Influence of Xylazine on the Plasma Concentration and Pharmacokinetics of Nefopam in Chickens

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Abstract
There are no prior studies on the pharmacological influence of xylazine on the plasma concentration and pharmacokinetics of nefopam in chickens. The median analgesic effective doses (ED_{50s}) of nefopam and xylazine were estimated individually as 9.75 and 1.65 mg/kg, i.m., respectively. Thereafter, their values were determined together in combination as 3.17 and 0.55 mg/kg, i.m. after administration at the ratio of 1:1 of their ED_{50s}. The pharmacodynamic interaction between nefopam and xylazine was designated as synergistic through the interaction index 0.66. The plasma concentration of nefopam alone (19.50 mg/kg, i.m.) estimated through different times (0.25, 0.5, 1, 2, 4, and 24 hours) were 349.83, 652.73, 564.38, 513.90, 406.63 and 349.83 µg/ml whereas the plasma concentration of nefopam and xylazine (19.50 and 3.30 mg/kg, i.m. respectively) was elevated to became 791.55, 1568.98, 854.65, 690.59, 488.66 and 400.31 µg/ml by 126, 140, 51, 34, 20 and 14 %, respectively. The changes in the pharmacokinetic parameters of nefopam included decreases in area under curve ($AUC_{0-\infty}$)(9%), area under moment curve ($AUMC_{0-\infty}$)(39%), mean residence time (MRT)(33%), half-life ($t_{1/2\beta}$)(33%) and volume of distribution at steady state (V_{ss})(26%), whereas other values were increased which included concentration maximum (C_{max}) (58%), elimination rate constant (K_{el})(32%), and clearance (Cl)(14%). The net results indicated a synergistic interaction between nefopam and xylazine in addition to an alteration in nefopam pharmacokinetic parameters which may enhance nefopam therapeutic efficacy in chicks.
Keywords: Chicks, Nefopam, Pharmacodynamics, Pharmacokinetics, Xylazine

1. Introduction

Nefopam belongs to non-narcotic analgesic drugs that cast-off for treatment of modest to severe pain sensation (Alfonsi et al., 2004; Girard et al., 2016) and to manage a neuropathic pain disorder (Kim and Abdi, 2014). Its analgesic action may be enhanced by acetaminophen coadministration (Li et al., 2018). By its centrally acting on the brain and spinal cord, nefopam might produce a well, more weighty and consistent analgesia devoid of producing respiratory inhibition likewise to morphine (Kapfer wt al., 2005; Zanjani et al., 2013; Kang et al., 2019) and oxycodone (Tigerstedt et al., 1979). Nefopam acts by an exclusive mode of action through producing analgesia by either change of sodium and calcium channels that reduce releasing of glutamate which considered a crucial neurotransmitter related to pain occurrence or it raises catecholamines (especially norepinephrine and dopamine) and serotonin action by decreasing their re-uptake to the presynaptic neurons, which are well-planned a pain signaling reliant neurotransmitters (Sanga et al., 2016).
Furthermore, xylazine considered to have sedative, analgesic and muscle relaxant effects that are the results of its action by stimulating α_2–adrenoceptor causing an inhibition of the release of noradrenaline neurotransmitter and leading to depression of the central nervous system (Pawson, 2008; Kleinz and Spence, 2008). Xylazine is used commonly with ketamine to produce balanced anesthesia characterized by good hypnotic, analgesic and muscle relaxant effects (Pawson, 2008; Kleinz and Spence, 2008).
The current objective was to examine the effect of xylazine on nefopam plasma concentration and its pharmacokinetic parameters as well as their analgesic interaction in chicks by the isobolographic analysis.

2. Materials and Methods

Experimental chicks and drugs preparation

Seven to ten-day broiler chicks of both genders used in the trials, with a bodyweight of 92-120 g. They well-kept-up in 30-35°C, with nonstop light and the ground litter consequent from shreds of wood though water and food of chicks delivered freely. The

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dilution of nefopam (1%, Nefopam chlorhydrate, France) and xylazine (2% Interchemei, Holland) in a physiological saline solution (0.9% NaCl) to get the wanted dosage be injected intramuscularly (i.m.) by 5 ml/kg.

Animal ethics

The study and the usage of the experimental animals have been authenticated through monitoring by means of the scientific board of the department of Pharmacology, Physiology and Biochemistry, Veterinary Medicine College / Tikrit University.

Determination of analgesic interaction between nefopam and xylazine by using isobolographic analysis

The analgesic ED_{50s} of either nefopam and xylazine were assessed for each drug alone. Thereafter, the analgesic ED_{50} values of nefopam and xylazine together (at 1:1 from their ED_{50} values) were measured by the up-and-down technique in the chicks (Dixon, 1980). The first dosage of nefopam and xylazine in isobolographic analysis were at 9.75 and 1.65 mg/kg, i.m., respectively. The chicks were measured individually prior, and post 30 minutes of treatment of the two drugs via using the electro-stimulator (Harvard apparatus, USA) (occurrence of distress call marked to pain sensation in the chicks) (Mousa and Mohammad, 2012; Mousa, 2019; 2020; Mousa and Al-Zubaidy, 2019; Mousa, 2021; Mousa et al., 2021). At this time, the dosage of both drugs was reduced or raised by 25% (2.4 and 0.4 mg/kg) of the first dose used of both drugs as to look or lack of the analgesic action.

Measuring the analgesic interaction between nefopam and xylazine in the chicks

The ED_{50} values of nefopam (9.75 mg/kg, i.m.) and xylazine (1.65 mg/kg, i.m.) administered alone be positioned on x and y axes, correspondingly. Direct line will depicted to gain the isobolographic analysis among the ED_{50} dosages of nefopam and xylazine each alone that produces analgesia in experimental chicks. The line indicates the line of additive effect (no interaction). The point below the line signifies a synergistic interaction whereas the point above the line indicates an antagonism pattern. The interaction index will marked as Y symbol which could be figured out through the following:

da/Da + db/Db which:

Da and Db were the analgesic ED_{50s} of nefopam and xylazine each alone; da and db were their coadministered analgesic ED_{50s}, respectively (as shown in Table 1). Y= 1 indicates additive (there is no interaction), <1 is indicates synergistic interaction, and > 1is antagonistic kinds of interaction (Tallarida, 1992; Valle et al., 2000; Gonzalez et al., 2011).

Estimation of plasma concentration of nefopam alone and its modification with xylazine coadministration in the chicks

One group was treated with nefopam alone at mg/kg, i.m. while the other group was injected with nefopam (19.50 mg/kg, i.m.) and xylazine (3.30 mg/kg, i.m.). Blood samples got from the jugular vein for 5 chicks per estimated time at 0.25, 0.5, 1, 2, 4, and 24 hours for both the groups that received nefopam alone or nefopam plus xylazine. Then, plasma was obtained by addition of heparin (B. Braun Medical Inc, USA) (used as 1:10 v/v) to the blood samples and undergoing centrifugation (Chalice, UK) at 3000 rpm for 15 minutes. Lastly, plasma samples were frozen at -18 ^{O}C till analysis for 72 hours by the spectrophotometric device (Lovibond, Germany) with Ultraviolet (UV) detector (Singh et al., 2016; Sharma et al., 2017).

Determination of nefopam plasma concentration and its alteration with xylazine coadministration in the chicks

Preparing the phosphate buffer

The phosphate buffer solution was set by dissolving 6.82 g of KH_2PO_4 in 250 ml purified water in a graded flask to yield KH_2PO_4 (0.2 M). Another graded flask was used to set of NaOH (0.2 M) by dissolving 2 g of NaOH in 250 ml purified water. 195.5 ml of NaOH prepared before was added to 250 ml of KH_2PO_4 solution and then the volume of the solution will be completed to 1000 ml by addition of purified water. The pH of the resulted solution was attuned to 7.4 by the addition of either NaOH or HCl (Sharma et al., 2017).

Preparing of nefopam standards

The nefopam standards are made of 80, 160, 320, 640, 1280, and 2560 µg/ml (Sharma et al., 2017) by dilution of nefopam with the phosphate buffer (pH 7.4) previously described. The solution will undergo filtration through a filter paper. The net solution was finally analyzed by spectrophotometer at a wavelength of 266 nanometers (nm). Through the equation of the simple linear regression of the nefopam standards with R^2= 0.9958, the concentration of nefopam in the plasma samples can be calculated in both groups of experimental chicks (included nefopam alone with or without xylazine) (Figure 1).

y= a + b x which:

y= absorbance of plasma samples (at 266 nm by spectrophotometer apparatus); a= intercept (0.0038); b= slope (0.00003) and x= the nefopam concentration (unknown) in the plasma.

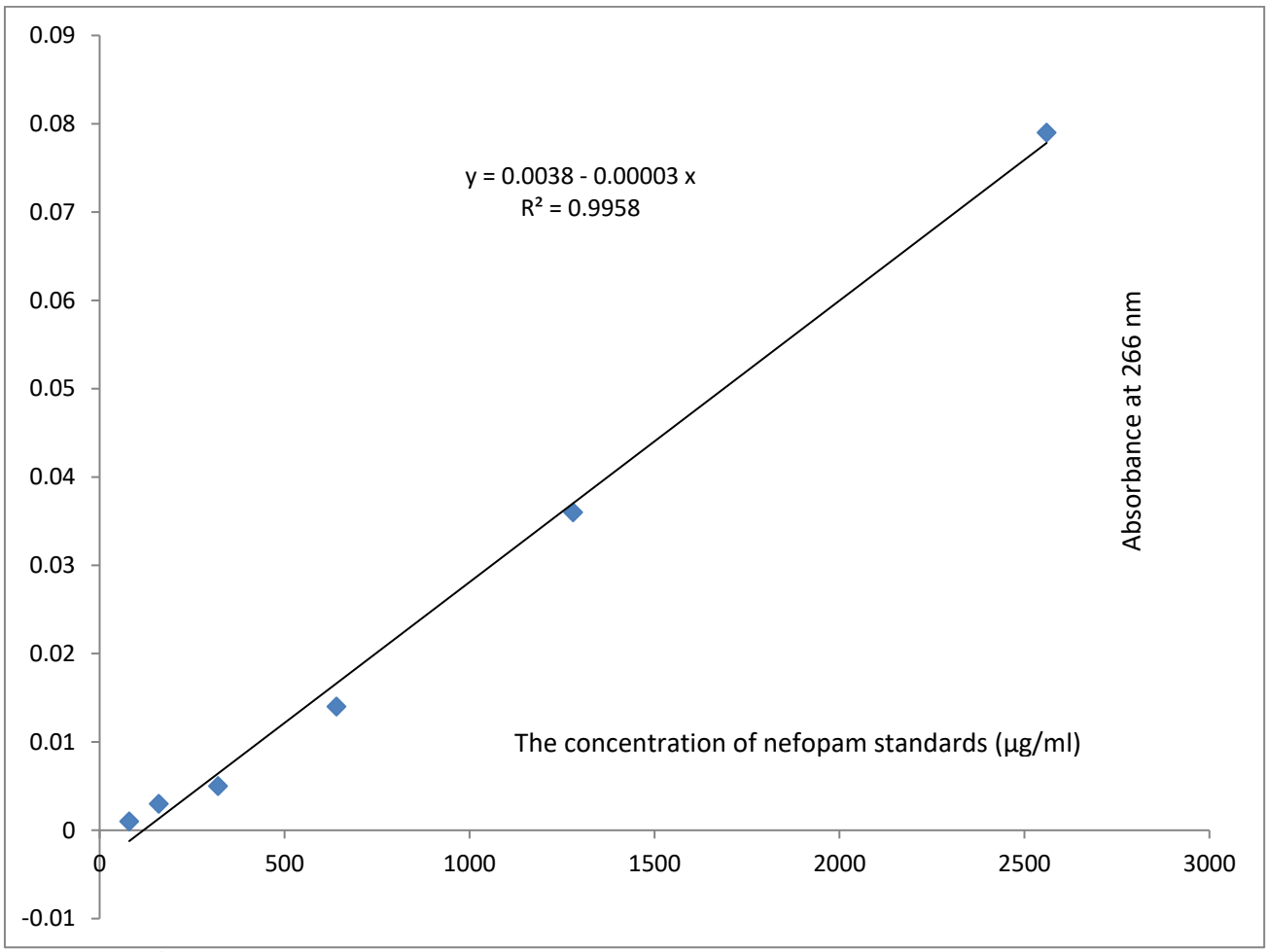

Figure (1): Nefopam's standards (80, 160, 320, 640, 1280, and 2560 µg/ml) and the absorbance at 266 nm represented in a simple linear regression

Extraction of nefopam from the plasma samples

A simple, authorized, and precise method for extraction of nefopam was set to the plasma samples (Singh et al., 2016). The technique was demonstrated by the addition of 1 ml of phosphate buffer (pH= 7.4) to 1 ml of plasma sample then after, the mixture was moved to a glass tube experiencing vortexing for 5 minutes. After that, centrifugation was conducted (3500 rpm for 10 minutes) to obtain the resultant solution that filtered through a filter paper. The final solution was detected by the spectrophotometer device combined with a UV-chromatographic detection at 266 nm. The absorbance of different dilutions was measured in contrast to the blank of phosphate buffer mentioned before.

Determination of nefopam pharmacokinetic parameters and its alteration with xylazine coadministration in the chicks

Non-compartmental model of pharmacokinetics was applied to obtain the pharmacokinetic parameters for nefopam alone or its combination with xylazine through a PKSolver program (Zhang et al., 2010). These parameters comprised of AUC $_{0-\infty}$ (µg×h/ml), $AUMC_{0-\infty}$ (µg×h^2/ml), MRT (AUMC/AUC)(h), $t_{1/2\beta}$ (h), T_{max} (h), C_{max} (µg), K_{el} (0.693/$t_{1/2\beta}$) (h^{-1}), V_{ss} [dose×AUMC/(AUC)^2](L/kg) and Cl (dose/AUC)(L/h/kg). The rise or reduction in the percentages obtained of these parameters was conducted in both groups that were treated with nefopam with or without xylazine.

Statistics

The parametric statistical examination was directed by an unpaired student T-test applied to relate the means of the two groups (Petrie and Watson, 2013). The level will be considered significant when $p < 0.05$.

3. Results

Analgesic interaction between nefopam and xylazine by isobolographic analysis

The analgesic ED_{50} value of nefopam alone was 9.75 mg/kg, i.m. and for xylazine alone was 1.65 mg/kg, i.m. The resulted analgesic ED_{50} values of nefopam and xylazine combinations were 3.17 and 0.55 mg/kg, i.m. when given together 1:1 from their ED_{50s}. Table 1 displays the various results gained from this experiment.

Analgesic interaction between nefopam and xylazine in the chicks

The interaction index (Y) is 0.66 (less than 1) so that, the pharmacodynamic interaction between nefopam and xylazine is synergistic as expressed in Table 1 and Figure 2.

Table (1): Analgesic interaction between nefopam and xylazine by using isobolographic analysis at a ratio of 1:1		
Parameters	Nefopam alone	Xylazine alone
ED_{50} value*	9.75 mg/kg, i.m.	1.65 mg/kg, i.m.
Initial dosage	10 mg/kg	2 mg/kg
Last dosage (xf)	10 mg/kg	2 mg/kg
± Dosage (d)	3 mg/kg	0.5 mg/kg
Range of the dosages	10-7= 2 mg/kg	2-1.5= 0.5mg/kg
Overall chicks	5 (XOXOO)	5 (XOXOX)
	Nefopam+xylazine (1:1)	
ED_{50} value*	3.17 mg/kg, i.m.	0.55 mg/kg, i.m.
Initial dosage	9.75 mg/kg	1.65 mg/kg
Last dosage (xf)	4.95 mg/kg	0.85 mg/kg
± Dosage (d)	2.4 mg/kg	0.4 mg/kg
Range of the dosages	9.75-2.55= 7.2 mg/kg	1.65-0.45= 1.2 mg/kg
Overall chicks	7 (XXXOXOX)	
#Y= da/Da + db/Db	0.66	
* ED50 value= xf + (k × d) X= result (analgesia), O= no result (no analgesia)		

Volts registered preinjection and after 30 minutes of nefopam and xylazine injection

Da and Db resembles ED50 results for nefopam versus xylazine alone while da and db resembles their coadministered ED50 results, respectively. An interaction index of 1 indicates additive, <1 synergism and > 1 antagonism interactions

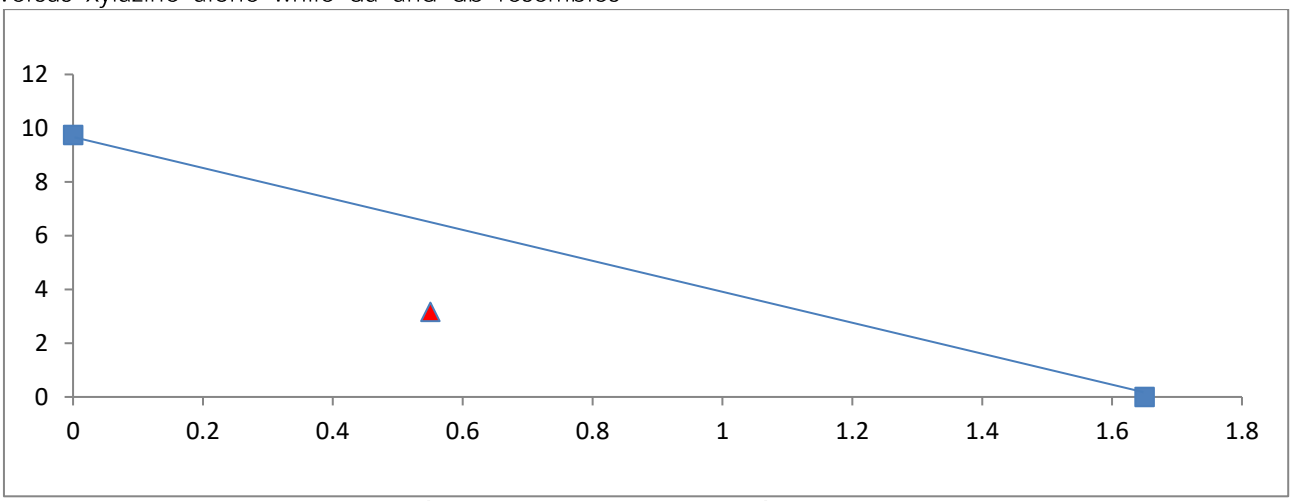

Figure (2): Isobolographic examination of analgesic interaction among nefopam and xylazine. The point on y-axis denotes the ED50 value of nefopam (9.75 mg/kg, i.m.) while the point on x-axis represents the ED50s of xylazine (1.65 mg/kg, i.m.). The triangular point represents 1:1 of ED50s combinations for both drugs (3.17 and 0.55 mg/kg, i.m. for nefopam and xylazine). The position of the triangular point indicates synergistic interaction between nefopam and xylazine.

Plasma concentration of nefopam (µg/ml) alone or its combination with xylazine in the chicks at different times

There is a significant rise in the nefopam plasma concentration (except at time 4 h after nefopam injection) when coadministered with xylazine in contrast to the group injected with nefopam alone. The plasma concentration of nefopam alone (19.50 mg/kg, i.m.) estimated through different times (0.25, 0.5, 1, 2, 4, and 24 hours) were 349.83, 652.73, 564.38, 513.90, 406.63 and 349.83 µg/ml whereas the plasma concentration of nefopam and xylazine (19.50 and 3.30 mg/kg, i.m. respectively) was elevated to became 791.55, 1568.98, 854.65, 690.59, 488.66 and 400.31 µg/ml by 126, 140, 51, 34, 20 and 14 %, respectively (Table 2 and Figure 3).

Table (2): Plasma concentration (µg/ml) of nefopam with or without xylazine in the chicks through different measured times			
Time (Hour)	Groups		Effect of xylazine on plasma concentration of nefopam (%) +
	Nefopam	Nefopam and xylazine	
0.25	349.83 ± 21.86	791.55 ± 33.39*	126
0.5	652.73 ± 189.31	1568.98 ± 164.33*	140
1	564.38 ± 45.51	854.65 ± 233.74*	51
2	513.90 ± 12.62	690.59 ± 139.11*	34
4	406.63 ± 28.94	488.66 ± 12.62	20
24	349.83 ± 21.85	400.31 ± 55.01*	14
Numbers characterized as mean ± SE (5 chicks/ time estimated) * Significantly dissimilar from the nefopam alone group ($p < 0.05$)			

Nefopam was injected at 19.50 mg/kg, i.m. alone or with xylazine at 3.30 mg/kg, i.m.
+ % of the effect of xylazine on plasma concentration of nefopam= nefopam plus xylazine - nefopam alone / nefopam alone × 100

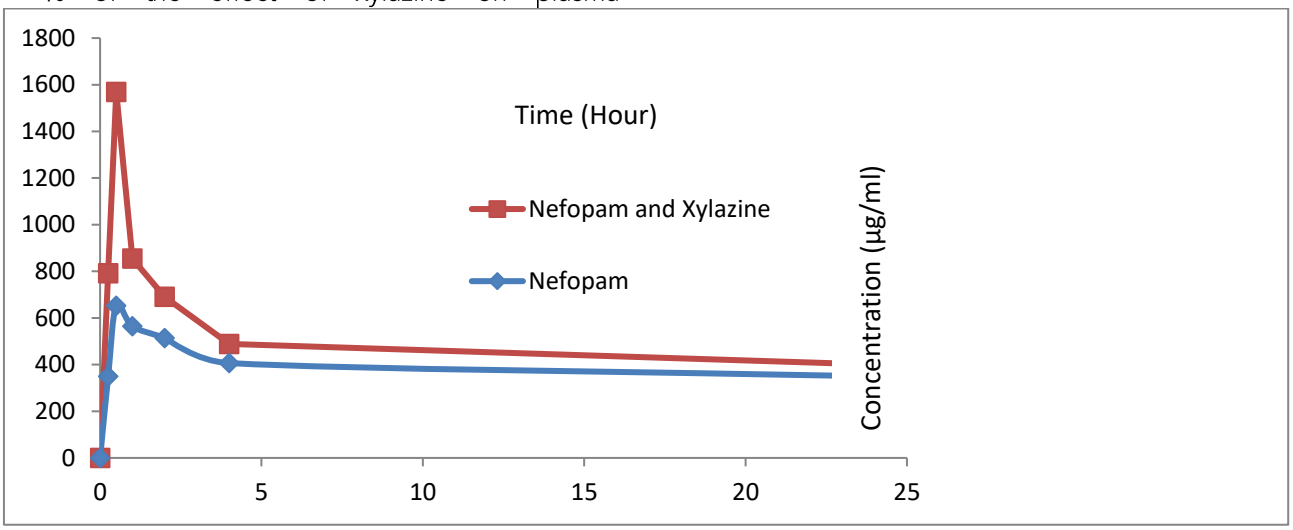

Figure (3): Plasma concentration of nefopam with or without xylazine in the chicks through different estimated times (0.25, 0.5, 1, 2, 4 and 24 Hour)

Pharmacokinetic profile of nefopam with or without xylazine in the chicks

Administration of nefopam alone elucidate the pharmacokinetic parameters included AUC 0-∞ (30284.23 µg×h/ml), AUMC 0-∞ (1837965.36 µg×h2/ml), MRT (60.69 h), t1/2β (41.19 h), Vss (0.038 L/kg), Tmax (0.5 h), Cmax (652.73 µg), Kel (0.017 h-1) and Cl (0.0006 L/h/kg). The values of AUC 0-∞, AUMC 0-∞, MRT, t1/2β and Vss in the chicks that administered nefopam plus xylazine were reduced to became 27704.17, 1129911.28, 40.78, 27.47 and 0.028 by 9, 39, 33, 33 and 26 %, respectively whereas other pharmacokinetic parameters included Cmax, Kel and Cl were elevated to became 1568.98, 0.025 and 0.0007 by 58, 32 and 14 % respectively in contrast to the group treated with nefopam alone (Table 3).

Table (3): Pharmacokinetic parameters of nefopam with or without xylazine in the chicks

Pharmacokinetic parameters	Units	Treated groups		Effect of xylazine (%)*
		Nefopam	Nefopam and xylazine	
$AUC_{0-\infty}$	µg×h/ml	30284.23	27704.17	(-) 9
$AUMC_{0-\infty}$	µg×h^2/ml	1837965.36	1129911.28	(-) 39
MRT = AUMC / AUC	h	60.69	40.78	(-) 33
$t_{1/2\beta}$	h	41.19	27.47	(-) 33
V_{ss}=dose×AUMC/$(AUC)^2$	L / kg	0.038	0.028	(-) 26
T_{max}	h	0.5	0.5	0
C_{max}	µg	652.73	1568.98	(+) 58
K_{el} = 0.693/ $t_{1/2\beta}$	h^{-1}	0.017	0.025	(+) 32
Cl = dose / AUC	L / h / kg	0.0006	0.0007	(+) 14

Nefopam was injected at 19.50 mg/kg, i.m. alone or with xylazine at 3.30 mg/kg, i.m.
Pharmacokinetic parameters gained are non-compartmental model measured using PKSolver program
* % of the effect of xylazine on plasma concentration of nefopam= nefopam plus xylazine - nefopam alone / nefopam alone × 100

4. Discussion

The objective was to examine the effect of xylazine on nefopam plasma concentration and its pharmacokinetic parameters besides their possible pharmacodynamics interaction (analgesic interaction) in the chicks as illustrated by isobolographic analysis. Nefopam is considered a good, profound, and non-narcotic analgesic medication used primarily to treat moderate and severe of acute or chronic nociception (Alfonsi et al., 2004; Girard et al., 2016), and its analgesic activity may be potentiated by certain drugs like acetaminophen (Li et al., 2018). By its centrally acting on the brain and spinal cord, nefopam could produce a better, more profound, and reliable analgesia without causing respiratory depression like morphine (Kapfer wt al., 2005; Zanjani et al., 2013; Kang et al., 2019). As found in this study, the values of ED50s for nefopam and xylazine combination were decreased in comparison for their values alone suggesting an increase in the analgesic efficacy which is required to produce analgesia in half of the population used as the experimental model. The isobolographic analysis considered a good tool for determining the type of pharmacological interaction between two drugs (Tallarida, 1992; Valle et al., 2000; Gonzalez et al., 2011) and as indicated here, there is a synergistic interaction between nefopam and tramadol through estimating their interaction index. This is thought to

be attributed to the different mechanisms of action of centrally acting drugs used in this study. The other important key for increase effectiveness and synergistic interaction between nefopam and xylazine was estimated here in this study which is the alteration in the different pharmacokinetic parameters of nefopam when coadministered with xylazine. The change in the pharmacokinetic profile of nefopam resulted from an increase in the plasma concentration (free drug) of nefopam affected by administering xylazine and this may be attributed to competition on the protein binding and the number of binding sites on plasma proteins (albumins) (direct effect of the apparent volume of distribution) because xylazine is considered to protein-bound drug (> %) which causes an elevation of nefopam free drugs available at the sites of action besides the direct effect of xylazine on the other crucial factors determining the pharmacokinetics included absorption, metabolism, and excretion.

5. Conclusions

The net results of this study indicated a synergistic interaction between nefopam and xylazine as well as an alteration in nefopam pharmacokinetic parameters which may enhance nefopam therapeutic efficacy in chicks.

6. Acknowledgments

We would like to acknowledge the University of Tikrit and Mosul, College of Veterinary Medicine, for the facilities delivered.

7. Conflict of Interest

The authors declare there is no conflict of interest.

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