



# Central depressant effects and toxicity of propofol in chicks



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## ABSTRACT

Propofol is an ultra-short acting anesthetic agent. The information on the pharmacological and toxicological effects of propofol in the chicken is rather limited. This study examines the toxicity and pharmaco-behavioral effects of propofol given intraperitoneally in 7–10 day-old chicks. The median effective doses of propofol for the induction of sedation, analgesia to electric stimulation and sleep in the chicks were 1.82, 2.21 and 5.71 mg/kg, respectively. The 24-h median lethal dose of propofol in chicks was 57.22 mg/kg. The therapeutic indices of propofol for sedation, analgesia and sleep were 31.4, 25.9 and 10, respectively. Propofol at 0.5 and 1 mg/kg reduced the locomotor activity and increased the duration of tonic immobility in chicks. Propofol at 2 and 4 mg/kg caused analgesia to electric stimulation as well as analgesia and anti-inflammatory responses against formalin test in chicks. Propofol at 5, 10 and 20 mg/kg induced sleep in chicks for 8.4 to 25 min. Physostigmine shortened the sleep duration of propofol. Data suggest that propofol induces anti-inflammatory action and central nervous system depression in chicks resulting in sedation, analgesia and anesthesia with wide safety margin. These effects could form the basis of further pharmacological and toxicological studies on propofol in the young chick model, and the drug could be safely applied clinically in the chicken.

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## 1. Introduction

Propofol, 2,6-diisopropylphenol, is an ultra-short acting anesthetic agent used intravenously in humans [1,2] and animals [3,4]. It is a non-narcotic and non-barbiturate anesthetic agent which is usually administered for maintenance of anesthesia with rapid induction and recovery phases [1]. Propofol has been shown to be increasingly used in veterinary practice [4], especially in dogs and cats [5]. Several studies have attempted to apply propofol as an anesthetic in birds such as wild turkeys [6], barn owl [7], free ranging wood ducks [8], mallard ducks [9], pigeons [10], ostriches [11] and Amazon parrots [12] with varying dosages and resultant effects. These studies reported that

general effects of propofol in birds were anesthesia of short duration of action, good muscle relaxation with minimal cardiovascular and respiratory adverse effects.

Other than the anesthetic effects of propofol which are well known, several studies have shown that the drug may induce various pharmacological effects not related to general anesthesia [13]. These include in vitro and in vivo antioxidant actions [14,15], treatment of erectile dysfunction [16], as well as sedative [17], anxiolytic [18], analgesic [2], antiemetic [2], immunomodulatory [19] and neuromodulatory effects [20]. There is no specific pharmacological antagonist of propofol. However, studies have shown that physostigmine enhances recovery from propofol anesthesia in humans [21,22]. This effect could be related to the central stimulatory action of physostigmine which inhibits acetylcholinesterase activity as found in case of isoflurane-induced anesthesia in horses [23].

Based on the studies of propofol effects in avian species [8,11,12] it appears that the potential chick model of

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propofol effects would be an addition to the already existing animal models for examining the pharmacological and toxicological effects of general anesthetics [13,24]. Neuropharmacological effects are vital issues to be taken into consideration in any study involving animal models used to examine anesthetic and non-anesthetic effects of general anesthetics [25]. Despite the reported central nervous system (CNS) depressant effects of propofol in the avian species [6–12], the information on the pharmacological and toxicological actions of propofol in the chicken from behavioral point of view is rather scarce [4,26]. One study reported the cardiopulmonary effects of propofol anesthesia in adult chickens preanesthetized with isoflurane [27]. More recently we reported an isobolographic analysis of combined sedative and hypnotic uses of propofol with the dissociative anesthetic ketamine and the sedative analgesic xylazine in chicks [28]. Therefore, the present study explores the toxicity and pharmacobehavioral effects of propofol in 7–10 day-old chicks. Specifically, the aim of the present study lies in two categories. First, we attempted to characterize and examine non-anesthetic and potential therapeutic actions (sedation, analgesia, anti-inflammation, hypoactivity and sleep) of propofol which would be an added information in the avian practice and the chick model of examining subanesthetic effects of the drug. Second, we examined the acute toxicity of propofol to set a benchmark of toxic response and the drug margin of safety in chicks and whether the central depressant effect can be antagonized by the acetylcholinesterase inhibitor physostigmine.

## 2. Materials and methods

### 2.1. Animals

Day old Ross broiler chicks of both sexes were purchased from a certified local hatchery and they were maintained until the age of 7–10 days when the experiments were done. The chicks were housed in a room with a temperature of 32–35 °C, constant lighting, and wood shavings as floor litter, with free access to drinking water and feed. The commercial injectable solution of propofol (10 mg/ml, Astra, Zeneca, UK) was further diluted in distilled water to obtain the concentrations needed for injection intraperitoneally (i.p.) in a volume of 10 ml/kg body weight. Chicks in control groups were injected i.p. with physiological saline solution at 10 ml/kg. All drug solutions were freshly prepared before each experiment. The Graduate Studies Committee of the College of Veterinary Medicine at the University of Mosul reviewed and approved the present study. All experiments complied with our institutional regulations addressing animal use, attention and humane care which are based on the guidelines of the National Research Council [29].

### 2.2. Determination of the median effective doses (ED50s) of propofol for the induction of sedation, analgesia and anesthesia in chicks

The up-and-down method [30] was used to determine separately the individual ED50s of propofol for the induction of sedation, analgesia and anesthesia in chicks.

The criteria for the occurrence of sedation in chicks were drooping of the head, closed eyelids, reduced motility or immotility, decreased distress calls, or recumbency [31,32]. Analgesia was assessed by the increase in pain threshold using an electric stimulator (SRI, Scientific and Research Instruments Ltd, UK) after setting the frequency at 50 Hz, the width at 5 ms and the pulse amplitude at 10 volts. The electrodes of the stimulator were gently inserted subcutaneously at an upper chest region, wetted with distilled water, under the wing. The response of the chick to pain after electric stimulation was in the form of distress calls and/or resisting with wing flapping [33]. Each chick was subjected to a minimum voltage that caused aversive pain response before the propofol injection and then 15 min after the injection. The increase or decrease in voltage that caused pain response was calculated for each group. Usually, the latency for positive analgesic response was apparent within 2 s after the electrical stimulation. The onset of anesthesia (sleep) was manifested as loss of righting reflex when the chick was gently placed on one side [34,35]. We monitored each chick within 20 min after the injection of propofol for the induction of sedation, analgesia or anesthesia. The initial dose of propofol was at 2 mg/kg for the induction of sedation and analgesia and it was 5 mg/kg for the induction of anesthesia. The choice of these doses based on preliminary experiments in chicks. The experiments were concluded using only 5 or 6 chicks/experiment within 5–6 days.

### 2.3. Determination of the median lethal dose (LD50) of propofol in chicks

We determined the acute (24-h) LD50 of propofol in chicks by the up-and-down method [30]. This experiment was done so that the relative pharmacological effects and behavioral outcome at the doses used could be compared to a standard benchmark of acute toxicity. The dosages of propofol ranged between 40 and 100 mg/kg with an initial dose at 100 mg/kg. The chicks were individually observed for signs of toxicosis for 1 h after the drug injection, and then we recorded the 24-h lethality. This experiment was concluded using only 6 chicks within 6 days.

### 2.4. Behavioral effects of propofol

Twenty seven chicks were randomly divided into three groups of nine birds each. The chicks were injected with either physiological saline solution (control) or with propofol at 0.5 and 1 mg/kg. These doses of propofol were below the ED50 of propofol for the induction of sedation and they did not produce overt signs of sedation in the chicks. The 3-min open-field activity of each chick was monitored 1 h after the propofol injection as described before [32]. The dimensions of the open-field box were 60 × 60 × 30 cm, and its arena was divided into 16 equal squares with 50 g of wheat grains scattered evenly on the surface. After the open-field activity test, each chick was subjected to tonic immobility test which is considered a kind of fear response in birds [34,36] and the duration of immobility was recorded.

### 2.5. Analgesic effect of propofol in chicks

Thirty two chicks were randomly divided into four groups of eight birds each. The chicks were injected with either physiological saline solution (control) or with propofol at 1, 2 and 4 mg/kg. The highest dose of propofol was almost two-fold of the analgesic ED50 of the drug. For each chick, we measured the minimum voltage that caused aversive pain response, as mentioned in the previous experiment, before propofol injection and then 15 min after the injection. The increase in the voltage in each group was assessed statistically to determine the analgesic response of the chicks to propofol.

Using another analgesic-anti-inflammatory protocol, 24 chicks were randomly divided into three groups of eight birds each. Pain and inflammatory responses were induced in the chicks by injecting 0.05 ml of 0.1% aqueous solution of formalin into the planter region of the right foot [37,38]. The planter of the left foot was injected with physiological saline solution (0.05 ml) as a control measure. The chicks were treated with either physiological saline solution (control) or with propofol at 2 and 4 mg/kg 15 min before the formalin injection. Immediately after the formalin injection, we recorded within 3 min the latency to lift the right foot and the frequency of lifting the right foot in response to formalin injection. Further, we determined the anti-inflammatory effect of propofol by measuring the foot thickness (mm) with an electronic digital caliper (Electronics Lab, China) before and 1 h after the formalin injection. The anti-inflammatory response (%) was calculated as follows:

$$\% \text{ anti-inflammatory response} = \left[ \frac{\text{change in foot thickness of control group} - \text{change in foot thickness of propofol group}}{\text{change in foot thickness of control group}} \right] \times 100$$

### 2.6. Propofol-induced sleep in chicks

Twenty seven chicks were randomly divided into three groups of nine birds each. The chicks were injected with propofol at 5, 10 or 20 mg/kg. The latency to onset of loss of righting reflex (sleep) and its duration after the propofol injection were recorded for each chick [34,39]. The sleep time was estimated from the time of loss of the righting reflex when the chick was placed on one side or from the

time of sternal recumbency with closed eyelids to the time of standing unaided.

### 2.7. Antagonism of propofol sleep in chicks by physostigmine

Thirty six chicks were randomly divided into four groups of nine birds each. The chicks were injected with propofol at 20 mg/kg. Immediately, after the propofol injection the chicks were treated intramuscularly (i.m.) with physostigmine (Fluka, Switzerland) at 0.05, 0.1 or 0.2 mg/kg. The doses of physostigmine were obtained from literature [40,41] and they were prepared in physiological saline solution with a volume of administration at 5 ml/kg. The duration of loss of righting reflex was recorded for each chick and the lethality in each treatment group was recorded 24 h after the propofol injection.

### 2.8. Statistics

We used the Past Statistics Package (<http://folk.uio.no/ohammer/past/index.html>) to statistically analyze the data. Parametric data as multiple means were analyzed by one way analysis of variance followed by the least significant difference test [42]. Non-parametric data were subjected to Mann–Whitney–U-test [42], whereas frequency data were analyzed by the Fisher's exact probability test [43]. The accepted level of statistical significance was at  $p < 0.05$ .

## 3. Results

The ED50 values of propofol for the induction of sedation, analgesia and anesthesia (sleep) in the chicks were 1.82, 2.21 and 5.71 mg/kg, i.p., respectively (Table 1). Chicks sedated with propofol were docile, showed head drooping, closed eyelids, immobility, reduced distress calls. The signs of sleep were characterized by recumbency and loss of the righting reflex. No death occurred during the ED50 experiments. The acute (24-h) LD50 value of propofol in chicks

**Table 1**

Median effective doses (ED50) of propofol injected intraperitoneally for induction of sedation, analgesia and sleep in 7–10 day-old chicks.

Variable	Sedation	Analgesia	Sleep
ED50 (mg/kg)	1.82	2.21	5.71
Range of the doses used (mg/kg)	2.0 – 1.75 = 0.25	2.5 – 2.0 = 0.5	7 – 5 = 2
Initial dose (mg/kg)	2	2	5
Last dose (mg/kg)	2	2.25	7
Number of chicks used	5 (XOXOX) <sup>a</sup>	6 (OXXOX) <sup>a</sup>	5 (XOOOX) <sup>a</sup>
Increase or decrease in the dose (mg/kg)	0.25	0.25	1
Latency in min to onset (minimum–maximum)	2–3	ND	2–3
Duration in min (minimum–maximum)	6–7	ND	6–9
Minimum–maximum voltage that caused pain	NA	9–13 before propofol 10–15 after propofol	NA

<sup>a</sup> X, positive response (sedation, analgesia or sleep); O, no response. NA, not applicable; ND, not determined. The ED50s were determined by the up-and-down method [30].

**Table 2**

The acute (24-h) median lethal dose (LD50) of propofol injected intraperitoneally in 7–10 day-old chicks and the calculated therapeutic indices for sedation, analgesia and sleep.

Variable	Result
LD50 (mg/kg)	57.22
Range of the doses used (mg/kg)	100 – 40 = 60
Initial dose (mg/kg)	100
Last dose (mg/kg)	40
Number of chicks used	6 (XXOXXO) <sup>a</sup>
Increase or decrease in the dose (mg/kg)	20
latency to onset of poisoning (minimum-maximum)	1–1.5
Therapeutic index	Sedation = 31.4 Analgesia = 25.9 Sleep = 10.0

X = death; O = survival. The LD50 was determined by the up-and-down method [30]. Therapeutic index = LD50/ED50. The ED50 values were obtained from Table 1.

was 57.22 mg/kg, i.p. (Table 2). Signs of poisoning appeared within less than 2 min in chicks, and they consisted of head drooping, closed eyelids, ataxia, recumbency on the sternum and tachypnea before death. The calculated therapeutic indices of propofol for the induction of sedation, analgesia and anesthesia in chicks were 31.4, 25.9 and 10, respectively (Table 2).

The 3-min open-field activities of chicks injected with a single dose of propofol at 0.5 or 1 mg/kg, i.p. are shown in Table 3. Generally, propofol dose-dependently reduced the locomotor activity of the chicks 1 h after each treatment as seen by a significant increase in the latency to move from the center of the open-field arena and decreases in the frequency of lines crossed and escape jumps in comparison with the control values (Table 3). Propofol treatments decreased the percentages of chicks moved in the open field test by 33 and 100%, respectively (Table 3). Propofol treatments also significantly and dose-dependently prolonged the durations of tonic immobility of the chicks when compared with the control value (Table 3).

Propofol at 2 and 4 mg/kg, i.p. significantly and dose-dependently caused analgesia by increasing the voltage needed to elicit pain in chicks in comparison with the control group (Table 4). Concurrently, the percentages of chicks treated with propofol at 1, 2 and 4 mg/kg, which showed analgesia were 25, 75 and 100%, respectively. Using

**Table 3**

Effects of propofol on 3-min open-field activity and tonic immobility test in chicks.

Variable	Propofol (mg/kg, intraperitoneally)		
	0 (Saline-control)	0.5	1.0
Latency to move (seconds)	27.0 ± 2.4	76.3 ± 20.9 <sup>*</sup>	–
Lines crossed	15.4 ± 2.1	2.8 ± 1.1 <sup>*</sup>	0 ± 0 <sup>*</sup>
Escape jumps	1.7 ± 0.8	0.8 ± 0.4	0 ± 0 <sup>*</sup>
Distress calls (scores)	2.8 ± 0.1	1.2 ± 0.1 <sup>†</sup>	0.7 ± 0.7 <sup>*,a</sup>
Pecking (scores)	0.7 ± 0.2	0 ± 0	0 ± 0
Defecations	1.2 ± 0.1	0.1 ± 0.1 <sup>*</sup>	0.9 ± 0.1 <sup>a</sup>
% Chicks moved	100	67	0 <sup>*,a</sup>
Duration of tonic immobility (s)	4.3 ± 0.9	23.8 ± 3.5 <sup>*</sup>	70.3 ± 17.4 <sup>*,a</sup>

Values are mean ± SE of 9 chicks/group. Each chick was subjected to open field activity test 1 h after the propofol injection, followed by the tonic immobility test.

<sup>\*</sup> Significantly different from the respective control value,  $p < 0.05$ .

<sup>a</sup> Significantly different from the respective value of the group treated with propofol at 0.5 mg/kg,  $p < 0.05$ .

**Table 4**

Analgesic effect of propofol injected intraperitoneally in chicks subjected to electric stimulation.

Propofol (mg/kg)	Increase or decrease in voltage causing pain (V) <sup>†</sup>	% Chicks showed analgesia
0 (saline-control)	–1.63 ± 0.42	0
1	–0.13 ± 0.52	25
2	1.75 ± 0.7 <sup>*</sup>	75 <sup>*</sup>
4	6.0 ± 0.71 <sup>*,ab</sup>	100 <sup>*</sup>

<sup>†</sup> Values are mean ± SE of 8 chicks/group.

<sup>\*</sup> Significantly different from the respective control value,  $p < 0.05$ .

<sup>ab</sup> Significantly different from the respective value of the groups treated with propofol at 1 and 2 mg/kg, respectively,  $p < 0.05$ .

a different analgesic and anti-inflammatory test, propofol (2 and 4 mg/kg, i.p.) also dose-dependently induced analgesia against formalin injected into the planter region of the foot of the chick. This was shown by the significant increase in the latency to lift the right foot as well as by the significant decrease in the frequency of foot lifting when compared with control values (Table 5). The anti-inflammatory response of propofol was shown by the significant reduction in foot thickness compared to the control value, with 89% positive responses in the chicks (Table 5).

Propofol at 5, 10 and 20 mg/kg, i.p. significantly and dose-dependently decreased the latency to onset of sleep in chicks (Table 6). The duration of sleep ranged between 8.4 and 25 min. The lowest dose of propofol (5 mg/kg) induced only sternal recumbency in chicks (100%), whereas the other doses (10 and 20 mg/kg) induced loss of the righting reflex in chicks by 44 and 100%, respectively (Table 6). Propofol at 5 mg/kg induced sternal recumbency with closed eyelids in chicks, whereas the other two doses caused loss of the righting reflex when the chicks were gently placed on one side.

Physostigmine at 0.05, 0.1 and 0.2 mg/kg, i.m. significantly and dose-dependently shortened the sleep time of the propofol (20 mg/kg, i.p.)-treated chicks (Table 7). However, physostigmine at 0.1 and 0.2 mg/kg, i.m. caused 78 and 100% lethality in the propofol treated chicks when examined 24 h later. No death occurred at the lowest dose of physostigmine (0.05 mg/kg, i.p.).

**Table 5**

Analgesic and anti-inflammatory effects of propofol injected intraperitoneally in chicks subjected to intraplantar formalin test.

Propofol (mg/kg)	Latency to lift right foot (s)	Frequency of right foot lifting (counts)	Increase in foot thickness (mm)	Anti-inflammatory response (%)
0 (saline-control)	1.0 ± 0	30.3 ± 1.6	0.9 ± 0.2	0
2	1.6 ± 0.9 <sup>*</sup>	23.4 ± 1.9 <sup>*</sup>	0.1 ± 0.03 <sup>*</sup>	89
4	3.4 ± 0.3 <sup>*,a</sup>	17.9 ± 1.4 <sup>*,a</sup>	0.1 ± 0.03 <sup>*</sup>	89

Values are mean ± SE of 8 chicks/group.

<sup>\*</sup> Significantly different from the respective control value,  $p < 0.05$ .<sup>a</sup> Significantly different from the respective value of the group treated with propofol at 2 mg/kg,  $p < 0.05$ .**Table 6**

Propofol-induced sleep in chicks.

Propofol (mg/kg, intraperitoneally)	Latency to sleep onset (min)	Sleep duration (min)	Type of sleep	% Sleep chicks
5	7.1 ± 0.3	8.4 ± 0.4	Sternal recumbency	100
10	4.3 ± 0.3 <sup>*</sup>	9.9 ± 0.7 <sup>*</sup>	Loss of righting reflex	44 <sup>*</sup>
20	2.3 ± 0.2 <sup>*,a</sup>	25.0 ± 1.5 <sup>*,a</sup>	Loss of righting reflex	100 <sup>a</sup>

Values are mean ± SE of 9 chicks/group. The chicks were monitored for the induction of sleep when they lost the righting reflex after placing them on one side or when they became recumbent on the sternum with closed eyelids.

<sup>\*</sup> Significantly different from the respective value of the group treated with propofol at 5 mg/kg,  $p < 0.05$ .<sup>a</sup> Significantly different from the respective value of the group treated with propofol at 10 mg/kg,  $p < 0.05$ .**Table 7**

Physostigmine reduction of propofol-induced sleep (loss of righting reflex) in chicks.

Physostigmine (mg/kg)	Sleep duration (min)	% 24-h death
0 (Saline-control)	19.6 ± 0.6	0
0.05	15.0 ± 0.5 <sup>*</sup>	0
0.1	8.6 ± 0.4 <sup>a</sup>	78 <sup>a</sup>
0.2	3.3 ± 0.3 <sup>ab</sup>	100 <sup>a</sup>

Values are mean ± SE of 9 chicks/group. Physostigmine was injected intramuscularly immediately after the intraperitoneal injection of propofol (20 mg/kg).

<sup>\*</sup> Significantly different the respective control value,  $p < 0.05$ .<sup>a,b</sup> Significantly different from the respective values of groups treated with physostigmine at 0.05 and 0.1 mg/kg, respectively,  $p < 0.05$ .

#### 4. Discussion

Propofol as an ultra short acting hypnotic was reported to induce sedation, analgesia and anesthesia in humans [2] and various animals species [3,4] including the avian species [6,8,10,12]. However, such effects have not been reported collectively in the chicken [27,28]. In the present study, we report and characterize propofol effects in young chicks. These effects included propofol sedation, analgesia and sleep quantitatively (ED50s with LD50s and therapeutic indices) as well dose response effects.

Depending on the signs seen in the propofol treated chicks, we have demonstrated the central nervous depressant action of the drug in the birds. Signs of sedation (drooping of the head, closed eyelids, immobility, or reduced motility) and sleep (recumbency or loss of the righting reflex) have been reported to occur in the avian species subjected to treatment with sedatives, hypnotics or anesthetics [31,39,44,45]. Our results further support the findings of others using propofol as an ultra short acting anesthetic agent in avian species [6,8,10,12,27,28]. Furthermore, propofol was found to be safe in the chicks depending on the calculated therapeutic indices which were high for the induction of sedation, analgesia and anesthesia in

chicks (Table 1). The induction of these effects was smooth in chicks and the recovery was also smooth and uneventful. In adult chickens, propofol was lethal when given three times the induction doses at 4.5–9.7 mg/kg, intravenously [27]. Age differences and variations in response due to dosage and route of administration are expected to contribute to the toxicity of propofol in the chicken. However, dosages vary according to animal species and the required therapeutic effect [3,4].

Further experiments we conducted demonstrated the dose-dependent effects of propofol and durations of action in producing sedation, analgesia and sleep in chicks (Tables 3–6). Such findings could be the basis of further clinical trials of propofol in this avian species. The mechanism of analgesic and anesthetic actions of propofol has been suggested to be related to modulation of the inhibitory effect of gamma-aminobutyric acid through GABA<sub>A</sub> and potentiation of the glycinergic neurotransmission in the central nervous system and the spinal cord [13,46].

We unequivocally demonstrated the analgesic effect of propofol by two different experimental protocols (electric stimulation and formalin test) suggesting the analgesic property of the drug at dosages well below the anesthetic ones in chicks (2 and 4 vs. 10 and 20 mg/kg, i.p.). In addition, the present study shows for the first time the anti-inflammatory action of propofol in the chick model of formalin test (reducing the foot edema). However, according to our results, one important limitation of the use of propofol as an anti-inflammatory agent is that it could possibly modify animal behavior at the potential effective anti-inflammatory doses (Table 3). It is possible that these doses (2 and 4 mg/kg—Table 5) have reached the maximum anti-inflammatory effect, since the attempt of the formalin test was primarily to examine the analgesic effect. Therefore, testing lower doses are warranted and further studies are needed to examine and expand this novel effect of propofol. This anti-inflammatory response

of propofol also supports the widely accepted notion that the drug exerts a wide-range of non-anesthetic actions at usually sub-anesthetic doses that could be useful therapeutically (for review see reference 13). To further support this notion, propofol at doses of 0.5 and 1 mg/kg, i.p. reduced the general locomotor activity of the chicks in the open-field activity paradigm (Table 3). These doses represent about 1/10–1/40 of the hypnotic doses of the drug. The decreases in open-field activities (delayed or absence of movement, decreased ambulation and jumping attempts) together with increased duration of tonic immobility–a fear related response further suggest the CNS depressant action of propofol at sub-anesthetic doses. Propofol at sedative/anesthetic doses (10, 20, 40 and 80 mg/kg) in Japanese quails delayed initiation of ambulation in the open-field test [47]. Such an effect would be expected because of the high dosage of propofol used. However, sedatives and anesthetics at sub-hypnotic doses were reported to exert central depressant actions, as found in the present study, on open-field locomotor activity and tonic immobility in chicks [31,32,35,48–50].

Physostigmine was attempted to be used in the present study to antagonize propofol-induced sleep in chicks, since it was reported to reverse propofol anesthesia in humans [22]. Further, preanesthetic administration of physostigmine increased the anesthetic dose of propofol in humans [21]. Physostigmine at 0.05, 0.1 and 0.2 mg/kg effectively reduced the duration of propofol sleep in the chicks by 23, 56 and 83%, respectively (Table 7). However, our findings indicate that caution should be practiced when using physostigmine in the avian species as lethality occurred at 0.1 and 0.2 mg/kg dose groups of physostigmine. This is apparently a phenomenon of drug interaction between physostigmine and propofol and its reason is not clear at present.

In conclusion, using the chick model presented here, the data suggest that propofol induces CNS depression in chicks resulting in sedation, analgesia, anti-inflammatory action and anesthesia dose dependently with wide safety margins. Physostigmine can be cautiously used to antagonize the central depressant action of propofol. Further clinical studies are needed to explore potential propofol applications in the chicken.

### Conflict of interest statement

The authors declare that they have no conflict of interests.

### Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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