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Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.3.593-597

# Investigation of serum biochemical responses in White Leghorn layer birds following acute avian toxicity screening using indoxacarb[#]

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***Citation:*** Aswathy, P.U., Suresh, N.N., Nisha, A.R., Sanis, J., Anitha, P., Asha, R. and Sajitha, I.S. 2024. Investigation of serum biochemical responses in White Leghorn layer birds following acute avian toxicity screening using indoxacarb. *J. Vet. Anim. Sci.* **55** (3):593-597

Received: 20.03.2024 Accepted: 17.05.2024 Published: 30.09.2024

**Abstract**

*The experiment was undertaken to study the effect of indoxacarb on various biochemical parameters in White Leghorn layer birds for a period of 14 days following a single oral administration. The study follows OECD guideline no. 223. White Leghorn layer birds (n=30) weighing between 1.2 to 1.6 kg were used for the study. Six birds were kept as the control group and approximately 2 ml of blood was collected in EDTA tubes on days 7 and 14 of the experiment. Biochemical parameters such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and blood urea nitrogen (BUN) were estimated. The mean values of ALP, AST, ALT and BUN were found significantly higher on days 7 and 14 of the acute toxicity study of indoxacarb in layers as compared to that of the control group.*

**Keywords**: *Biochemical, layers, indoxacarb*

Indoxacarb is an oxadiazine insecticide, introduced in the early 2000s, which is widely used nowadays to control a variety of pests including lepidopteran insects (moths and butterflies) (Tsurubuchi and Kono, 2003). It is a pro-insecticide which requires metabolic activation to exert its mode of action as described by Mudaraddi and Kaliwal (2009). It acts by disrupting the nervous system of insects, leading to paralysis, cessation of feeding and ultimately death (Mabrouk *et al*., 2016).

Indoxacarb has a unique mode of action in which it blocks the voltage-gated sodium channel of insects thereby suppressing action potential generation and propagation (Lapied *et al*., 2001). It has been used in agriculture for controlling pests on crops like cotton, tobacco, soya beans, vegetables and fruits also in residential settings for controlling pests like ants, cockroaches and fleas (Mccann *et al*., 2001). Though indoxacarb is very effective against many pests, it's important to use it with proper safety precautions and according to labelling instructions.

Kumar *et al*. (2013) observed that the intoxication of male broiler chicks with indoxacarb over a 28-day period led to notable alterations in serum biochemical parameters. The levels of alkaline phosphatase (ALP), alanine

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aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine, and albumin were significantly elevated in the indoxacarb-treated groups. Choudhary and Singh (2017) documented the reproductive toxicity of indoxacarb in cockerels. Histopathologically, the ultrastructure of the testes revealed decapitated spermatozoa, and the seminiferous tubule lumen was filled with numerous cross-sections of sperm tails. Additionally, the indoxacarb-treated groups exhibited a reduced number of spermatogonial cells in the testes. Birds may acquire toxicity when exposed to agricultural fields or under wild conditions or by the ingestion of contaminated pests bearing the active metabolites (Stafford *et al*., 2003). In this context, the present study was undertaken to analyze the effects of indoxacarb on various biochemical parameters of White Leghorn layer birds.

## Materials and methods

### *Test chemicals and kits*

Indoxacarb was purchased from Urban Crop Sciences Ltd., Haryana, India. The kits for biochemical analysis were purchased from AGAPPE diagnostics having LOT numbers 11408003 (AST), 11401003 (ALP), 11409003 (ALT), 11412002 (BUN), and 11420003 (Creatinine).

### *Experimental birds and housing conditions*

Thirty White Leghorn layer birds (*Gallus gallus domesticus*) within the age group of 30-35 weeks were divided into nine groups of which the dosed group contained three birds each and the control group had six birds. The birds were weighing between 1.2-1.6 kg and were housed at the All India Co-ordinated Research Project on Poultry (AICRP) farm, College of Veterinary and Animal Sciences, Mannuthy. Each bird was individually caged and received a standard chicken layer diet, along with unrestricted access to water. Two weeks before experimentation, all birds were randomly assigned to cages and acclimated to experimental conditions. The birds underwent an overnight fast with only *ad libitum* access to water before individual dosing. The study design was approved by the institutional animal ethics committee (IAEC/CVASMTY12/21).

### *Experimental design*

In this study, guideline 223 of the Organization for Economic Cooperation and Development (OECD, 2004) was followed, offering three testing options—namely, the limit dose test, LD50 alone test, and LD50-slope test, with the LD50-only test selected for implementation. This test procedure is sequential and is designed to minimize the number of birds. The primary endpoint of the test was mortality or permanent morbidity. The doses were selected based on reported toxicity studies of indoxacarb in quails and ducks (Dias, 2006).

At each stage, three birds received a single oral dose of indoxacarb in doses ranging from 500, 750, 1000, 1100, 1250, 1300, 1500 and 2000 mg/kg body weight. Indoxacarb was mixed in coconut oil to make a paste and was smeared on the tongue of individual birds for swallowing. The control group were provided with water *ad libitum* and a standard diet. Birds displaying evident pain or distress were euthanised. The blood samples were collected on the 7th and 14th days, processed for serum separation, and stored at -20ºC for subsequent analysis.

### *Biochemical analysis*

For biochemical analysis, approximately 2mL of blood was aseptically collected from the brachial veins of each bird using spirit and absorbable cotton in 2mL centrifuge tubes. The analyzed biochemical parameters included alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine on both the 7th and 14th days of the study.

### *Statistical analysis*

All the data are presented as mean ± SEM. Statistical analysis was performed using GraphPad Prism version V. Statistical significance of the differences between the treatment groups was analysed using one-way ANOVA followed by Bonferroni post-test ($P < 0.05$).

## Results and discussion

The acute (24 h) oral LD50 of indoxacarb in layers, as determined by the up-and-down method utilising AOT 425 software, was found to be 1250 mg/kg. Since birds dosed at higher doses which are 1500 and 2000 showed acute death their data could not be included in statistical analysis.

**Table 1.** Changes in the levels of AST of birds treated with indoxacarb @ 500, 750, 850, 1000, 1100, 1250 and 1300 mg/kg body weight, U/L

| Treatments | Day 7 | Day 14 |
|---|---|---|
| Control | 219.0 ± 2.82 | 226.9 ± 1.50 |
| Indoxacarb @ 500 mg/kg | 203.8 ± 0.63 | 193.7 ± 0.70 |
| Indoxacarb @ 750 mg/kg | 209.85 ± 1.5 | 199.15 ± 1.9 |
| Indoxacarb @ 1000 mg/kg | 298.25 ± 0.49* | 210.40 ± 0.68 |
| Indoxacarb @ 1100 mg/kg | 283.2 ± 0.31* | 170.9 ± 0.27 |
| Indoxacarb @ 1250 mg/kg | 225.9 ± 0.54* | 195.9 ± 0.57 |
| Indoxacarb @ 1300 mg/kg | 300.9 ± 1.23* | 270.1 ± 0.98 |

The values are Mean ± SE of 3 birds and values bearing * differ significantly from others in the same column (P<0.05)

### *Effect on aspartate aminotransferase*

Table 1 represents the variations in serum aspartate aminotransferase of birds subjected to indoxacarb treatment. Indoxacarb-treated birds exhibited a noteworthy increase in the level of AST as compared to the control birds. On the 7th day, birds dosed with indoxacarb @ 1300 mg/kg showed the highest concentration of 300.9 ± 1.23 U/L of enzyme whereas it was 219.0 ± 2.82 U/L in control birds. However, the elevated enzyme levels returned to normal concentrations by the 14th day.

### *Effect on alanine aminotransferase*

Table 2 represents the variations in serum alanine aminotransferase of birds subjected to indoxacarb treatment. Indoxacarb-treated birds exhibited a notable increase in the level of ALT as compared to the control birds. Birds dosed with indoxacarb @ 1000 mg/kg showed the highest concentration as 12.3 ± 0.33 U/L of enzyme whereas it remained at 6.37 ± 0.62 U/L in control birds. However, the elevated enzyme levels returned to normal concentrations by the 14th day except in birds treated with indoxacarb @ 1000 mg/kg and indoxacarb @ 1250 mg/kg.

### *Effect on alkaline phosphatase*

**Table 2.** Changes in the levels of ALT of birds treated with indoxacarb @ 500, 750, 850, 1000, 1100, 1250 and 1300 mg/kg body weight, U/L

| Treatments | Day 7 | Day 14 |
|---|---|---|
| Control | 6.37 ± 0.62 | 6.40 ± 0.65 |
| Indoxacarb @ 500 mg/kg | 6.33 ± 0.14 | 5.93 ± 0.03 |
| Indoxacarb @ 750 mg/kg | 8.28 ± 0.23 | 5.26 ± 0.60 |
| Indoxacarb @ 1000 mg/kg | 12.3 ± 0.33* | 9.21 ± 0.85* |
| Indoxacarb @ 1100 mg/kg | 10.87 ± 0.97* | 4.94 ± 0.47 |
| Indoxacarb @ 1250 mg/kg | 9.74 ± 0.28* | 9.24 ± 0.98* |
| Indoxacarb @ 1300 mg/kg | 5.48 ± 0.87 | 6.13 ± 0.65 |

The values are Mean ± SE of 3 birds and values bearing * differ significantly from others in the same column (P<0.05)

**Table 3**. Changes in the levels of ALP of birds treated with indoxacarb @ 500, 750, 850, 1000, 1100, 1250 and 1300 mg/kg body weight, U/L

| Treatments | Day 7 | Day 14 |
|---|---|---|
| Control | 1059.3 ± 0.39 | 1053.8 ± 0.30 |
| Indoxacarb @ 500 mg/kg | 998.3 ± 0.63 | 1002.9 ± 1.9 |
| Indoxacarb @ 750 mg/kg | 1109.15 ± 0.90* | 1111.85 ± 0.41* |
| Indoxacarb @ 1000 mg/kg | 1223.7 ± 0.75* | 1298.00 ± 0.15* |
| Indoxacarb @ 1100 mg/kg | 1143.66 ± 0.46* | 946.40 ± 0.39* |
| Indoxacarb @ 1250 mg/kg | 1119.3 ± 0.95 | 604.90 ± 0.62* |
| Indoxacarb @ 1300 mg/kg | 1404.30 ± 0.12* | 951.40 ± 0.33* |

The values are Mean ± SE of 3 birds and values bearing * differ significantly from others in the same column(p<0.05).

**Table 4.** Changes in the levels of BUN of birds treated with indoxacarb @ 500, 750, 850, 1000, 1100, 1250, 1300 and 1500 mg/kg body weight, mg/dL

| Treatments | Day 7 | Day 14 |
|---|---|---|
| Control | 27.31 ± 0.43 | 27.65 ± 0.21 |
| Indoxacarb @ 500 mg/kg | 34.80 ± 0.61 | 29.95 ± 0.19 |
| Indoxacarb @ 750 mg/kg | 67.66 ± 0.37* | 28.92 ± 0.98 |
| Indoxacarb @ 1000 mg/kg | 58.25 ± 0.15* | 39.26 ± 0.65* |
| Indoxacarb @ 1100 mg/kg | 36.45 ± 0.60* | 28.75 ± 0.21 |
| Indoxacarb @ 1250 mg/kg | 70.24 ± 0.69* | 61.11 ± 0.46* |
| Indoxacarb @ 1300 mg/kg | 51.64 ± 0.68* | 59.34 ± 0.89* |

The values are Mean ± SE of 3 birds and values bearing * differ significantly from others in the same column (P<0.05)

Table 3 represents the variations in serum alkaline phosphatase of birds subjected to indoxacarb treatment. Indoxacarb-treated birds exhibited a significant increase in the level of ALP as compared to the control birds. Birds dosed with indoxacarb @ 750 mg/kg and higher doses showed elevated concentration of enzyme whereas it remained 1059.3 ± 0.39 in control birds during the period of acute study. Moreover, unlike the case of AST and ALT, ALP concentration remained elevated during the whole period of the study except in birds treated with indoxacarb @ 1250 mg/kg.

### *Effect on blood urea nitrogen*

Table 4 represents the variations in BUN of birds subjected to indoxacarb treatment. Indoxacarb-treated birds exhibited a significant increase in the level of BUN as compared to the control birds. Birds dosed with indoxacarb @ 750 mg/kg exhibited 67.66 ± 0.37 mg/dL and higher doses showed similarly elevated levels whereas it remained at 27.31 ± 0.43 mg/dL in control birds during the period of acute study. However, the BUN concentration remained high during the whole period of the study except in birds treated with indoxacarb @ 750 mg/kg and indoxacarb @1100 mg/kg.

### *Effect on creatinine*

Table 5 represents the creatinine concentrations of birds subjected to indoxacarb treatment. From the data, it is clear that there was no significant variation in the creatinine concentration for the birds subjected to indoxacarb treatment compared to that of the control birds.

The liver is the major organ involved in the detoxification of xenobiotics. Any liver injury could be

**Table 5.** Changes in the levels of creatinine of birds treated with indoxacarb @ 500, 750, 850, 1000, 1100, 1250, 1300 and 1500 mg/kg body weight, mg/dL

| Treatments | Day 7 | Day 14 |
|---|---|---|
| Control | 1.97 ± 0.24 | 2.0 ± 0.31 |
| Indoxacarb @ 500 mg/kg | 1.33 ± 0.01 | 1.22 ± 0.07 |
| Indoxacarb @ 750 mg/kg | 1.64 ± 0.06 | 1.31 ± 0.07 |
| Indoxacarb @ 1000 mg/kg | 1.54 ± 0.98 | 1.44 ± 0.74 |
| Indoxacarb @ 1100 mg/kg | 2.89 ± 1.19 | 1.19 ± 0.01 |
| Indoxacarb @ 1250 mg/kg | 1.31 ± 1.34 | 0.94 ± 1.21 |
| Indoxacarb @ 1300 mg/kg | 1.98 ± 0.32 | 1.85 ± 0.65 |

The values are Mean ± SE of 3 birds and values bearing * differ significantly from others in the same column ($P<0.05$)

detected by monitoring the liver biomarker enzymes AST, ALT, and ALP which are widely used to estimate liver function (Foster *et al*., 2020). In the present study, exposure of layers to indoxacarb caused a significant increase in ALP, AST and ALT. The present finding agrees with the study of Goyal and Sandhu (2009) in buffalo calves. They observed that a 38.3 per cent increase in ALT was found due to sub-chronic oral exposure of indoxacarb toxicity in buffalo calves. Kumar *et al*. 2013 also reported a significant elevation of ALT and AST enzymes in indoxacarb-treated broiler chicken. The exposure of layers to indoxacarb caused significant elevation in ALT and AST in the treated birds which was returned to normal concentration on day 14. This explains the possibility of probable destruction of the hepatocytes with subsequent leakage of these enzymes into the circulation. Cellular leakage following loss of hepatocyte membrane integrity was reported by Baz, (2013).

However, in the present study, it is clear that the responses of the hepatic enzymes to treatments were in a phase-dependent manner involving two phases. In phase 1 the enzyme concentration increases and in phase 2 it returns to normal concentration. This could be due to the response of the liver to acute injury in which the enzymes are actively secreted into the circulation. In phase 2, the enzymes released in phase 1 after undergoing detoxification become stable in their release. Hence, it could be concluded that the liver tends to exert metabolic activity to a certain extent for detoxifying indoxacarb. This explanation agrees with Abdelrasoul (2018) who found the increased activity of these enzymes in male albino rats exposed to indoxacarb.

Similarly, Shit *et al*. (2008) conducted an experiment to study indoxacarb-induced sub-chronic toxicity in Wister albino male rats administered 12 mg/kg and 24 mg/kg orally for 28 days. In the study, they found an increase in kidney biomarkers like creatinine and BUN in rats at 24 mg/kg, indicating the toxic effect of indoxacarb on the kidney. In the present study BUN level of birds was elevated on day 7 after treatment with indoxacarb at high doses. However, in this particular study, there was no significant change in the level of creatinine concentrations in indoxacarb-treated layers.

## Conclusion

The above findings suggest that the oxadiazine insecticide is toxic to layer birds when administered at a dose rate ≥ 750 mg/kg. Also, it could be concluded from the study that indoxacarb is potentially hepatotoxic as well as nephrotoxic to layer birds.

## Conflict of interest

The authors declare that they have no conflict of interest.

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