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ORIGINAL ARTICLE

OXIDATIVE STRESS ALTERS THE THERAPEUTIC EFFECTS OF KETOROLAC IN THE CHICKS MODEL

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Summary

The purpose of the research consisted of assessing the modification produced by hydrogen peroxide (H_2O_2)-induced oxidative stress (OS) on the ketorolac therapeutic effects in the chickens which are the analgesic, antipyretic, and anti-inflammatory. A significant decrease in the total antioxidant capacity (T-AOC) and subsequent occurrence of OS was observed in the stressed (H_2O_2) group on days 7th, 10th, and 14th by 39, 29, and 41%, respectively in comparison to the control (non-stressed) group. The analgesic effect of ketorolac in the stressed group had more intense in comparison to the non-stressed group, the analgesic effectiveness of ketorolac raised by 16% in that group. In the non-stressed and stressed groups, ketorolac produces its antipyretic effect at 3 and 4 hours after fever induction by baker's yeast while it shows the effect significantly at 1, 2, and 4 hours. Furthermore, ketorolac has the superiority of antipyretic action in stressed group over the non-stressed group. Ketorolac carries out anti-inflammatory activity in the stressed and non-stressed groups by 61 and 75%, respectively. Ketorolac has a significant anti-inflammatory property in the stressed group through a significant decrease in the delta thickness compared to the non-stressed group. The stressed group was treated with ketorolac for five consecutive days significantly affect the kidney and liver function concerning the non-stressed group. The net findings proposed the ability of H_2O_2-induced OS to alter ketorolac's analgesic, antipyretic, and anti-inflammatory properties in the chickens thus, it is recommended to reduce the dose of ketorolac intended to be given to stressed animals involved.

Key words: Analgesic; Antipyretic; Antiinflammatory; Oxidative stress; Ketorolac

Introduction

Ketorolac belongs to the most famous drugs of the non-steroidal anti-inflammatory drugs (NSAIDs) which has a therapeutic benefit for preventing pain thus producing analgesia as well as its effect on lowering the fever and preventing inflammation (1, 2). Ketorolac pharmacological action was achieved through its ability to reduce both types

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of cyclooxygenases (COX) (which is COX_1 and COX_2) by the non-selective mechanism, thus blocking the conversion of arachidonic acid to prostaglandins inflammatory mediator leading to diminishing of the pain, fever, and inflammation, (1, 3). Ketorolac is considered an excellent analgesic drug used to relieve moderate and severe pain with some degree of adverse effects therefore, it is considered an exceptional, cheaper, and more effective drug rather than the centrally acting opioids, (like morphine) (3). The opioids causes a seriously adverse effects which include respiratory depression and addiction for example (3). Ketorolac produces less deleterious adverse effects involved like the respiratory depression, hemodynamic, and central effects which makes it of benefit for post-operative pain treatment (4). Stressful agents like using chemical (e.g. H_2O_2) stressors were documented to alter the therapeutic effects of the drugs (5). H_2O_2 was formerly identified to modify diazepam (6) and xylazine (7, 8) sedation with altering of anesthesia induced by ketamine in the chickens and causes seriously toxic effects in the laboratory animals (9). Besides the modifying of the of xylazine and diazepam pharmacological responses (10). H_2O_2 is known to induce OS by elevating the reactive oxygen species (ROS) thus rises the free radicals contents leading to interactions and altering the physiological functions of the viable cellular constituents particularly the proteins (i.e. receptors) accountable for pharmacodynamic interaction as well its ability to destruct the plasma proteins binding and cytochrome P_{450} enzymes (11-13) responsible for pharmacokinetic alterations of the drugs used. Other more reasons assumed to the damaging pattern of the viable blood-brain barrier (BBB) leading to changes of the free drug concentration inside the central nervous system (CNS) (11-13).

The purpose of this study consisted of assessing the alteration due to H_2O_2-induced OS status on the ketorolac therapeutic effects which were the analgesic, antipyretic, and anti-inflammatory in the chicks model.

Materials and methods

Animals and drug preparation

Broiler Ross chicks of both sexes were used at 7-14 day-olds in all the experiments provided by a local hatchery. Their bodyweights were between 73-122 g and kept at 29-36°C of temperature with continuous lighting. The animals had free access to water and food rations ad libitum. Ketorolac (3% ketorolac trometamol, Spain) was prepared with a 0.9% sodium chloride intended to be administered parenterally by intramuscular (IM) route of injection.

Induction of OS by using of H_2O_2 as a stressor agent

At one-day-old chicks, the animals (36 total chicks) were separated randomly to the control (H_2O) group which were supplied the tap water while the cluster of the chicks (stressed group) were given a daily 0.5% of H_2O_2 (Scharlau, Spain) in drinking water (7, 8, 10). On days 7^{th}, 10^{th}, and 14^{th} of the chicks, they were undergoing blood collection (6 chicks per group in each estimated day) to acquire the plasma using heparin (1:10 v/v). Plasma was then measured by a specific kit (Solarbio, China) for determining the total antioxidant capacity (T-AOC) (Catalog No. BC1310) for both the stressed and non-stressed groups of chickens (14). The age of OS occurrence in the chickens then is selected in the next experiments of this study.

Assessing the analgesic median effective dose (ED_{50}) of ketorolac in the stressed and non-stressed groups

First of all, the ketorolac analgesic ED_{50} was estimated by using the up-and-down method (15) applied to the stressed and non-stressed groups to select the ketorolac dosage to be applied for the subsequent experiments. In this method, the first dose of ketorolac was given at 13 mg/kg, IM (16) to both groups, and then by increasing and decreasing the dose by 3 mg/kg. Ketorolac analgesia was measured by using the electro-stimulator apparatus (Harvard, USA) as a pain inducer (10, 16). Thirty minutes after ketorolac administration, the distress call generated because of pain sensation was recorded as a voltage for each chick separately. At that point, ketorolac was considered to have analgesia when the voltage increased at post-injection record compared to the voltage recorded pre-injection and designated as X symbol and the O symbol denotes for lacking analgesia. The below equation was used to assess the impact of the H_2O_2- induced OS on the ketorolac's ED_{50} (17):

% effects of OS on ketorolac's ED_{50} = ED_{50} of non-stressed group - ED_{50} of stressed group / ED_{50} of non-stressed group × 100.

Estimating of the ketorolac analgesia in the stressed and non-stressed groups

According to the ED_{50} of ketorolac measured above, two groups (6 chicks/group) were treated with 14 mg/kg, IM (that represented the ED_{100} of ketorolac) for examining the analgesia in both of the stressed and non-stressed groups of chickens. The voltage (volt) induced by electro-stimulation for each chicken was recorded before and after 30 minutes of ketorolac injection. Later, the percentage of analgesia in each group of chickens in addition to the delta voltage was also recorded (7, 16, 18, 19).

Measuring the antipyretic effects of ketorolac in the stressed and non-stressed groups

The antipyretic effect of ketorolac was assessed and recorded by injecting the baker's yeast at 135 mg/kg, IP (20) for induction of fever in the experimental chicks. Both stressed and non-stressed groups (6 chickens/group) were treated with 14 mg/kg, IM of ketorolac. The temperature was recorded before and after 1, 2, 3, and 4 hours by using the digital thermometer inserted via the rectum.

Estimating the anti-inflammatory effects of ketorolac in the stressed and non-stressed groups

Two groups (6 chicks/group) of the stressed and non-stressed groups have been selected. Ketorolac was injected at 14 mg/kg, IM 30 min before formaldehyde injection (0.05 ml of 0.1%) in the right paw (May and Baker, UK) for induction of inflammation (21). At that time, measuring the right paw thickness with a digital caliper (vernia) in millimeters (mm) pre and post-60 min. of formaldehyde injection.

Finally, the delta-thickness of the injected paw will reveal the inflammation occurrence (22) was measured and its alteration in percentages was recorded to elucidate the anti-inflammatory action of ketorolac in both the stressed and non-stressed groups of chickens.

Measurement of the kidney and liver function in the stressed and non-stressed groups after treatment with ketorolac for five consecutive days

Both the stressed and non-stressed groups of chicks were treated with ketorolac (14 mg/kg, IM) for five consecutive days. After that, the blood was taken from the jugular vein of the two groups of chicks (6 chicks/group) for estimating the kidney function (creatinine and uric acid) in addition to liver function (alkaline phosphatase) in the serum which were all determined with the specified kit (23).

Statistical analysis

The analysis of parametric data of two groups was conducted by using the paired and unpaired student T-test for comparing the means. The one-way analysis of variance test was applied to relate the means of three groups of chicks (24). The level was considered significantly different when p less than 0.05.

Results

Induction of OS with H_2O_2 in the chicks

Figure 1 shows there is a significant decrease in the total antioxidant capacity and subsequent occurrence of OS in the stressed group of chicks at days 7^{th}, 10^{th}, and 14^{th} of treatment with H_2O_2 by 39, 29, and 41%, respectively in comparison to the non-stressed group.

Ketorolac analgesic ED_{50} in the stressed and non-stressed groups

Ketorolac analgesia was elevated in the stressed chicks by assessing the analgesic ED_{50} of ketorolac (Table 1). The ketorolac ED_{50} in the non-stressed group was measured to be 7.79 mg/kg, IM and it reduced by 16% in the stressed group of chicks to became 6.58 mg/kg, IM.

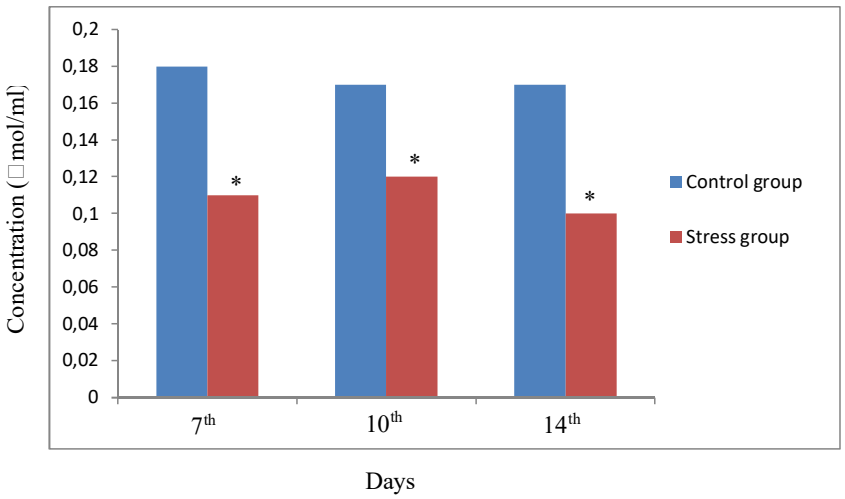

Figure 1. Total antioxidant capacity (T-AOC) in the stressed and non-stressed chickens. Non-stressed group was supplied by the tab water and stressed group was given 0.5% H_2O_2 (from 1st to 14th day-old of chicks' age)
* significantly different in comparison to the non-stressed group ($p < 0.05$)

Table 1. ED_{50} ketorolac analgesia in the stressed and non-stressed chicks.

Variable	Groups	
	Non-stressed	Stressed
ED_{50}	7.79 mg/kg, IM	6.58 mg/kg, IM
Range of the doses used	13-7= 6 mg/kg	13-4= 9 mg/kg
The first dose used	13 mg/kg	13 mg/kg
The final dose used	10 mg/kg	4 mg/kg
± in the doses	3 mg/kg	3 mg/kg
Chickens used	6 (XXOXOX)*	6 (XXOXXO)*
OS effect on ketorolac analgesic ED_{50} = non-stressed - stressed / non-stressed ×100 = 16%		

Non-stressed group was supplied by the tab water and stressed group was given 0.5% H_2O_2 (from 1st to 14th day-old of chicks' age).
*X symbol was the analgesia while the O symbol indicated no analgesia.

The analgesic effects of ketorolac in the stressed and non-stressed groups of chicks

The analgesic percentages of ketorolac in the H_2O and stressed groups were similar in the chickens while ketorolac treatment in the stressed group had a more potent analgesic effect in comparison to the non-stressed group regarding the post voltage of analgesia measured beside the difference in delta voltage between the two groups of chickens (Table 2).

Table 2. The analgesic effects of ketorolac in the stressed and non-stressed chicks.

Parameters	Non-stressed	Stressed
Analgesia (%)	100 (6/6)	100 (6/6)
Pre-voltage	10.00 ± 0.26	9.67 ± 0.49
Post-voltage	11.67 ± 0.33 #	12.50 ± 0.67 #
Delta voltage	1.67 ± 0.33	2.33 ± 0.21

Numbers represented as Mean ± SE (6 chicks/group).
Non-stressed group was supplied by the tab water and stressed group was given 0.5% H_2O_2 (from 1st to 14th day-old of chicks' age).
Ketorolac was given at 14 mg/kg, IM and voltage recorded pre and 30 minutes post-ketorolac treatment.
* significantly different in comparison to the non-stressed group ($p < 0.05$).
# significantly different in comparison to the pre-voltage (at the same group) ($p < 0.05$).

The antipyretic effects of ketorolac in the stressed and non-stressed groups of chicks

Table 3 shows that ketorolac injection at 14 mg/kg, IM significantly reduces the body temperature after 3 and 4 hours of baker's yeast induction for both the stressed and non-stressed chicks. Ketorolac antipyretic efficacy was greater in the stressed group at 1, 2, and 4 hours after its induction by baker's yeast compared to the non-stressed chicks.

Table 3. Ketorolac antipyretic effect in the stressed and non-stressed chicks.

Parameters	Non-stressed	Stressed
Pre-temperature	40.67 ± 0.13	40.22 ± 0.25
Post-temperature (1h)	40.72 ± 0.17	39.58 ± 0.54 *
Post-temperature (2h)	40.72 ± 0.16	39.88 ± 0.36 *
Post-temperature (3h)	39.55 ± 0.43 #	39.15 ± 0.26 #
Post-temperature (4h)	39.12 ± 0.44 #	38.43 ± 0.20 *,#

Numbers represented as Mean ± SE (6 chicks/group).
Non-stressed group was supplied by the tab water and stressed group was given 0.5% H_2O_2 (from 1st to 14th day-old of chicks' age).
Ketorolac was given at 14 mg/kg, IM and temperature recorded by using the digital thermometer.
* significantly different from the non-stressed group ($p < 0.05$).
# significantly different from the pre-temperature at the same group ($p < 0.05$).

The anti-inflammatory effects of ketorolac in the stressed and non-stressed chickens

Table 4 illustrates that ketorolac exerts its anti-inflammatory activity in the stressed and non-stressed groups by 61 and 75%, respectively. Ketorolac has a significant anti-inflammatory property in stressed group through a significant decrease in the delta thickness compared to non-stressed chicks.

Table 4. Ketorolac anti-inflammatory effect in stressed and non-stressed chicks.

Parameters	Non-stressed group		Stressed group	
	Formaldehyde (+ve control)	Ketorolac	Formaldehyde (+ve control)	Ketorolac
Anti-inflammatory effect (%)	-	61	-	75
Pre-thickness	8.65 ± 0.18	8.33 ± 0.12	8.75 ± 0.40	9.33 ± 0.15
Post-thickness	9.28 ± 0.16#	8.50 ± 0.11	9.52 ± 0.33#	9.48 ± 0.13#,+
Delta thickness	64 ± 0.13	0.30 ± 0.04*	0.77 ± 0.08	0.16 ± 0.03*,+

Numbers represented as Mean ± SE (6 chicks/group).
Non-stressed group was supplied by the tab water and stressed group was given 0.5% H_2O_2 (from 1st to 14th day-old of chicks' age).
Ketorolac was injected at 14 mg/kg, IM 30 min before formaldehyde injection (0.05 ml of 0.1%) in the right paw.
* significant different in relation to the +ve control (at the same cluster) ($p < 0.05$).
# significantly different from pre-thickness in the same group ($p < 0.05$).
+ significant different in relation to respective +ve control group ($p < 0.05$).

Measurement of kidney and liver function in the stressed and non-stressed chickens

Table 5 shows that both kidney function (creatinine and uric acid) and liver function (alkaline phosphatase) concentrations, of stressed groups of chickens that were treated with ketorolac for five consecutive days, have a significant elevation in their concentrations compared to the non-stressed chicks.

Table 5. Kidney and liver function in the stressed and non-stressed chickens after treatment with ketorolac for five consecutive days.

Variables	Non-stressed group	Stressed group
Creatinine (mg/dl)	1.22 ± 0.18	3.28 ± 0.74*
Uric acid (mg/dl)	70.37 ± 7.45	90.54 ± 2.26*
Alkaline phosphatase (IU/100 ml)	111.17 ± 1.02	116.62 ± 1.24*

Numbers represented as Mean ± SE (6 chicks/group).
Non-stressed group was supplied by the tab water and stressed group was given 0.5% H_2O_2 (from 1st to 14th day-old of chicks' age).
Ketorolac injected at 14 mg/kg, IM for five consecutive days.
* significant different compared to the non-stressed group ($p < 0.05$).

Discussion

The aim of the present study consisted of determining the alteration and impact of H_2O_2-induced OS on ketorolac therapeutic effects which were the analgesic, antipyretic, and anti-inflammatory in the chickens. The use of chickens as a research model has become prevalent throughout the history of biology and pharmacology. Research on analgesia, anesthesia, neurobehavior, and toxicity has extensively used chicks as a laboratory model (25, 26). The study used H_2O_2 for induction of OS status and for simulation of the abnormal and diseased condition because it is well known that the disease itself, in some conditions, will induce a status of stress. In a manner of speaking, stress will be induced by physical (like heat), chemical (i.e., H_2O_2 used in our study), and miscellaneous agents (including some diseased conditions, especially if they affect the body metabolism) (27). Ketorolac is considered one of the most used agents that belong to the NSAIDs which have a therapeutic benefits for preventing pain, fever, and inflammation (1, 2). The mechanism of action of ketorolac was achieved through the reduction of both types of COX (COX_1 and COX_2) by a non-selective mechanism, thus blocking the conversion of arachidonic acid to prostaglandins (a vital autacoid mediator responsible for pain, fever, and inflammation produced) (1, 3). This experimental study used H_2O_2 for the induction of OS because it is a well-known stressor belongs to chemical oxidants which is used for experimentally inducing OS in laboratory animals (5). To detect OS status in the chickens, the total antioxidant capacity (T-AOC) is used which considered a beneficial indicator for inspecting the OS occurrence (28, 29) and used to specify on which day of treatment with H_2O_2 was OS occur. Based on these days explored, the next subsequent experiments are conducted. The reasons assumed for the amplification in the ketorolac actions denoted the H_2O_2 capability to induce OS by increasing Reactive oxygen species; therefore rise of free radicals production that cooperates and modifies the physiological functions of the cellular vital constituents particularly the receptor which composed of protein (like COX target receptor) that elaborate in drug pharmacodynamics as a result, the destruction of the receptors will cause an increase in the binding sites on the receptors as a physiological defense mechanism, which leads to an increase in the therapeutic effects of the drugs (9, 27). H_2O_2 can destruct of the cytochrome P_{450} enzymatic system, reduced the elimination of the ketorolac by interfering metabolism and rising the ketorolac plasma concentration. Consequently, the destruction anticipated to the particular ketorolac protein binding sites on the plasma proteins (albumins) due to ketorolac is broadly bound $\geq$ 99% to the plasma albumins and having a low distribution volume to the target site of achievement in contrast to those of other NSAIDs (11-13, 25). All of these reasons assumed can anticipated and responsible for the alteration of ketorolac's therapeutic effects. The kidney and liver functions indicated through the estimation of the levels of creatinine, uric acid, and alkaline phosphatase examined in the non-stressed group of chicks are considered within the normal ranges of chicken criteria (9) while they increased significantly in the stressed group of chicks due to the direct affection of the reactive oxygen species produced by the OS, which causes the destruction of the vital cellular components and impairs the functions of the kidney and liver (11–13).

Conclusion

The net findings of this study proposed the ability of H_2O_2-induced OS to alter ketorolac's analgesia, antipyresis, and anti-inflammation for the chickens therefore, it is recommended to reduce the dose of ketorolac intended to be given to the stressed animals in medical practice.

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Adherence to Ethical Standards

The study was approved by the scientific Ethical Committee in the University of Mosul/ College of Veterinary Medicine according to approval number UM.VET.2022.37 on 15/09/2022.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Hilal-Dandan R, Brunton LL. Pharmacotherapy of Inflammation, Fever, Pain, and Gout. 2nd ed., McGraw-Hill Companies, Inc.; 2014.
2. Soleimanpour M, Imani F, Safari S, et al. The Role of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in the Treatment of Patients With Hepatic Disease: A Review Article. Anesth Pain Med. 2016;6(4):e37822. https://doi.org/10.5812/aapm.37822
3. Botting RM. Inhibitors of cyclooxygenases: Mechanisms, Selectivity and Uses. J Physiol Pharmacol. 2006;57(5):113-124. https://pubmed.ncbi.nlm.nih.gov/17218763/
4. Shankariah M, Mishra M, Kamath RA. Tramadol Versus Ketorolac in the Treatment of Postoperative Pain Following Maxillofacial Surgery. J Maxillofac Oral Surg. 2012;11(3):264-270. https://doi.org/10.1007/s12663-011-0321-y
5. Yaribeygi H, Panahi Y, Sahraei H, et al. The impact of stress on body function: A review. Exper Clin J. 2017;16:1057-1072. https://doi.org/10.17179/excli2017-480
6. Mousa YJ, Mohammad FK. Influence of hydrogen peroxide in drinking water on diazepam pharmacokinetics in chicks. Vet World. 2012;5(11):658-662. https://doi.org/10.5455/vetworld.2012.658-662
7. Mousa YJ. (2020): Etomidate anesthesia in chicks: Effect of xylazine. J Hell Vet Med Soc. 2020;71:2463-2470. https://doi.org/10.12681/jhvms.25921
8. Mousa YJ, Mohammad FK. The analgesic efficacy of xylazine and dipyrone in hydrogen peroxide–induced oxidative stress in chicks. Iraq J Vet Sci. 2012;26(2):69-76. https://doi.org/10.33899/ijvs.2012.67444
9. Mousa YJ. Anaesthetic properties of ketamine in chicks stressed with hydrogen peroxide. Vet Med. 2014;59(8):369-375. https://doi.org/10.17221/7656-VETMED
10. Mousa YJ, Mohammad FK. Effects of hydrogen peroxide on diazepam and xylazine sedation in chicks. Interdiscip Toxicol. 2012;5(4):179-183. https://doi.org/10.2478/v10102-012-0030-5
11. Lee KJ, Jeong HG. Protective effect of kahweol and cafestol against hydrogen peroxide-induced oxidative stress and DNA damage. Toxicol Lett. 2007;173:80-87. https://doi.org/10.1016/j.toxlet.2007.06.008
12. Nade VS, Yadav AV. Anti–stress effect of ethyl acetate soluble fraction of Morus alba in chronic restraint stress. Pharmaceutical Biol. 2010;48(9):1038-1046. https://doi.org/10.3109/13880200903473741
13. Nita M, Grzybowski A. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Long. 2016;2016:3164734. https://doi.org/10.1155/2016/3164734
14. Africa PE, Emine DGT, Nwachuku EO, et al. Assessment of the Antioxidant Potential of Hypoestes rosea Leafin Streptozotocin-induced Diabetic Albino Rats. J Complem Altern Med Res. 2020;9(4):35-43. https://doi.org/10.9734/jocamr/2020/v9i430149
15. Dixon WJ. Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol. 1980;20:441-462. https://doi.org/10.1146/annurev.pa.20.040180.002301
16. Mousa YJ. Effect of nefopam in normal chickens and its relationship to hydrogen peroxide-induced oxidative stress. Iraq J Vet Sci. 2021;35:7-12. https://doi.org/10.33899/ijvs.2021.127013.1433
17. Alias AS, Al-Zubaidy MHI, Mousa YJ, et al. Plasma and whole brain cholinesterase activities in three wild bird species in Mosul, Iraq: In vitro inhibition by insecticides. Interdiscip Toxicol. 2011,4:144-148. https://doi.org/10.2478/v10102-011-0022-x
18. Mousa YJ, Mahmood MB. Pharmacodynamics and pharmacokinetics interaction between nefopam and tramadol in the broiler chicks model. Iraq J Vet Sci. 2022;36(2):327-332. https://doi.org/10.33899/ijvs.2021.130163.1746
19. Mousa YJ, Mahmood MB and Mohammad MS. Administration of ketamine with the central and peripheral analgesics for induction of balanced anesthesia in the chicks. IOP Conference Series: Earth Envir Sci. 2019,388:012021. https://doi.org/10.1088/1755-1315/388/1/012021
20. Abotsi WK, Lamptey SB, Afrane S, et al. An evaluation of the anti-inflammatory, antipyretic and analgesic effects of hydroethanol leaf extract of Albizia zygia in animal models. Pharmaceut Biol. 2016;55(1):338-348. https://doi.org/10.1080/13880209.2016.1262434
21. Sufka KJ, Roach JT, Chambliss Jr. WG, et yl. Anxiolytic properties of botanical extracts in the chick social separation–stress procedure. Psycopharmacol. 2001;153:219-224. https://doi.org/10.1007/s002130000571

22. Collin X, Robert JM, Duflos M, et al. Synthesis of N-Pyridinyl (methyl)-1,2-dihydro-4-hydroxyl-2-oxoquinolone-3-carboxamides and analogues and their anti-inflammatory activity in mice and rats. J Pharmacy Pharmacol. 2001;53:417-423. https://doi.org/10.1211/0022357011775505
23. Plummer DT. An introduction to practical Biochemistry. 3rd ed. New York: McGrew-Hill Co Inc, USA; 1987, pp. 182-188. https://doi.org/10.1016/0307-4412(88)90082-9
24. Petrie A, Watson P. Statistics for Veterinary and Animal Sciences. Oxford: Blackwell Science; 2013. 90-140 p. https://doi.org/10.1136/vr.f7415
25. Albadrany YM, Naser AS, Hasan MM. Study the analgesic effect of diclofenac and silymarin coadministration in chicks. Iraqi J Vet Sci. 2021;35(4):833-839. https://doi.org/10.33899/IJVS.2021.127065.1453
26. Naser AS, Albadrany YM. The neurobehavioral effects of flumazenil in chicks. Iraqi J Vet Sci. 202;35(4):783-788. https://doi.org/10.33899/IJVS.2020.128443.1577
27. Mousa YJ. Neuroacting drugs and its pharmacological response in relation to different stress status: A review. J Hell Vet Med Soc. 2021;72(3):3007-3014. https://doi.org/10.12681/jhvms.28481
28. Lobo V, Patil A, Phatak A, et al. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-126. https://doi.org/10.4103/0973-7847.70902
29. Hendarman I, Triratna S, Kamaludin MT (2014). Ketorolac vs. tramadol for pain management after abdominal surgery in children. Paed Indon. 2014;54(2):118-121. https://doi.org/10.14238/pi54.2.2014.118-21