics and pharmacodynamics of fentanyl administered via transdermal patch in horses. *Front. Pain Res.,* **5**: 1373759.

Sancak T (2023). The effects of repeated doses of xylazine-ketamine and medetomidine-ketamine anesthesia on DNA damage in the liver and kidney. *Acta Cirúrgica Bras.* **38**: e385723.

Sayin HU (2019). Getting high on dopamine: Neuroscientific aspects of pleasure. *SexuS J.,* **04**(11): 883-906.

Taghizadeh S M, Soroushnia A, and Mohamadnia F (2010). Preparation and *in vitro* evaluation of a new fentanyl patch based on functional and non-functional pressure-sensitive adhesives. *AAPS PharmSciTech,* **11**(1): 278-284.

Wang X-X, Cui L-L, Gan S-F, Zhang Z-R, Xiao J, Li C-H, and Luo F (2022). Inhibition of oligodendrocyte apoptosis in the prelimbic medial prefrontal cortex prevents fentanyl-induced hyperalgesia in rats. *J. Pain,* **23**(6): 1035-1050.

Xu Q, Li QG, Fan GR, Liu QH, Mi FL and Liu B (2017). Protective effects of fentanyl preconditioning on cardiomyocyte apoptosis induced by ischemia-reperfusion in rats. *Braz. J. Med. Biol. Res.,* **50**(2): e5286.

Yazdani I, Majdani R, Ghasemnejad-berenji M, and Dehpour AR (2022). Beneficial effects of cyclosporine A in combination with nortriptyline on germ cell-specific apoptosis, oxidative stress, and epididymal sperm qualities following testicular

ischemia/reperfusion in rats: A comparative study. *BMC Pharmacol. Toxicol.,* **23**: 59.

Zecca E, Manzoni A, Centurioni F, Farina A, Bonizzoni E, Seiler D, Perrone T and Caraceni A (2015). Pharmacokinetic study between a bilayer matrix fentanyl patch and a monolayer matrix fentanyl patch: single dose administration in healthy volunteers. *Br. J. Clin. Pharmacol.,* **80**(1): 110-115.

Zhang XL, Chen ML and Zhou SL (2014). Fentanyl increases colorectal carcinoma cell apoptosis by inhibition of NF-κB in a Sirt1-dependent manner. *Asian Pac. J. Cancer Prev.,* **15**(22): 10015-10020.

Zheng R (2022). Pleasure and Achievement: Dopamine and Endorphins *Highlights Sci. Eng. Technol.,* **6**: 83-89.