Drug Safety and Analgesic Interaction of Nefopam and Xylazine in Chicks Model

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Abstract
The aim of the study was to investigate the drug safety besides the analgesic interaction of nefopam (NFM) and xylazine (XZN) in chicks model. The median lethal and effective (analgesic) doses (LD50 and ED50) of NFM were 47.89 and 9.75 mg/kg, IM, while for XZN were 64.93 and 1.65 mg/kg, IM, respectively. XZN was the safer drug than NFM in chicks as represented by the therapeutic index of 39 and 5, respectively. Subsequently, the values of NFM and XZN were estimated together in combination as 2.88 and 0.44 mg/kg, IM after administration at the ratio of 0.5:0.5 of their ED50s which reveals a pharmacodynamic interaction between them as synergism through the interaction index 0.56. NFM and XZN, alone or together, increases the analgesic efficiency of ketamine (KTN) anesthesia. The administration of NFM and XZN have no significantly deleterious effects on the liver (AST and ALT) and kidney (creatinine and uric acid) functions. The results suggested a drug safety and synergism between NFM and XZN in addition to their benefits of using these drugs as preanesthetics to enhance KTN anesthesia in chicks model.
Keywords: Chicks, Interaction, Ketamine, Nefopam, Xylazine

1. Introduction

NFM is a non-narcotic analgesic drugs that used for treatment of moderate to severe pain issues (Alfonsi et al., 2004; Girard et al., 2016) and to manage a neuropathic pain disorder (Kim and Abdi, 2014). Its analgesic action may be improved by administration of other drugs such as acetaminophen (Li et al., 2018). NFM acts centrally on the brain and spinal cord, and might produce a well, more profound and consistent analgesia without producing a respiratory depression likewise to other narcotic opioids such as morphine (Kapfer wt al., 2005; Zanjani et al., 2013; Kang et al., 2019) and oxycodone (Tigerstedt et al., 1979). NFM acts by a unique mode of action to produce analgesia by either blocking of sodium and calcium channels that reduce releasing of glutamate which considered a crucial neurotransmitter considered for pain occurrence or it raises norepinephrine, dopamine and serotonin by decreasing their re-uptake to the presynaptic neurons, which are considered a pain signaling dependent neurotransmitters (Sanga et al., 2016).
On the other hand, XZN well-thought-out to have sedative, analgesic and muscle relaxant effects that are the results of its action as agonist by stimulating α_2–adrenergic receptor so causes an inhibition of the release of noradrenaline neurotransmitter and leading to depression of the central nervous system (Pawson, 2008; Kleinz and Spence, 2008). XZN is used commonly with KTN in the veterinary medicine to produce balanced anesthesia characterized by good hypnotic, analgesic and muscle relaxant effects (Pawson, 2008; Kleinz and Spence, 2008).
The aim of the study was to investigate the drug safety besides the analgesic interaction of NFM and XZN in chick's model.

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 90-125 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 dilution of NFM (1%, Nefopam chlorhydrate, France) and XZN (2% Interchemei, Holland) in a physiological saline solution (0.9% NaCl) to get the wanted dosage be injected intramuscularly (IM).

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 median lethal doses (LD_{50}s) of NFM and XZN

The acute LD_{50s} of either NFM and XZN were assessed for each drug alone. The first dosage of NFM and XZN 100 mg/kg, IM for each drug estimated by the up-and-down method (Dixon, 1980). The chicks were assessed separately during 24

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hours for the occurrence of death and toxic effects. Then, the doses of the drugs will be decreased or increased 30 mg/kg for each drug according to the first doses used.

Estimation of median Effective doses (ED_{50}s) of NFM and XZN

The analgesic ED50s of either NFM and XZN were assessed for each drug alone. The first dosage of NFM and XZN were at 10 and 2 mg/kg, IM as illustrated according to the up-and-down technique (Dixon, 1980). The electro-stimulator device (Harvard apparatus, USA) was used to assess the occurrence of distress call in the chicks (as indicator to pain sensation). The chicks were assessed separately pre and post 30 minutes of IM medications treatment (Mousa, 2019; 2020; Mousa and Al-Zubaidy, 2019; Mousa, 2021; Mousa et al., 2019;2021a). Then, according to the look or lack of the analgesia, the doses of the drugs will be decreased or increased as 3 and 0.5 mg/kg, correspondingly according to the initial dose used.

Determination of drug safety of NFM and XZN

According to the first and second experiments mentioned above, the drug safety was estimated by using the equation of the therapeutic index which is LD_{50} / ED_{50}. The results from this equation will reveal the drug safety of the medications used in this study (Trevor et al., 2013).

Determination of analgesic interaction between NFM and XZN by using isobolographic analysis

The analgesic ED50s of either NFM and XZN were assessed for each drug alone. Thereafter, the analgesic ED50 values of NFM and XZN together (at 0.5:0.5 from their ED50 values) were measured by the up-and-down technique (Dixon, 1980). The first dosage of NFM and XZN in isobolographic analysis were at 4.88 and 0.83 mg/kg, IM, respectively. The chicks were measured individually prior, and post 30 minutes of treatment of the two drugs via using the electro-stimulator (occurrence of distress call marked to pain sensation in the chicks) (Mousa, 2019; 2020; Mousa and Al-Zubaidy, 2019; Mousa, 2021; Mousa et al., 2019;2021b). At this time, the dosage of both drugs was reduced or raised by 25% (1.22 and 0.21 mg/kg) of the first dose used of both drugs as to look or lack of the analgesic action.

Measuring the analgesic interaction between NFM and XZN

The ED50 values of NFM (9.75 mg/kg, IM) and XZN (1.65 mg/kg, IM) administered alone be positioned on x and y axes, correspondingly. Direct line will depicted to gain the isobolographic analysis among the ED50 dosages of NFM and XZN 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 ED50s of NFM and XZN each alone; da and db were their coadministered analgesic ED50s, 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).

Influence of NFM and XZN on KTN analgesia

This experiment included the use of 24 chicks of 7-15 days, and their weight ranged from 65-100 g. This experiment was divided into four groups, with six chicks per group. The dose of NFM and XZN was determined and used based on previous experiments, which are 19.5 mg/kg each, and the dose of KTN was 25 mg/kg based on previous studies (Mohammad and Faris, 2006).

The first group (positive control): KTN was injected at a dose of 25 mg/kg into the chest muscle.

The second group: KTN was injected with NFM (25 and 19.5 mg/kg IM, respectively), at the same time and separately.

The third group: KTN and XZN (25 and 3.3 mg/kg IM, respectively) were injected at the same time and each separately.

The fourth group: KTN was injected with NFM and XZN (25, 19.5 and 3.3 mg/kg IM, respectively).

The analgesic efficacy of KTN was recorded when it was given with NFM or XZN or both, using an electrical stimulator device, and the voltages of each chick were measured before injection and 30 minutes after injection of the used drugs.

Effects of NFM and XZN on the liver and kidney functions

After treatment with NFM (19.5 mg/kg, IM) and XZN (3.3 mg/kg, IM) for five consecutive days, the blood were taken from the four groups of chicks (6 chicks for each) in addition to the control group. Serum creatinine and uric acid were used to extrapolate the kidney function while the serum AST and ALT indicated the liver function (Plummer, 1987).

Statistics

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

3. Results

Determination of acute LD_{50}s of NFM and XZN

Table 1 illustrate the results gained from up-and down method. The acute LD_{50} value of NFM was

47.89 mg/kg, IM that causes death in 50 % of the chicks whereas XZN was 64.93 mg/kg, IM with some toxic effects like ataxia, torticollis, recumbency, convulsions and the death.

Table (1): The results of acute LD_{50}s of NFM and XZN in the chicks		
Parameters	NFM	XZN
ED_{50} *	47.89 mg/kg, IM	64.93 mg/kg, IM
First dosage	100 mg/kg	100 mg/kg
Latest dosage	70 mg/kg	70 mg/kg
± in the doses	30 mg/kg	30 mg/kg
Range of the dosages	100-40= 60 mg/kg	100-40= 60 mg/kg
Overall chicks	6 (XXOXOX)	6 (XXOXOO)
*LD_{50} = Latest dosage + (table value of Dixon × increased or decreased in the doses) X indicate death while O means survival		

Estimation of analgesic ED_{50}s of NFM and XZN

Table 2 demonstrate the several results obtained from up-and down method. The analgesic ED_{50} of NFM was 9.75 mg/kg, IM that exhibit analgesia in 50 % of the chicks whereas XZN was 1.65 mg/kg, IM.

Table (2): The results of analgesic ED_{50}s of NFM and XZN in the chicks		
Parameters	NFM	XZN
ED_{50} *	9.75 mg/kg, IM	1.65 mg/kg, IM
First dosage	10 mg/kg	2 mg/kg
Latest dosage	10 mg/kg	2 mg/kg
± in the doses	3 mg/kg	0.5 mg/kg
Range of the dosages	10-7= 3 mg/kg	2-1.5= 0.5 mg/kg
Overall chicks	5 (XOXOO)	5 (XOXOX)
*ED_{50} = Latest dosage + (table value of Dixon × increased or decreased in the doses) X indicate analgesia while O means no analgesia)		

Volt documented pre, and post 30 min. of NFM and XZN treatment

Determination of drug safety of NFM and XZN

Depending on the first experiment (LD50) and the second experiment (ED50) for NFM and XZN, the equation of therapeutic index was applied mathematically and the result was 5 and 39 (Table 3).

Table (3): Drug safety of NFM and XZN in the chicks		
Parameter	NFM	XZN
Therapeutic index*	5	39
*Therapeutic index = LD_{50} / ED_{50}		

Measuring the analgesic interaction between NFM and XZN

The analgesic ED50 value of NFM alone was 9.75 mg/kg, IM and for XZN alone was 1.65 mg/kg, IM. The resulted analgesic ED50 values of NFM and XZN combinations were 2.88 and 0.44 mg/kg, IM when given together 0.5:0.5 from their ED50s. Table 4 displays the various results gained from this experiment.

The interaction index (Y) is 0.56 (less than 1) so that, the pharmacodynamic interaction between NFM and XZN is synergistic as expressed in Table 4 and Figure 1.

Table (4): Analgesic interaction between NFM and XZN by using isobolographic analysis at a ratio of 0.5:0.5		
Parameters	NFM	XZN
ED_{50} value*	9.75 mg/kg, IM	1.65 mg/kg, IM
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)
	NFM+XZN (0.5:0.5)	
ED50 value*	2.88 mg/kg, IM	0.44 mg/kg, IM
Initial dosage	4.88 mg/kg	0.44 mg/kg
Last dosage (xf)	3.78 mg/kg	0.59 mg/kg
± Dosage (d)	1.22 mg/kg	0.21 mg/kg
Range of the dosages	4.88-2.68= 2.2 mg/kg	0.83-0.35= 0.48 mg/kg
Overall chicks	6 (XXOXOX)	
#Y= da/Da + db/Db	0.56	
* ED50 value= xf + (k × d) X= result (analgesia), O= no result (no analgesia)		

Volts registered preinjection and after 30 minutes of NFM and XZN injection

Da and Db resembles ED_{50} results for NFM versus XZN alone while da and db resembles their combined ED_{50} results, respectively. An interaction index of 1 indicates additive, <1 synergism and > 1 antagonism interactions

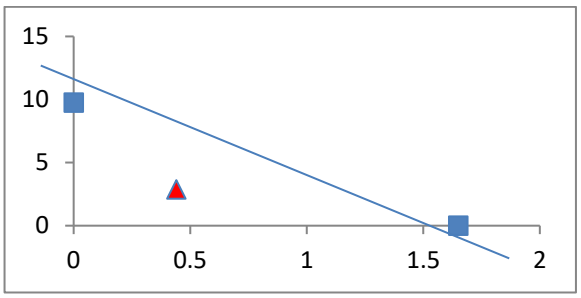

Figure (2): Isobolographic examination of analgesic interaction among NFM and XZN. The point on y-axis denotes the ED50 value of NFM (9.75 mg/kg, IM) while the point on x-axis represents the ED50s of XZN (1.65 mg/kg, IM). The triangular point represents 0.5:0.5 of ED50s combinations for both drugs (2.88 and 0.44 mg/kg, IM for NFM and XZN). The position of the triangular point indicates synergistic interaction between NFM and XZN.

Influence of NFM and XZN on KTN analgesia

It is noted from Table 5 that the positive control group injected with KTN alone produced significant pain relief in chicks when comparing the post-injection voltage with the pre-injection voltage, while NFM and XZN alone or together with KTN led to a significant increase in the analgesic efficacy of KTN and better when comparing the voltage after injection with the voltage before injecting the drugs, and the best significant increase in pain relief was when giving each of NFM and XZN together with KTN in relation to the voltage after injection as well as the change in voltage as it increased significantly compared with the positive control group injected with KTN alone.

Table (5): Influence of NFM and XZN on KTN analgesia

Groups	Pre-voltage	Post-voltage	Delta-voltage
KTN (positive control)	5.17 ± 0.31	8.50 ± 0.50 $^{\#}$	3.33 ± 0.61
KTN+NFM	5.00 ± 0.26	11.83 ± 0.31$^{*,\#}$	6.83 ± 0.31 *
KTN+XZN	5.50 ± 0.22	13.67 ± 0.61$^{*,a,\#}$	8.17 ± 0.48 $^{\text{l},*}$
KTN+NFM+XZN	5.17 ± 0.31	16.50± 0.56 $^{*,a,b,\#}$	11.33 ± 0.61$^{*,a,\#}$

Numbers categorized for mean ± Std.E (6 chicks/ group)
* Dissimilar significantly than the control group at $p < 0.05$
[a] dissimilar significantly than the KTN+NFM group at $p < 0.05$
b dissimilar significantly than the KTN+XZN group at $p < 0.05$
[#] Dissimilar significantly than the pre-voltage for the similar group at $p < 0.05$
KTN, NFM and XZN were injected at 25, 19.5 and 3.3 mg/kg, IM, respectively

Effects of NFM and XZN on the liver and kidney functions

Table 6 explains that creatinine and uric acid (kidney function) in addition to AST and ALT (liver function) concentrations for the groups of chicks which were treated with NFM and XZN (alone or together for five consecutive days) have no significant difference in the concentrations in comparison to the control group.

Table (6): Kidney and liver function in chicks after treatment with NFM and XZN for five consecutive days

Groups	AST (U/L)	ALT (U/L)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	244.20 ± 33.1	1.05±0.40	0.93±0.41	0.08±0.008
NFM	242.20 ± 38.8	1.07±0.51	0.80±0.20	0.12±0.005
XZN	247.50 ± 34.7	0.82±0.56	0.76±0.33	0.10±0.001
NFM+XZN	186.70 ± 21.5 *	1.25±0.53	0.68±0.28	0.07±0.006

Numbers characterized as mean ± SE for 6 chicks per group
* Dissimilar significantly than the control group at $p < 0.05$
NFM and XZN were injected at 19.5 and 3.3 mg/kg, IM, respectively for five consecutive days

4. Discussion

The aim of the study was to investigate the drug safety besides the analgesic interaction of NFM and XZN in chicks model. NFM 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, NFM 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 NFM and XZN 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 NFM 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 NFM and XZN was estimated here in this study which is the alteration in the different pharmacokinetic parameters of NFM when combined with XZN. The enhancement in the efficacy of NFM resulted from an increase in the plasma concentration (free drug) of NFM affected by

administering XZN 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 XZN is considered to protein-bound drug (>99%) which causes an elevation of NFM free drugs available at the sites of action besides the direct effect of XZN on the other crucial factors included absorption, metabolism, and excretion.

5. Conclusions

The results suggested a drug safety and synergism between NFM and XZN in addition to their benefits of using these drugs as preanesthetics to enhance KTN anesthesia in chicks model.

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Conflict of Interest

The authors declare there is no conflict of interest.

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