Bulgarian Journal of Veterinary Medicine, 2023 ONLINE FIRST
ISSN 1311-1477; DOI: 10.15547/bjvm.2023-0066

Original article

INTERACTION OF PHYSOSTIGMINE WITH THREE INJECTABLE ANAESTHETICS IN A YOUNG CHICK MODEL

H. M. S. GARMAVY[1] & F. K. MOHAMMAD[2]

[1]Department of Pharmacology, College of Pharmacy, University of Duhok, Duhok, Kurdistan Region, Iraq, [2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Summary

Garmavy, H. M. S. & F. K. Mohammad, 2023. Interaction of physostigmine with three injectable anaesthetics in a young chick model. *Bulg. J. Vet. Med.* (online first).

Propofol, thiopental and ketamine are injectable general anaesthetics with different mechanisms of action. Reports vary with respect to the antagonistic action of physostigmine against these anaesthetics. The purpose of the present study was to examine the possible interaction of physostigmine with the anaesthetic action of the three anaesthetics in a model of young chicks (7–14 days old). Chicks (8/group) were anaesthetised with propofol at 10 mg/kg, intraperitoneally (i.p.), thiopental at 20 mg/kg, i.p. and ketamine at 10 mg/kg, intramuscularly (i.m.). The anaesthetised chicks were subjected to treatment challenges with physostigmine (0.25 mg/kg, i.p.) or neostigmine (0.125 mg/kg, i.p.), 5 minutes before the anaesthetic injection or after the induction of anaesthesia. When physostigmine was injected before anaesthesia, it prevented propofol but not thiopental or ketamine anaesthesia. Physostigmine given after the anaesthesia reduced the sleep time of propofol, but not those of thiopental or ketamine in chicks. Neostigmine treatments did not significantly affect the anaesthesia induced by the three anaesthetics in chicks. The median effective doses (ED50) of the anaesthetics in chicks were determined by the up-and-down method with or without concomitant physostigmine (0.25 mg/kg, i.p.) after the loss of the righting reflex. The ED50 values of propofol, thiopental and ketamine in chicks were 7.712 mg/kg, i.p., 14.744 mg/kg, i.p. and 10.168 mg/kg, i.m., respectively. Physostigmine differentially increased the ED50 of propofol by 137%, and did not affect those of the thiopental and ketamine. Plasma cholinesterase activity was significantly reduced in the propofol and thiopental anaesthetic groups of chicks, whereas that of the ketamine group was not affected. In conclusion, the results suggest that physostigmine, being a cholinergic stimulant, could specifically antagonise propofol anaesthesia in the young chick model, with clinical trial awaiting further studies.

Key words: antidote, cholinesterase, general anaesthesia, ketamine, neostigmine, propofol, thiopental

INTRODUCTION

Injectable general anaesthetics such as propofol, thiopental and ketamine are commonly used in human medicine (Brohan & Goudra, 2017; Sahinovic *et al.*,

2018; Kohtala, 2021) and in veterinary clinical practice (Gozalo-Marcilla & Ringer, 2021; Gomes *et al.*, 2022). The mechanism of anaesthetic action of propofol is mostly related to potentiation of $GABA_A$-receptor-mediated central inhibitory effect and also, inhibition of the N-methyl-d-aspartate (NMDA) receptor (Kotani *et al.*, 2008; Sahinovic *et al.*, 2018), whereas thiopental causes anaesthesia via a GABA-mimetic action (Brohan & Goudra, 2017). Ketamine antagonises N-methyl-d-aspartate receptors (NMDARs) and ion channels mainly related to the excitatory glutamatergic neurotransmission (Sinner & Graf, 2008; Kohtala, 2021). All these anaesthetic actions are mediated centrally (Brohan & Goudra, 2017; Sahinovic *et al.*, 2018; Tripathi, 2019; Barrett *et al.*, 2020).

No specific antidotes are yet available for propofol, thiopental or ketamine anaesthetics. It has been reported that general anaesthesia can be modulated by manipulating the cholinergic tone by physostigmine (Plourde *et al.*, 2003; Hölle *et al.*, 2023). However, physostigmine by inhibiting central cholinesterase (ChE) activity was reported to partially antagonise propofol and isoflurane anaesthesia in rats (Reed *et al.*, 2013; Kenny *et al.*, 2016) and the centrally-mediated propofol toxic depression in chicks (Naser & Mohammad, 2014a), and its antagonistic effect on other anaesthetics such as sevoflurane appears to be controversial (Paraskeva *et al.*, 2005). Based on these studies, it is apparent that any antagonistic effect of physostigmine against anaesthesia is not specific to a particular anaesthetic. Within this context, the reversible ChE inhibiting carbamate compounds physostigmine and neostigmine can be used to manipulate the general anaesthetics (Tripathi, 2019; Andrade & Zafar Gondal, 2023; Neely *et al.*, 2022; Hölle *et al.*, 2023). Physostigmine gains access to the central nervous system, whereas neostigmine does not penetrate the blood brain barrier (Tripathi, 2019; Andrade & Zafar Gondal, 2023).

In the light of the uncertainty regarding the antidotal action of physostigmine against propofol, thiopental or ketamine, the purpose of the present study was to examine the possible interaction of physostigmine with the anaesthetic action of the three general injectable anaesthetics using the model of young chicks which was proven to be a useful animal model for assessing the interaction of various anaesthetics or analgesics therapeutically or toxicologically (Mohammad *et al.*, 2007; Naser & Mohammad, 2014a,b; Mousa & Mahmood, 2022). The developed young chick model and anaesthetic-antidotal manipulations can be applied in the avian species as found in previous reports using the central depressant metoclopramide (Al-Zubaidy & Mohammad, 2005; Mohammad *et al.*, 2007), the general anaesthetic propofol alone (Naser & Mohammad, 2014a) or in combination with the sedative xylazine and the general anaesthetic ketamine (Naser & Mohammad, 2014b) as well as in cases of examining the hypnotic actions of ketamine (Mohammad *et al.,* 2007), xylazine-ketamine (Rashid & Mohammad, 2023) or thiopental (Mousa & Mahmood, 2022). Whether physostigmine or neostigmine can influence anaesthetic effects in chicks, when given before or after anaesthesia, needs further elucidation, a possibility which was addressed in the present report taking into account the determination of plasma ChE activity, which is synthesised in the liver and affects metabolism of neuromuscular blocking agents (Benner *et al.*, 2022).

MATERIALS AND METHODS

Animals

One day old unsexed Ross broiler chicks were obtained from a certified local hatchery in Duhok, Iraq. They were raised in batches of 20 to 30 chicks at a time in the animal house of the College of Medicine, University of Duhok at a temperature of 30–34 °C controlled by electric heaters with a constant lighting. The chicks were used as an animal model for the anaesthetic action in the experiments when they were 7 to 14 day-old with body weights of 75–120 g (Naser & Mohammad, 2014a,b). The litter consisted of wood shavings; water and feed were supplied *ad libitum*. The Committee of Postgraduate Studies at the College of Medicine, University of Duhok, Iraq has approved the present study according to the institutional regulations and ethics on the animal use and handling in biomedical research in accordance with ARRIVE guidelines (https://www.nc3rs.org. uk/arrive-guidelines) and the Guide for the Care and Use of Laboratory Animals (https://www.ncbi.nlm.nih.gov/books/NBK54050/).

Drugs used

Drugs used were as follows: propofol, 1%, 20 mL emulsion ampoule (Astra Zeneca, Switzerland), thiopental sodium 1 g vial (Sandoz GmbH, Austria), ketamine HCl (50 mg/mL), 10 mL vial (Rotexmedica, Germany), physostigmine salicylate (1 mg/mL) 2 mg vial (Apotheek UMCG, Netherlands), neostigmine methyl sulfate, 2.5 mg/mL (Laboratoire Renaudin, France). Before each experiment, the required drug concentrations were freshly prepared using distilled water (propofol) or physiological saline solution (thiopental and ketamine), and the volume of administration was 10 mL/kg of body weight (Mohammad *et al.*, 2007; Naser & Mohammad, 2014a,b). Ketamine was injected intramuscularly (i.m.), whereas other drugs were injected intraperitoneally (i.p.). Pilot experiments were conducted in chicks to determine appropriate doses of the drugs to be used in the present study, and they were also comparable to those reported in the literature (Mohammad et *al.*, 2005; 2007; Naser & Mohammad, 2014a,b).

Effects of physostigmine and neostigmine on injectable anaesthesia

Chicks (8/group) were treated with propofol (10 mg/kg, i.p.), thiopental (20 mg/kg, i.p.) and ketamine (10 mg/kg, i.m.). The anaesthetised chicks with loss of the righting reflex, were subjected to treatment challenges with either physostigmine (0.25 mg/kg, i.p.) or neostigmine (0.125 mg/kg, i.p.) at two time points: 5 minutes before the anaesthetic injection or after the induction of anaesthesia. The latency to onset of righting reflex and the duration of the loss of the righting reflex (sleep) were recorded for each chick.

Median effective anaesthetic doses (ED50) of injectable anaesthetics and the influence of physostigmine

The up-and-down method (Dixon, 1980) was used to determine the ED50 values of propofol, thiopental and ketamine for the induction of anaesthesia (sleep) as judged by the loss of the righting reflex with sternal or lateral recumbency (Al-Zubaidy & Mohammad, 2005). Physostigmine (0.25 mg/kg) was given i.p. 5 minutes before each anaesthetic was injected. Each ED50 value of the anaesthetics with or without physostigmine was computed using the formula of Dixon (1980):

$$LD50 = xf + kd,$$

where *xf* was the last dose of anaesthetic administered, *d* was the increase or decrease in the anaesthetic dose and *k* is a value from the table of Dixon (1980) with a standard error of 0.61. The 95% confidence interval (95% C.I.) of each ED50 value was determined according to Zhang *et al.* (2022) as outlined earlier (Mohammad, 2022). Each ED50 experiment, as determined by the up-and-down method, can be concluded using < 10 chicks/test (Mohammad, 2022; Mohammed & Mohammad, 2022).

Determination of plasma ChE activity

Chicks (8/group) were treated with single doses of each of the anaesthetics, propofol (10 mg/kg, i.p.), thiopental (20 mg/kg, i.p.) and ketamine (10 mg/kg, i.m.), with a control group chicks given physiological saline solution (10 ml/kg, i.p.). Heparinised blood samples were obtained from chicks two hours after treatments by cardiac puncture (Kelly & Alworth, 2013). Plasma ChE activity was determined by a modified electrometric method as described before (Mohammad *et al.*, 2014). The enzymatic reaction mixture of the electrometric method for ChE determination in the plasma consisted of 3 mL distilled water, 0.2 mL plasma sample and 3 mL of barbital-phosphate buffer (1.237 g sodium barbital, 0.163 g potassium dihydrogen phosphate and 35.07 g sodium chloride/L of distilled water, pH 8.1). The pH1 of the mixture was measured with the electrode of a pH meter (CamLab Co., Cambridge, U.K.) before the addition of 0.1 mL of the substrate acetylcholine iodide (7.1%). The enzymatic reaction mixture was incubated at 37 ^{o}C for 30 min. Thereafter, the pH2 of the reaction mixture was measured for a second time. The plasma ChE activity was estimated as follows: ChE activity (Δ pH 30/min) = (pH1 – pH2) – Δ pH of blank (no plasma sample)

The percentage of ChE inhibition was calculated as follows: % ChE inhibition= [ChE activity (without anaesthesia) – ChE activity (with anaesthesia) / ChE activity (without anaesthesia)] ×100.

Statistical analysis

Multiple means were statistically analysed by one way analysis of variance followed by the least significant difference test (Petrie & Watson, 2013), using the statistics software program SPSS (IBM). The level of statistical significance was $P<0.05$.

RESULTS

The initial anaesthetic analysis in the young chick model included examination of the nature of interaction of the ChE inhibitors physostigmine and neostigmine with the three anaesthetics, taking into account the possibility of their antidotal action against the anaesthetic response. Physostigmine, given after the onset of propofol anaesthesia, reduced significantly ($P<0.05$) the duration of sleep in chicks in comparison with the corresponding control group (Table 1). Physostigmine injection after the onset of thiopental or ketamine anaesthesia had no significant effects on sleep durations in chicks in comparison with the corresponding control groups (Table 1). When physostigmine was injected before the anaesthetic dose of propofol, loss of the righting reflex did not occur and the chicks were not anaesthetised. Aside from propofol, physostigmine injection did not prevent the onset of thiopental or ketamine anaesthesia, and it did not significantly affect their durations of sleep (Table 1). In contrast to physostigmine, neostigmine did not sig-

Table 1. Effects of physostigmine on injectable anaesthesia in chicks (mean ± SE of 8 chicks/ treatment group)

Treatment groups (mg/kg)	Latency to onset of loss of righting reflex (s)	Duration of sleep (min)
Propofol (10, i.p.) alone	30.0 ± 3.3	10.0 ± 0.7
Propofol (10, i.p.) + Physostigmine (0.25, i.p.)[a]	30.0 ± 2.2	8.6 ± 0.3[*]
Physostigmine (0.25, i.p.) + propofol (10, i.p.)[b]	Nil[†]	Nil[†]
Thiopental (20, i.p.) alone	35.0 ± 2.9	9.3 ± 0.6
Thiopental (20, i.p.) + physostigmine (0.25, i.p.)[a]	30.0 ± 3.5	10.9 ± 0.9
Physostigmine (0.25, i.p.) + thiopental (20, i.p.)[b]	32.0 ± 2.4	10.1 ± 0.6
Ketamine (10, i.m.) alone	138 ± 5.5	10.7 ± 0.7
Ketamine (10, i.m.) + physostigmine (0.25, i.p.)[a]	120 ± 5.0	10.3 ± 0.5
Physostigmine (0.25, i.p.) + ketamine (10, i.m.)[b]	150 ± 6.0	11.6 ± 0.8

[a]Physostigmine was given after the loss of the righting reflex induced by the anaesthetics; [b]Physostigmine was given 5 minutes before the anaesthetic injection. [*]Significantly different from the corresponding control value, $P<0.05$; [†]Physostigmine pretreatment prevented propofol anaesthesia.

nificantly affect the onset and duration of anaesthesia induced by the three anaesthetics in chicks in comparison with respective control values (Table 2).

The anaesthetic ED50 values of propofol, thiopental and ketamine as determined by the up-and-down method were 7.712 mg/kg, i.p.; 14.744 mg/kg, i.p. and 10.168 mg/kg, i.m. respectively, which caused loss of the righting reflex and sleep (Table 3). When physostigmine (0.25 mg/kg, i.p.) was administered after the induction of anaesthesia (loss of the righting reflex), the ED50 value of propofol was differentially increased by 137% to 18.280 mg/kg, i.p., and those of the thiopental and ketamine were not affected. Concomitantly, physostigmine delayed the latency to the onset of propofol anaesthesia by 100%, and prolonged the duration of anaesthetic action (sleep) by 48% (Table 3).

Plasma ChE activity was significantly reduced in the propofol (33%) and thiopental (39%) anaesthetic groups of chicks compared to the respective control value, whereas that of the ketamine group was not significantly affected, as it was reduced by only 8% (Table 4).

DISCUSSION

The main finding of the present study is that physostigmine differentially affected propofol anaesthesia (increased ED50, reduced duration) when given after the injection of the anaesthetic, and it prevented the anaesthetic action of propofol

Table 2. Effects of neostigmine on injectable anaesthesia in chicks (mean ± SE of 8 chicks/treatment group)

Treatment groups (mg/kg)	Latency to onset of loss of righting reflex (s)	Duration of sleep (mins)
Propofol (10, i.p.) alone	33.6 ± 2.6	9.3 ± 0.4
Propofol (10, i.p.) + neostigmine (0.125, i.p.)[a]	32.1 ± 1.8	10.1 ± 0.6
Neostigmine (0.125, i.p. + propofol (10, i.p.)[b]	31.4 ± 2.6	9.6 ± 0.4
Thiopental (20, i.p.) alone	32.1 ± 3.8	9.6 ± 0.9
Thiopental (20, i.p.) + neostigmine (0.125, i.p.)[a]	31.4 ± 2.6	8.3 ± 0.4
Neostigmine (0.125, i.p.) + thiopental (20, i.p.)[b]	31.4 ± 1.8	8.9 ± 0.3
Ketamine (10, i.m.) alone	135.7 ± 6.6	10.3 ± 0.6
Ketamine (10, i.m.) + neostigmine (0.125, i.p.)[a]	131.4 ± 5.2	10.1 ± 0.5
Neostigmine (0.125, i.p.) + ketamine (10, i.m.)[b]	133.6 ± 5.8	10.9 ± 0.6

[a]Neostigmine was given after the loss of the righting reflex induced by the anaesthetics; [b]Neostigmine was given 5 minutes before the anaesthetic injection.

when given before its administration. This effect was specific to propofol and not to the other anaesthetics thiopental and ketamine used in the present study.

Such a selective action of physostigmine has been reported during deep isoflurane anaesthesia in rats (Kenny *et al.*, 2016). However, physostigmine did not affect the early recovery from sevoflurane in human patients (Paraskeva *et al.*, 2005). Part of the reason for this discrepancy could be related to anaesthetics used in different studies as well as to the species variations in response to physostigmine when administered during anaesthesia (Naser & Mohammad, 2014a; Kenny *et al.*, 2016).The effect of physostigmine on propofol anaesthesia in the present study correlates with previous findings in young chicks, in which it shortened the propofol sleep time in the birds (Naser & Mohammad, 2014a). Furthermore, in humans, physostigmine was reported to antagonise propofol anaesthesia (Meuret *et al.*, 2000), and increase the dose of propofol needed to induce anaesthesia (Fassoulaki *et al.*, 1997). In the present study, physostigmine increased the ED50 anaesthetic dose of propofol in chicks almost 1.4 fold. In contrast, thiopental or ketamine anaesthesia in chicks was not affected by physostigmine treatments, suggesting a differential specificity of physostigmine in modulating propofol anaesthesia in our experimental-anaesthetic paradigms in chicks. This effect of physostigmine appeared to be related to the central cholinergic effects of physostigmine,

Table 3. Median effective anesthetic doses (ED50) of injectable anaesthetics and influence of physostigmine in chicks

Variables	Propofol	Physostigmine + propofol	Thiopental	Physostigmine + thiopental	Ketamine	Physostigmine + ketamine
Route of anaesthetic injection	i.p.	i.p.	i.p.	i.p.	i.m.	i.m.
ED50 (mg/kg)	7.712	18.280	14.744	14.744	10.168	10.168
95% C.I.	6.669; 8.755	16.611; 19.949	13.701; 15.787	13.701; 15.787	9.475; 10.861	9.475; 10.861
Number of chicks used	7 (OOOXXOX)	8 (OOOOXOOX)	7 (OOOXXOO)	7 (OOOXXOO)	5 (OXOXX)	5 (OXOXX)
Range of used doses (mg/kg) (max–min)	10–4= 6	20–10= 10	16–10= 6	16–10= 6	12–10= 2	12–10= 2
Initial dose (mg/kg)	4	10	10	10	10	10
Last dose (mg/kg)	8	20	14	14	10	10
Increase or decrease in dose (mg/kg)*	2	2	2	2	2	2
Onset of anaesthetic action (mean ± SE), s	30.0 ± 3.3	60.0 ± 2.4	35.0 ± 2.9	40.0 ± 2.4	123.0 ± 0.7	125.0 ± 0.6
Duration of anaesthesia (mean ± SE), min	8.33 ± 0.36	12.30 ± 0.62	9.50 ± 0.57	9.80 ± 0.51	7.33 ± 0.21	8.50 ± 0.60

X: anaesthesia; O: no anaesthesia; i.p.: intraperitoneal; i.m.: intramuscular. Physostigmine (0.25 mg/kg) was injected i.p. in chicks after the loss of the righting reflex induced by the anaesthetics. *The increase or decrease in the dose of anesthetics was at 2 mg/kg, depending on the response of the previous chick (O: no anaesthesia or X: anaesthesia, respectively).

Table 4. Plasma cholinesterase activity (Δ pH/30 min) of anaesthetised chicks (mean ± SE of 8 chicks/treatment group)

Anaesthesia	Cholinesterase activity	% decrease from control
Control (saline)	0.438 ± 0.052	
Propofol (10 mg/kg, i.p.)	0.293 ± 0.027*	33
Thiopental (20 mg/kg, i.p.)	0.269 ± 0.024*	39
Ketamine (10 mg/kg, i.m.)	0.403 ± 0.033	8

i.p: intraperitoneal; i.m.: intramuscular; *significantly different from the control value, $P<0.05$.

as it crosses the blood brain barrier to inhibit neuronal ChE activity reversibly (Tripathi, 2019; Andrade & Zafar Gondal, 2023; Hölle *et al.*, 2023). Neostigmine, as expected, did not affect any anaesthetic effects (Table 2), because being a quaternary ammonium compound, it does not cross the blood brain barrier with only a peripherally inhibitory action on the ChE activity (Tripathi, 2019; Neely *et al.*, 2022).

In the present study, physostigmine, in contrast to its action on propofol anaesthesia, did not affect anaesthesia produced by thiopental or ketamine in chicks as seen by the three experimental protocols, which were the ED50 of the anaesthetic, and pre- and post-anaesthetic treatments with physostigmine. Such results, especially those of propofol have not been reported in the avian species before, and it is difficult to extrapolate the present findings to humans or other animal species because of the considerable limitations of species variations in response to drugs including the anaesthetics. Therefore, our interpretation on the possible antagonistic effect of physostigmine against propofol anaesthesia should be dealt with cautiously, and it should be taken as a guide towards the avian species at present. Further studies are needed on other animal species regarding the antagonistic effects of physostigmine on different types of anaesthetics or their adjuvants. Similarly, contradictory results were reported about effects of physostigmine on the antagonism of other types of anaesthetics, including the injectable ones (Mimura *et al.*, 1990; Kenny *et al.*, 2016; Hölle *et al.*, 2023). Such antagonistic discrepancies regarding the antidotal effects of physostigmine could be attributed to the species variations involved in the studies, drug dosages, routes of administration and to the differences in the experimental protocols regarding the timing of physostigmine administration relative to the onset of anaesthesia (Naser & Mohammad, 2014a,b; Gozalo-Marcilla & Ringer, 2021; Kohtala, 2021; Louro *et al.*, 2022; Mousa & Mahmood, 2022; Hölle *et al.*, 2023). Furthermore, different regions of the brain are affected by the anaesthetics and hence possibly a differential reponse to physostigmine is to be expected (Pedersen *et al.*, 2004; Kushikata & Hirota, 2014).

The plasma ChE activity is usually monitored during general anaesthesia (Brzezinski-Sinai *et al.*, 2021). ChE is synthesised in the liver to be secreted later into the plasma (Benner *et al.,* 2022). In the present study, the reductions in plasma ChE activity in propofol and thiopental anaesthetised chicks call for careful consideration of the fact that that animals anaesthetised with propofol or thiopental could be at risk of plasma ChE inhibition.

This enzyme is important in the metabolism of various anaesthetics and neuromuscular blocking agents (Zhang *et al.*, 2018; Andersson *et al.*, 2019; Brzezinski-Sinai *et al.*, 2021). The benefit of monitoring plasma ChE activity lies in the fact that it is considered a biomarker of exposure to antiChE compounds (Odisho & Mohammad, 2022; Mohammad *et al.*, 2023), and it is associated with the effects of anaesthetic and neuromuscular blocking agents at the level of the autonomic nervous system (Zhang *et al.*, 2018; Tripathi, 2019; Athiraman *et al.*, 2021; Brzezinski-Sinai *et al.*, 2021). Additional studies, however, are needed for biomonitoring plasma ChE activity during propofol or thiopental anaesthesia, preferably when combined with various adjuvants usually used with anaesthetics (Brown *et al.*, 2018; Athiraman *et al.*, 2021; Karam & Mohammad, 2022; 2023).

The present study successfully used the young-chick animal model to find out differential antidotal effect of physostigmine against propofol anaesthesia. Overall, the results of the present study suggest that physostigmine, being a cholinergic stimulant, could specifically antagonise propofol anaesthesia in the young chick model, with clinical trial awaiting further studies in the chicken and possibly other animal species.

ACKNOWLEDGEMENTS

This report represents a part of a dissertation submitted by the first author to the University of Duhok, Iraq as partial fulfillment of the requirements for the Ph.D. degree in Pharmacology. The authors thank the Colleges of Medicine and Pharmacy, University of Duhok for support and providing facilities and supplies to conduct this study.

REFERENCES

Al-Zubaidy, M. H. & F. K. Mohammad, 2005. Metoclopramide-induced central nervous system depression in the chicken. *BMC Veterinary Research*, **1**, 6.

Andersson, M. L., A. M. Møller & K. Wildgaard, 2019. Butyrylcholinesterase deficiency and its clinical importance in anaesthesia: A systematic review. *Anaesthesia*, **74**, 518–528.

Andrade, O. A. & A. Zafar Gondal, 2023. Physostigmine. In: *StatPearls*. StatPearls Publishing, Treasure Island, FL, USA, PMID: 31424845, https://www.ncbi.nlm.nih.gov/ books/NBK545261/ (27 May 2023, date last accessed).

Athiraman, U. & G. J. Zipfel, 2021. Role of anesthetics and their adjuvants in neurovascular protection in secondary brain injury after aneurysmal subarachnoid hemorrhage. *International Journal of Molecular Sciences*, **22**, 6550.

Barrett, W., M. Buxhoeveden & S. Dhillon, 2020. Ketamine: A versatile tool for anesthesia and analgesia. *Current Opinion in Anaesthesiology*, **33**, 633–638.

Benner A., N. F. Lewallen & N. M. Sadiq, 2022. Biochemistry, Pseudocholinesterase. In: *StatPearls*. StatPearls Publishing, Treasure Island, FL., USA. PMID: 31424868, https://www.ncbi.nlm.nih.gov /books/NBK545284/ (3 July 2023, date last accessed).

Brohan, J. & B. G. Goudra, 2017. The role of GABA receptor agonists in anesthesia and sedation. *CNS Drugs*, **31**, 845–856.

Brown, E. N., K. J. Pavone & M. Naranjo, 2018. Multimodal general anesthesia: Theory and practice. *Anesthesia and Analgesia*, **127**, 1246–1258.

Brzezinski-Sinai, Y., E. Zwang, E. Plotnikova, E. Halizov, I. Shapira, D. Zeltser, O. Rogowski, S. Berliner, I. Matot & S. Shenhar-Tsarfaty, 2021. Cholinesterase activity in serum during general anesthesia in patients with or without vascular disease. *Scientific Reports*, **11**, 16687.

Dixon, W. J., 1980. Efficient analysis of experimental observations. *Annual Review of Pharmacology and Toxicology*, **20**, 441–462.

Fassoulaki, A., C. Sarantopoulos & C. Dervenіotis, 1997. Physostigmine increases the dose of propofol required to induce anaesthesia. *Canadian Journal of Anaesthesia = Journal Canadien d'Anesthesie*, **44**, 1148–1151.

Gomes, V. H., W. T. S. de Carvalho, V. C. Pimentel, N. Cappelli, B. T. G. Mignani & M. F. A. da Silva, 2022. Ketamine-dexmedetomidine combined with local anesthesia, with or without different doses of atipamezole in the postoperative period, for orchiectomy in cats. *Journal of the American Veterinary Medical Association*, **261**, 217–222.

Gozalo-Marcilla, M. & S. K. Ringer, 2021. Recovery after general anaesthesia in adult horses: A structured summary of the literature. *Animals (Basel)*, **11**, 1777.

Hölle, T., J. C. Purrucker, B. Morath, M. A. Weigand & F. C. F. Schmitt, 2023. Zentrales anticholinerges, malignes neuroleptisches und Serotoninsyndrom: Wichtige Differenzialdiagnosen bei postoperativen Bewusstseinsstörungen [Central anticholinergic, neuroleptic malignant and serotonin syndromes]. *Wiener Klinisches Magazin : Beilage zur Wiener klinischen Wochenschrift*, **26**, 124–132.

Karam, R. S. & F. K. Mohammad, 2022. The use of anesthetics for cesarean section delivery in women in Duhok, Kurdistan region, Iraq. *Journal of Ideas in Health,* **5**, 755−759.

Karam, R. S. & F. K. Mohammad, 2023. Changes in blood oxidative stress biomarker and cholinesterase activity after general vs spinal anesthesia for elective cesarean sections. *Anaesthesia, Pain and Intensive Care*, **27**, 396−404.

Kelly, L. M. & L. C. Alworth, 2013. Techniques for collecting blood from the domestic chicken. *Lab Animal*, **42**, 359–361.

Kenny, J. D., J. J. Chemali, J. F. Cotton, C. J. Van Dort, S. E. Kim, D. Ba, N. E. Taylor, E. N. Brown & K. Solt, 2016. Physostigmine and methylphenidate induce distinct arousal states during isoflurane general anesthesia in rats. *Anesthesia and Analgesia*, **123**, 1210–1219.

Kohtala, S., 2021. Ketamine – 50 years in use: From anesthesia to rapid antidepressant effects and neurobiological mechanisms. *Pharmacological Reports,* **73**, 323–345.

Kotani, Y., M. Shimazawa, S. Yoshimura, T. Iwama & H. Hara, 2008. The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties. *CNS Neuroscience and Therapeutics*, **14**, 95–106.

Kushikata, T. & K. Hirota, 2014. Mechanisms of anesthetic emergence: Evidence for active reanimation. *Current Anesthesiology Reports*, **4**, 49–56.

Louro, L. F., K. Robson, J. Hughes, K. Loomes & M. Senior, 2022. Head and tail rope-assisted recovery improves quality of recovery from general anaesthesia in horses undergoing emergency exploratory laparotomy. *Equine Veterinary Journal*, **54**, 875–884.

Meuret, P., S. B. Backman, V. Bonhomme, G. Plourde & P. Fiset, 2000. Physostigmine reverses propofol-induced unconsciousness and attenuation of the auditory steady state response and bispectral index in human volunteers. *Anesthesiology*, **93**, 708–717.

Mimura, M., A. Namiki, R. Kishi, T. Ikeda & H. Miyake, 1990. Antagonistic effect of physostigmine on ketamine-induced anesthesia. Psychopharmacology, 102, 399–403.

Mohammad, F. K., 2022. Calculation of 95% confidence interval of the median lethal dose determined by the up-and-down procedure: A letter to editor. *Journal of Ideas in Health,* **5**, 725–726.

Mohammad, F. K., G. A. M. Faris & B. Kh. Al-Baggou, 2005. Some neurobehavioral

effects of ketamine in chicks. *Iraqi Journal of Veterinary Sciences,* **19**, 13–19.

Mohammad, F. K., M. H. Al-Zubaidy & A. S. Alias, 2007. Sedative and hypnotic effects of combined administration of metoclopramide and ketamine in chickens. *Lab Animal*, **36**, 35–39.

Mohammad, F. K., B. K. Al-Baggou, A. S. Naser & M. A. Fadel, 2014. *In vitro* inhibition of plasma and brain cholinesterases of growing chicks by chlorpyrifos and dichlorvos. *Journal of Applied Animal Research,* **42**, 423–428.

Mohammad, F. K., H. M. S. Garmavy, A. A. Mohammed & H. M. Rashid, 2023. First meta-analysis study of cholinesterase inhibition in experimental animals by organophosphate or carbamate insecticides under the influence of diphenhydramine. *Veterinary World*, **16**, 118–125.

Mohammed, A. A. & F. K. Mohammad, 2022. Recognition and assessment of antidotal effects of diphenhydramine against acute carbaryl insecticide poisoning in a chick model. *Toxicology International*, **29**, 339–352.

Mousa, Y. J. & M. B. Mahmood, 2022. Effect of meloxicam coadministration on the anaesthetic potency of thiopental sodium in a chick model. *Veterinarska Stanica,* **53**, 155–163.

Naser, A. S. & F. K. Mohammad, 2014a. Central depressant effects and toxicity of propofol in chicks. *Toxicology Reports*, **1**, 562–568.

Naser, A. S. & F. K. Mohammad, 2014b. Isobolographic analysis of sedative and hypnotic interactions of propofol with ketamine and xylazine in chicks. *Human and Veterinary Medicine Bioflux, 6*, 56–60.

Neely, G. A., S. Sabir & A. Kohli, 2022. Neostigmine. In: *StatPearls*. StatPearls Publishing, Treasure Island, FL. PMID: 9261883, https://www.ncbi.nlm.nih.gov/books/NBK470596/ (27 May 2023, date last accessed).

Odisho, S. K. & F. K. Mohammad, 2022. Blood cholinesterase activities and oxidative stress status among farmworkers using pesticides in Duhok, KRG, Iraq. *Journal of Ideas in Health,* **5**, 786–793.

Paraskeva, A., C. Staikou, M. Diamadis, I. Siafaka & A. Fassoulaki, 2005. Anesthesia with 1.5 minimum alveolar concentration sevoflurane is not altered by physostigmine as measured by bispectral and clinical indices. *Journal of Clinical Anesthesia*, **17**, 581–585.

Pedersen, J. L., J. Lillesø, N. A. Hammer, M. U. Werner, K. Holte, P. G. Lacouture & H. Kehlet, 2004. Thiopental and propofol affect different regions of the brain at similar pharmacologic effects. *Anesthesia and Analgesia*, **99**, 912–918.

Petrie, A. & P. Watson, 2013. Statistics for Veterinary and Animal Sciences, 3rd ed. Wiley-Blackwell, West Sussex, U.K.

Plourde, G., D. Chartrand, P. Fiset, S. Font & S. B. Backman, 2003. Antagonism of sevoflurane anaesthesia by physostigmine: Effects on the auditory steady-state response and bispectral index. *British Journal of Anaesthesia*, **91**, 583–586.

Rashid, H. M. & F. K. Mohammad, 2023. Statins modify response of chicks to challenges with xylazine-ketamine and carbaryl. *Veterinarski Arhiv* (in press).

Reed, S. J., G. Plourde, S. Tobin & C. A. Chapman, 2013. Partial antagonism of propofol anaesthesia by physostigmine in rats is associated with potentiation of fast (80–200 Hz) oscillations in the thalamus. *British Journal of Anaesthesia*, **110**, 646–653.

Sahinovic, M. M., M. M. R. F. Struys & A. R. Absalom, 2018. Clinical pharmacokinetics and pharmacodynamics of propofol. *Clinical Pharmacokinetics*, **57**, 1539–1558.

Sinner, B. & B. M. Graf, 2008. Ketamine. *Handbook of Experimental Pharmacology*, **182**, 313–333.

Tripathi, K. D., 2019. Essentials of Medical Pharmacology, 8th edn. The Health Sciences Publisher, New Delhi, India.

Zhang, C., H. Cao, Z. G. Wan & J. Wang, 2018. Prolonged neuromuscular block as-

sociated with cholinesterase deficiency. *Medicine*, **97**, e13714.

Zhang, Y. Y., Y. F. Huang, J. Liang & H. Zhou, 2022. Improved up-and-down procedure for acute toxicity measurement with reliable LD_{50} verified by typical toxic alkaloids and modified Karber method. *BMC Pharmacology and Toxicology*, **23**, 3.

Paper received 27.05.2023; accepted for publication 04.08.2023

Correspondence:

Fouad K. Mohammad
Department of Physiology, Biochemistry and Pharmacology,
College of Veterinary Medicine,
University of Mosul, Mosul, Iraq,
e-mail: fouadmohammad@yahoo.com
https://orcid.org/0000-0002-5715-4823