RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.2.510-515

Determination of the median lethal dose of indoxacarb pesticide in Wistar rat#

Archana Raj[1], N. Suresh Nair[2*], A.R. Nisha[3], S. Sujith[4], C. Divya[5] and R. Shankar[1]
Department of Veterinary Pharmacology and Toxicology
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651
Kerala Veterinary and Animal Sciences University
Kerala, India

Citation: Raj, A., Nair, N.S., Nisha, A.R., Sujith, S., Divya C. and Shankar R. Determination of the median lethal dose of indoxacarb pesticide in Wistar rat. *J. Vet. Anim. Sci.* **54**(2):510-515
DOI: https://doi.org/10.51966/jvas.2023.54.2.510-515

Received: 14.11.2022 *Accepted: 13.02.2023* *Published: 30.06.2023*

Abstract

Indoxacarb is a novel organophosphate substitute and is considered a "lower risk" pesticide in agriculture. However, in humans and animals, indoxacarb exposure had been linked to substantial health problems. This study was carried out to investigate the acute oral toxicity and histopathological changes of indoxacarb in female Wistar albino rats. The acute oral toxicity of indoxacarb was performed as per the OECD guidelines No. 425, (Acute Oral Toxicity - Up-and-down procedure). The LD_{50} of indoxacarb was estimated using AOT425StatPgm (version: 1.0) statistical program and found to be 1098 mg/kg. Hence the pesticide is categorized as Type II class of pesticide according to the WHO standards. All the animals dosed at 2000 mg/kg died within 12 h exhibiting clinical signs of intermittent episodes of tremors, incoordination, respiratory distress and open-mouth breathing. Histopathological examination of indoxacarb treated rats at 2000mg/kg degeneration of hepatocytes and necrosis in haematoxylin and eosin staining.

Keywords: *Indoxacarb, oral LD_{50}, wistar rats, acute oral toxicity, hepatotoxicity*

Since ancient times, mankind has employed various chemicals such as sulphur, mercury, copper, and plant extract to kill insects and other unwanted pests. After the Second World War, the use of pesticides became popular with the advent of different classes of pesticides into the public domain. Pesticides have enhanced human and animal health by eliminating vector-borne diseases; yet, their long-term and indiscriminate usage have resulted in major health impacts (Lewis *et al*., 2016).

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

1. *MVSc Scholar*
2. *Associate Professor*
3. *Associate Professor and Head*
4. *Assistant Professor*
5. *Assistant Professor, Department of Veterinary Pathology*

**Corresponding author: suresh@kvasu.ac.in, Ph. 9446919146*

Indoxacarb is a novel organophosphate substitute and is considered a "lower risk" pesticide in the industry. However, in humans and animals, indoxacarb exposure had been linked to substantial health problems. In mammals, indoxacarb is metabolised by cytochrome P450 1A1 (CYP 1A1) converting to toxic metabolites that generate intracellular reactive oxygen species (ROS) and induce a state of oxidative stress (Silver *et al*., 2010; Mabrouk *et al.,* 2016). Several studies on mammalian toxicity were published since this pesticide was introduced into the market. The present study was undertaken to assess the acute toxicity of indoxacarb in female Wistar albino rats and to establish the median lethal dose (LD_{50}) of indoxacarb.

Materials and methods

Technical grade indoxacarb was procured from M/s Urban crop science, India.

Animals and experimental design

Nine numbers of female Wistar albino rats weighing 150 - 200g were procured from Small Animal Breeding Station, Mannuthy. All the animals were kept in well-ventilated polypropylene cages under standard laboratory conditions of 12 h/12 h light/dark cycle at 25°C ± 2°C with 30-70 per cent relative humidity and were acclimatized for 14 days prior to experimentation. They were given standard rat feed procured from School of Animal Nutrition and Feed Technology, College of Veterinary and Animal Sciences, Mannuthy and drinking water was provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy (No. IAEC/CVASMTY 22/18 dated 29/06/2022) and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

Acute oral toxicity study of indoxacarb

The acute oral toxicity study of indoxacarb was performed as per the Organisation for Economic Co-operation Development (OECD) guidelines No. 425, Acute Oral Toxicity - Up-and-down procedure for the main test. The test compound, indoxacarb was solubilized in one percent carboxy methyl cellulose (CMC) and administered orally in a sequential manner, at an interval of 48 hours. Animals were fasted overnight prior to the day of dosing. On the next day, the fasting body weight of the animal was recorded and the dose was calculated according to the body weight. Later the rats were dosed orally through oral feeding needles. All the rats were observed for mortality and clinical signs, such as behavioural patterns and changes in the various body systems like skin, mucous membrane, respiratory, cardiovascular, central nervous system and somatomotor functions. The mortality and clinical signs were observed for the first 10 min, 30 min, 1 h, 2 h, 4 h, and 6 h after dosing and thereafter twice daily for mortality and once a day for clinical signs.

The study was conducted in nine female Wistar albino rats by dosing them sequentially at 175, 550 and 2000 mg/kg with an interval of 48 h (sigma: 0.5 mg/kg) . Three rats were taken as control animals for the statistical comparison of relative organ weight with the indoxacarb treated animals and administered with the vehicle (1% CMC) alone. The test was stopped when the LD_{50} determination criterion as per AOT425 was met. The body weight, feed intake and water intake of the rats were recorded on day 0 (after overnight fasting) and daily thereafter. After the observation period of 14 days, all surviving animals were euthanized and subjected to a detailed gross pathology examination. The organs such as the brain, heart, liver, spleen, kidney, lung, uterus and ovary were collected and the weight of organs was taken for the estimation of relative organ weight. The histopathological examination of representative samples of the above organs was conducted at the end of the experiment.

Statistical analysis

LD_{50} was estimated by AOT425StatPgm (version: 1.0) statistical program. All the results were expressed as Mean ± SEM with 'n' as the number of replicates. The statistical analysis was performed by Graphpad Prism software version 5.01. The data were analyzed using two-way analysis of variance (ANOVA) followed by

Bonferroni post hoc test. Statistical significance was set at $p<0.05$.

Results and discussion

All the animals dosed at 2000 mg/kg died within 12 h exhibiting clinical signs of intermittent episodes of tremors, incoordination, respiratory distress and open-mouth breathing. The behaviour of animals treated with 550 and 175 mg/kg of indoxacarb was apparently normal. Body weight, feed and water intake of the surviving rats were recorded for 14 days. There was no significant variation in mean weekly body weight gain, feed and water intake between the control group and the treated group at the dose of 550 mg/kg of indoxacarb.

All the animals were subjected to detailed necropsy at the time of death in 2000 mg/kg treated animals and at the end of the study in surviving animals. The weight of different organs was taken during the necropsy and relative organ weights were estimated. No significant variations were observed in the relative organ weight of different organs except in the liver and lungs. There was a significant decrease in the relative weight of the liver in the group treated with 2000 mg/kg compared to the control group while there was a significant increase in the weight of lungs in the indoxacarb-treated group at the dose of 2000 mg/kg.

The preliminary findings of the acute oral toxicity study of indoxacarb in female Wistar albino rats were the appearance of neurotoxic signs such as intermittent episodes of tremors, head tilt, staggering gait and incoordination. Gross observable symptoms started between 60-90 min in rats following the administration of indoxacarb at the dose of 2000 mg/kg. These findings were similar to the previous reports of Shit *et al.* (2008), where toxic signs such as staggering gait; motor incoordination and prostrations were observed in rats between 40-60 min after the administration of indoxacarb. The neurotoxicity of indoxacarb was reported in a self-poisoning case of a human where seizures developed in the initial stage, followed by paralysis on the eighth day of poisoning (Viswanathan *et al*., 2013).

Table 1. Mean weekly feed intake of control and indoxacarb treated group @ 550 mg/kg

Dose (mg/kg)	Mean weekly feed intake (g)	
	Week 1	Week 2
550	10.798 ± 2.414[a]	12.47 ± 2.613
Vehicle	12.086 ± 1.086[a]	12.214 ± 1.545

Values are expressed as Mean ± SEM; n = 3.

Values bearing different superscripts vary significantly at $p<0.05$ in the column

Table 2. Mean weekly water intake of control and indoxacarb-treated group @ 550 mg/kg

Dose (mg/kg)	Mean weekly water intake (g)	
	Week 1	Week 2
550	13.333 ± 4.047	17.333 ± 3.002
Vehicle	14.286 ± 2.062	15 ± 2.944

Values are expressed as Mean ± SEM; n = 3

Values bearing different superscripts vary significantly at $p<0.05$ in the column

Table 3. Mean weekly body weight gain of control and indoxacarb-treated group @ 550 mg/kg

Dose (mg/kg)	Mean weekly body weight gain (g)	
	Week 1	Week 2
550	1.143 ± 4.972	-0.143 ± 3.375
Vehicle	1.286 ± 3.208	0.143 ± 3.182

Values are expressed as Mean ± SEM; n = 3

Values bearing different superscripts vary significantly at $p<0.05$ in the column

Table 4. Relative organ weight of vehicle control and indoxacarb-treated groups

Dose (mg/kg)	Relative organ weight (g/100 g of body weight)		
	Vehicle	550	2000
Liver	3.595 ± 0.055[a]	3.531 ± 0.103[ab]	3.352 ± 0.03[b]
Spleen	0.192 ± 0.006	0.199 ± 0.01	0.158 ± 0.034
Kidney	0.633 ± 0.001	0.676 ± 0.078	0.728 ± 0.09
Uterus	0.301 ± 0.011	0.224 ± 0.042	0.256 ± 0.032
Ovary	0.05 ± 0.006	0.055 ± 0.003	0.061 ± 0.001
Heart	0.276 ± 0.007	0.277 ± 0.01	0.364 ± 0.029
Lungs	0.62 ± 0.009[b]	0.67 ± 0.065[b]	1.265 ± 0.131[a]
Brain	0.864 ± 0.006	0.903 ± 0.033	0.837 ± 0.109

The voltage-gated sodium channel (VGSC) is the primary target of action of indoxacarb due to the formation of methyl 7-chloro-2 -[[4-(trifluoromethoxy) phenyl] carbamoyl] -3,5-dihydroindeno[1,2-e] [1,3,4] oxadiazine-4a-carboxylate (DCJW). The sodium-dependent compound action potentials are blocked by the extremely powerful, voltage-dependent compound DCJW. DCJW bind to the slow inactivated state of the sodium channel, reduces the number of channels for activation and inhibits the sodium current (von Stein *et al*., 2013). Tsurubuchi *et al*. (2003) reported the suppressive effects of indoxacarb on VGSC in rat dorsal root ganglion neurons. According to Wang and Wang (2003), animals that are exposed to neurotoxins that alter Na^+ channel gating frequently exhibit hyperexcitability, convulsions, paralysis and even death. Thus in the present study, the neurotoxic signs of indoxacarb can be due to the blocking action of DCJW on VGSC in the neurons of rats.

There was no significant difference in mean weekly feed intake, water intake and body weight gain between the control and indoxacarb-treated animals at the dose of 550 mg/kg. While the administration of indoxacarb at the dose of 2000 mg/kg induced death in rats which showed that the toxicity of indoxacarb is dose-dependent. This was in agreement with the previous studies of Shit *et al.* (2008), where they reported dose-dependent onset and severity of toxic symptoms in rats and mice.

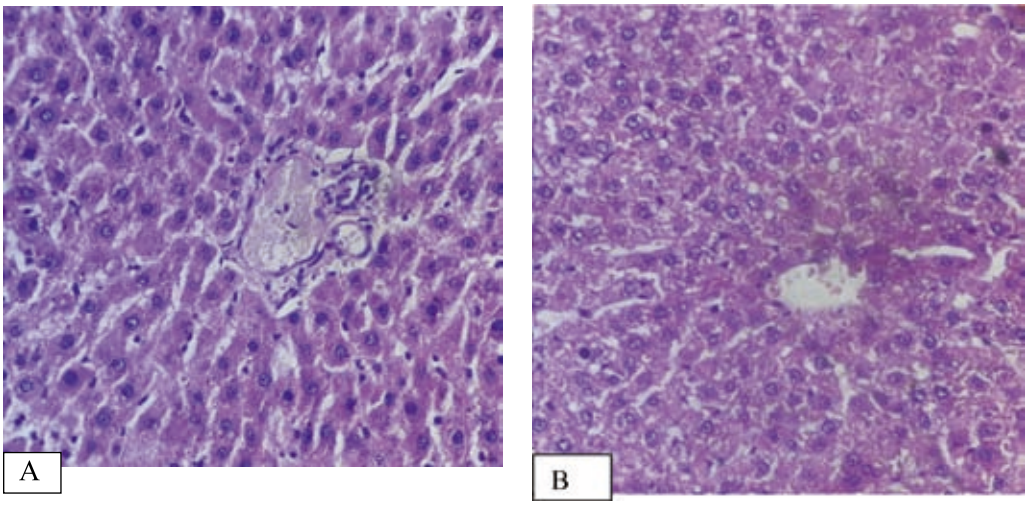

Plate 1. Section of liver from control and indoxacarb @ 2000 mg/kg (H & E)

(A) Control – 40X showing normal architecture of lungs (B) Indoxacarb @ 2000 mg/kg - 40X showing degeneration of hepatocytes

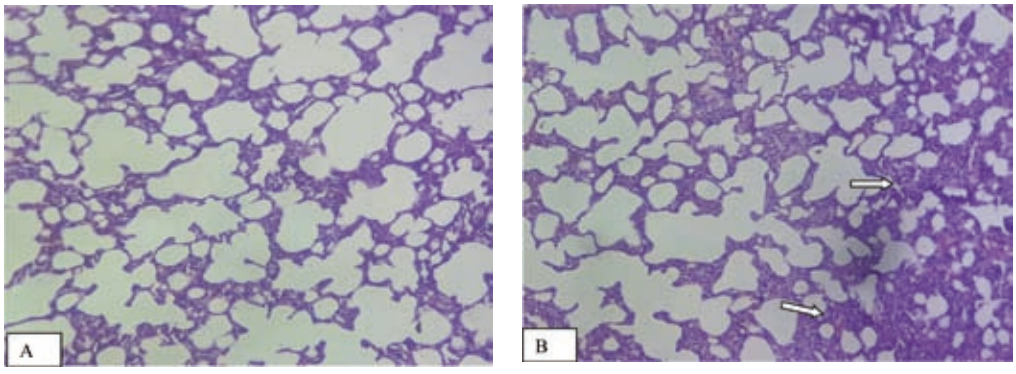

Plate 2. Section of lungs from control and indoxacarb @ 2000 mg/kg (H & E)

(A) Control – 40X showing normal architecture of lungs (B) Indoxacarb @ 2000 mg/kg - 40X showing oedematous area with cellular infiltration (white arrows)

On histopathological examination of different organs collected, noticeable lesions were observed in liver and lungs. In animals treated with indoxacarb at the dose of 2000 mg/kg, degeneration of hepatocytes was noticed in liver (Plate 1) and cellular infiltrations with oedematous areas were observed in the lungs (Plate 2).

In the present study, there was a significant decrease in the relative weight of the liver in the indoxacarb-treated group at the dose of 2000 mg/kg compared to the control group while there was a significant increase in the weight of lungs in the indoxacarb-treated group at the dose of 2000 mg/kg. The significant changes in the relative weight of the liver and lungs may indicate the hepatotoxicity and pulmonotoxicity of indoxacarb. Absolute weight is less predictive of toxicity than relative weight, which is further corroborated by the indoxacarb-treated organs showing a considerable impact on histological and gross evaluations. The increase in lung weight in indoxacarb-treated animals at 2000 mg/kg can be due to the oedema and cell infiltration that occurred in the lungs, which was revealed in the histopathological evaluation. (Van den Steen *et al*., 2010).

Table 5. Estimation of LD_{50} at the different doses of indoxacarb

Dose (mg/kg)	No. of animals dosed	Live	Death
175	1	1	0
550	3	3	0
2000	3	0	3
All doses	7	4	3

Values are expressed as Mean ± SEM; n = 3

Values bearing different superscripts vary significantly at $p<0.05$ in the row

Acute LD50 of indoxacarb

The LD_{50} of indoxacarb was estimated by AOT425StatPgm (version: 1.0) statistical program and found to be 1098 mg/kg. Hence it can be categorized as a Type II class of pesticide according to the WHO classification of pesticides.

Based on toxicity of pesticides, WHO classified them into four classes: extremely dangerous, highly dangerous, moderately dangerous and slightly dangerous. The classification is primarily based on the acute oral and dermal toxicity to the rat because these assessments are standard toxicology procedures. Pesticides can be classified into 4 types based on the oral LD_{50} respectively type Ia (<5 mg/kg), type Ib (5-50 mg/kg), type II (50-2000 mg/kg) and type III (>2000 mg/kg) (Yadav and Devi, 2017). In the present study, the oral LD_{50} of indoxacarb was estimated as 1098 mg/kg and hence the pesticide can be classified as to type II category with respect to the WHO classification of pesticides. Thus, the pesticide is moderately hazardous according to OECD guideline 425, Acute oral toxicity – up and down procedure.

Conclusion

The present study concluded with the findings that indoxacarb is a moderately hazardous compound that falls into the type II category as per the WHO classification of pesticides. Besides, the research also revealed the potential of indoxacarb to induce hepatotoxicity and pulmonotoxicity in Wistar rats. This research is the first comprehensive investigation of the LD_{50} of indoxacarb in Wistar rats. The observations from the investigation facilitated a better evaluation of the toxic characteristic of indoxacarb and also measured its short-term poisoning potential. Further studies should be carried out to assess the long-term poisoning potential of indoxacarb.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Lewis, K.A., Tzilivakis, J., Warner, D.J. and Green, A. 2016. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess.* **22**: 1050-1064.

Mabrouk, Z.E., Abusrer, S., Shibani, N. and El Jaafari, H. 2016. The effect of Indoxacarb

on blood parameters and liver tissues in Mice. *Libyan J. Vet. Med. Sci.* **2**: 23-30.

Shit, S.P., Panghal, R.S., Kumar, V and Rana, R.D. 2008. Acute toxicity and gross behavioural effects of indoxacarb in laboratory animals. *Haryana Vet*. **47**: 49-51.

Silver, K.S., Song, W., Nomura, Y., Salgado, V.L. and Dong, K. 2010. Mechanism of action of sodium channel blocker insecticides (SCBIs) on insect sodium channels. *Pestic. Biochem. Physiol.* **97**: 87-92.

Tsurubuchi, Y., Kagaya, Y. and Kono, Y. 2003. Suppression of tetrodotoxin-resistant voltage-gated sodium channels by enantiomers of the oxadiazine insecticide indoxacarb in rat dorsal root ganglion neurons. *J. Pestic. Sci.* **28**: 315-317.

Van den Steen, P.E., Geurts, N., Deroost, K., Van Aelst, I., Verhenne, S., Heremans, H., Van Damme, J. and Opdenakker, G. 2010. Immunopathology and dexamethasone therapy in a new model for malaria-associated acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* **181**: 957-968.

Viswanathan, S., Kumar, S. and Kandan, B. 2013. Indoxacarb-Related ARDS, Neurotoxicity and Orange Urine. *Eurasian J. Med.* **45**: 135-143.

von Stein, R.T., Silver, K.S. and Soderlund, D.M. 2013. Indoxacarb, metaflumizone, and other sodium channel inhibitor insecticides: mechanism and site of action on mammalian voltage-gated sodium channels. *Pestic. Biochem. Physiol.* **106***:* 101-112.

Wang, S.Y. and Wang, G.K. 2003. Voltage-gated sodium channels as primary targets of diverse lipid-soluble neurotoxins. *Cell. Signal.***15**: 151-159.

Yadav, I.C. and Devi, N.L. 2017. Pesticides classification and its impact on human and environment. *Environ. Sci. Eng*. **6**: 140-158. ■