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Pharmacological Challenge with Xylazine and Ketamine After Repeated the Different Doses of Arsenic in Duck Chicks

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Abstract

Arsenic trichloride ($AsCl_3$) is a highly toxic inorganic compound that adversely affects multiple organ systems, including the liver, kidneys, and brain. As a widespread environmental pollutant, arsenic poses significant health risks to both humans and animals, particularly poultry. Young birds, such as duck chicks, are especially vulnerable due to their developing physiological systems. Chronic arsenic exposure in poultry can lead to metabolic disturbances, organ damage, and impaired immune function, ultimately affecting growth, survival, and response to veterinary interventions. This study determined the median lethal dose (LD_{50}) of arsenic trichloride in duck chicks, which was established at 6.53 mg/kg. Additionally, the research evaluated the impact of arsenic exposure on anesthesia induced by xylazine (5 mg/kg) and ketamine (20 mg/kg). Arsenic exposure at doses of 4 and 8 mg/kg significantly prolonged the duration of anesthesia compared to lower doses (0 and 2 mg/kg), while the onset of anesthesia became significantly more rapid at 8 mg/kg. The percentage of birds exhibiting anesthesia also increased in a dose-dependent manner. These findings suggest that arsenic exposure alters drug metabolism and neurophysiological responses, which could complicate anesthetic management in birds. Understanding the effects of arsenic toxicity on anesthetic response is crucial for veterinary professionals working with poultry. This study emphasizes the need to consider environmental toxicant exposure when administering anesthetic agents in birds to ensure safe and effective anesthesia. Additionally, monitoring arsenic contamination in poultry farming is essential to prevent long-term health consequences, optimize veterinary anesthesia protocols, and safeguard food safety.

Keywords: Arsenic, Xylazine, Ketamine, Anesthetic Response, Duck Chicks.

Introduction

Numerous studies have examined the harmful impacts of arsenic trichloride ($AsCl_3$), an extremely poisonous inorganic chemical, on a range of biological systems(1). Arsenic is a potent environmental contaminant that can be absorbed through the skin, inhaled, or consumed(2). It is harmful to both humans and animals, particularly birds(3). And it's a serious problem in the aquatic environment(4). Its toxicological profile has attracted attention in toxicology studies, primarily because of its capacity to accumulate in important organs(5).The liver, kidneys, and brain are the main vulnerable organs to arsenic-induced poisoning, and the effects of exposure vary according to the dose, length of time, and species(6). Poultry, particularly duck chicks, are more susceptible to the adverse effects of arsenic due to their young age and growing physiological systems(7). According to research, arsenic damages several organs, including the kidneys and liver, which exhibit oxidative stress, inflammation, and cellular destruction(8). Arsenic's detrimental effects, which include hepatocellular damage, disruption of normal liver function, and altered chemical reactions, are especially dangerous for the liver, a key organ for metabolism and detoxification(9). Arsenic exposure also has a major impact on the kidneys, which are in charge of excretion and fluid balance, this can result in nephrotoxicity, renal dysfunction, and, in extreme situations, acute kidney failure(10,11). Besides these essential organs, it has been found that arsenic affects the central nervous system, causing neurotoxic effects on the brain. These consequences may result in neurological impairments, behavioral abnormalities, and in severe situations, may cause death(12). Oxidative stress, neurotransmitter imbalances, and disruption of the blood-brain barrier have been suggested to be the mechanisms behind arsenic-induced neurotoxicity(13)Understanding the toxicological effects of arsenic trichloride in poultry species like duck chicks is essential because of the substantial impact it has on these vital organs and the young age of these birds, which makes them especially susceptible to neurological impairments that can have long-lasting effects on their development(14). With an emphasis on the onset and duration of anaesthesia brought on by xylazine "Alpha 2 adrenergic agonist" and ketamine "aspartate antagonist"(15,16) , This study aims to determine the median lethal dose (LD_{50}) of arsenic trichloride in duck chicks and examine its impact on anaesthetic response.

Materials and Methods

In this study forty-six healthy local duck chicks aged between (2-3) week, and (100-200 g) body weight were used after being bought from the hatchery, the chicks were introduced to standard laboratory conditions for a week before the experiment, a commercial starting feed and free water were given to them with sanitary conditions, with well-ventilated cages with a 12-hour light/dark cycle and a managed at

temperature (28–30°C), Ketamine (5% Hameln pharmaceuticals gmbh, Germany), Xylazine 1 (2% G.L. Pharma GmbH, Austria), and Arsenic (0.1% BDH chemicals Ltd Poole England) were used. All experiments complied with our institutional regulations addressing animal use, and the chicks received proper attention and humane care. The Scientific Committee of the College of Veterinary Medicine at the University of Duhok reviewed and approved the protocol of this study and its related ethical considerations for the use of experimental animals.

Experiments

The study was divided into two phases:

1-Determination of Median Lethal Dose (LD_{50}) of oral administration of Arsenic in duck chicks

In this study, 6 healthy local duck chicks were used. The dose of arsenic was administered at (10mg/kg) of body weight by oral route, as the initial dose depended on a pilot study that was done on one animal given the same dose orally.

The final result of the Arsenic response was all alive-or-none (O for alive and X for dead) was assessed for each bird after 24 hours of administration. The increase and decrease in arsenic dose was in constant value (2mg/kg), by repeating this method up and down for dose value in different chicks, we could estimate the median lethal dose (LD_{50}) of arsenic according to (17). Using the following equation: $LD_{50} = Xf + Kd$

Where:- LD_{50} : Median lethal dose, Xf: Last dose used , K: From Dixon table and d: Constant dose range (up and down dose).

2-Pharmacological Challenge of Xylazine and Ketamine After Arsenic Exposure in Duck Chicks

Forty duck chicks were randomly assigned to four groups, each containing 10 chicks, the dosage of Arsenic was determined based on the previous experiment. The dose of Xylazine (alpha-2 adrenergic agonist) and ketamine (NMDA receptor antagonist) were 5 , 20 mg/kg i.m respectively,(18,19).

Group I (Control): Administered distilled water (5 ml/kg) orally. Group II: Administered arsenic (2 mg/kg body weight) orally. Group III: Administered arsenic (4 mg/kg body weight) orally. Group IV: Administered arsenic (8 mg/kg body weight) orally. Each group received the respective dose of arsenic to assess the impact of exposure on anesthetic response. All groups were administered Xylazine 5 mg/kg, and Ketamine 20 mg/kg, intramuscularly into the thigh muscles two hours after receiving arsenic to determine the challenging effects of arsenic in the onset (loss of righting reflex means; time of sleeping) and duration effects (length of sleep) individually, of the anesthesia status.

Statistical Analysis

The data were analyzed using IBM SPSS statistics (Version 27). One-way ANOVA was used to compare the effects of arsenic on the health parameters and anaesthetic response in duck chicks. Post-hoc tests were performed using Tukey's HSD to determine significant differences between groups.

Results were expressed as mean ± standard error (S.E), and a p-value of less than 0.05 was considered statistically significant.

Results

Experiment 1: - Determination of Median Lethal Dose (LD_{50}) of Arsenic

The median lethal dose (LD_{50}) of arsenic administered via oral gavage in duck chicks was determined using the up-and-down dosing method. The dose that resulted in 50% mortality of the chicks was found to be 6.53 mg\kg of body weight. Toxicological signs were observed incoordination, tremors, wing drooping, shortness of breath, gasping due to fluid accumulation in the respiratory tract, sternal recumbency, excessive salivation, lacrimation, and eventually death (Table:1).

Experiment2: Pharmacological Challenge with Xylazine and Ketamine After Arsenic Exposure in Chick Ducks

The injection of Xylazine at a dose of 5 mg/kg of body weight into the thigh muscle and ketamine at a dose of 20 mg/kg of body weight into the opposite thigh muscle in chicks two hours previously treated with different arsenic doses at 0, 2 , 4 and 8 mg\kg. resulted in a significant decrease in the onset of sleep compared to the control group. Additionally, a significant increase in the duration of sleep was observed compared to the control group.as dose depended. Table 2.

Discussion

Arsenic is considered an environmental contaminant with widespread distribution worldwide Arsenic is considered an environmental contaminant with widespread distribution worldwide(20). It is known to cause cancer, dermatitis, liver damage, and disorders of the nervous and cardiovascular systems in humans(21).

In animals, arsenic exposure leads to gastrointestinal disturbances in addition to its effects on the nervous system, liver, and kidneys. Numerous studies have utilized laboratory animals, particularly rodents, as models for acute and chronic arsenic poisoning(22,23).

This study, duck chicks were used as a model for arsenic toxicity to investigate challenging effects following arsenic exposure, due to the limited number of studies available in this field. In the first experiment of this study, we determined the median lethal dose (LD_{50}) of arsenic in duck chicks following oral gavage, which was found to be 6.53 mg/kg of body weight., this result is close to the approximate lethal dose of 5 mg/kg of body weight that we observed in broiler chicks(24)Based on these dose estimates, we used similar or lower doses of arsenic in the next experiment to avoid severe toxicity symptoms or significant mortality within the first two hours post-administration, allowing us to study bird behaviour and collect the necessary measurements.

The pharmacological challenge is one of the methods used to investigate and reveal latent or hidden effects of drugs and toxins on the neurological and behavioral functions of

animals, this challenge induces additional unexpected stress on the nervous system, thereby highlighting functional impairments(25,26). This study, the pharmacological challenge of xylazine and ketamine, which are commonly used as an anaesthetic combination in mammals and poultry(27).

As well as playing an additional role in demonstrating the depressant effects of arsenic dose, the anaesthetic response in duck chicks was markedly changed by arsenic exposure, most likely due to its harmful effects on important physiological systems (28). It's believed that hepatic impairment decreased xylazine and ketamine metabolism, resulting in prolonged anaesthesia(29).

As well as by interfering with enzyme function, arsenic-induced liver damage lowers medication clearance and increases anaesthetic retention(30). Another factor that might have contributed to the prolonged drug elimination was renal impairment. Since anaesthetic metabolites accumulate due to arsenic's nephrotoxic effects, kidney filtration is compromised, resulting in protracted drowsiness(31).

A possible explanation for the quick onset of anesthesia is neurotoxicity, Arsenic increases neuronal susceptibility to anaesthetic drugs by interfering with neurotransmitter balance, causing oxidative stress, and disrupting the blood-brain barrier(32). These results demonstrate the necessity of cautious anaesthetic management in birds exposed to arsenic, as well as the effects of arsenic poisoning on drug metabolism and central nervous system function.

Conclusions

This study determined the median lethal dose (LD_{50}) of arsenic trichloride in duck chicks to be 6.53 mg/kg. Exposure to arsenic doses of 4 and 8 mg/kg significantly affected anesthetic responses, resulting in quicker onset and extended anesthesia duration. These findings suggest that arsenic not only impacts survival rates but also interferes with anesthetic management, emphasizing the importance of adjusting anesthesia protocols in arsenic-exposed poultry.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

Table 1: Median lethal dose (LD_{50}) of arsenic in duck chicks

Measure	Result
LD_{50}	6.53 mg/kg
Dose range	10–6 mg/kg
Initial dose	10 mg/kg
Last dose	8 mg/kg
Up-and-down increment	2 mg/kg
Number of chicks	6 (XX0X0X)
Onset of toxic effects	8–20 minutes
Signs of poisoning	Incoordination, tremors, gasping, Salivation, lacrimation, death

O represent for alive, X for dead

Table2: Pharmacological interactions for Xylazine and Ketamine After Arsenic Exposure in Chick Ducks.

Arsenic dose (mg\kg)	Onset of sleeping (minute)	Duration of sleeping (minute)	Percentage increase sleeping time %
0	3.69±0.15	26.4±1.38	----------
2	2.85±0.18	30.0±1.29	14
4	2.14±0.11	37.1±1.29*a	30
8	1.08±0.18 *	42.6±1.62*ab	39

The values represent the mean ± SE for (10) chicks \ group.

(*) Significant difference vs. control ($p \leq 0.05$)
(a) Significant difference vs. 2 mg/kg group ($p \leq 0.05$)
(b) Significant difference vs. 4 mg/kg group ($p \leq 0.05$)

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لتحدي الدوائي للزايلازين مع الكيتامين عند استخدام جرع متعدده من الزرنيخ في افراخ البط

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الخلاصة

يعتبر مركب ثلاثي كلوريد الزرنيخ ($AsCl_3$)من المركبات الغير عضوية شديدة السمية حسث يؤثر سلبًا على العديد من الأجهزة الحيوية في الجسم، بما في ذلك الكبد والكلى والدماغ. يُعد الزرنيخ من الملوثات البيئية واسعة الانتشار، مما يشكل مخاطر صحية كبيرة على البشر والحيوانات على حد سواء ، وخاصة الدواجن. وتكون الطيور الصغيرة، مثل أفراخ البط، أكثر عرضة للتأثر بسبب أنظمتها الفسيولوجية غير المكتملة التطور. يمكن أن يؤدي التعرض المزمن للزرنيخ في الدواجن إلى اضطرابات استقلابية، وتلف في اعضاء الجسم ، وضعف الوظائف المناعية، مما يؤثر سلبًا على النمو، والبقاء على قيد الحياة، والاستجابة للتدخلات البيطرية. حددت هذه الدراسة الجرعة القاتلة الوسطية لثلاثي كلوريد الزرنيخ في أفراخ البط، حيث بلغت 6.53 ملغ/كغ. بالإضافة إلى ذلك، قامت الدراسة بتقييم تأثير التعرض للزرنيخ على التخدير المستحث بالزيلازين (5 ملغ/كغم) والكيتامين (20 ملغ/كغم). حيث لوحظ أن التعرض للزرنيخ بجرعات 4 و8 ملغ/كغ أدى إلى إطالة مدة التخدير بشكل ملحوظ ومعنوي مقارنة بالجرعات المنخفضة (0 و2 ملغ/كغم)، في حين أصبح بدء التخدير أسرع بشكل ملحوظ ومعنوي عند جرعة 8 ملغ/كغم وبطريقة تعتمد على الجرعة. تشير هذه النتائج إلى أن التعرض للزرنيخ يؤثرعلى استقلاب الأدوية والاستجابات العصبية الفسيولوجية، مما قد يؤثر على عمل التخدير.واظهرت الدراسه بان التداخل الدوائي لاستخدام الزيلازين والكيتامين في افراخ البط والتي تعرضت بشكل متكرر للزرنيخ يمثل سيناريو معقدًا يتطلب دراسة دقيقة للتفاعلات المحتملة والتأثيرات السمية وقد تؤدي الى التغيرات في الحركية الدوائية والديناميكية الدوائية الناتجة عن التعرض للزرنيخ إلى استجابات غير متوقعة لهذه الأدوية، مما يستدعي مراقبة دقيقة اثناء وبعد التخدير.

لكلمات المفتاحية: الزرنيخ, الزايلازين,الكيتامين, التداخلات الدوائية,افراخ البط