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ORIGINAL RESEARCH

Experimental Investigation of Fentanyl Analgesic Potency and Its Relationship with Endogenous Adrenaline Levels in Rats

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Purpose: Fentanyl is a synthetic opioid analgesic widely used in perioperative medicine due to its high analgesic potency and short duration of action. Previous studies suggest that the analgesic and anesthetic effects of fentanyl may be associated with endogenous catecholamine levels. This study aimed to evaluate the relationship between fentanyl's analgesic potency and anesthesia duration and endogenous levels of adrenaline (ADR) and noradrenaline (NDR) in rats, as well as changes in oxidative stress and inflammation markers including malondialdehyde (MDA), total glutathione (tGSH), and interleukin-6 (IL-6).

Patients and Methods: Three groups of six rats each were formed: an intact group receiving fentanyl, an intact group treated with metyrosine, and an adrenalectomized group receiving fentanyl. Fentanyl was administered at doses of 2, 15, 30, and 75 µg/kg, and anesthesia duration was recorded following intraperitoneal injection. Analgesic activity was assessed by measuring pain threshold using the paw pressure method, and immobility in the supine position was evaluated as an observational parameter. ADR, NDR, MDA, tGSH, and IL-6 levels were biochemically measured in blood and tissue samples.

Results: Fentanyl produced analgesic effects in all experimental groups. However, no distinct anesthetic effect sufficient for surgical procedures was observed. Although the durations of immobility in the supine position were recorded, these observations were not evaluated as a direct measure of anesthetic duration. Different doses of fentanyl did not lead to significant alterations in oxidative stress parameters, antioxidant capacity, or anti-inflammatory cytokine levels across the groups.

Conclusion: In conclusion, although the analgesic effect of fentanyl increased in a dose-dependent manner, it was found to be independent of serum ADR and NDR levels. High-dose fentanyl (75 µg/kg) did not induce anesthesia and did not significantly affect oxidative stress markers (MDA, tGSH) or IL-6 levels. The increase in MDA and the decrease in tGSH observed in the absence of adrenal hormones support their indirect antioxidant role. Overall, the findings indicate that fentanyl modulates catecholamine responses depending on dose and hormonal status, while its analgesic effect appears to be primarily mediated through µ-opioid receptor activation.

Keywords: ADR, analgesic effect, fentanyl, µ-opioid receptor, NDR, rats

Introduction

Fentanyl is the prototype of the 4-anilidopiperidine class of synthetic opioid analgesics.[1] Fentanyl, a µ-receptor agonist, was synthesized in 1960 and introduced into clinical medicine in 1963 as a potent intravenous analgesic agent with a relatively short duration of action.[2] It also has the ability to activate other opioid system receptors such as delta and kappa receptors.[3] Fentanyl remains one of the most widely used opioids worldwide in the perioperative period.[4] In clinical medicine, fentanyl derivatives are used in the treatment of persistent and severe cancer pain and to produce balanced intravenous anesthesia.[5] Fentanyl has various routes of administration, including intravenous, intramuscular, transdermal, intranasal, intrathecal, and sublingual tablets.[3] When administered as a single dose, fentanyl has an average

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duration of action of about 30 minutes.[6] Despite this, due to its short duration, fentanyl must be administered at appropriate intervals to achieve the desired effect.[3] In animals, intraperitoneal administration of fentanyl at a dose of 5 μg/kg has been shown to produce significant analgesia.[7] Higher doses, such as 55–75 μg/kg, are considered primary anesthetic doses.[8] It has been reported that fentanyl, at anesthetic doses (75 μg/kg), decreases epinephrine levels and causes reductions in heart rate and arterial blood pressure.[9] In contrast, at small doses that produce marked analgesia (2 μg/kg), it is known to increase epinephrine levels.[10] These findings suggest that the analgesic potency and anesthetic duration of fentanyl may be associated with endogenous ADR levels. Corroborating this view, one study demonstrated that the analgesia induced by thiopental sodium results from its ability to reduce endogenous adrenaline levels.[11] Furthermore, it has been reported that adrenaline plays a significant role in determining the duration of ketamine anesthesia; in rats with suppressed endogenous adrenaline production (adrenalectomized rats), the duration of ketamine anesthesia was observed to increase sixfold.[12] The short duration of fentanyl's action makes it preferable for brief surgical procedures, whereas for longer surgical interventions the dose is administered repeatedly. Achieving adequate anesthesia with small doses of fentanyl may reduce its adverse effects and enhance its clinical value in prolonged surgical procedures. In addition to their analgesic and anesthetic effects, opioids have been reported to influence inflammatory responses and oxidative stress status. Therefore, the evaluation of biochemical markers such as IL-6, MDA, and tGSH may provide important information about the systemic effects of fentanyl. IL-6 is a pro-inflammatory cytokine; MDA is an indicator of oxidative damage and lipid peroxidation; and tGSH represents endogenous antioxidant capacity. Consequently, the aim of our study is to investigate the relationship between the analgesic potency and anesthetic duration of fentanyl and endogenous levels of ADR and NDR in rats.

Materials and Methods

Animals

The animals used in this study were obtained from the Medical Experimental Practice and Research Center of Binali Yıldırım University. A total of 78 male albino Wistar rats, weighing between 270 and 282 grams, were used for the experiment. Ahead of the study, the animals were housed in groups under standard laboratory conditions at normal room temperature (22 °C) with a 12-hour light/12-hour dark cycle. All animals in the groups were provided with standard laboratory chow and tap water ad libitum under appropriate conditions. All experimental procedures involving animals were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals and were approved by the [Erzincan Binali Yıldırım University Ethics Committee] (Approval No: 2025/04, Date: 27/04/2025).

Chemical Agents

Fentanyl used in the experiment was obtained from Haver Pharma (Turkey), and metyrosine was purchased from Sigma-Aldrich.

Experimental Design and Experimental Groups

Experimental Design

In accordance with the 4R principles (Reduction, Refinement, Replacement, and Responsibility),[13] the sample size was determined to ensure the use of the minimum number of animals required. Although animals showing signs such as hunching, reduced mobility, or injuries caused by cage mates were evaluated as potential candidates for exclusion from the study and analyses, no animals were excluded. Randomization was performed using a random number table, and numerical labeling was applied to both cages and animals in order to minimize potential confounding factors.

Experimental Groups

Intact Animal Groups

Intact animals were divided into four groups according to the fentanyl dose administered (IF-2, IF-15, IF-30 and IF-75). Additionally, intact animals were allocated into nine groups in total, including those receiving metyrosine alone (IMR), and those receiving metyrosine plus fentanyl (IMF-2, IMF-15, IMF-30, IMF-75), as well as a healthy control group (HG) (Table 1).

Table 1 Summary of Experimental Animal Groups and Treatments

Main Model	Group Code	Treatment Description	Fentanyl Dose	Metyrosine
Intact	HG	Healthy control	–	–
Intact	IF-2	Fentanyl	2 µg/kg	–
Intact	IF-15	Fentanyl	15 µg/kg	–
Intact	IF-30	Fentanyl	30 µg/kg	–
Intact	IF-75	Fentanyl	75 µg/kg	–
Intact	IMR	Metyrosine alone	–	✓
Intact	IMF-2	Metyrosine + Fentanyl	2 µg/kg	✓
Intact	IMF-15	Metyrosine + Fentanyl	15 µg/kg	✓
Intact	IMF-30	Metyrosine + Fentanyl	30 µg/kg	✓
Intact	IMF-75	Metyrosine + Fentanyl	75 µg/kg	✓
Adrenalectomized	ADC	Adrenalectomized control	–	–
Adrenalectomized	AF-2	Fentanyl	2 µg/kg	–
Adrenalectomized	AF-15	Fentanyl	15 µg/kg	–
Adrenalectomized	AF-30	Fentanyl	30 µg/kg	–
Adrenalectomized	AF-75	Fentanyl	75 µg/kg	–

Abbreviations: HG, Healthy group; IMR, Intact + metyrosine; IMF, Intact + metyrosine + fentanyl; ADC, Adrenalectomized control.

Adrenalectomized Animal Groups

Adrenalectomized animals were divided into five groups: a control group (ADC) and four groups receiving fentanyl at different doses (AF-2, AF-15, AF-30 and AF-75) (Table 1).

Experimental Procedure

Intact Animals

At the beginning of the experimental procedure, the baseline paw pain thresholds of all animal groups were measured using a Basile Algesimeter.[14] In our study, intact rats in the IF-2 (n=6), IF-15 (n=6), IF-30 (n=6) and IF-75 (n=6) groups received fentanyl intraperitoneally (i.p.) at doses of 2, 15, 30 and 75 µg/kg, respectively. Additionally, intact animals in the IMR (n=6), IMF-2 (n=6), IMF-15 (n=6), IMF-30 (n=6), and IMF-75 (n=6) groups were administered 100 mg/kg metyrosine orally via gavage. The metyrosine dose (100 mg/kg), oral route of administration, and 1-hour pretreatment interval were selected based on previous studies reporting effective inhibition of catecholamine synthesis.[14] One hour after metyrosine administration, the animals in these groups received fentanyl intraperitoneally at doses of 2, 15, 30 and 75 µg/kg, correspondingly. Ten minutes after fentanyl injection, paw pain thresholds were measured once more. Furthermore, the anesthetic-inducing dose and the duration of anesthesia were evaluated. Anesthetic onset was defined as the loss of righting reflex. The duration of anesthesia was recorded as the time elapsed until recovery of this reflex, while immobility in the supine position was evaluated as an observational parameter.[15] However, this measure was not considered a definitive indicator of surgical anesthetic duration. Tail vein blood was used for MDA, tGSH, and IL-6 analyses, whereas catecholamines (ADR and NDR) were measured in cardiac blood samples. Biochemical measurements were performed at the 10th minute following fentanyl administration to reflect early systemic and neuroendocrine responses. All experimental data were evaluated and compared among the groups.

Adrenalectomy Procedure

Bilateral adrenalectomy was performed under sterile surgical conditions. Animals were anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p). After shaving and disinfecting the dorsolateral area, bilateral incisions were made to access the adrenal glands, which were then removed. Postoperative care was provided, and 0.9% saline was administered to prevent adrenal insufficiency. Animals were allowed to recover for one week before experimental procedures.

Adrenalectomized Animals

One week after adrenalectomy, the baseline paw pain thresholds of all animals were measured using a Basile Algesimeter.[14] Subsequently, animals in the AF-2 (n=6), AF-15 (n=6), AF-30 (n=6), and AF-75 (n=6) groups received intraperitoneal fentanyl at doses of 2, 15, 30, and 75 µg/kg, respectively. Animals in the ADC group (n=6) were administered distilled water as a vehicle control. Ten minutes after vehicle or fentanyl administration, paw pain thresholds were measured again. In addition, the anesthetic-inducing dose of fentanyl and the duration of anesthesia were evaluated. Ten minutes following fentanyl injection, blood samples were collected from the tail veins, and the levels of MDA, tGSH, IL-6, ADR and NDR were determined. All results obtained from the experiment were evaluated and compared among the groups.

Biochemical Analyses

Determination of Blood MDA and tGSH Levels

Determination of MDA and GSH in blood (ELISA) was performed by measuring each assay according to kit instructions (product nos. 10009055 and 703002, respectively, Cayman Chemical Company).

Determination of Blood IL-6 Levels

The levels of IL-6 were measured using according to commercial kits supplied by Eastbiopharm Co Ltd ELISA kit, China.

Determination of Blood ADR and NDR Levels

Blood samples were collected from the hearts of rats in 2 mL EDTA vacuum tubes to determine the ADR and NDR levels. Within 15 min of venesection, the EDTA samples for the ADR and NDR measurements were placed on ice and centrifuged at 3500 g for 5 min. After centrifugation, the plasma ADR and NDR concentrations were measured by an isocratic system using a high-performance liquid chromatography (HPLC) pump (model Hewlett Packard Agilent 1100) (flow rate: 1 mL/min; injection volume: 40 µL; analytical run time: 20 min) and an electrochemical detector. We used a reagent kit for HPLC analysis of the catechol-amines in the plasma serum (Chromsystems, Munich, Germany).

Statistical Analysis

The statistical analyses of the biochemical parameters were performed using IBM SPSS Statistics for Windows (Version 27.0; IBM Corp., Armonk, NY, USA, 2020). Graphical visualizations were created using GraphPad Prism (Version 8.0.1; GraphPad Software, San Diego, CA, USA, 2018). Biochemical results were presented as mean ± standard deviation (SD). The distribution of the data was examined using the Shapiro–Wilk test, and homogeneity of variances was assessed with Levene's test. Comparisons between groups were conducted using one-way ANOVA, and Tukey's honest significant difference (HSD) test was used for pairwise comparisons. Paired *t*-test was used to compare paw pain threshold values before and after treatment within each experimental group. Statistical analyses for pain threshold were conducted using raw paw pressure values, while percentage baseline changes were calculated only for descriptive presentation in the Results section. For multiple comparisons, the Dunn test with Bonferroni correction was applied, and adjusted p-values were reported. A p-value < 0.05 was considered statistically significant.

Results

Pain Threshold Results in IF, IMF and AF Groups

As shown in Figure 1, a significant increase in pain threshold was observed following fentanyl administration. The greatest elevation was detected in the IF-75 group (175%). The IF-15 (90.2%) and IF-30 (122.7%) groups also exhibited a strong analgesic response, whereas the increase in the IF-2 group was more limited (40.4%). An increase in pain threshold was observed across all IMF groups. The most pronounced elevation occurred in the IMF-75 group (202.4%), followed by IMF-30 (153.3%) and IMF-15 (127.5%). Although the increase in the IMF-2 group was comparatively lower (81.4%), it still reflected an analgesic response. Similarly, dose-dependent increases were observed in adrenalectomized

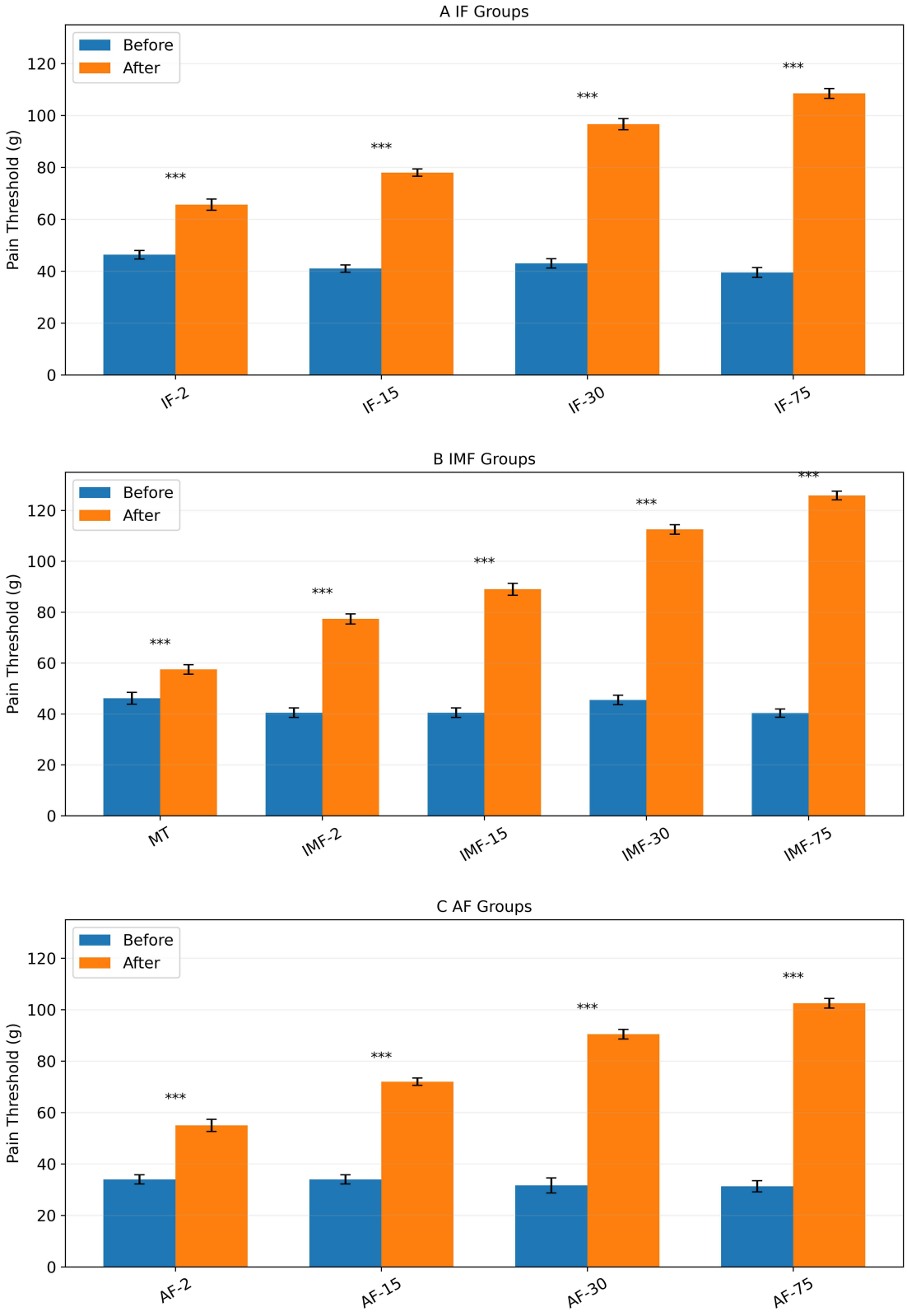

Figure 1 Paw pain threshold values measured before and after treatment in experimental groups. (**A**) Intact (IF) groups, (**B**) metyrosine-treated (IMF) groups, and (**C**) adrenalectomized (AF) groups. Data are expressed as mean ± SD (n = 6 per group). Bars represent Before (blue) and After (Orange) measurements. Statistical analysis was performed using paired *t*-test. ***p < 0.001 vs before treatment.

Abbreviations: HG, healthy control; IF, fentanyl-treated group; MT, Metyrosine alone group; IMF, metyrosine + fentanyl group; AF, adrenalectomized fentanyl-treated group.

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animals. The highest response was detected in the AF-75 group (246.7%), followed by AF-30 (190.3%) and AF-15 (121.2%), while the AF-2 group showed an increase of approximately 60%.

Results of Biochemical Analyses

Results of Blood MDA and tGSH Determination in IF Groups

No significant MDA levels differences were detected among the groups ($p > 0.05$). The mean MDA levels in the IF-2 (3.33 ± 0.21), IF-15 (3.34 ± 0.17), and IF-30 (3.32 ± 0.19) groups were similar and did not show statistically significant variation. Fentanyl administration did not produce a marked change in MDA levels; only a slight decreasing trend was observed in the IF-75 group. Overall, the data indicate that different fentanyl doses did not exert a significant effect on MDA levels in intact rats. Similarly, no statistically significant differences were found in tGSH levels among the IF groups ($p > 0.05$). The tGSH levels in the IF-2 (6.39 ± 0.24), IF-15 (6.31 ± 0.14), and IF-30 (6.28 ± 0.17) groups were comparable (Figure 2 and Table 2).

Results of Blood MDA and tGSH Determination in IMF Groups

A partially significant difference was detected within the IMF groups ($p < 0.05$). The MDA level in the IMF-75 group (3.30 ± 0.28) was slightly higher than that of the IMR group (3.27 ± 0.18). No statistically significant differences were observed among the IMF-2 (3.53 ± 0.16) and IMF-15 (3.43 ± 0.17) groups ($p > 0.05$). Overall, the results indicate that the combination of metyrosine and fentanyl produced a modest increase in MDA levels; however, this increase did not reach statistical significance. The measured tGSH levels in the IMR group (6.39 ± 0.16) and the IMF groups (ranging between 6.15 and 6.40) were comparable ($p > 0.05$). Although increasing doses of fentanyl produced a slight decrease in tGSH levels, this change was not statistically significant (Figure 3 and Table 3).

Results of Blood MDA and tGSH Determination in AF Groups

The MDA levels in the AF-30 group (4.59 ± 0.14) were similar to those of the other AF groups, and no statistically significant differences were observed. Increasing doses of fentanyl did not produce a significant change in MDA values. These findings indicate that, in the absence of adrenal glands, fentanyl does not exert a notable effect on MDA levels ($p > 0.05$). There were no statistically significant differences among the groups ($p > 0.05$). The tGSH level in the AF-2 group (5.39 ± 0.18) was comparable to those of the other groups. Although variations in tGSH levels occurred with increasing fentanyl doses, no substantial decreasing trend was detected (Figure 4 and Table 4).

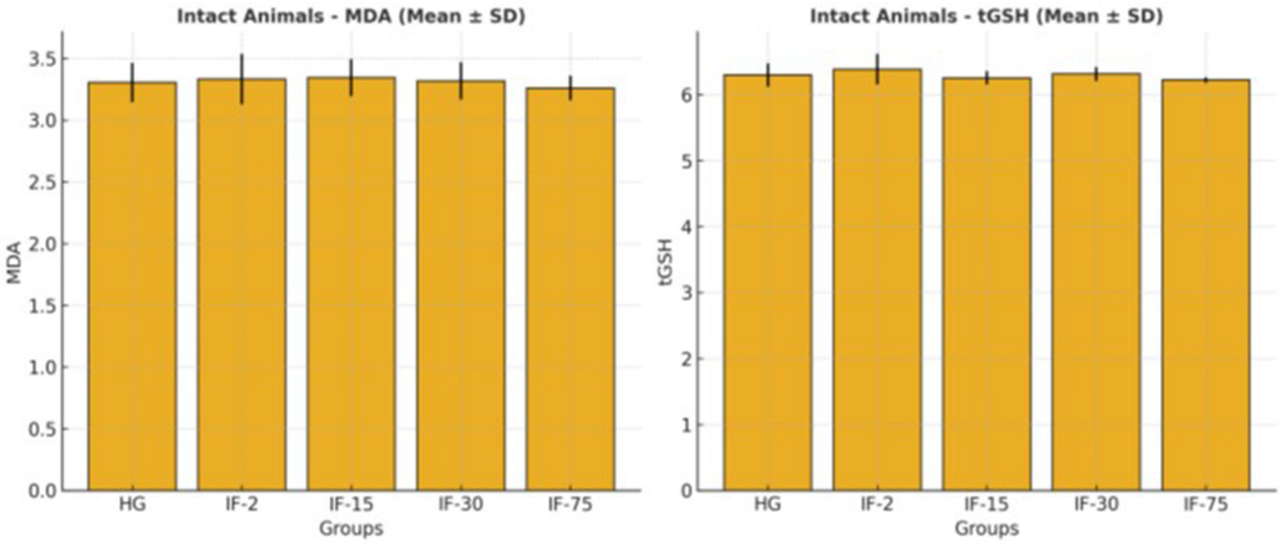

Figure 2 The results of the effects of fentanyl in intact rats on blood MDA and tGSH levels.
Notes: Data are expressed as mean ± SD (n = 6 per group). Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test.
Abbreviations: HG, healthy control; IF, fentanyl-treated group; MDA, malondialdehyde; tGSH, total glutathione.

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Table 2 Statistical Comparisons of Oxidative Stress Markers (MDA, tGSH), Inflammatory Marker (IL-6) and Catecholamines (ADR, NDR) in Intact Rat Group

Groups	MDA[a] (nmol/mg protein)	tGSH[a] (nmol/mg protein)	IL-6[a] (pg/mg protein)	ADR[a] (pg/mg)	NDR[a] (pg/mg)
HG	3.30 ± 0.17	6.30 ± 0.19	2.39 ± 0.17	302.50 ± 9.18	329.50 ± 8.98
IF-2	3.33 ± 0.23	6.39 ± 0.25	2.40 ± 0.18	482.17 ± 8.89	563.67 ± 13.82
IF-15	3.34 ± 0.16	6.25 ± 0.11	2.43 ± 0.13	471.67 ± 11.09	555.50 ± 13.71
IF-30	3.32 ± 0.17	6.32 ± 0.12	2.34 ± 0.17	458.17 ± 23.76	551.17 ± 9.35
IF-75	3.26 ± 0.11	6.22 ± 0.05	2.33 ± 0.15	210.83 ± 7.47	245.00 ± 8.12
Group comparisons	**p values**				
HG vs IF-2	>0.05	>0.05	>0.05	<0.001	<0.001
HG vs IF-15	>0.05	>0.05	>0.05	<0.001	<0.001
HG vs IF-30	>0.05	>0.05	>0.05	<0.001	<0.001
HG vs IF-75	>0.05	>0.05	>0.05	<0.001	<0.001
IF-2 vs IF-15	>0.05	>0.05	>0.05	>0.05	>0.05
IF-2 vs IF-30	>0.05	>0.05	>0.05	<0.05	>0.05
IF-2 vs IF-75	>0.05	>0.05	>0.05	<0.001	<0.001
IF-15 vs IF-30	>0.05	>0.05	>0.05	>0.05	>0.05
IF-15 vs IF-75	>0.05	>0.05	>0.05	<0.001	<0.001
IF-30 vs IF-75	>0.05	>0.05	>0.05	<0.001	<0.001
F value	0.21	0.92	0.35	491.74	1111.17
***df* (df1/df2)**	4/25	4/25	4/25	4/25	4/25
p	<0.001	<0.001	<0.001	<0.001	<0.001

Notes: Results are expressed as mean ± SD (n=6 per group). [a]Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test for post-hoc comparisons, as the assumption of homogeneity of variances was met.
Abbreviations: HG, healthy group; IF, intact fentanyl group; MDA, malondialdehyde; tGSH, total glutathione; IL-6, interleukin-6; ADR, adrenaline; NDR, noradrenaline; F, F statistic; df, degrees of freedom; p, probability value.

Results of Blood IL-6 Determination

No statistically significant differences were observed among the IF groups ($p > 0.05$). The mean IL-6 levels in the IF-2 (2.29 ± 0.17), IF-15 (2.33 ± 0.15), and IF-30 (2.22 ± 0.14) groups were found to be similar. Increasing doses of fentanyl did not produce a marked change in IL-6 levels (Figure 5 and Table 2).

No significant differences were observed among the IMF groups either ($p > 0.05$). The IL-6 levels in the IMR group (2.00 ± 0.11) and in the IMF-2 and IMF-75 groups (ranging between 2.10 and 2.32) were comparable (Figure 5 and Table 3).

Similarly, the IL-6 levels in the AF-15 (2.59 ± 0.13) and AF-75 (2.62 ± 0.15) groups were comparable, and no statistically significant differences were detected between the groups ($p > 0.05$) (Figure 5 and Table 4).

Results of Blood ADR Determination

A statistically significant difference was detected among the IF groups ($p < 0.01$).

Compared to the SG group (302 ± 10.3), the mean ADR level in the IF-2 group (474 ± 8.9) was found to be significantly higher ($p < 0.001$). In the IF-75 group (213 ± 7.4), a statistically significant decrease was observed ($p < 0.001$) (Figure 6 and Table 2).

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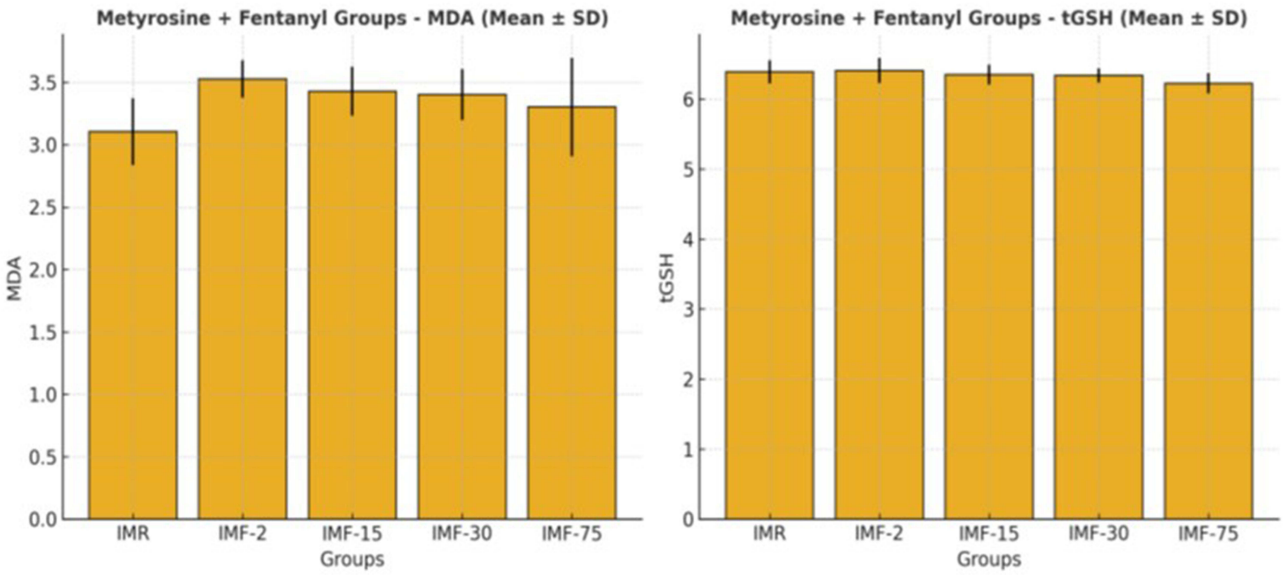

Figure 3 The results of blood MDA and tGSH levels in rats administered metyrosine alone or in combination with fentanyl.
Notes: Data are expressed as mean ± SD (n = 6 per group). Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test.
Abbreviations: MR, metyrosine alone group; IMF, metyrosine + fentanyl group; MDA, malondialdehyde; tGSH, total glutathione.

A statistically significant difference was also observed between the IMF groups ($p < 0.05$). Compared to the IMR group (158 ± 8.5), the IMF-2 group (183 ± 9.2) showed a slight increase; however, this difference was not statistically significant ($p > 0.05$). In the IMF-75 group (112 ± 6.8), ADR levels showed a significant decrease ($p < 0.05$) (Figure 6 and Table 3).

No statistically significant difference was found among the AF groups ($p > 0.05$). In adrenalectomized rats, blood adrenaline levels following fentanyl administration were too low to be measured (Figure 6 and Table 4).

Table 3 Statistical Comparisons of Oxidative Stress Markers (MDA, tGSH), Inflammatory Marker (IL-6) and Catecholamines (ADR, NDR) in Methyrosine-Treated Group

Groups	MDA[a] (nmol/mg protein)	tGSH[a] (nmol/mg protein)	IL-6[a] (pg/mg protein) (U/mg protein)	ADR[a] (pg/mg)	NDR[a] (pg/mg)
IMR	3.10 ± 0.30	6.39 ± 0.18	2.21 ± 0.16	158.83 ± 8.70	155.50 ± 9.57
IMF-2	3.53 ± 0.17	6.41 ± 0.20	2.22 ± 0.08	167.83 ± 10.50	167.17 ± 13.70
IMF-15	3.43 ± 0.21	6.35 ± 0.16	2.23 ± 0.1	159.33 ± 8.24	176.67 ± 9.27
IMF-30	3.40 ± 0.22	6.34 ± 0.11	2.24 ± 0.13	153.00 ± 11.93	172.00 ± 7.97
IMF-75	3.30 ± 0.43	6.22 ± 0.16	2.27 ± 0.14	111.67 ± 6.28	110.67 ± 8.12
Group comparisons	**p values**				
IMR vs IMF-2	>0.05	>0.05	>0.05	>0.05	>0.05
IMR vs IMF-15	>0.05	>0.05	>0.05	>0.05	<0.01
IMR vs IMF-30	>0.05	>0.05	>0.05	>0.05	>0.05
IMR vs IMF-75	>0.05	>0.05	>0.05	<0.001	<0.001

(*Continued*)

Table 3 (Continued).

Groups	MDA[a] (nmol/mg protein)	tGSH[a] (nmol/mg protein)	IL-6[a] (pg/mg protein) (U/mg protein)	ADR[a] (pg/mg)	NDR[a] (pg/mg)
IMF-2 vs IMF-15	>0.05	>0.05	>0.05	>0.05	>0.05
IMF-2 vs IMF-30	>0.05	>0.05	>0.05	>0.05	>0.05
IMF-2 vs IMF-75	>0.05	>0.05	>0.05	<0.001	<0.001
IMF-15 vs IMF-30	>0.05	>0.05	>0.05	>0.05	>0.05
IMF-15 vs IMF-75	>0.05	>0.05	>0.05	<0.001	<0.001
IMF-30 vs IMF-75	>0.05	>0.05	>0.05	<0.001	<0.001
F value	1.93	1.11	0.23	33.77	43.40
***df* (df1/df2)**	4/25	4/25	4/25	4/25	4/25
p	>0.05	>0.05	>0.05	<0.001	<0.001

Notes: Results are expressed as mean ± SD (n=6 per group). [a]Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test for post-hoc comparisons, as the assumption of homogeneity of variances was met.
Abbreviations: HG, healthy group; MR, metyrosine alone group; IMF, metyrosine + fentanyl group; MDA, malondialdehyde; tGSH, total glutathione; IL-6, interleukin-6; ADR, adrenaline; NDR, noradrenaline; F, F statistic; df, degrees of freedom; p, probability value.

Results of Blood NDR Determination

As shown in Figure 7 and Table 2, a statistically significant difference was detected among the IF groups ($p < 0.001$). Compared to the SG group (329 ± 9.5), the mean NDR levels in the IF-2 (565 ± 12.3), IF-15 (556 ± 10.8), and IF-30 (551 ± 10.8) groups were found to be significantly higher ($p < 0.001$). In the IF-75 group (245 ± 8.7), a statistically significant decrease was observed ($p < 0.001$).

A statistically significant difference was detected among the IMF groups ($p < 0.05$). Compared to the IMR group (155 ± 6.5), the IMF-2 (170 ± 7.1) and IMF-15 (176 ± 6.9) groups showed a slight increase in mean NDR levels, but this

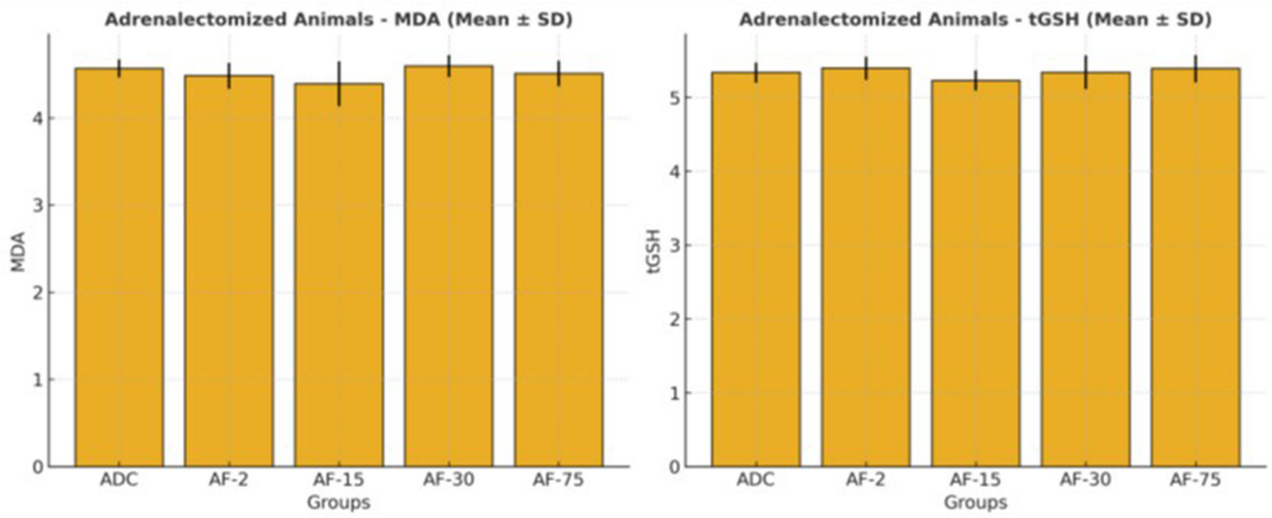

Figure 4 The results of the effects of fentanyl in adrenalectomized rats on blood MDA and tGSH levels.
Notes: Data are expressed as mean ± SD (n = 6 per group). Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test.
Abbreviations: ADC, adrenalectomized rats control; AF, adrenalectomized fentanyl-treated group; MDA, malondialdehyde; tGSH, total glutathione.

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Table 4 Statistical Comparisons of Oxidative Stress Markers (MDA, tGSH), Inflammatory Marker (IL-6) and Catecholamines (ADR, NDR) in Adrenalectomized Group

Groups	MDA[a] (nmol/mg protein)	tGSH[a] (nmol/mg protein)	IL-6[a] (pg/mg protein) (U/mg protein)	ADR[b] (pg/mg)	NDR[a] (pg/mg)
ADC	4.57 ± 0.11	5.34 ± 0.15	3.20 ± 0.36	0.00 ± 0.00	138.83 ± 7.65
AF-2	4.48 ± 0.16	5.40 ± 0.17	2.56 ± 0.10	0.00 ± 0.00	199.33 ± 4.27
AF-15	4.48 ± 0.32	5.23 ± 0.15	2.56 ± 0.17	0.00 ± 0.00	185.50 ± 6.28
AF-30	4.59 ± 0.14	5.34 ± 0.25	2.57 ± 0.17	0.00 ± 0.00	153.17 ± 8.66
AF-75	4.51 ± 0.16	5.39 ± 0.20	2.57 ± 0.09	0.00 ± 0.00	103.67 ± 5.39
Group comparisons	**p values**				
ADC vs AF-2	>0.05	>0.05	<0.001	NA	<0.001
ADC vs AF-15	>0.05	>0.05	<0.001	NA	<0.001
ADC vs AF-30	>0.05	>0.05	<0.001	NA	<0.01
ADC vs AF-75	>0.05	>0.05	<0.001	NA	<0.001
AF-2 vs AF-15	>0.05	>0.05	>0.05	NA	<0.05
AF-2 vs AF-30	>0.05	>0.05	>0.05	NA	<0.001
AF-2 vs IMF-75	>0.05	>0.05	>0.05	NA	<0.001
AF-15 vs IMF-30	>0.05	>0.05	>0.05	NA	<0.001
AF-15 vs IMF-75	>0.05	>0.05	>0.05	NA	<0.001
AF-30 vs AF-75	>0.05	>0.05	>0.05	NA	<0.001
F value	0.42	0.76	11.70	NA	197.04
***df* (df1/df2)**	4/25	4/25	4/25	4/25	4/25
p	>0.05	>0.05	<0.001	NA	<0.001

Notes: Results are expressed as mean ± SD (n=6 per group). [a]Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test for post-hoc comparisons, as the assumption of homogeneity of variances was met. [b]For ADR in AF, all measured values were zero; therefore ANOVA and Tukey's tests were not applicable (NA).

Abbreviations: HG, healthy group; ADC, adrenalectomized control; AF, adrenalectomized fentanyl-treated group; MDA, malondialdehyde; tGSH, total glutathione; IL-6, interleukin-6; ADR, adrenaline; NDR, noradrenaline; F, F statistic; df, degrees of freedom; p, probability value.

difference was not statistically significant ($p > 0.05$). In the IMF-75 group (112 ± 5.9), however, a statistically significant decrease in NDR levels was observed ($p < 0.01$) (Figure 7 and Table 3).

No statistically significant difference was found between the AF-2 (198 ± 9.3) and AF-75 (105 ± 6.7) groups. Fentanyl administration did not lead to a notable change in noradrenaline levels in the absence of adrenal glands. No statistically significant differences were observed among the AF groups ($p > 0.05$) (Figure 7 and Table 4).

Discussion

In our study, the relationship between the analgesic potency and anesthetic duration of fentanyl and endogenous ADR and NDR levels was investigated in rats. In addition, serum MDA, tGSH, IL-6, ADR, and NDR levels were measured in all animals.

It is well established that, opioids acting on the μ-opioid receptor represent the gold standard in analgesia.[16] Fentanyl, a μ-receptor agonist, is used therapeutically as a potent intravenous analgesic agent with a relatively short duration of action.[2] Our findings confirmed the analgesic effect of fentanyl, demonstrating a dose-dependent increase in paw pain

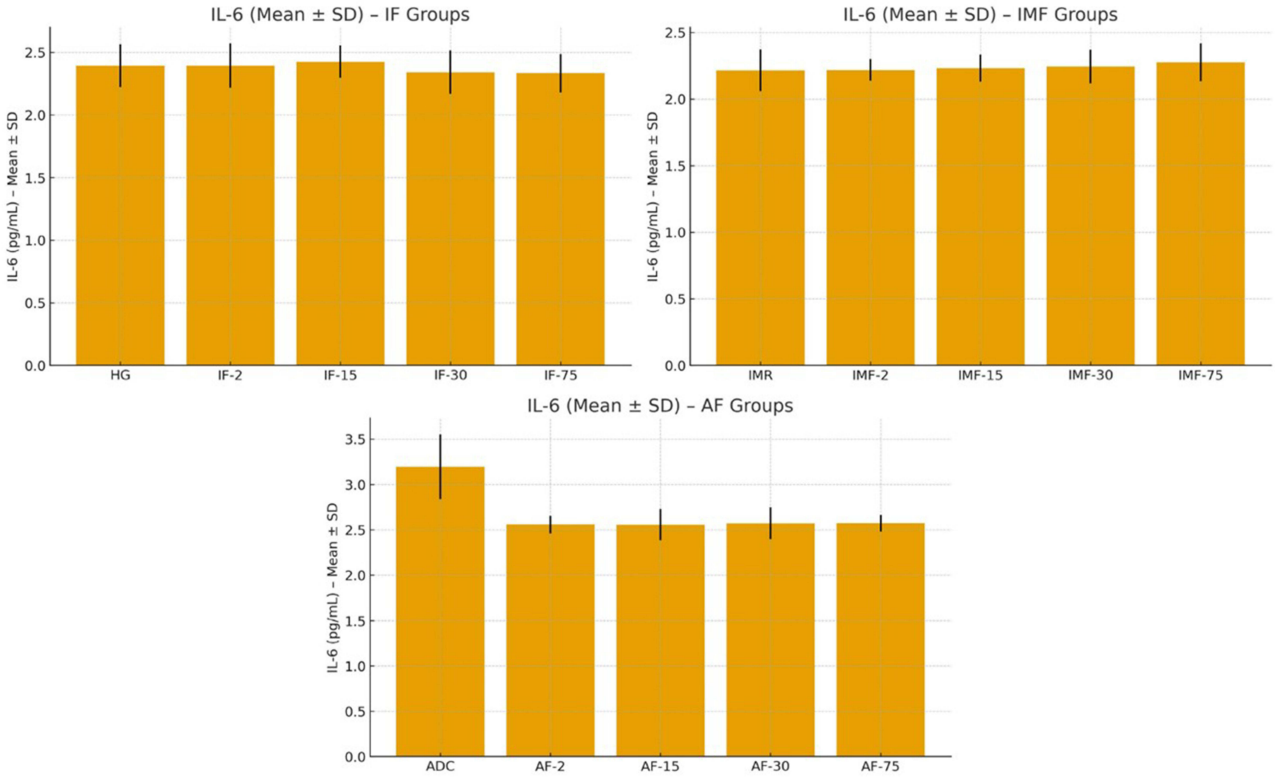

Figure 5 The results of IL-6 levels following fentanyl administration in intact rats, in rats administered metyrosine alone or in combination with fentanyl and in adrenalectomized rats.

Notes: Data are presented as mean ± SD (n = 6 per group). Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test.

Abbreviations: HG, healthy control; IF, fentanyl-treated groups; IMR, metyrosine alone group; IMF, metyrosine + fentanyl groups; ADC, adrenalectomized rats control; AF, fentanyl-treated groups; IL-6, interleukin-6.

threshold in the intact animal groups. On the other hand, no anesthetic effect was observed in any of the intact animals administered fentanyl, including those receiving doses within the anesthetic range reported in the literature.[8] There is evidence in the literature indicating that adrenaline increases pain threshold.[14] For this reason, in our study, we also investigated whether the analgesic potency and anesthetic duration of fentanyl were associated with ADR and NDR levels. Metyrosine was used to reduce serum ADR and NDR concentrations. As is well established, metyrosine is an agent that decreases catecholamine levels.[17] In animals administered metyrosine alone, an increase in pain threshold was observed. Although the paw pain threshold in the metyrosine–fentanyl combination group showed a greater dose-dependent increase compared to the group receiving fentanyl alone, no anesthetic effect was observed. We consider the more pronounced increase in pain threshold in the combination group to be attributable to the analgesic effect of metyrosine. In accordance with our results, Albayrak et al reported that metyrosine produced analgesia by reducing catecholamine levels without affecting corticosterone concentrations.[18] In adrenalectomized animals, the baseline paw pain threshold measured prior to fentanyl administration was found to be lower compared to the intact groups. This finding is thought to be attributable to insufficient cortisol production. Similarly, Trevino et al reported that resistance to pain decreases when cortisol synthesis is reduced.[19] In the adrenalectomized groups, fentanyl also increased the paw pain threshold in a dose-dependent manner; however, anesthesia did not occur. These findings indicate that the analgesic effect of fentanyl is independent of adrenal hormones. Our results are in line with the literature reporting that the analgesic action of opioids largely depends on μ-opioid receptor activation.[20]

Reactive oxygen species (ROS) are generated in response to various environmental stressors, and their excessive production can lead to lipid peroxidation (LPO). LPO is commonly monitored by measuring MDA, a well-established biomarker of oxidative stress.[21,22] Opioids have been reported to increase oxidative stress under certain conditions, while

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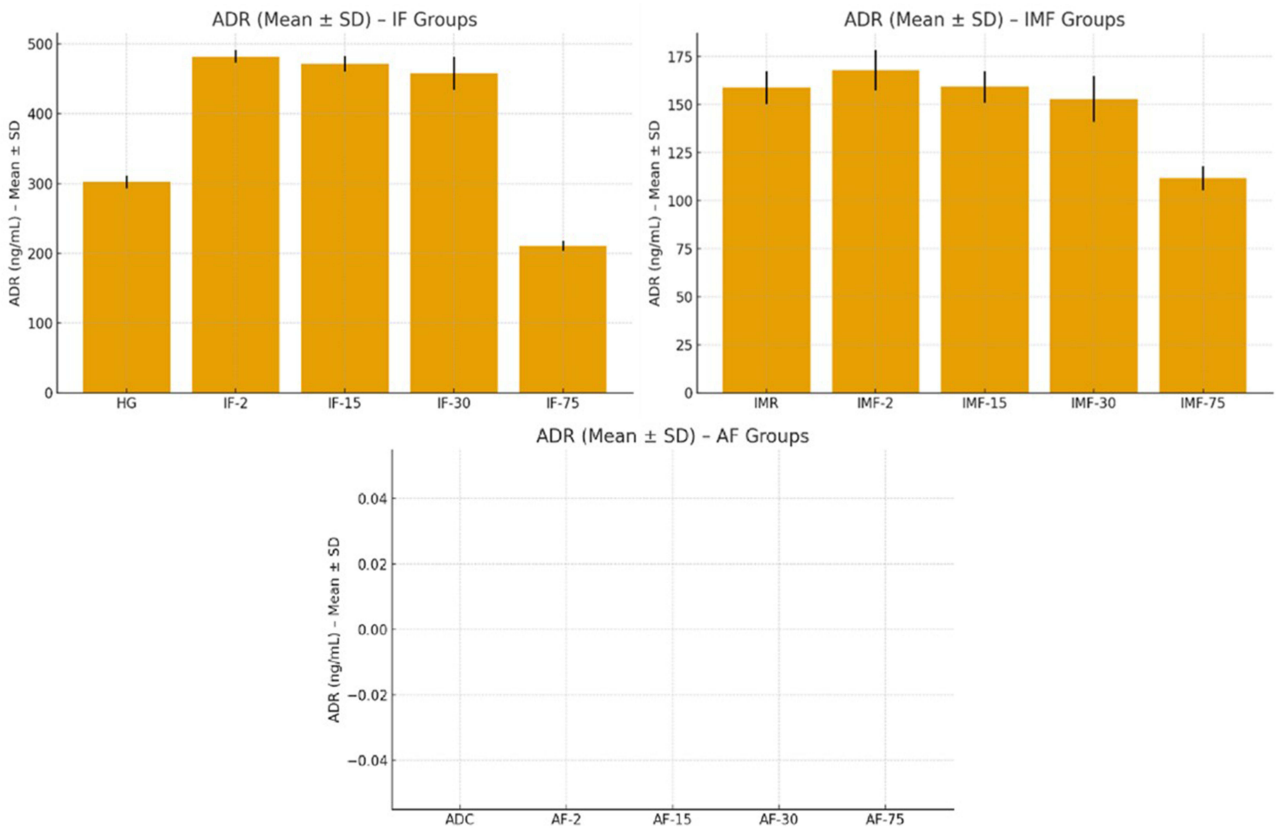

Figure 6 The results of the effects of fentanyl in intact rats, metyrosine-treated rats and adrenalectomized rats on blood ADR levels.
Notes: Data are expressed as mean ± SD (n = 6 per group). Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test.
Abbreviations: HG, healthy control; IF, fentanyl-treated group; IMR, metyrosine alone group; IMF, metyrosine + fentanyl group; ADC, adrenalectomized rats control; AF, fentanyl-treated group; ADR, adrenalin.

in other contexts they may enhance antioxidant defense mechanisms.[23] Chronic fentanyl administration has been shown to induce oxidative stress in the brain[24] and elevate MDA levels.[25] Yadav et al demonstrated that fentanyl and several of its analogs exert oxidative stress–inducing effects in rodents, reporting increased MDA levels in brain tissue and decreased GSH levels in some organs.[26] Similarly, Kosal et al indicated that fentanyl treatment leads to oxidative damage, evidenced by significant reductions in CAT and SOD levels.[27] In contrast, our data revealed no significant differences in MDA or tGSH concentrations at any of the fentanyl doses administered to intact animals. This suggests that the oxidative damage–inducing potential of fentanyl does not become pronounced in a dose-dependent manner, or alternatively, that the antioxidant system may compensate for this effect. Similarly, neither metyrosine alone nor the metyrosine–fentanyl combination produced significant alterations in oxidative or antioxidant parameters. Çimen et al reported that metyrosine significantly prevented the ischemia-reperfusion–induced increase in MDA and the decrease in GSH and SOD levels.[28] Nevertheless, that study did not investigate the effects of metyrosine on oxidative or antioxidant markers in intact animals. Removal of the adrenal glands results in the complete elimination of adrenal-derived hormones from the circulation, particularly glucocorticoids and catecholamines. Because steroid compounds function as natural antioxidants,[29] the organism's anti-inflammatory and antioxidant defense capacity is consequently reduced. Prasad et al reported that oxidative stress is associated with adrenal insufficiency and that the deficiency of adrenal hormones may lead to oxidative damage.[30] In parallel with these findings, our study demonstrated that all adrenalectomized animal groups exhibited significantly elevated oxidant levels and reduced antioxidant levels compared to the intact groups.

As is well established, IL-6 is a cytokine that stimulates the synthesis of acute-phase proteins.[31] De Cosmo et al examined postoperative IL-6 levels in patients receiving fentanyl and reported no significant reduction.[32] In contrast,

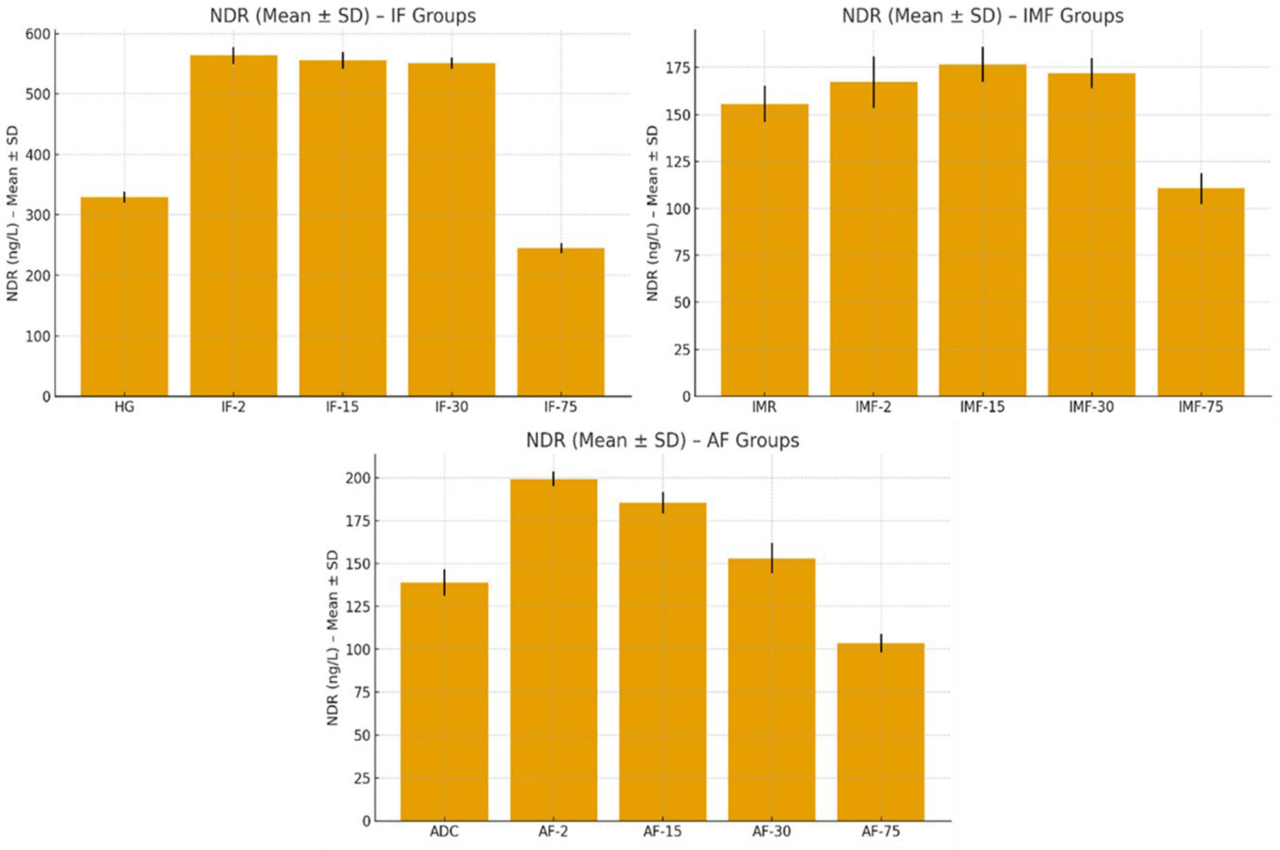

Figure 7 The results of the effects of fentanyl in intact rats, metyrosine-treated rats and adrenalectomized rats on blood NDR levels.
Notes: Data are expressed as mean ± SD (n = 6 per group). Statistical analyses were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test.
Abbreviations: HG, healthy control; IF, fentanyl-treated group; IMR, metyrosine alone group; IMF, metyrosine + fentanyl group; ADC, adrenalectomized rats control; AF, adrenalectomized fentanyl-treated group; NDR, noradrenalin.

Kwon et al demonstrated that preoperative transdermal fentanyl attenuated the postoperative increase in IL-6 levels and suppressed inflammation[33] Previous studies therefore suggest that fentanyl does not exert a unidirectional effect in this context and may display both anti-inflammatory and pro-inflammatory properties. In our study, no significant change in IL-6 levels was observed in the groups receiving fentanyl. There are reports indicating that metyrosine exerts protective effects against oxidative damage and reduces inflammation.[28,34] However, our findings showed that the combination of metyrosine and fentanyl did not produce any significant alteration in IL-6 concentrations. These results indicate that metyrosine does not influence basal cytokine levels and that the analgesic effect of fentanyl is independent of IL-6. Our data further suggest that increases in IL-6 production may be partially related to adrenal gland hormones. Fentanyl administration in adrenalectomized groups likewise did not significantly alter IL-6 levels. The literature indicates that cortisol possesses anti-inflammatory properties and plays an essential role in regulating immune responses,[35,36] and reduced cortisol levels may contribute to enhanced inflammation.[37] Our findings show that in the absence of adrenal hormones, fentanyl administration does not affect IL-6 elevation, thereby supporting the notion that its analgesic effect is independent of adrenal gland hormones. The concept that opioids may reduce inflammation[38] does not align with the results obtained for fentanyl in our study.

It is known that fentanyl, even at small doses that produce pronounced analgesia, increases adrenaline levels.[10] High-dose fentanyl anesthesia, on the other hand, suppresses elevations in catecholamine concentrations and reduces stress-related circulatory disturbances observed in balanced anesthesia protocols.[39] In our study, the significant increase in ADR levels in the intact groups receiving relatively low doses of fentanyl is consistent with the literature.[10] Moreover, the reduction in ADR levels observed at the higher fentanyl dose (75 μg/kg) aligns with reports indicating that high-dose

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fentanyl suppresses sympathetic activity.[39] ADR levels were significantly reduced in the group receiving metyrosine alone. Fentanyl at doses of 2, 15, and 30 μg/kg did not significantly prevent the metyrosine-induced decrease in ADR levels. However, the combination of metyrosine with the 75 μg/kg dose of fentanyl resulted in a further reduction in ADR concentrations. This finding may be explained by the inhibitory effect of metyrosine on adrenaline synthesis and the ability of fentanyl to suppress the release of existing adrenaline stores.[40] The dose-dependent increase in the analgesic activity of fentanyl did not significantly differ between animals with high and low ADR levels.

In our study, NDR levels increased in the groups of intact animals receiving relatively low doses of fentanyl (2, 15, 30 μg/kg), whereas they decreased at the higher dose. The reduction in NDR concentrations at the high dose is consistent with the literature.[10] Our data also demonstrated that metyrosine inhibits noradrenaline synthesis. Fentanyl at doses of 2, 15, and 30 μg/kg did not significantly antagonize the effect of metyrosine on NDR levels; however, the high dose produced a significant decrease. The literature contains evidence that μ-opioid receptor agonists reduce NDR release.[40] Because the adrenal medulla is absent in adrenalectomized animals, circulating ADR is completely eliminated.[41] In accordance with this, we observed that adrenaline production was entirely abolished in the adrenalectomized group. The relatively higher levels of NDR compared to ADR in these animals may be attributed to the fact that NDR is not solely derived from the adrenal glands but is also released by the sympathetic nervous system.[42] In adrenalectomized animals, low-dose fentanyl initially increased NDR through sympathetic nervous system activation; however, circulating noradrenaline levels subsequently decreased due to central sympathetic inhibition and the absence of an adrenal medullary response.

Limitations

Several limitations of this study should be acknowledged. The use of rats as the experimental model restricts the generalizability of the findings directly to humans. Additionally, only the anesthetic duration and analgesic potency of fentanyl were evaluated, without comparison to other opioid agents. This confines the conclusions specifically to fentanyl. Because ADR and NDR levels were measured only in peripheral serum, they may not accurately reflect the actual neurotransmitter concentrations within the synaptic cleft. Similarly, although the measurements of MDA, tGSH, and IL-6 provide a general indication of oxidative stress and inflammation, they are insufficient to fully elucidate these processes. Another limitation of the study is the use of one-way ANOVA instead of a two-way model. We acknowledge that this may have limited the evaluation of potential interaction effects between fentanyl dose and treatment condition. Furthermore, the absence of statistically significant differences in some parameters should be interpreted with caution, as this may result from limited statistical power or the timing of measurements rather than the true absence of a biological effect. In addition, future studies employing larger sample sizes and longer exposure and observation periods may help to identify meaningful changes in inflammatory and oxidative stress markers that may not be detectable at early time points. To clarify the underlying mechanisms and validate the current results, future studies should include different doses of fentanyl and incorporate central-level measurements.

Conclusion

In conclusion, our findings indicate that the analgesic effect of fentanyl is dose-dependent, yet occurs independently of serum ADR and NDR levels. High-dose fentanyl (75 μg/kg) did not produce an anesthetic effect. Evaluation of oxidative stress parameters revealed that fentanyl did not induce significant changes in MDA or tGSH levels. The increase in MDA and decrease in tGSH observed in the absence of adrenal hormones support the notion that these hormones play an indirect role in antioxidant defense. The inflammatory marker IL-6 did not change significantly in any group, suggesting that short-term fentanyl administration does not alter pro-inflammatory IL-6 production. The influence of fentanyl on the adrenergic system appeared to vary depending on dose and hormonal status: while low doses increased adrenaline levels, high doses suppressed them. Changes in noradrenaline concentrations may be attributable to both adrenal and sympathetic sources. Metyrosine and adrenalectomy attenuated these effects, indicating that fentanyl modulates catecholamine responses at both central and peripheral levels. Overall, the analgesic effect of fentanyl is likely mediated through μ-opioid receptor activation. It was further concluded that adrenal hormone deficiency partially affects pain threshold and

oxidative balance. These findings are based on acute, peripheral measurements obtained in a rat model and should be interpreted within this experimental scope.

Ethics Approval and Informed Consent

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020). Rats were anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg). At the end of the experiment, euthanasia was performed under deep anesthesia by administering an intraperitoneal overdose of sodium pentobarbital (150 mg/kg). All efforts were made to minimize pain, distress, and the number of animals used. The study protocol was approved by the Institutional Animal Ethics Committee (Date: 27/04/2025; Meeting No: 2025/04). Human participants were not involved in this study.

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Disclosure

The authors declare that there are no conflicts of interest for this work.

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