

1 **Nefopam as a Central Analgesic in the Chick Model: Dose-Response**
2 **Efficacy, Synergistic Interaction with Ketorolac, and Potentiation of**
3 **Ketamine Anesthesia**

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ABSTRACT

Introduction: The aim of this study was to reveal the analgesic efficacy of nefopam in the chicks model and to investigate the extent and type of interaction between nefopam and ketorolac.

Materials & Methods: Analgesia and drug interactions were evaluated in chicks model. Nefopam was administered at doses of 4, 8, and 16 mg/kg (ED25, ED50, and ED100, respectively). Nefopam (16 mg/kg) was combined with ketorolac (16 mg/kg) using a formaldehyde-induced paw edema model in chicks and compared against control groups. Nefopam (16 mg/kg) was evaluated in combination with ketamine (25 mg/kg), both with and without ketorolac (16 mg/kg), to measure analgesia and hypnosis. Nefopam and ketorolac were administered at a 0.5:0.5 ratio of their respective ED50 values, and the interaction index was calculated to characterize the nature of the drug interaction.

Results: Nefopam produced dose-dependent analgesia in chicks at rates of 35%, 50%, and 100% at doses of 4, 8, and 16 mg/kg, i.m., respectively. Combining nefopam (16 mg/kg) with ketorolac (16 mg/kg) significantly enhanced anti-inflammatory activity compared to the control group. Nefopam combined with ketamine significantly increased both analgesia and hypnosis compared to ketamine alone. The triple combination of nefopam, ketorolac, and ketamine resulted in even greater anesthetic efficacy. Isobolographic analysis yielded interaction index values confirming a synergistic interaction between nefopam and ketorolac at the 0.5:0.5 ED50 ratio.

Conclusion: Nefopam acts as a potent, dose-dependent analgesic in chicks that synergizes with ketorolac and enhances ketamine's anesthetic effects, demonstrating strong potential as a veterinary anesthetic adjunct at reduced doses.

Keywords: Analgesia, Isobolographic analysis, Ketamine, Ketorolac, Nefopam

1. Introduction

Nefopam is a non-opioid, non-specialized analgesic primarily utilized for the management of moderate to severe acute or chronic pain. It is also effective in controlling neuropathic pain. When co-administered with analgesics such as acetaminophen, the analgesic efficacy of nefopam is significantly improved [1].

As a centrally acting analgesic, nefopam is distinct from opioid medications such as morphine and tramadol in that it does not induce respiratory depression. Nefopam exerts its antinociceptive effects through unique mechanisms in the brain and spinal cord, including the inhibition of neuronal sodium and calcium channels, which subsequently prevents the release of glutamate an excitatory neurotransmitter vital to pain signaling. Additionally, it increases the concentration of serotonin, norepinephrine, and dopamine within the synaptic cleft by inhibiting their re-uptake and suppressing monoamine oxidase (MAO) enzymes [1].

Nefopam is classified as a non-opioid, non-steroidal, centrally acting analgesic. It is distinct from traditional analgesic classes, as it does not bind to opioid receptors nor does it exhibit the classic anti-inflammatory properties associated with non-steroidal anti-inflammatory drugs (NSAIDs). Due to its robust pharmacological profile, nefopam is widely utilized for the management of moderate to severe pain, encompassing both acute and chronic conditions. Its primary clinical applications include postoperative analgesia, frequently administered to mitigate acute pain following surgical procedures and for specialized pain management in treating dental pain, malignancy-associated (cancer) pain, and musculoskeletal disorders [2].

Nefopam offers several significant clinical advantages over traditional analgesics:

Respiratory safety: Unlike opioids such as morphine and tramadol, nefopam provides potent central analgesia without inducing respiratory depression. Neurological and cardiovascular stability: Although it acts centrally, it is not classified as a general

anesthetic and does not produce inhibitory or depressive effects on the central nervous system or the cardiovascular system. Gastrointestinal and hematological integrity: A critical advantage of nefopam over NSAIDs like aspirin and diclofenac is its safety profile regarding the gastrointestinal tract. It does not cause erosion of the gastric mucosa or subsequent hemorrhage, as it has no effect on platelet [3].

Nefopam is pharmacologically classified as a non-opioid, non-steroidal, centrally acting analgesic that is distinct from traditional analgesic classes as it does not bind to opioid receptors nor exhibit the anti-inflammatory properties associated with non-steroidal anti-inflammatory drugs. Due to its robust profile, it is widely utilized to manage moderate to severe acute and chronic pain, including postoperative, dental, musculoskeletal, and malignancy-associated pain [2]. Clinically, nefopam offers significant advantages over opioids and NSAIDs, as it provides potent analgesia without inducing respiratory depression, cardiovascular or CNS inhibition, gastric mucosal erosion, or interference with platelet aggregation. Pharmacokinetically, it possesses a half-life of approximately four to five hours and is approximately 73% plasma protein-bound, undergoing primary biotransformation in the liver with secondary renal excretion [3]. However, its clearance can be compromised in patients with renal impairment affecting enzymes like Cytochrome P450 or glucuronidation pathways requiring dose reductions in end-stage renal failure to prevent toxic accumulation. Despite its safety benefits, nefopam is associated with antimuscarinic side effects such as dry mouth and cardiovascular stimulation including tachycardia, palpitations, and rare instances of angina or arrhythmia, particularly at high doses [4].

Ketorolac is a prominent analgesic belonging to the first generation of non-steroidal anti-inflammatory drugs (NSAIDs). It provides analgesia via a peripheral mechanism involving the reversible, non-specific inhibition of cyclooxygenase enzymes (COX-1

and COX-2). This prevents the conversion of arachidonic acid into prostaglandins, which are key mediators of pain, fever, and inflammation [5].

Ketorolac is characterized by its use in treating moderate to severe pain, its relatively low side-effect profile, and its affordability. Unlike opioids, it does not produce adverse effects on the cardiovascular or central nervous systems, nor does it cause respiratory depression. In postoperative settings, ketorolac effectively manages pain while offering anti-inflammatory benefits and a higher therapeutic safety margin compared to opioids [5].

Ketorolac is clinically indicated for the management of moderate to severe postoperative pain, particularly following abdominal, orthopedic, and urological surgeries. Furthermore, it is utilized for the relief of renal colic, trauma-induced pain, and visceral pain associated with malignancies, as well as the alleviation of pain secondary to primary dysmenorrhea [5].

Ketorolac effectively serves as an opioid-sparing agent, significantly reducing the required dosage of opioids when administered concurrently. Research indicates that the co-administration of ketorolac with morphine allows for a lower effective dose of morphine compared to its use as a monotherapy. This dosage reduction consequently mitigates severe opioid-related adverse effects, such as respiratory depression, urinary retention, and emesis [5].

Studies demonstrate that the combination of nefopam and ketoprofen produces a potent synergistic analgesic effect, as confirmed by isobolographic analysis, which allows for enhanced pain management at reduced dosages [6]. When integrated into a balanced anesthesia protocol with ketamine, nefopam effectively serves as a preanesthetic agent by prolonging hypnotic duration.

The hypothesis of this study is based on the premise that nefopam possesses central analgesic properties that may be effective in the chick model. Furthermore, nefopam

may interact with the peripherally acting analgesic ketorolac, potentially leading to enhanced efficacy. This interaction may be attributed to pharmacodynamic or pharmacokinetic mechanisms, or a combination of both.

The aim of this study was to reveal the analgesic efficacy of nefopam in the chicks model and to indicate the degree of its safety for future use on other animals and to investigate the extent and type of interaction between nefopam and the analgesic drug ketorolac, as well as to study the change at pharmacokinetic level of nefopam when given with ketorolac.

2. Materials and methods

2.1. Animals

This research utilized Ross (Belgian strain) broiler chicks of both sexes, sourced from the Al-Diyar hatchery in Erbil Governorate. A total of 242 chicks were employed in the experiments, with an allocation of six chicks per experimental unit. Upon arrival at one day of age, the subjects were housed in specialized rearing cages within the animal house facility at the College of Veterinary Medicine, University of Mosul. The dimensions of the enclosures were 180 × 150 × 150 cm. Standardized environmental conditions essential for avian development were rigorously maintained, including optimized ventilation and a controlled ambient temperature ranging from 32°C to 35°C. The photoperiodic regimen consisted of 23 hours of light followed by 1 hour of darkness. The subjects were provided with consistent bedding, concentrated feed, and ad libitum access to water, and were reared under these conditions until the commencement of experimental procedures at 7-15 days of age.

2.2. Drugs preparation

The required dilutions of the experimental pharmacological agents specifically nefopam (1%, Medisol, France), ketorolac (3%, Normon, Spain), and ketamine (5%,

Hameln Pharmaceuticals gmbh, Germany) were prepared to achieve the precise target dosages. This was accomplished by diluting the stock high-concentration solutions with physiological saline (0.9 %, NaCl) to a final volume of 5 ml. The administration volume for nefopam, ketorolac, and ketamine was standardized at 5 ml/kg of body weight. The substances were administered via intramuscular (i.m.) injection into the pectoral muscle (*Musculus pectoralis*) of the broiler chicks.

2.3. Assessment of the analgesic dose-response relationship of nefopam

This experiment was conducted to identify the optimal analgesic dosage of nefopam in broiler chicks. The selection of multiple dosage levels was informed by the previously determined median effective analgesic dose [7-9], which was established as 8.39 mg/kg via intramuscular administration. The study utilized 18 broiler chicks (aged 7-15 days; weighing 90-130 g), which were randomly allocated into three experimental groups (n = 6 per group).

Based on the calculated ED₅₀, the dosages were standardized as follows:

Group I (ED₂₅): Administered 4 mg/kg of nefopam.

Group II (ED₅₀): Administered 8 mg/kg of nefopam.

Group III (ED₁₀₀): Administered 16 mg/kg of nefopam.

Pharmacological agents were administered via intramuscular injection into the pectoral muscle. Analgesic efficacy was evaluated using an electrical stimulator (Harvard Apparatus, USA) by measuring the voltage threshold required to elicit a pain response. Following established protocols for avian models [10], the stimulator electrodes were attached sub-axillary (under the wing). The area was moistened with water to ensure optimal electrical conductivity.

The voltage was increased incrementally until a nociceptive response characterized by vocalization was observed. Baseline (pre-injection) thresholds were recorded for each subject. Subsequently, post-treatment thresholds were measured 15 minutes after the

administration of the assigned nefopam dosage to quantify the degree of antinociception.

2.4. Assessment of anti-inflammatory efficacy of nefopam and ketorolac individually and in combination via formaldehyde-induced paw edema

This experiment aimed to evaluate the anti-inflammatory properties of nefopam and ketorolac using the formaldehyde-induced paw edema model in avian subjects, following the methodology described by Sondhi et al. [11]. The study utilized 30 broiler chicks (aged 7-15 days; weighing 110-170 g), distributed into five experimental groups (n = 6 per group).

Inflammation was induced by the subplantar injection of 0.05 ml of 0.1% formaldehyde (37% stock, Agrikima, Malizia) into the right paw. This procedure elicits a localized inflammatory response characterized by edema, erythema, and swelling. The experimental groups were designated as follows:

Group I (Negative Control): Subjects received physiological saline (5 ml/kg, i.m.) 15 minutes prior to a subplantar injection of saline (0.05 ml).

Group II (Positive Control): Subjects received physiological saline (5 ml/kg, i.m.) 15 minutes prior to the formaldehyde challenge (0.05 ml, subplantar).

Group III: Subjects were pre-treated with nefopam (16 mg/kg, i.m.) 15 minutes before the formaldehyde injection. This dosage was selected based on its previously demonstrated 100% analgesic efficacy.

Group IV: Subjects were pre-treated with ketorolac (16 mg/kg, i.m.) 15 minutes before the formaldehyde injection. This dosage was also selected for its 100% analgesic efficacy.

Group V (Combination): Subjects received a co-administration of nefopam (16 mg/kg) and ketorolac (16 mg/kg) via intramuscular injection 15 minutes prior to the formaldehyde challenge.

Paw thickness was quantified in millimeters (mm) using a digital vernier caliper. Measurements were recorded at baseline (pre-injection) and 30 minutes following the administration of the pharmacological agents. The anti-inflammatory activity was calculated using the following percentage inhibition formula:

$$\text{Anti-inflammatory activity (\%)} = \frac{\text{delta paw thickness (control)} - \text{delta paw thickness (treated)}}{\text{delta paw thickness (control)}} \times 100$$

Where delta represents the change in paw thickness relative to the baseline measurement.

2.5. Evaluation of nefopam and ketorolac, individually and in combination, on balanced anesthesia induced by ketamine:

This experiment investigated the potentiation of ketamine-induced balanced anesthesia specifically analgesic efficacy and hypnotic activity by nefopam and ketorolac. The study utilized 24 broiler chicks (aged 7-15 days; weighing 70-120 g), divided into four experimental groups (n = 6 per group). The dosages for nefopam and ketorolac were standardized at 16 mg/kg based on previous results, while the ketamine dosage was set at 25 mg/kg in accordance with established avian pharmacological literature [12].

The experimental groups were organized as follows:

Group I (Positive Control): Administered ketamine (25 mg/kg, i.m.) as a single agent.

Group II: Administered a combination of ketamine and nefopam (25 and 16 mg/kg, i.m., respectively), injected simultaneously at separate sites.

Group III: Administered a combination of ketamine and ketorolac (25 and 16 mg/kg, i.m., respectively), injected simultaneously at separate sites.

Group IV: Administered a triple combination of ketamine, nefopam, and ketorolac (25, 16, and 16 mg/kg, i.m., respectively).

Pharmacodynamic Parameters Measured [13]:

Analgesic Efficacy: Evaluated using an electrical stimulator. Voltage thresholds were recorded for each subject at baseline (pre-injection) and 15 minutes post-administration.

Onset of Anesthesia (Hypnosis): Defined as the latency period from the moment of ketamine injection to the loss of the righting reflex.

Duration of Anesthesia (Sleep Time): Defined as the interval between the loss of the righting reflex and its subsequent recovery (return to normal posture).

Recovery Period: Defined as the duration from the initial onset of anesthesia until the subject regained full motor activity and normal behavioral patterns.

The methodology for assessing these anesthetic stages followed protocols established [10, 14-16].

2.6. Isobolographic analysis of the analgesic interaction between nefopam and ketorolac

This experiment was designed to characterize the nature of the pharmacological interaction between nefopam and ketorolac when administered in a fixed-ratio combination (0.5:0.5). The study utilized seven broiler chicks (aged 7-15 days; weighing 100–130 g).

2.7. Determination of the combined ED50

Based on prior experimental data, the individual median effective analgesic doses (ED50) [7] for nefopam and ketorolac were established as 8.39 mg/kg each. To evaluate the combination, an initial dose representing 50% of the ED50 for each drug (4.195 mg/kg nefopam + 4.195 mg/kg ketorolac) was administered.

The Up-and-Down method [17] was employed using an electrical stimulator to assess nociception. Dose adjustments (increments or decrements) were standardized at 25% (1.05 mg/kg) of the initial combined dose based on the observed analgesic response.

2.8. Quantitative assessment of interaction

The nature of the interaction was determined graphically via an isobologram and mathematically using the Interaction Index (Y), calculated as follows:

$$Y = \frac{d_a}{D_s} + \frac{d_b}{D_b}$$

Where D_a and D_b represent the ED₅₀ values of nefopam and ketorolac when administered individually; d_a and d_b represent the specific doses of each drug within the combination that produce the same 50% effect.

Interpretation Criteria:

$Y < 1$: Synergistic Interaction; $Y = 1$: Additive Interaction and $Y > 1$: Antagonistic Interaction.

This analysis follows established pharmacological frameworks for drug synergy [18].

2.9. Statistical Analysis

The parametric data were analyzed for more than two experimental groups were statistically analyzed using One-Way Analysis of Variance (ANOVA). Post-hoc comparisons to determine significant differences between means were conducted using the Least Significant Difference (LSD) Test. For comparisons involving only two parametric groups, Student's Paired or Unpaired t-Tests were employed as appropriate while non-parametric data analyzed using the Mann-Whitney U-Test for pairwise comparisons between specific groups. Categorical data and percentage distributions were statistically evaluated using the Fisher's Exact Probability Test. For all statistical procedures, the threshold for significance was set at a probability level of $P < 0.05$ [19].

3. Results

3.1. Assessment of the dose-response relationship for nefopam analgesia

The experimental results demonstrated that the analgesic response to nefopam in broiler chicks is strictly dose-dependent. The optimal analgesic efficacy was achieved at a dosage of 16 mg/kg of body weight (Group III), which elicited a 100% response rate,

effectively inducing antinociception in all subjects within this group. Statistical analysis revealed that Group III (16 mg/kg) exhibited significantly higher values ($P < 0.05$) compared to Group II (8 mg/kg) and Group I (4 mg/kg) across all measured parameters, including post-injection voltage thresholds, the net change in voltage (delta voltage), and the overall percentage of analgesia (Table 1).

Table 1. Effect of multi-dose analgesia of nefopam in chicks

Dose (mg/kg, i.m.)	Analgesia (%)	Pre-injection Voltage (V)	Post-injection Voltage (V)	Voltage Change (ΔV)
4	33.33 (2/6)	6.83 ± 0.54	7.50 ± 0.62	0.67 ± 0.42
8	50.00 (3/6)	7.33 ± 0.42	8.50 ± 0.62	1.17 ± 0.54
16	100.00 (6/6) *a	6.66 ± 0.49	13.33 ± 0.80 *a#	6.67 ± 0.42 *a

Values represent Mean $\pm$ SE (n=6). * $P < 0.05$ vs. 4 mg/kg; a $P < 0.05$ vs. 8 mg/kg; # $P < 0.05$ vs. pre-injection.

3.2. Assessment of anti-inflammatory efficacy of nefopam and ketorolac individually and in combination via formaldehyde-induced paw edema

The subplantar administration of 0.05 ml of formaldehyde induced a significant increase in paw thickness, indicative of an acute inflammatory response. Pre-treatment with ketorolac (16 mg/kg), both as a monotherapy and in combination with nefopam (16 mg/kg each), resulted in a significant reduction in paw edema, demonstrating potent anti-inflammatory activity. In contrast, nefopam administered alone failed to elicit any significant anti-inflammatory effect. Furthermore, the co-administration of nefopam and

ketorolac did not result in a synergistic or additive increase in the anti-inflammatory efficacy of ketorolac in this avian model. These findings, summarized in Table (2), suggest that while ketorolac possesses substantial anti-inflammatory properties, nefopam's pharmacological profile is primarily restricted to its analgesic activity.

Table 2. Anti-inflammatory efficacy of ketorolac and nefopam interaction

Groups	Pre-injection Paw Thickness (mm)	Post-injection Paw Thickness (mm)	Change in Thickness (mm)
Positive Control	8.42 ± 0.14	10.35 ± 0.18 #	1.92 ± 0.19
Nefopam	8.39 ± 0.13	9.95 ± 0.18 #	1.56 ± 0.15
Ketorolac	8.46 ± 0.12	8.67 ± 0.12 *a	0.20 ± 0.02 *a
Ketorolac + Nefopam	8.38 ± 0.16	8.57 ± 0.15 *a	0.19 ± 0.02 *a

* P < 0.05 vs. Positive Control; a P < 0.05 vs. Nefopam 16 mg/kg; # P < 0.05 vs. pre-injection.

3.3. The role of nefopam and ketorolac, individually or in combination, in potentiating ketamine-induced balanced anesthesia

3.3.A. Analgesic efficacy of ketamine

The administration of ketamine as a monotherapy (25 mg/kg), as well as its administration in combination with nefopam (16 mg/kg), ketorolac (16 mg/kg), or both agents concurrently, resulted in significant antinociceptive effects. Furthermore, the inclusion of either nefopam or ketorolac significantly enhanced the analgesic potency of

ketamine compared to the control group receiving ketamine alone. The most profound analgesic response was observed in the triple-combination group (ketamine + nefopam + ketorolac), which exhibited statistically significant differences ($P < 0.05$) in post-injection voltage thresholds and net voltage change (delta voltage) relative to all other experimental groups (Table 3-A).

Table 3-A. Effect of nefopam and ketorolac, individually or concurrently, on the analgesic efficacy of ketamine

Groups	Pre-injection Voltage (V)	Post-injection Voltage (V)	Voltage Change (V)
Ketamine (Positive Control)	8.33 ± 0.61	$12.50 \pm 0.62 \#$	4.16 ± 0.54
Ketamine + Nefopam	7.33 ± 0.42	$15.50 \pm 0.50^*, \#$	$8.17 \pm 0.54^*$
Ketamine + Ketorolac	8.33 ± 0.21	$14.50 \pm 0.34^*, \#$	$6.16 \pm 0.40^*, a$
Ketamine + Nefopam + Ketorolac	8.50 ± 0.34	$17.66 \pm 0.61^*, a, b, \#$	$9.17 \pm 0.65^*, b$

Values represent the Mean $\pm$ SE for six chicks per group.

Ketamine, Nefopam, and Ketorolac were administered intramuscularly (i.m.) at doses of 25, 16, and 16 mg/kg respectively, 15 minutes prior to analgesic evaluation.

* $P < 0.05$ vs. Ketamine; a $P < 0.05$ vs. Ketamine + Nefopam; b $P < 0.05$ vs. Ketamine + Ketorolac; # $P < 0.05$ vs. pre-injection voltage in the same group.

3.3.B. Hypnotic efficacy of ketamine

Following the intramuscular administration of ketamine (25 mg/kg), the onset of hypnosis in broiler chicks occurred within 1 minute, with a mean sleep duration of 23 minutes and a recovery period of 50 minutes. The concurrent administration of either nefopam or ketorolac (16 mg/kg, i.m.) significantly prolonged both the duration of hypnosis and the recovery time compared to the group receiving ketamine as a monotherapy. The most substantial and statistically significant ($P < 0.05$) extensions in sleep duration and recovery periods were observed in the groups where ketamine was combined with nefopam alone or with a mixture of both nefopam and ketorolac (Table 3-B).

Table 3-B. Effect of nefopam and ketorolac on ketamine-induced hypnosis

Groups	Onset of Sleep (min)	Sleep Duration (min)	Recovery Duration (min)
Ketamine	1.00 ± 0.00	23.33 ± 1.39	50.17 ± 2.29
Ketamine + Nefopam	1.00 ± 0.00	46.33 ± 2.40 *	82.83 ± 5.94 *
Ketamine + Ketorolac	1.00 ± 0.00	30.50 ± 2.06 a	50.00 ± 1.39 a
Ketamine + Nefopam + Ketorolac	1.00 ± 0.00	61.83 ± 3.85 *ab	99.17 ± 3.78 *ab

* $P < 0.05$ vs. Ketamine; a $P < 0.05$ vs. Ketamine + Nefopam; b $P < 0.05$ vs. Ketamine + Ketorolac.

3.4. Isobolographic analysis of the analgesic interaction between nefopam and ketorolac (analysis of the analgesic interaction at a 0.5:0.5 fixed-ratio)

Based on preceding experimental data, the individual median effective analgesic doses (ED₅₀) for nefopam and ketorolac were established at 8.39 mg/kg (i.m.) for each agent. Consequently, the initial theoretical additive dose at a 0.5:0.5 ratio was calculated to be 4.19 mg/kg (i.m.) for each drug. Upon experimental determination of the actual ED₅₀ for the combination at this fixed ratio, the value was found to be 3.45 mg/kg (i.m.). The nature of the pharmacological interaction between nefopam and ketorolac at the 0.5:0.5 ratio was characterized as synergistic. This was confirmed by the calculation of the interaction index (Y), which yielded a value of 0.82. Since $Y < 1$, the results quantitatively demonstrate a synergistic potentiation of the analgesic effect (Table 4, Figure 1).

Table 4. Qualitative analysis of the analgesic interaction between nefopam and ketorolac at a 0.5:0.5 ratio

Measurements	Nefopam	Ketorolac
ED ₅₀ of combination	3.45 mg/kg, i.m.	3.45 mg/kg, i.m.
Initial dose	4.19 mg/kg	4.19 mg/kg
Final dose	4.19 mg/kg	4.19 mg/kg
Dose increment/decrement	1.05 mg/kg	1.05 mg/kg

Number of chicks used 5 (XOXOX)

Interaction index (Y) 0.82

Drugs were administered 15 minutes before evaluation via electrical stimulation.

X: Occurrence of analgesia; O: Absence of analgesia.

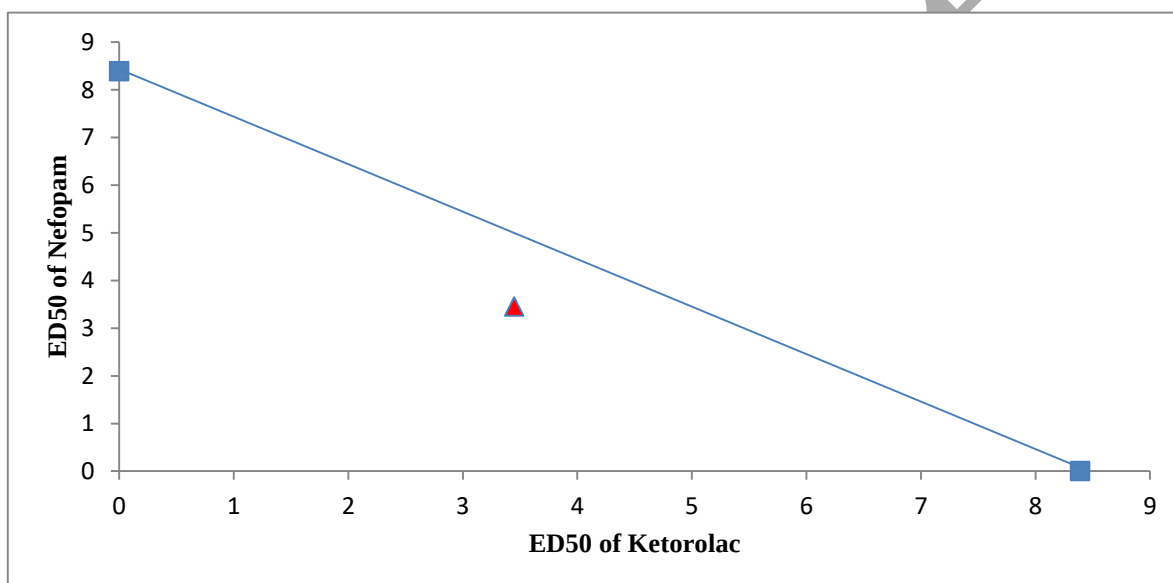


Figure 1. Isobolographic analysis demonstrates synergistic interaction between nefopam (Y-axis) and ketorolac (X-axis) when combined at 0.5:0.5 of their respective ED50 values.

3.5. Comparative analysis of analgesic efficacy between 0.5:0.5 and 1:1 ED50 ratios of nefopam and ketorolac

The analgesic potency of the nefopam-ketorolac combination was evaluated at two different dosage levels based on their previously determined ED50 values (8.39 mg/kg each).

Group I (0.5:0.5 ratio): Administration of 4.195 mg/kg of each drug (i.m.) resulted in an analgesic response rate of 83.33% (5/6 subjects).

Group II (1:1 ratio): Administration of 8.39 mg/kg of each drug (i.m.) achieved a 100% analgesic response rate (6/6 subjects).

Statistical analysis revealed that the 1:1 ratio group (Group II) exhibited a significantly higher analgesic effect ($P < 0.05$) compared to the 0.5:0.5 ratio group (Group I). This was evidenced by a substantial increase in post-injection voltage thresholds and a greater net change in voltage (delta voltage) confirming that increasing the dose proportions of the combination leads to a more robust antinociceptive response (Table 5).

Table 5. Comparison of analgesic efficacy at 0.5:0.5 and 1:1 ED50 ratios

Ratio (ED50)	Groups	Analgesia (%)	Rate	Pre-injection Voltage (V)	Post-injection Voltage (V)	Voltage Change (ΔV)
0.5 : 0.5		83.33 (5/6)		10.33 ± 0.33	12.67 ± 0.71	2.33 ± 0.56
1 : 1		100 (6/6)		10.00 ± 0.45	$17.17 \pm 1.01 \#$	$7.17 \pm 0.87 *$

* $P < 0.05$ vs. 0.5:0.5; # $P < 0.05$ vs. pre-injection.

4. Discussion

The primary objective of this study was to evaluate the analgesic efficacy and safety profile of nefopam individually and in combination with ketorolac using a chick model. The study aimed to establish the potential for future clinical applications in veterinary

medicine by investigating drug interactions and their impact on balanced anesthesia, as well as evaluating the efficiency of these models for research and clinical purposes.

Nefopam, a centrally acting analgesic that exerts its effects on the central nervous system (CNS), specifically the brain and spinal cord. It functions by inhibiting the reuptake of serotonin, noradrenaline, and dopamine. Furthermore, it modulates neuronal excitability by inhibiting sodium and calcium channels, thereby preventing the release of glutamate, a primary excitatory neurotransmitter and mediator of pain [20] while ketorolac, a non-steroidal anti-inflammatory drug (NSAID) characterized by analgesic, antipyretic, and anti-inflammatory properties. It functions primarily through the peripheral inhibition of prostaglandin synthesis [21].

The study established a clear dose-dependent analgesic relationship for nefopam using an electro-stimulation model. Intramuscular doses of 4, 8, and 16 mg/kg significantly increased pain-evoking voltage thresholds compared to baseline. The optimal dosage was 16 elicited a 100% analgesic response rate, while 8 mg/kg and 4 mg/kg resulted in 50% and 35% response rates, respectively.

Inflammation was induced using the formaldehyde-induced paw edema model, which triggers erythema and swelling as a result of the substance's irritant properties [22]. Ketorolac successfully reduced paw thickness, consistent with its role in reducing inflammatory prostaglandins [21]. Nefopam demonstrated no significant anti-inflammatory activity when administered alone, due to its lack of effect on the prostaglandins responsible for localized inflammation [1] while their the co-administration of nefopam and ketorolac reduced paw edema. This may be attributed to a pharmacokinetic interaction where Nefopam increases the concentration of free ketorolac by competing for plasma protein binding sites or metabolic pathways.

Ketamine acts as an N-methyl-D-aspartate (NMDA) receptor antagonist, reducing glutamate release and preventing hyperalgesia [23]. Both nefopam and ketorolac

enhanced ketamine's (25 mg/kg) analgesic effects [12]. The synergy between Nefopam and Ketamine is likely due to their convergent mechanisms in reducing excitatory glutamate [1]. Ketorolac further improved the effect by inhibiting prostaglandin-mediated pain [21].

Nefopam significantly prolonged sleep duration and recovery time, suggesting its efficacy as a preanesthetic drug. Ketorolac's impact on hypnosis was minimal due to its peripheral mechanism[21].

The combination of all three agents significantly increased the duration of hypnosis and analgesia. This is supported by the fact that ketamine blocks NMDA receptors and reduces ionic influx [24], while nefopam acts as a glutamate antagonist. Ketorolac may further increase Ketamine's plasma concentration by reducing available protein binding sites [25].

The study utilized isobolographic analysis a graphical and mathematical method to determine drug interactions regardless of individual mechanisms. At a 0.5:0.5 ratio of their respective ED₅₀ values, the combination demonstrated a significant reduction in the required effective dose. The interaction index ($Y = 0.82$) confirmed a strong synergistic interaction, aligning with previous findings regarding Nefopam and other NSAIDs like ketoprofen [6].

Repeated administration over five days showed a progressive increase in analgesic efficacy. The significantly higher thresholds on the fifth day compared to the first suggest a cumulative effect or pharmacokinetic competition for metabolic and excretory pathways.

5. Conclusion

In conclusion, nefopam exhibits potent, dose-dependent analgesia in chicks and significantly enhances both the anti-inflammatory activity of ketorolac and the

anesthetic efficacy of ketamine. Isobolographic analysis confirms a synergistic interaction between nefopam and ketorolac, allowing for superior therapeutic outcomes at reduced individual doses. These findings establish nefopam as a highly effective analgesic and anesthetic adjunct in the avian model, supporting its clinical utility in broader veterinary applications.

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Authors' Contribution (Name in abbreviation)

Conceptualization, study design, and final approval: All authors; Data acquisition, analysis, and interpretation: OYJ; Writing the original draft: YJM; Review and editing: YJM.

Conflict of interests

The authors declared there is no conflict of interest.

Ethics approval

The research on the laboratory mice has been valid as by reviewing and approval via the technical and scientific committee at College of Veterinary Medicine/University of Mosul.

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Data Availability

Data of the present study will be available upon request from the corresponding author.

Usage of generative AI declaration

Used for possible correction of writing grammar and searching for references related to the manuscript.

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