Neurobehavioral and Biochemical Toxicity of Atrazine in Chicks

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Abstract. *The aim of the study was to examine the neurobehavioral and biochemical toxicity of atrazine herbicides in chicks. This is a model of poisoning we explored recently to further elucidate the toxic action of atrazine. The acute oral LD50 of atrazine was determined by the Dixon method; acute toxic symptoms of atrazine were recorded. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (liver enzymes), as well as blood glucose, were determined, and the effects of atrazine on open field activity and body weight were also examined. Atrazine's LD_{50} was 1435.25 mg/kg when given orally. The oral administration of atrazine at doses of 1300 mg/kg and 1000 mg/kg to chicks resulted in the appearance of acute poisoning symptoms such as depression, dyspnea, frequent defecation, lacrimation, ataxia (which appeared in both treatments at 100%), as well as profuse salivation, nasal discharge followed by tremors, convulsions, recumbency, and death (which appeared in the first treatment at a percentage higher than the second treatment). The oral dosing of atrazine at 574 mg/kg and 861 mg/kg caused a significant increase in AST, ALT, and glucose levels in the plasma of treated chicks after 24 hours compared with the control group and with treated groups after 4 hours. The oral administration of atrazine at doses 71.75 mg/kg, 143.5 mg/kg, and 287 mg/kg, twice a week for 2 weeks, led to an increase in motor activity through the significant increase in squares crossed and jumping frequency, and decrease in the bodyweight of chick. Our results conclude that atrazine has toxic effects on chicks through its effects on liver enzymes, body weight, and neurobehavioral activity.*

Introduction

The atrazine herbicide is commonly used to control broadleaf and grass weeds in farming around the world because of its low cost and effectiveness in agricultural crops protection (Konstantinou et al., 2006; Adams, 2017). Because of its ability to accumulate in the environment, detection of atrazine in both communities and humans has been recorded (Foradori et al., 2011; Campos-Pereira et al., 2012). Atrazine is stored in many organs and tissues in case of chronic exposure (Foradori et al., 2013; Liu et al., 2017). Several studies have shown that atrazine had adverse effects on animals and humans (Li et al., 2014; 2015; Ma et al., 2015). An increase in the number of workers affected by Parkinson's disease has been recorded in areas exposed to atrazine contamination (Priyadarshi et al., 2000; Brown et al., 2005). Atrazine causes neurotoxic effects by alteration in the neurochemical transmitters (Coban & Filipov, 2007), as well as disturbance function of hypothalamic and pituitary-ovarian (Cooper et al., 2000). Atrazine causes a decrease in the level of dopamine suggesting atrazine-induced neurodegenerative disorder (Rodriguez et al., 2013). It is metabolized by CYP450s and excreted in urine and feces (Dutheil et al., 2008). Atrazine has an elimination half-life of about 31 hours in humans (Campbell et al., 2016). In mammals, atrazine causes endocrine imbalance, such as increased corticosterone levels in rats (Good, 1961; Laws et al., 2009), leading to a reduction in the level of luteinizing hormones in rats (Foradori et al., 2011). Atrazine causes immunotoxicity through oxidative stress and calcium hemostasis imbalance (Gao et al., 2016).

The central nervous system is one of the main target sites of atrazine, in addition to the atrazine effects on the reproductive function (Giusi et al., 2006; Foradori et al., 2009). Also, in rodents, atrazine has toxic effects on developmental and immune system activity as well as causes Parkinson disease-like symptoms (Stoker et al., 2000; Rowe et al., 2007). In addition, several studies have reported changes in neurobehavioral and sensorimotor functions in mice (Belloni et al., 2007).

Previous findings of rodents have shown that atrazine causes alteration in brain dopamine, and serotonin balance and possible change the metabolism of tyrosine and tryptophan (Coban & Filipov, 2007; Rodr´ıguez et al., 2013). Atrazine exposure has also been linked to the progression of neurological diseases, including Parkinson's disease (Ascherio et al., 2006). The United States Environmental Protection Agency has classified atrazine as endocrine-disrupting herbicides (Morales-Pe´rez et al., 2016). Atrazine has different toxic effects on plants, animals, and humans (Simranjeet et al., 2018). The mechanism of action of

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atrazine in plants is carried out through impairing the process of photosynthesis (Simranjeet et al., 2018). Young chicks have been used as a model to assess the toxicity of xenobiotics (Al-Zubaidy & Mohammed 2013; Alrawe & Alzubaidy, 2022). Due to the limited information on the toxicity of atrazine in chicks, the aim of the present study was to examine the acute toxicity of atrazine in chicks through investigating the effects on the central nervous system and liver function, after determining the median lethal dose.

Materials and Methods

Animals

The broiler chicks (type Ross) were treated between the ages of 10 and 14 days, with a body weight (B.Wt) of 125–295 g. They were placed in the animal house at laboratory conditions with a 12/12-hour light-dark cycle at a temperature of 35°C and provided with water and food ad libitum. All of the procedures were carried out in accordance with institutional guidelines for animal use in biomedical research. The protocol of this study was evaluated and approved by the Scientific Committee of the College of Veterinary Medicine, University of Mosul, Iraq, as of 27-11-2019.

Dose preparation

Different concentrations of a commercial wettable powder of the insecticide atrazine (50% Lizz Agrochem, China) were dissolved in freshly made distilled water each day before dosing, with a volume of administration of 10 mL/kg of body weight given orally via a gavage needle.

1. Determination of the oral median lethal dose (LD_{50}) of atrazine in chicks by Dixon method.

Seven chicks weighing between 125 g and 153 g were used, and atrazine was dosed orally at 750 mg/kg based on previous studies and trials; the result was read as death (X) or survival (O) after 24 hours, and the quantity of the dose increased or decreased at a constant rate of 250 mg/kg according to the Dixon method (Dixon, 1980). The following equation was used to calculate the LD_{50} value:

LD50 =xf +Kd

where xf – last dose, K – tabular value at a variance of 0.56, d – the values of a dose increase or decrease.

2. The acute toxic effects of atrazine in chicks

Twenty chicks were tested, with body weights ranging from 170 g to 295 g. Groups of chicks were orally dosed, and acute toxic symptoms of atrazine were recorded for 4 hours following oral dosage.

In this experiment, the chicks were divided into two groups of 10 birds each and treated with 90% and 70% of the LD_{50} of atrazine. The first group was given 1300 mg/kg body weight (B.W.) of atrazine orally. The second group was administered 1000 mg/kg B.W. orally.

3. The effects of different toxic doses of atrazine at different times on levels of glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in plasma of treated chicks.

We used 42 chicks at the age of 14 days. Their body weight ranged within 121–205 g, and they were dosed orally by using a gavage needle with different doses as follows.

a) The effects of 40% of LD_{50} of atrazine after 4 and 24 hours of treatment on glucose, AST, and ALT levels

Chicks were divided into three groups, 7 chicks each. The first group (control) was dosed with distilled water, the second group was dosed with atrazine at 574 mg/kg B.W. and killed after 4 hours, and the third group was dosed with the same dose but killed after 24 hours.

b) The effects of 60% of LD_{50} of atrazine after 4 and 24 hours of treatment on glucose, AST, and ALT levels:

Chicks were divided into 3 groups of 7 birds each. The first group (control) was dosed with distilled water; the second group was dosed with atrazine at 861 mg/kg B.W. and killed after 4 hours, and the third group was dosed with the same dose and killed after 24 hours.

Glucose, AST, and ALT were measured by using specific kits (Biolabo, France) with a colorimetric method by using a spectrophotometer for absorption measuring and then make calculations by using specific equations to find concentrations.

4. Neurobehavioral effects of many low doses of atrazine in chicks

We used 28 chicks 7 days aged; their body weight ranged within 93–135 g; they were treated orally twice a week for 2 weeks. Chicks were divided into 4 groups, 7 chick each. The first group (control) was dosed with distilled water, the second group was dosed with atrazine at 71.75 mg/kg B.W. (5%) LD_{50}, the third group was dosed with atrazine at 143 mg/kg B.W. (10%) LD_{50}, and the last group was dosed with atrazine at 28 7mg/kg B.W. (20%) LD_{50}. When 2 weeks of treatments ended, all chicks were subjected to neurobehavioral tests.

Chicks were subjected to the open-field activity test by using a 60 x 60 x 30 cm box. The arena of the box was divided into 16 equal squares, and each chick was placed in the middle of the arena to record the latency time (time spent moving from the center of arena), the number of squares crossed, jumping, defecation, as well as watching pecking and vocalization scores within 3 minutes (Al-Zubaidy & Mohammed, 2013; Al-Zubaidy, 2021; Alrawe & Alzubaidy, 2022) .

The tonic immobility response test measures the sensorimotor activity; after completing the open-field activity test, the same chick was subjected to tonic immobility response test (Hennig et al., 1984).

Statistical Analysis

The parametric data were statistically analyzed using the one-way analysis of variance (ANOVA)

test, followed by the least significant difference test using the SPSS (version 16). The nonparametric data were statistically examined using the Mann-Whitney U test, with a significance level of $p < 0.05$.

Results

1. Determination of the oral median lethal dose LD_{50} of atrazine in chicks by the Dixon method

Atrazine's oral LD_{50} was 1435.25 mg/kg body weight, resulting in toxic symptoms which included depression, dyspnea, salivation, lacrimation, defecation, recumbency, tremors, convulsions, and death at high toxic doses (Table 1).

The following equation was used to calculate the LD_{50} value:

LD50 =xf +Kd

where xf – last dose; K – tabular value at a variance of 0.56; d – the value of a dose increase or decrease.

2. Acute toxic effects of high doses of atrazine in chicks

In chicks, oral administration of atrazine at doses of 1300 mg/kg and 1000 mg/kg B.W. resulted in the appearance of acute poisoning symptoms such as depression, dyspnea, defecation, lacrimation, ataxia (which appeared in both treatments at 100%), profuse salivation, nasal discharge accompanied by tremors, convulsions, recumbency, and death (which appeared in the first treatment; Table 2).

3. The effects of different toxic doses of atrazine with different times on levels of glucose, aspartate aminotransferase and alanine aminotransferase in plasma of treated chicks

Table 1. Atrazine LD_{50} and toxic symptoms in chicks

Measurements	Result
Atrazine LD50	1435.25 mg/kg orally
Doses range	750–1500 mg/kg
First dose	750 mg/kg
Last dose	1250 mg/kg
Up and down dose	250 mg/kg
No. of chicks	7 (OOOXOXO)
Onset of toxic symptoms	29–40 minutes

O: a chick still alive during 24 hours, X: a chick dead during 24 hours.

a) The effects of 40% of LD_{50} of atrazine after 4 and 24 hours of treatment on glucose, AST, and ALT levels

The oral dosing of atrazine at 574 mg/kg causes a significant increase in AST, ALT, and glucose levels in the plasma of treated chicks after 24 hours compared with the control group and with a treated group at 574 mg/kg after 4 hours (Table 3).

b) The effects of 60% of LD_{50} (861 mg/kg) of atrazine after 4 and 24 hours of treatment on glucose, AST, and ALT levels

The oral dosing of atrazine at 861 mg/kg after 24 hours causes a significant increase in AST, ALT, and glucose levels in the plasma compared with the control group, and with the treated group at 861 mg/kg after 4 hours (Table 4).

4. Neurobehavioral effects of low doses of atrazine in chicks

After the end of the second week of the treatment, chicks were subjected to neurobehavioral tests and behavior changes were measured, which were represented by a significant prolongation in time spent by the chick to move from the center square

Table 2. Acute toxic symptoms induced by atrazine doses of 1300 mg/kg and 1000 mg/kg PO in chicks

Symptoms of toxicosis	1300 mg/kg %	1000 mg/kg %
Depression	100%	100%
Dyspnea	100%	100%
Ataxia	100%	100%
Defecation	100%	100%
Lacrimation	100%	100%
Salivation and nasal discharge	60%	20% *
Tremor	40%	10% *
Convulsions	40%	10% *
Recumbence	40%	10% *
Dead after 24 hours	40%	10% *
Onset of symptoms time	31.8 ± 0.71 minutes	36.8 ± 0.67 minutes*

Values are mean ± SE for 10 chicks /group.
* The value significantly different from the group treated with 1000 mg/kg atrazine at $p < 0.05$.

Table 3 Plasma levels of AST, ALT, and glucose of atrazine at a dose of 574 after 4 h and 24 h

Treatments	AST activity IU/L	ALT activity IU/L	Glucose level mg/dL
Control group (distilled water)	106.55 ± 1.7	78.98 ± 3.16	163.12 ± 4.6
Atrazine at a dose of 574 mg/kg after 4 h	137.14 ± 2.02 *a	123.88 ± 5.38 *a	222.95 ± 4.75 *a
Atrazine at a dose of 574 mg/kg after 24 h	143.57 ± 2.55*	155.29 ± 3.38 *	304.76 ± 5.12*

Values are mean ± SE for 7 chicks /group.
* The value significantly different from the control group at $p < 0.05$.
a the value is significantly different compared with the same dose with 24 h at $p < 0.05$.

Table 4. Plasma levels of AST, ALT, and glucose of atrazine at a dose of 862 mg/kg after 4 h and 24 h

Treatment	AST activity IU/L	ALT activity IU/L	Glucose level Mg/dL
Control group (distilled water)	108.79 ± 1.58	78.98 ± 3.16	164.88 ± 3.5
Atrazine at a dose of 861 mg/kg after 4 h	150.38 ± 1.59*	183.53 ± 3.40 *	263.12 ± 4.72 *
Atrazine at a dose of 861 mg/kg after 24 h	177.17 ± 5.03* a	237.48 ± 11.39 * a	371.25 ± 12.88 * a

Values are mean ± SE for 7 chicks /group.
* The value significantly different from the control group at $p < 0.05$.
a – the value significantly different compared with the same dose with 4 h at $p < 0.05$.

Table 5. Neurobehavioral measures of 71.75 mg/kg, 143 mg/kg and 287 mg/kg of atrazine

Variables	Treatments			
	Control	71.75 mg/kg	143 mg/kg	287 mg/kg
Latency (seconds)	5.71 ± 0.77	19.85 ± 2.34*	34.71 ± 2.46*[a]	38.29 ± 2.31*[a]
Squares crossed / 3min	15.0 ± 0.92	17.0 ± 1.35	22.42 ± 2.44*[a]	27.0 ± 1.96*[a]
Jumping times / 3 min	0.28 ± 0.18	0.57 ± 0.29	1.57 ± 0.61*	0.85 ± 0.34
Pecking scores / 3 min	2.00 ± 0.3	1.5 ± 0.22*	1.28 ± 0.18	1.57 ± 0.42*
Vocal scores / 3 min	1.42 ± 0.2	1.85 ± 0.34	2.0 ± 0.37[a]	1.71 ± 0.35[ab]
Defecation / 3 min	1.29 ± 0.28	0.57 ± 0.29	0.71 ± 0.28	0.29 ± 0.18*
Tonic immobility (seconds)	4.0 ± 0.43	14.57 ± 1.63*	32.85 ± 2.66*[a]	51.57 ± 3.94*[ab]
Chick weight at end of the experiment	469.6 ± 27.2	350.0 ± 26.9*	326.0 ± 12.7*	335.5 ± 12.9*

Values are mean ± SE for 7 chicks /group.
* The value significantly different from the control group at $p < 0.05$.
a – the value significantly different from the group treated with 5% of LD_{50}.
b – the value significantly different from the group treated with 10% of LD_{50}.

of the arena (latency) in comparison with the control group and with other groups treated with atrazine at 71.75 mg/kg, 143 mg/kg and 287 mg/kg. The open-field activity showed a significant increase in squares crossed compared with the control group and with 5% of the LD_{50} treated group. The jumping time shows a significant increase for the group treated with 10% of LD_{50} in comparison with the control group. The defecation time decreased only in 20% of the LD_{50} treated group compared with the control group. The tonic immobility time was significantly prolonged in comparison with the control group, and with 5% and 10% of the LD_{50} treated groups. The chick weight was decreased significantly in comparison with the control group.

Discussion

Atrazine is one of the widely used herbicides in Iraq and many countries of the world. Due to the lack of behavioral toxicological assessment of atrazine toxicity in poultry, our current study revealed the neurobehavioral and biochemical toxicity in chicks. The median lethal dose of atrazine was 1435.25 mg/kg orally with the appearance of toxic symptoms, which were represented by depression, dyspnea, salivation, lacrimation, defecation, tremors, convulsions, recumbency and eventually death at a high toxic dose. Based on our current results, atrazine is a moderately toxic herbicide, which is in agreement with the guidelines of British Columbia Ministry of Agriculture (2017).

The significantly higher liver marker (ALT and AST) activities in chicks dosed with atrazine at 571 mg/kg and 861 mg/kg orally are attributed to the leaking of AST enzymes from injured liver cells when compared with the control group. These findings are consistent with those of other investigations (Campos-Pereira et al., 2012; Fowler et al., 2012). Because ALT is found mostly in the cytosol of the liver and in modest amounts in other tissues, it is regarded to be more selective for hepatic damage (Zilva et al., 1988). The increase in ALT in the blood plasma of the current investigation was explicitly ascribed to the harmful effect of atrazine on liver cells (Campos-Pereira et al., 2012; Fowler et al., 2012). The increased serum AST is thought to be caused by mitochondrial dysfunction. Atrazine may induce damage by the reactive oxygen species (Grasiela et al., 2012). Our research with chicks given 574 mg/kg and 861 mg/kg of atrazine orally after 4 and 24 hours showed a significant increase in glucose level plasma. The hepatotoxic action of atrazine, which inhibits the activity of important glyconeogenesis enzymes such as hexokinase, glycogen synthase, and glucokinase, could be responsible for this effect (Curic et al., 1999; Gluzczak et al., 2006). The decrease in the body weight

of the chicks in our study is in accordance with other findings (Gluzczak et al., 2006; Dinesh et al.,2014) on fish and mice exposed to ATZ. They noticed a decrease in glycogen and an increase in lipids of the liver (Gluzczak et al., 2006; Dinesh et al., 2014). Current findings provide evidence that exposure to low doses of atrazine in chicks can cause behavioral alteration, which is represented by a significant delay in the latency to move in the open–field arena, which could be attributed to the effect of the toxicant on the curiosity of the chick to move in the novel environment, however, the chicks manifested hyperactivity which could have been resulted from a direct effect of toxicants on the CNS (Belloni et al., 2007; 2011). The disturbances in the neurobehavioral activity of atrazine–treated chicks could be due to the ability of atrazine to induce neurochemical changes in the brain regions (Giusi et al., 2006; Walters, 2014).

Conclusion

This study used chicks as a model of poisoning that we explored recently to further elucidate the acute toxic action of atrazine. Our results show that atrazine had toxic effects in a chick model represented by LD_{50}, liver enzyme activity, neurobehavioral test, and body weight. Further studies are needed to explain the precise neurotoxic mechanism of atrazine.

Conflict of interest

There are no conflicts of interest declared by the author.

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