Original Article

Oxidative Stress Modifies the Activity of Cyclooxygenase2, a Target of Nimesulide, in Chickens

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ABSTRACT

Background: Nowadays, there is a need to investigate the potential impact of oxidative stress (OS), particularly as it relates to drug response, to ensure it is warranted and applied in veterinary medicine.

Objectives: We aimed to evaluate the effect of hydrogen peroxide-induced OS on the pharmacological efficacy of nimesulide (NIM) in chickens.

Methods: We assessed the NIM median effective dose (ED_{50}) using the up-and-down method, measured cyclooxygenase 2 (COX2) activity via ELISA, and performed spectrophotometric analysis of NIM concentrations and its pharmacokinetics.

Results: Stressed chickens showed a significant ($P<0.05$) reduction in total antioxidant capacity (T-AOC) at the 7th, 10th, and 14th days of hydrogen peroxide treatment by 46, 58, and 22%, compared with the control group (tap water). The NIM antinociceptive efficacy increased due to a 31% reduction in ED_{50} in the stressed chickens. Consequently, there was a significant ($P<0.05$) inhibition in COX2 activity in both stressed and control chickens by 24 and 30%, respectively. The data also showed a significant ($P<0.05$) modification in NIM plasma concentration in the stressed chickens compared with the control group at different measurement times. Regarding the NIM pharmacokinetic parameters, OS caused an elevation of the $AUC_{0-\infty}$, $AUMC_{0-\infty}$, C_{max}, MRT, $t_{1/2\beta}$, and V_{ss} by 11, 42, 11, 35, 46, and 39%, respectively, whereas K_{el} and Cl were decreased by 45 and 8%, respectively.

Conclusion: These results showed that OS modifies the pharmacodynamics and pharmacokinetics of NIM, thereby modifying its pharmacological efficacy; thus, care and adjustment of the NIM dosage are warranted for its use in stressed animals.

Keywords: Hydrogen peroxide, COX2, Nimesulide (NIM), Pharmacodynamics Pharmacokinetics

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Introduction

Nimesulide (NIM) is one of the famous non-steroidal anti-inflammatory drugs (NSAIDs) used in human and veterinary medicine, which relieves pain, lowers fever, and prevents inflammation by inhibiting cyclooxygenase2 (COX2), through reducing the production of prostaglandin (a chemical mediator produced from arachidonic acid), which is responsible for pain, inflammation, and fever (Botting, 2006; Suleyman et al., 2008; Gao et al., 2018; Gunaydin & Bilge, 2018; Caiazzo et al., 2019).

NIM has unrivaled beneficial pharmacological uses that distinguish it from other NSAIDs, particularly in its low toxic effects on the digestive system and kidneys. Compared with other NSAIDs, NIM causes fewer and less severe gastrointestinal problems in patients with acute pain, and it can be administered to patients suffering from respiratory issues. The onset of pain relief is relatively rapid, as NIM is used to treat a various musculoskeletal and low back pain conditions, post-traumatic or postoperative pain, migraine, acute gout attacks, and dysmenorrhea (Kress et al., 2016; Patil et al., 2024). COX2 inhibitors increase the risk of cardiovascular disease, while NIM does not cause substantial side effects on the cardiovascular system (Suleyman et al., 2008; Patrono, 2016). NIM inhibits cancer cells (Liang et al., 2009; Afzal et al., 2018; Vunnam et al., 2023) and has also been considered a hypoglycemic agent for use in diabetics (Rasheed et al., 2018; Caiazzo et al., 2019).

Oxidative stress (OS) is defined as an elevation in the formation of free radicals inside the cells of an organism that occurs as a result of exposure to oxidant compounds known to increase their production within the body, such as hydrogen peroxide. The state of OS indicates a defect in the organism's antioxidant biological defenses within its cells (Dalle-Donne et al., 2006; Lee & Jeong, 2007; Chandimali et al., 2025).

Hydrogen peroxide is a powerful oxidizing agent capable of forming free radicals, especially the hydroxyl radical through the Fenton reaction. This is an indicator of OS because of its ability to break down cellular molecules and proteins that make up drug receptors and to oxidize lipids and nucleic acids (Lee & Jeong, 2007; Davies, 2005; Hassan et al., 2024). The use of hydrogen peroxide at low concentrations also stimulates natural cell death (Choi et al., 2006).

Antioxidants protect the body's cells from OS products. For example, glutathione is composed of three peptide chains—L-glutamyl-L-cysteinylglycine—linked via a sulfur group and is widespread in the cells of the organism, especially the liver. It plays a major role in removing toxicity resulting from hydrogen peroxide through the enzyme glutathione peroxidase (Aranda-Rivera et al., 2023; Georgiou-Siafis & Tsiftsoglou, 2023).

Previous findings have shown the ability of OS to alter the pharmacological response through its effects on receptors and pharmacokinetics. OS can destroy protein receptors, leading to changes in drug efficacy (Mousa & Mohammad, 2012; Mousa, 2014; Mousa, 2021a; Mousa, 2021b). OS can be assessed by measuring the status of total antioxidants (TAS), which involves evaluating the activities of the enzymes glutathione peroxidase, superoxide dismutase, and catalase. It also involves assessing OS occurrence by measuring the concentrations of glutathione and malondialdehyde in tissues and blood fluids such as plasma and blood serum (Dalle-Donne et al., 2006).

This study aimed to investigate the effect of hydrogen peroxide-induced OS on the pharmacological efficacy of NIM, specifically its COX2 inhibition, as well as on its pharmacokinetic alteration in chickens as a model.

Materials and Methods

Experimental chickens

Both genders of chickens were used in the experiments and were obtained from a local hatchery in Nineveh Governorate. The total number of chickens used in the experiments was 109. The chickens were housed in the animal facility belonging to the College of Veterinary Medicine, Mosul University. The breeding cages measured 180×150×150 cm (with a maximum of 12–18 chickens per cage, depending on the number of animals used in the specific experiment and experimental design). Each cage included bedding and concentrated feed. The chickens were placed in the cages at one day old and randomly assigned as control and stressed chickens according to the treatment paradigm. Appropriate climatic conditions were maintained, including ventilation and a temperature range of 24-33 ˚C, with 23 hours of light and 1 hour of darkness. The chickens were raised until the experiments were conducted at 7–14 days of age. For the hydrogen peroxide group (stressed chickens), the water was changed daily with hydrogen peroxide diluted to a concentration of 0.5%, while the control group was given tap water (non-stressed chickens) (Mohammad, 2000; Mousa, 2021a; Mousa, 2021b).

Drugs and chemicals used

1. Hydrogen peroxide with a concentration of 50%, produced by Scharlau, Spain.

2. NIM with a concentration of 10%, produced by Pharmaceuticals Instant, India.

3. Heparin (5000 IU/mL), produced by LEO, Denmark.

4. Normal saline solution at 0.90% w/v NaCl concentration, produced by Pioneer Chemical Pharmaceuticals, Iraq.

Devices and instruments used

1. Centrifuge (Chalice, England).

2. Electro-stimulator apparatus (Harvard Apparatus, USA).

3. Sensitive balance (AeADM, England).

4. Spectrophotometer (Lovibond Company, Germany).

5. Special measurement kit (Catalog No. BC1310) for measuring TASs in plasma.

6. ELISA kit for chicken (Solarbio, China; Catalog NO. SEKCN-0103) for measuring the concentration of COX2 in serum.

Preparation of medications and method of administration

The required doses of NIM were obtained by diluting with normal saline and administered through the intramuscular (IM) route. The injection volume was 5 mL/kg in all trials.

Induction of OS and measurement of TAS status in normal and stressed chickens

In this experiment, hydrogen peroxide (0.5%) was administered daily in the drinking water from the first day until the fourteenth day of the chickens' age, while the chickens in the control group were given normal drinking water. Blood was collected on the seventh, tenth, and fourteenth days of chicken's age from the jugular vein using heparin (1:10 volume/volume) to obtain plasma from six chickens in each group (the hydrogen peroxide group and the control group). The plasma was separated by placing the blood tubes in a centrifuge (3000 rpm for 15 minutes). The plasma samples were then stored at -18 °C until TAS was measured on the seventh, tenth, and fourteenth days of the chickens' age for both groups using a special measurement kit (Catalog No. BC1310) to determine antioxidant concentrations (Pellegrini et al., 2006: Alias et al., 2011; Madhubalaji et al., 2021; Quan et al., 2021; Zhou et al., 2023).

Determination of the analgesic ED_{50} for NIM using the up-and-down method (Dixon, 1980) and the effect of OS in chickens

In this experiment, chickens aged 7-14 days from both the stress and the control groups were used because OS occurs with hydrogen peroxide during these days. ED_{50} was determined using the up-and-down method (Dixon, 1980). At the beginning, and based on previous research (Caiazzo et al., 2016), an initial dose of NIM was 15 mg/kg administered IM. The analgesic effect was obtained using the electro-stimulator device by measuring the device's voltage before administering NIM.

After 30 minutes, the device's electrodes were connected under the wing to deliver the electric current. The area under the wing in contact with the electrodes was moistened with water, and the voltage was gradually increased until pain occurred. Pain was determined by distress calls and other signs of pain, such as wing flapping and calling. This was the first reading of the initial result before injection.

Then, NIM was administered at a dosage of 15 mg/kg, IM according to previous experiments. The time for the second reading was set at 30 minutes after injection. The device's electrodes were again connected under the wing, the area was moistened with water, and the voltage was increased until pain occurred. The final result was recorded, which was characterized by an increase in the volts applied after injection of NIM compared with the volts recorded before administration.

The occurrence of pain relief is indicated by the symbol X, and in the case of no pain relief or the voltage remained the same, it is indicated by the symbol O (Mousa, 2009; Mousa, 2020; Mousa et al., 2019; Mousa et al., 2022). Then, the ascending and descending dose method was applied, with a decrease and increase in the dose (3 mg/kg) in the case of analgesia or no pain relief in the non-stressed and stressed chickens. When a change occurred, this process was repeated using ascending and descending doses for three chickens for both groups, and then the analgesic ED_{50} was calculated (Mousa & Mohammad, 2012; Mousa & Mahmood, 2022; Mohammed et al., 2022).

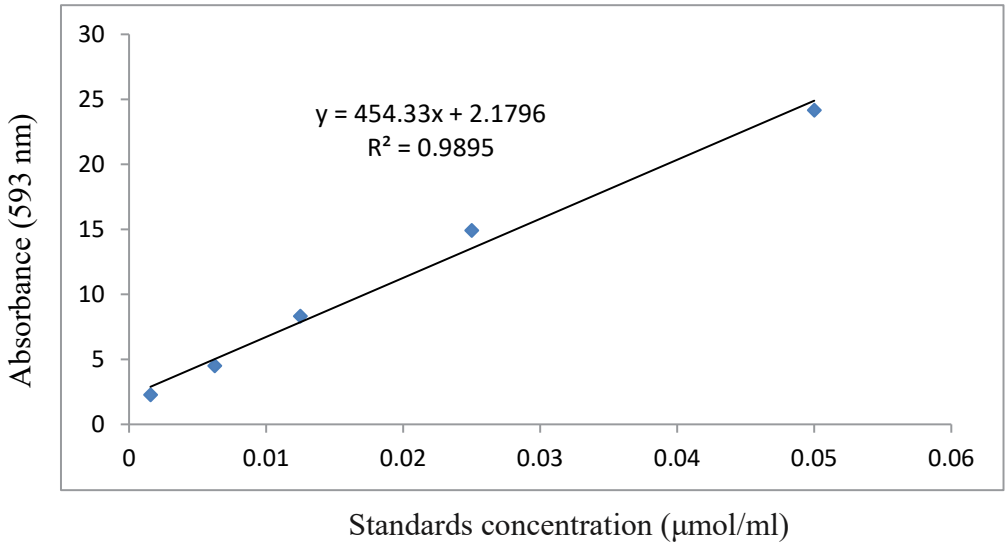

Figure 1. Standard concentration curve used to measure antioxidant status in chickens

Measurement of COX2 activity in stressed and normal chickens using the ELISA technique

In this experiment, 24 chickens weighing 65-120 g and aged 7-14 days were used. They were divided into four groups, with 6 chickens per group, consisting of two control groups. In the control groups, chickens were injected with normal saline solution at 0.9% in unstressed and stressed chickens with hydrogen peroxide. Two additional groups received NIM injections, represented by NIM injections (20 mg/kg, IM) (ED100) for five consecutive days.

On the fifth day of NIM treatment, blood was collected through the jugular vein 30 minutes after the injection of NIM and normal saline solution into anticoagulant-free test tubes (gel tubes) to obtain serum. Serum separation was done by blood centrifugation (3000 rpm/min for 15 minutes). The COX2 concentration (activity) was measured by an ELISA Kit.

The method was applied by determining the absorbance of standard solutions of COX2 at a wavelength of 450 nm, consisting of the following concentrations: 3.75, 7.5, 15, 30, and 60 ng/L. The standard titration curve was applied to obtain a simple linearity equation (y=0.1188 + 0.0325x (correlation coefficient R^2=0.9641)), which was used to determine the COX2 concentration in chicken serum samples (Figure 2).

For COX2 measurement in serum, the serum was incubated at 37 ˚C for 30 minutes. Then, the diluted washing solution was added; the samples were washed and left for 30 seconds, and then filtered using a repeated process five times. Next, the enzyme was added and incubated at 37 ˚C for up to 30 minutes. After that, the samples were washed and chromogen A and B solution was added and incubated at 37 ˚C for up to 10 minutes, followed by the addition of the stop solution. Finally, a microplate reader was used to measure absorbance within 15 minutes at 450 nm (Kurumbail et al., 2001).

Effect of OS on the concentration of NIM in the plasma of stressed and normal chickens

Thirty-six chicks aged 7-14 days were used in this experiment, with weights ranging from 73 to 130 g. They were divided into two parts: (1) the non-stress (control) group, which was injected with NIM (20 mg/kg, IM) and had blood collected at six different times—15, 30, 60, 120, 240, and 480 minutes after injection—at a rate of three chickens per time; and (2) the stressed group with hydrogen peroxide chickens, which received NIM injections (20 mg/kg, IM) and had blood collected at the same times as those mentioned above. After collecting blood samples to obtain blood plasma at each of the specified times, the NIM plasma concentration of the non-stressed and stressed chickens (with hydrogen peroxide) was measured, and the effect of OS on the NIM plasma concentration was then determined.

Preparation of NIM standards

Concentrations of NIM standards were prepared at 15, 30, 60, 120, and 240 by diluting them in distilled water, and then the optical density absorption was determined using a UV-spectrophotometer device (wavelength of 300 nm) (Patel et al., 2013). The simple regression line

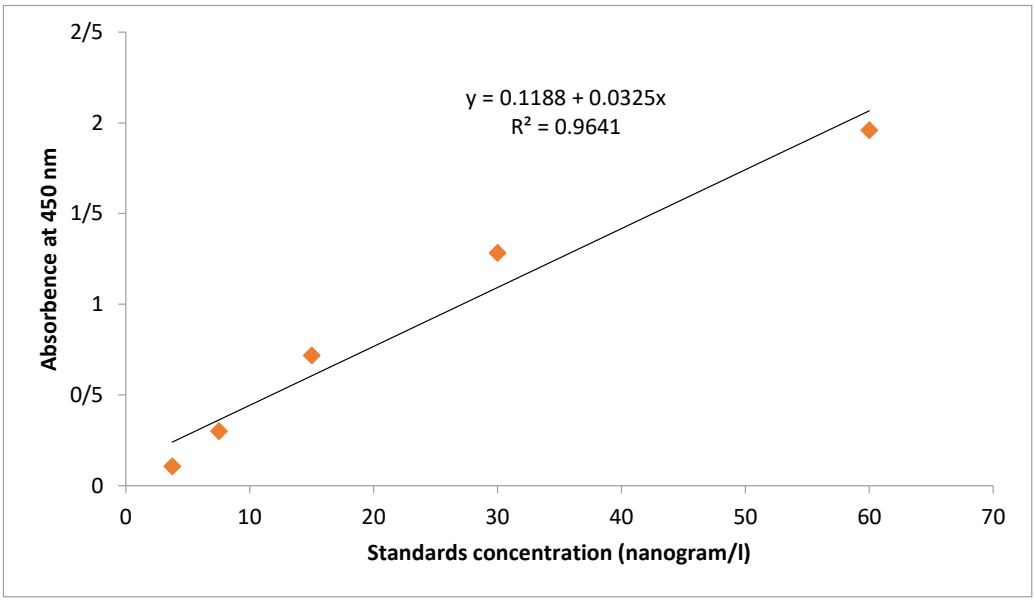

Figure 2. Standard concentration curve used to measure COX2 (ng/L)

curve of the standard solutions was applied to determine the NIM concentration in plasma, where the coefficient of determination (R^2) was 0.9969 (Figure 3). The NIM concentration in the plasma of the chicken groups was estimated according to the Equation 1:

1. $y = a + bx$

Where, y is the absorption of NIM in blood plasma samples at a wavelength of 300 nm, a is the intercept of 0.1205, b is the slope of 0.0099, and x is the unknown NIM concentration (µg) in the blood plasma sample.

Measurement the pharmacokinetics of NIM and the effect of OS on them in chickens

The pharmacokinetics of NIM were estimated by detecting the plasma NIM concentration from the preceding trial and at various times using an optical spectrophotometer in both the aborted and non-hydrogen peroxide groups. The pharmacokinetic criteria were calculated in the PKSlover program and integrated with the Excel program (Zhang et al., 2010).

Pharmacokinetic parameters were calculated manually and compared with the program results using the Equations 2-10:

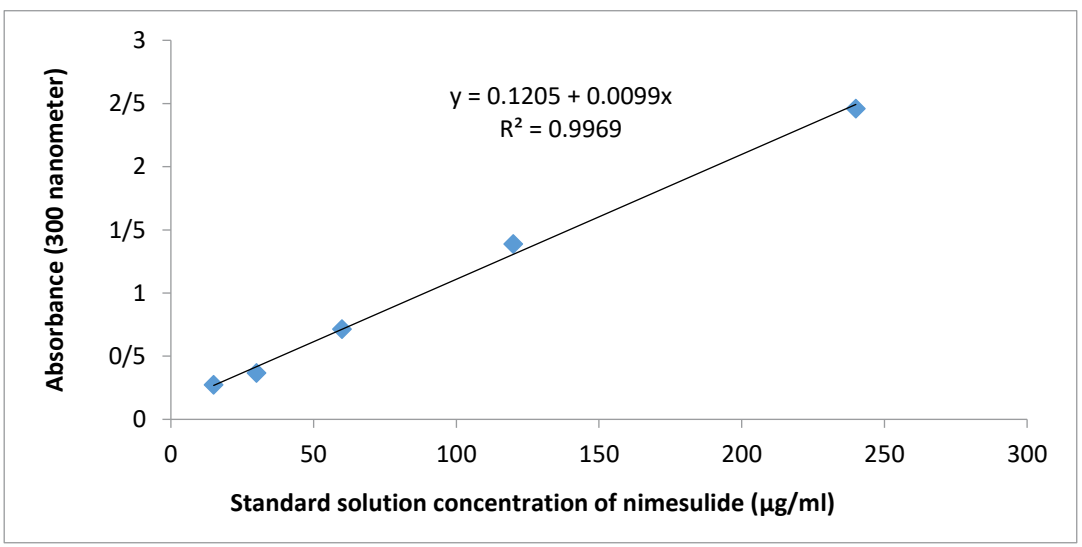

Figure 3. Equation of the regression line of a standard solution of NIM in µg/mL

2. K_{el} is the elimination rate constant, which represents the percentage of NIM eliminated from the plasma per hour.

K_{el} (h^{-1}) = (slope of the regression line) × 2.303

3. $t_{1/2\beta}$ (elimination half-life): It signifies the time, in hours, required for the plasma NIM concentration to decrease by 50%.

$t_{1/2\beta} = 0.693 / K_{el}$

4. V_{ss} is the volume of distribution at steady state, which is the apparent volume of the body fluids that serves to hold the drug.

V_{ss} (L/kg) = dose (mg) / NIM concentration at time zero

5. C_{max} is the maximal concentration (µg/mL), which represents the highest concentration of NIM in the plasma at a specific time.

6. T_{max} (maximal time) (h) is the time when the NIM concentration reaches its highest level in the plasma.

7. MRT is the mean residence time (hours), which is the predictable duration of the drug's stay in the plasma.

8. Cl (total clearance) is the ability of various body organs to excrete and clear NIM. Cl (L/h/kg) = Vss × K_{el}

9. AUC (area under the curve) is the concentration of NIM found in the plasma at various periods.

AUC (µg/h/mL) = dose (mg) / Cl

10. AUMC (area under the moment curve) represents the concentration of NIM present in the plasma at the time of quantification.

AUMC (µg × h^2/mL) = Vss × $(AUC)^2$ /dose (mg)

Statistics

One-way analysis of variance was used for the statistical examination of parametric data, which was achieved through the comparison of multiple means. The t-test was used to analyze the means of the two groups of chickens (Berke, 2007). Statistical significance was set at $P<0.05$.

Results

Measurement of T-AOC in the plasma of stressed and normal chickens

Treatment with hydrogen peroxide from the first day until the fourteenth day of the chickens' age at a concentration of 0.5% in drinking water led to a significant reduction ($P<0.05$) in the total concentration of antioxidants in the plasma of these chickens on the seventh, tenth, and fourteenth days by 46%, 58% and 22%, respectively, compared to the control group (normal drinking water) (Figure 4).

Determination of the ED_{50}s of pain-relieving for NIM and the effect of OS with hydrogen peroxide in chickens

NIM analgesic ED_{50} was determined to be 9.79 mg/kg IM, which is the dose required for pain relief in 50% of chickens in the control group (tap water) 30 minutes after injection with NIM (Table 1). While the analgesic ED_{50} of NIM was recorded to be 6.78 mg/kg IM, which is the dose required for pain relief in 50% of chicken in the hydrogen peroxide group (0.5% in drinking water) by measuring the voltages before and after NIM injection at a time of 30 minutes using an electro stimulator (Table 1). When comparing the ED_{50}s of NIM in the chickens of both the control group and those treated with hydrogen peroxide, OS was observed to increase the effectiveness of NIM pain relief in chickens treated with hydrogen peroxide compared to the control group by reducing the ED_{50} value by 31% (Table 1).

Measurement of COX2 Activity in stressed and normal chickens

Injecting NIM (20 mg/kg, IM) for five consequent days significantly ($P<0.05$) reduced the concentration of COX2 in the serum of the chickens in the control group injected with NIM with an inhibition rate of 30% compared to the same group injected with physiological normal saline, while the administration of NIM (20 mg/kg, IM) led to a significant ($P<0.05$) reduction in the concentration of COX2 in the serum of the chickens in the hydrogen peroxide group injected with NIM with an inhibition rate of 24% compared to the respective control group (Table 2).

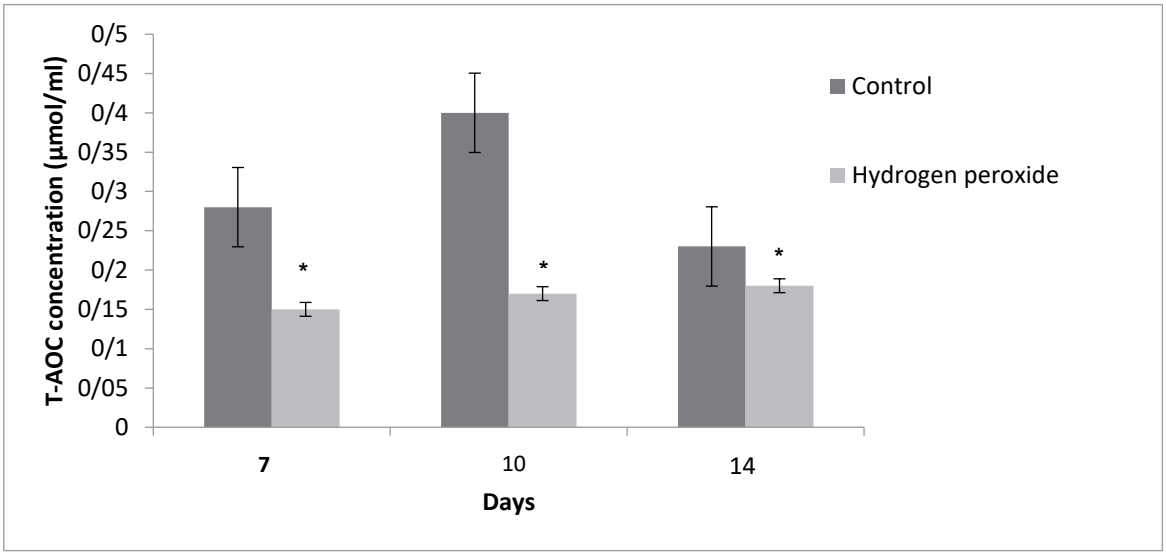

Figure 4. Antioxidant concentration in the blood plasma of chickens stressed with hydrogen peroxide during different days of measurement

*Varies significantly in comparison to the similar control group on the same day (treatment with normal drinking water) at $P<0.05$.

Note: Values are presented as mean±standard error (six chickens per group). Hydrogen peroxide at a concentration of 0.5% was added to the drinking water of the chickens from the first day until the fourteenth day of their life. Percentage of decrease in T-AOC = (control group – hydrogen peroxide group / control group) ×100.

Table 1. Determination of NIM analgesic ED_{50}s and the effect of OS in chickens

Measurements		Result
Control group (normal drinking water)	ED_{50}	9.79 (mg/kg, IM)
	Dosing range	15-9=6 (mg/kg)
	Initial dose	15 (mg/kg)
	Final dose	12 (mg/kg)
	± Doses	3 (mg/kg)
	Number of chickens	6 (XXOXOX)
Hydrogen peroxide group (0.5% in drinking water)	ED_{50}	6.78 (mg/kg, IM)
	Dosing range	15-6=9 (mg/kg)
	Initial dose	15 (mg/kg)
	Final dose	9 (mg/kg)
	Amount of dose decrease and increase	3 (mg/kg)
	Number of chickens	7 (XXXOXOX)
	Reduction in ED_{50} of NIM	31%

Note: Hydrogen peroxide (0.5%) was added to the drinking water of the chickens from the first day to the fourteenth day of their life. Percentage of decrease in ED_{50} for NIM (%) = ED_{50} (control group) – ED_{50} (hydrogen peroxide group / ED_{50} (control group) ×100. X: Antinociceptive effect occurred after 30 minutes of NIM injection. O: No antinociception occurred after 30 minutes of NIM injection.

Table 2. Determination of COX2 activity in normal chickens and the effect of hydrogen peroxide-induced OS in chickens

Groups		Mean±SE COX2 Activity (ng/L)	Inhibition Percentage (%)
Control group	Normal saline	15.77±1.56	30
	NIM	11±0.75*	
Hydrogen peroxide group	Normal saline	8.92±1.67+	24
	NIM	6.77±0.54+	

*Significantly differs compared to the chickens injected with physiological saline in the same section at P<0.05, +Significantly differs compared to the control group at P<0.05.

Note: Hydrogen peroxide at a concentration of 0.5% in drinking water was given to chickens from the first day until the fourteenth day of their life. NIM was injected at a dose of 20 mg/kg intramuscularly for five consequent days. Percentage of COX2 inhibition (%) by NIM = (Control – Treated) / Control ×100.

Effect of OS with hydrogen peroxide on the plasma NIM concentration of chickens

The concentration of NIM when injected at 20 mg/kg IM in the control group at different times—15, 30, 60, 120, 240, and 480 minutes—was 20.25, 66.42, 16.98, 19.01, 19.78, and 3.35, respectively. Meanwhile, the NIM plasma concentration of the stressed chickens was modified to become 17.05, 74.97, 24.43, 11.60, 17.56, and 5.84, respectively, with a ratio of 16, 11, 30, 39, 11, and 43%, respectively. There was an elevation in the NIM concentration of the stressed chickens at 30, 60 and 480 minutes after NIM injection compared to the control group (normal drinking water). For both the control and stressed groups, the best time for maximal NIM concentration was 30 minute after injection, which differed significantly (P<0.05) from the other measured times. In addition, there was no significant difference (P>0.05) among the measured times of 60, 120, 240, and 480 minutes after NIM injection (Table 3) and (Figure 5).

Measurement of pharmacokinetic parameters of NIM and the effect of OS in the chickens

The pharmacokinetic parameters for NIM injected (20 mg/kg, IM) in the control group (normal drinking water)

Table 3. Effect of OS on the plasma NIM concentration of chickens

Time (minute)	Mean±SE (3 chickens/measured time)		Effect of OS on Plasma NIM Concentration (%)+
	Control Group (Normal Drinking Water) (ug/mL)	Hydrogen Peroxide Group (0.5% in Drinking Water) (µg/mL)	
15	20.25±8.12	17.05±4.51	16 (-)
30	66.42±24.6[a]	74.97±7.15[a]	11 (+)
60	16.98±1.5[b]	24.43±13.21[b]	30 (+)
120	19.01±9.70[b]	11.6±1.14[b]	39 (-)
240	19.78±5.38[b]	17.56±5.3[b]	11 (-)
480	3.35±0.3[b]	5.84±2.58[b]	43 (+)

[a]Significantly differs from the time after 15 minutes in the similar group at P<0.05, [b]Significantly differs from the time after 30 minutes in the similar group P<0.05.

Note: Hydrogen peroxide (0.5%) was supplemented to the chickens' drinking from the first day to the fourteenth day of their life. NIM was injected at a dose of 20 mg/kg IM. + OS influence on plasma NIM concentration (%) = (control group − hydrogen peroxide group) / control group ×100.

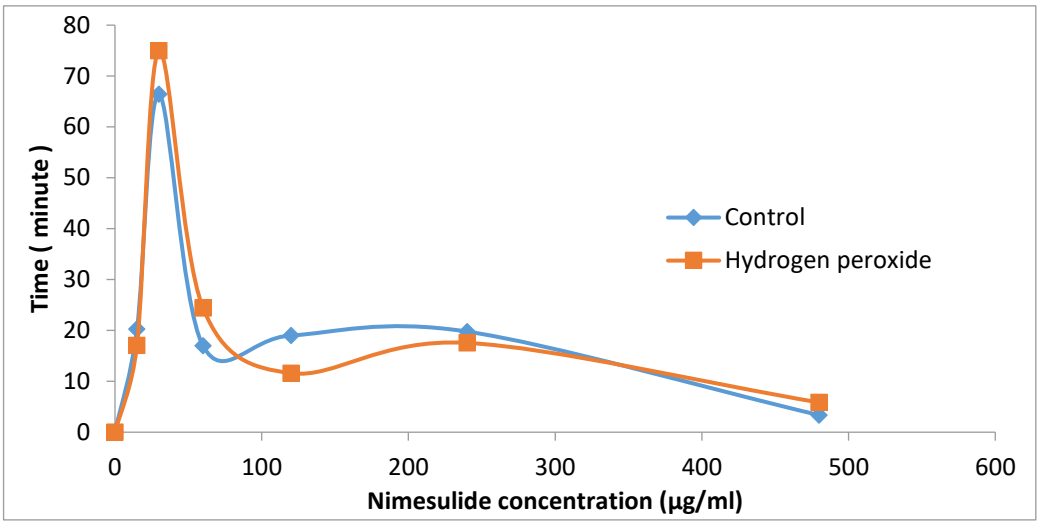

Figure 5. Effect of OS on the plasma NIM concentration of chickens

Note: Values are presented Mean±SE (3 chickens/measured time). Hydrogen peroxide (0.5%) was supplemented to the chickens' drinking water from the first day to the fourteenth day of their life. NIM was injected at a dose of 20 mg/kg IM. NIM concentration was modified in the stressed chickens compared to the control and reflects first-order kinetics for both groups of chickens.

were the area under the curve 148.01 µg×hr/mL, the area under the moment curve 495.04 µg×hr²/mL, the highest concentration 66.42, the mean residence time 3.34 hours, the half-life 2.23 h, and the volume of distribution 0.42 l/kg. The OS induced by hydrogen peroxide increased these parameters to 166.99 µg×hr/mL, 852.98 µg×h²/mL, 74.97, 5.11 h, 4.10 h, and 0.69 l/kg, with increases of 11, 42, 11, 35, 46, and 39%, respectively (Table 4).

The pharmacokinetic criteria of NIM in the control group (tap water) were represented by the K_{el} 0.31 h-1 and the total clearance of 0.13 l/h/kg. The OS induced by hydrogen peroxide decreased these two variables to 0.17 h-1 and 0.12 l/h / kg, with decreases of 45 and 8%, respectively (Table 4).

Table 4. Effect of OS on the pharmacokinetic criteria of NIM in chickens

Pharmacokinetic Parameters	Unit	Control Group (Drinking Water Group)	Hydrogen Peroxide Group (0.5% In Drinking Water)	Increase or Decrease in Parameter (%) +
$AUC_{0-\infty}$	µg× h/mL	148.01	166.99	11 (+)
$AUMC_{0-\infty}$	µg× h²/mL	495.04	852.98	42 (+)
C_{max}	µg/mL	66.42	74.97	11 (+)
K_{el}	h-1	0.31	0.17	45 (-)
T_{max}	h	0.5	0.5	0
MRT	h	3.34	5.11	35 (+)
$T_{1/2\beta}$	h	2.23	4.10	46 (+)
V_{ss}	L /kg	0.42	0.69	39 (+)
Cl	L /h/kg	0.13	0.12	8 (-)

Note: + Effect of OS on pharmacokinetic parameters of plasma NIM (%) = (control group - hydrogen peroxide group) / control group ×100. PKSolver program was used to assess pharmacokinetic variables of a non-compartmental model of pharmacokinetics.

Discussion

This study aimed to assess the effect of OS (induced by hydrogen peroxide) on the pharmacokinetics of NIM and the concentration of COX2 in chickens. Hydrogen peroxide (0.5%) supplied to chickens for 14 days induced OS, which was precluded in the first trial, by a significant reduction in the plasma TAS concentration of chickens stressed with hydrogen peroxide on days 7, 10 and 14 by 46, 58, and 22%, respectively, compared with the control group given normal drinking water. This result is compatible with previous studies (Mousa, 2014; Mousa, 2021a; Mousa, 2021b).

Hydrogen peroxide that causes OS affects the pharmacological response to various drugs because it was found to cause the breakdown of lipids, nucleic acids, and proteins (including enzymes such as COX2, which is considered the receptor for NIM in this study). It also breaks down the cell membrane via lipid peroxidation as a result of OS, which changes the vital properties of the membrane, including the permeability of substances and receptors located inside the cell membrane and on its surface.

This leads to an imbalance in the cellular drug response (Dalle-Donne et al., 2006). Therefore, the NIM analgesic efficacy increased in the stressed chickens, and the ED_{50} of NIM was determined in the chickens of the control group that received normal drinking water. This value was similar to a previous study conducted in mice (Yahya & Mousa, 2024) and in chickens (Yahya & Mousa, 2024) and chickens (Mousa, 2019).

This study showed that injection of NIM (20 mg/kg, IM) significantly decreased the concentration of COX2 in the control group of chickens given normal drinking water, in which OS was inhibited (induced by hydrogen peroxide) (Zheng et al., 2000; Kullich et al., 2007), or as a result of the accumulation of large amounts of hydrogen peroxide, which stimulated its decomposition into water and oxygen (Woods et al., 1998).

It was noted that the OS induced in chickens with hydrogen peroxide (0.5% in drinking water) led to an elevation in the NIM plasma concentration and that the changes were significant at different measurement times of 15, 30, 60, 120, 240, and 480 minutes. The highest concentration occurred at 30, 60, and 480 minutes after injection with NIM as a single dose (20 mg/kg, IM) compared with the control group given ordinary drinking water. The reason is due to the ability of hydrogen peroxide to break down cells and affect cellular processes (Dalle-Donne et al., 2006; Lee & Jeong, 2007; Chandimali et al., 2025), and the most important of these occur in the organs responsible for drug metabolism pathways and the excretion paradigm, such as the liver and kidneys.

It has also been observed that administration of NIM directly affects important pharmacokinetic parameters. The pharmacokinetics of NIM in chickens stressed with hydrogen peroxide were changed compared with the control, especially for the AUC, AUMC, maximal concentration, elimination half-life, mean residence time, and volume of distribution at steady state. This was accompanied by a decrease in the elimination rate constant, in addition to the total clearance, which contributes to the increased effectiveness of NIM. These alterations in valuable pharmacokinetic parameters, e.g. volume of distribution, elimination half-life, and clearance could be due to the direct effect of OS in hepatocytes, renal cells, and plasma proteins (Lee & Jeong, 2007; Mousa, 2021a; Aranda-Rivera et al., 2023; Georgiou-Siafis & Tsiftsoglou, 2023), which may interfere with and enhance NIM efficacy and subsequent toxicity. On the other hand, the half-life of NIM was found in this study to be modified and raised by doubled due to the effect of OS, and this require a modification in the treatment protocol in the stressed animals by reducing the dose and the subsequent dosing interval to achieve the required therapeutic efficacy without causing notable toxicity (Mousa, 2014; Mousa, 2021a; Mousa, 2021b).

Conclusion

Hydrogen peroxide-induced OS modifies the pharmacodynamics and pharmacokinetics of NIM, which modifies its pharmacological efficacy in chickens. Careful selection and adjustment of the NIM dosage must be taken when planning treatment with NIM in stressed animals. Other studies in different stressed animals besides its application in veterinary practice are required to provide a clear representation and support our results.

Ethical Considerations

Compliance with ethical guidelines

The study was reviewed and approved by the Animal Use and Care Committee of the University of Mosul/Veterinary Medicine College, Mosul, Iraq (Code: UM.VET.2024.005).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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