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Serum biochemistry of white leghorn layer birds following subacute avian dietary toxicity test

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Abstract
The experiment was undertaken to study the effect of indoxacarb, an oxadiazine insecticide, on various biochemical parameters in White Leghorn layer birds for a period of 21 days following incorporation in feed at five different concentrations for five days. The study follows OECD guideline no. 205, White Leghorn layer birds (n=60) weighing between 1.2 to 1.6 kg were utilized for the study. Ten birds were kept as control group and approximately 1.0 ml blood was collected in EDTA tubes on days 0, 7, 14 and 21 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 in indoxacarb treated groups as compared to the control group.

Keywords: Biochemical, layers, indoxacarb, sub-acute

Introduction
Indoxacarb is the ISO common name for methyl (S)-N-[7-chloro-2, 3, 4a, 5-tetrahydro-4a-(methoxycarbonyl) indeno [1, 2-e] [1, 3, 4] oxadiazin-2-ylcarbonyl]-40(trifluoromethoxy) carbanilate and is the only commercially available oxadiazine insecticide [1]. It is a pro-insecticide which gets actively metabolized to its decarbomethoxyllated product, DCJW which is 10 times more potent than the parent compound [2]. It acts by blocking the voltage-gated sodium channels that eventually hampers the initiation of action potential generation in nerve fibres resulting in impaired nerve function, feeding cessation, paralysis, and death [3]. HPLC analysis and mass spectrometry analysis revealed that the time course of development of neurotoxic symptoms and metabolic conversion of indoxacarb to DCJW by whole larvae coincides [4].

Indoxacarb is mainly used against agriculture and horticulture lepidopteran and coleopteran pests [5]. Indoxacarb is highly toxic to aquatic organisms and less toxic to mammals [6]. Indoxacarb is metabolised to its active form rapidly in insects, but limited in mammals which results in favourable selective toxicity to non-target organisms [7]. Hence, it is considered as a safe substitute for organophosphate insecticide with activity against a wide range of pests [8]. Technically, the S-enantiomer of indoxacarb is having the insecticidal activity with the R-enantiomer being insecticidally inactive. It is also effective against insects that are resistant to other neurotoxic insecticides such as pyrethroids, carbamates, and organophosphates [9].

Indoxacarb has been extensively used as an insecticide to control tobacco budworms, cotton bollworms, pod-borers in pulses, pests in vegetables, and forage crops. Consequently, animals and humans are more likely to get exposed to this insecticide in day-to-day life [10]. The objective of the current study was to evaluate the biochemical parameters following subacute dietary toxicity of indoxacarb in White leghorn layer birds.

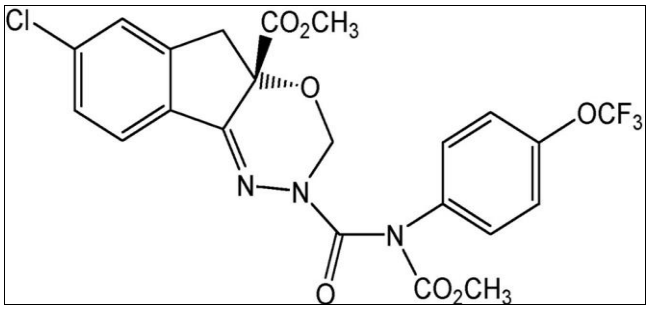

Fig 1: Structure of indoxacarb

Materials and Methods

Chemicals and kits

Indoxacarb was purchased from Urban Crop Science 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).

OECD 205

Principle of the test method

Birds are fed a diet containing the test substance at a range of concentrations for a period of five days. Beginning on day 6, the birds are fed the basal diet, free of the test substance, for a minimum of three additional days. Mortalities and signs of toxicity are recorded daily. The guideline describes treatment groups for each of the, at least, five dietary levels of the test substance along with a control group. Each group should consist of 10 birds. Control diets or diets containing the test substance should be available ad libitum.

The minimum duration of the test is eight days: five days on the test diet followed by a minimum of three days on normal diet. If mortalities occur on days seven or eight, or if signs of toxicity remain on day eight and are not clearly in remission, the test should be continued until two successive days pass without a mortality and it is assured that the birds will recover or until 21 days after the beginning of the test, whichever comes first. Observations should be made twice on day one and daily thereafter for signs of intoxication and other abnormal behaviour twice daily and daily thereafter for mortality, body weights on days 0, 5, 8, and end of test, days 0-5, 5-8, and 8-end of test (if extended) for food consumption.

Experimental birds and dosage

A total of 60-layer birds (*Gallus gallus* White leghorn strain) between 30-35 weeks age group weighing between 1.2-1.6 kg were used for the experiment and were maintained in AICRP (All India Co-ordinated Research Project on Poultry) farm, College of Veterinary and Animal Sciences, Mannuthy. All the birds were individually caged and fed with a standard chicken breeder diet (100 g per day) and water *ad libitum*. Birds were acclimatized for one week prior to experimentation. The birds were fasted overnight with only water being provided *ad libitum* before dosing individually.

Approval from the institutional animal ethics committee was obtained for the use of birds IAEC/CVASMTY12/21 for the study. Sixty birds were divided into 6 groups of ten birds each where one group served as the control group and other groups were administered with indoxacarb containing feed, after uniform mixing, for the first 5 days of the subacute dietary toxicity study at the doses ranging from 125, 250, 500, 1000 and 2000 µg/g of feed. Blood samples were collected on days 0, 7, 14 and 21 days of the research work for biochemical analysis. The vacutainers were kept in a slanting position at room temperature for clotting to take place and then centrifuged at 3000 rpm for 5 minutes. The separated serum was collected in microcentrifuge tubes and was kept at -20ºC till further use. The feed intake of birds was recorded every day until 21st day.

Biochemical analysis

For biochemical analysis, approximately 2 ml of blood was collected from the brachial veins of each bird aseptically by using spirit and absorbable cotton in 2 ml centrifuge tubes. The various biochemical parameters estimated were alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine on 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 were analyzed by TWO WAY ANOVA followed by post-test using Bonferroni comparison tests (p<0.05).

Results

Serum biochemical analysis

Effect on Aspartate aminotransferase

The effect of indoxacarb treatment on concentration of AST in birds are represented in Table 1 and Figure 2. The results indicate that there was a significant increase in AST level on day 7 in IND treated groups, irrespective of the dose administered, compared to that of the control group in which the enzyme concentration remained in the normal range during the entire duration of the study. However, AST concentration returned to normal range during the end of the study in all the groups except birds treated with higher doses of indoxacarb *viz*. IND 1000 and IND 2000.

Table 1: Changes in AST (U/L) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Treatments	Day 0	Day 7	Day 14	Day 21
Control	170.95 ± 2.15^{Aa}	166.41 ± 1.67^{Ba}	161.32 ± 3.34^{Ba}	163.21 ± 2.69^{ABa}
IND 125	120.38 ± 3.99^{Bb}	152.58 ± 4.42^{Ba}	131.22 ± 3.53^{Cab}	128.91 ± 5.09^{Cab}
IND 250	135.3 ± 6.34^{Bb}	169.61 ± 9.02^{Ba}	159.91 ± 11.18^{Bab}	134.52 ± 7.63^{Cab}
IND 500	121.46 ± 2.65^{Bb}	156.98 ± 11.37^{Ba}	144.7 ± 10.44^{Cab}	125.61 ± 4.43^{Cb}
IND 1000	169.4 ± 10.83^{Ac}	228.16 ± 3.82^{Aa}	202.7 ± 5.18^{Ab}	195.71 ± 1.99^{Ab}
IND 2000	131.65 ± 7.07^{Bd}	210.7 ± 5.35^{Aa}	189.35 ± 6.82^{Aab}	155.33 ± 7.83^{Bc}

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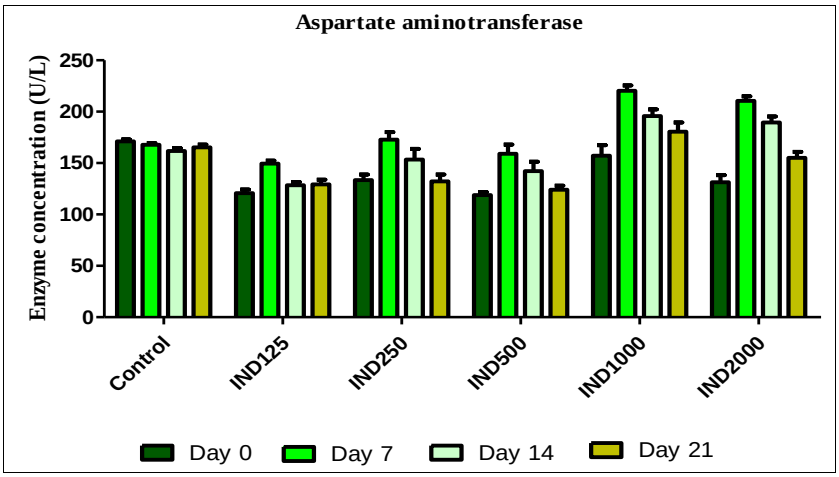

Fig 2: Changes in AST (U/L) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Effect on Alanine aminotransferase

The effect of indoxacarb treatment on concentration of ALT in birds treated with indoxacarb are represented in Table 2 and Figure 3. The analysis of data on effect of ALT indicates that there was significant increase in the concentration of ALT on day 7 as observed in the case of AST. Similarly, the enzyme concentration remained significantly high on day 21 as well in higher dose Groups *viz*. IND 1000 and IND 2000.

Table 2: Changes in ALT (U/L) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Treatments	Day 0	Day 7	Day 14	Day 21
Control	5.9 ± 0.29^{Aa}	6.06 ± 0.24^{Ba}	5.98 ± 0.28^{Ba}	5.92 ± 0.24^{Ba}
IND 125	4.43 ± 0.48^{Aa}	12.67 ± 1.68^{Ba}	11.02 ± 1.97^{Ba}	8.05 ± 0.95^{Ba}
IND 250	8.38 ± 1.23^{Ab}	23.62 ± 4.76^{Aa}	15.31 ± 3.01^{Bb}	12.82 ± 3.03^{Ab}
IND 500	7.55 ± 1.31^{Ab}	31.71 ± 4.14^{Aa}	18.43 ± 3.09^{Ab}	16.88 ± 4.39^{Ab}
IND 1000	7.4 ± 0.82^{Ab}	26.76 ± 2.28^{Aa}	15.32 ± 2.24^{Bb}	10.5 ± 1.47^{Abc}
IND 2000	10.21 ± 1.63^{Ac}	28.06 ± 14.19^{Aa}	26.78 ± 0.6^{Aa}	19.64 ± 3.70^{Aab}

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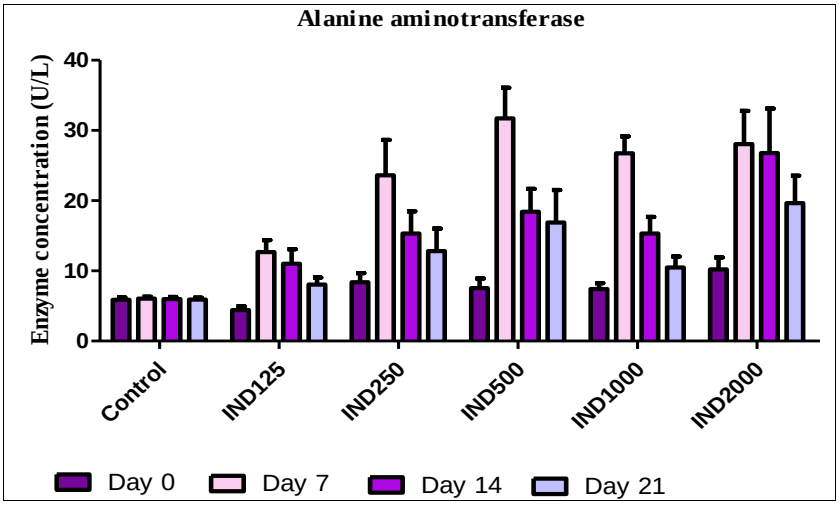

Fig 3: Changes in ALT (U/L) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Effect on Alkaline Phosphatase

The effect of indoxacarb treatment on concentration of ALP in birds are represented in Table 3 and Figure 4. The analysis of data on effect of ALP indicates that there was significant increase in the concentration of the enzyme on day 7 which remained high on day 14 as well but returned to almost normal concentration on day 21in all the treatment groups.

Table 3: Changes in ALP (U/L) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Treatments	Day 0	Day 7	Day 14	Day 21
Control	227.51 ± 9.93^{ABa}	232.22 ± 11.88^{Da}	225.15 ± 12.4^{Ca}	228.26 ± 12.81^{Ca}
IND 125	265.53 ± 12.78^{ABb}	311.03 ± 15.79^{BCa}	277.35 ± 21.73^{BCab}	267.92 ± 13.4^{Cb}
IND 250	275.56 ± 9.89^{Ab}	383.03 ± 12.5^{Aa}	334.1 ± 20.48^{Aac}	293.7 ± 6.98^{ABbc}
IND 500	230.08 ± 6.69^{ABb}	314.85 ± 16.96^{Ba}	268.45 ± 8.62^{BCab}	243.53 ± 9.55^{Cb}
IND 1000	202.66 ± 10.26^{CDb}	304.6 ± 13.47^{Ba}	239.36 ± 14.38^{BCab}	222.11 ± 12.65^{Cb}
IND 2000	260.85 ± 6.74^{ABc}	315.92 ± 6.39^{Ba}	306.72 ± 3.21^{Ba}	298.46 ± 2.89^{Ac}

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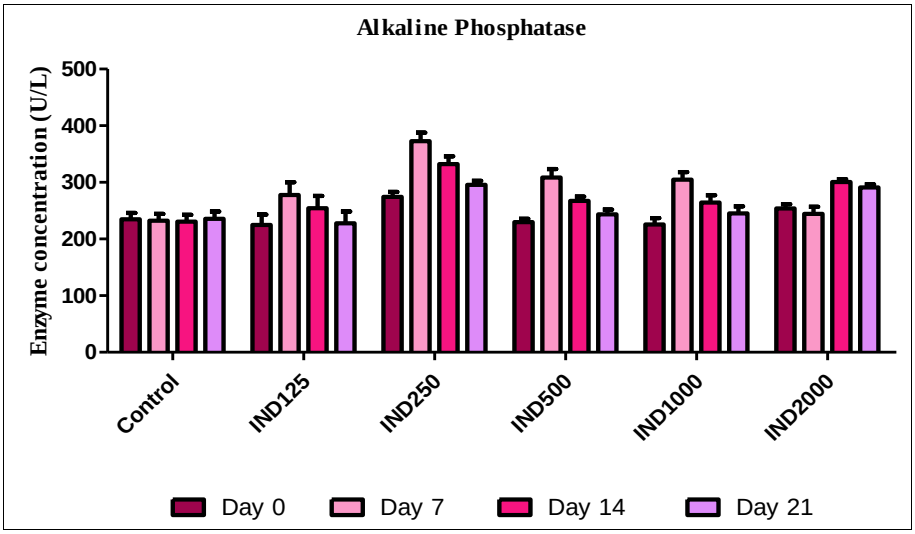

Fig 4: Changes in ALP (U/L) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Effect on BUN

The effect of indoxacarb treatment on concentration of BUN in birds are represented in Table 4 and Figure 5. The data regarding effect of various treatments on the concentration of BUN revealed that BUN level also follows the same pattern of increase in the concentration on day 7 and 14 followed by return to normal concentration on day 21 of the experiment.

Table 4: Changes in BUN (mg/dl) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Treatments	Day 0	Day 7	Day 14	Day 21
Control	19.11 ± 0.58^{Aa}	18.91 ± 0.47^{Ca}	19.05 ± 0.92^{ABa}	18.80 ± 0.82^{ABa}
IND 125	16.23 ± 0.97^{Ab}	30.11 ± 3.11^{ABa}	16.76 ± 0.60^{Bb}	16.20 ± 0.81^{ABb}
IND 250	17.69 ± 1.06^{Ab}	27.01 ± 1.58^{Ba}	16.96 ± 1.43^{Bb}	15.85 ± 1.62^{ABb}
IND 500	20.46 ± 0.65^{Abc}	26.53 ± 2.87^{Ba}	17.62 ± 1.72^{ABc}	12.49 ± 1.54^{Ab}
IND 1000	18.26 ± 0.83^{Ab}	33.70 ± 2.34^{Aa}	22.22 ± 1.45^{Ab}	20.07 ± 2.02^{Ab}
IND 2000	17.64 ± 0.54^{Ab}	28.00 ± 1.33^{ABa}	18.73 ± 1.42^{ABb}	15.20 ± 1.19^{ABb}

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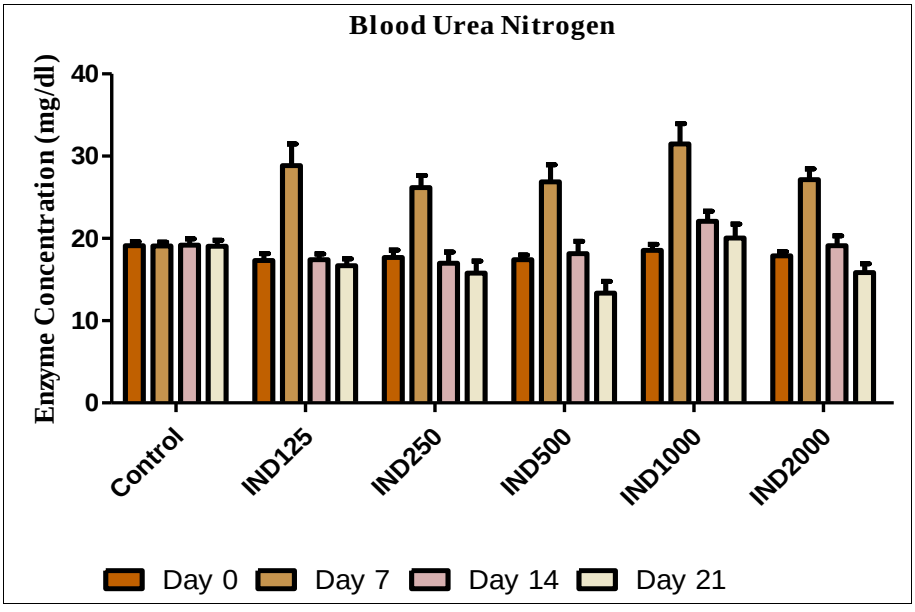

Fig 5: Changes in the concentration of BUN (mg/dl) of birds treated with indoxacarb 125, 250, 500, 1000 and 2000 µg/g in feed

Effect on Creatinine

The effect of indoxacarb treatment on concentration of creatinine in birds are represented in Table 5 and Figure 6. The data regarding effect of various treatments on creatinine concentration indicates that there was a significant increase on day 7 which remained elevated on day 14 as well in higher dose groups only whereas it returned to normal concentrations on day 14 of the experiment in lower doses and day 21 of the experiment in higher doses.

Table 5: Changes in creatinine (mg/dl) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Treatments	Day 0	Day 7	Day 14	Day 21
Control	1.74 ± 0.27^{Aa}	1.87 ± 0.23^{CDa}	1.83 ± 0.21^{Ca}	1.81 ± 0.17^{Aa}
IND 125	1.37 ± 0.12^{Aa}	1.93 ± 0.22^{Ca}	1.91 ± 0.21^{BCa}	1.92 ± 0.25^{Aa}
IND 250	1.70 ± 0.24^{Ab}	2.72 ± 0.12^{Ba}	2.30 ± 0.18^{ABCa}	2.19 ± 0.21^{ACa}
IND 500	1.48 ± 0.14^{Ab}	3.16 ± 0.3^{ABa}	1.60 ± 0.13^{Cb}	1.61 ± 0.10^{Ab}
IND 1000	1.96 ± 0.19^{Ab}	3.51 ± 0.30^{Aa}	2.62 ± 0.36^{Ab}	2.05 ± 0.22^{Ab}
IND 2000	1.67 ± 0.27^{Ab}	2.97 ± 0.21^{ABa}	2.54 ± 0.21^{ABa}	2.25 ± 0.17^{Aab}

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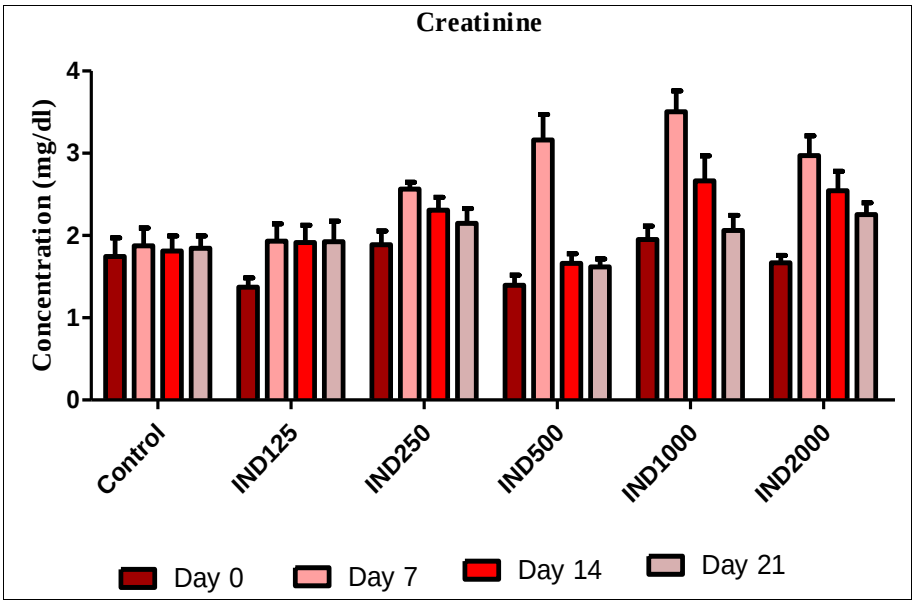

Fig 6: Changes in the concentration of creatinine (mg/dl) of birds treated with indoxacarb @ 125, 250, 500, 1000 and 2000 µg/g in feed

Discussion

Indoxacarb, an oxadiazine pesticide which is being widely and extensively used in agriculture, horticulture and some veterinary preparations, exhibits strong activity against lepidopteran pests infesting vegetables and fruits. Indoxacarb does not have any reported toxicity in layer birds, therefore, the study was extended to assess the sub-acute toxicity and thereby evaluating the biochemical parameters.

A set of chemical tests are now considered as classic tests to assess liver injury and dysfunction *viz*. estimation of total bilirubin and serum activities of ALP, AST and ALT. Elevated serum enzyme activities indicate injury to hepatocytes or biliary cells whereas elevated bilirubin is the prime indicator of loss of liver function. These two transaminases not only indicate liver injury but also are used by cardiologists for decades to assess postoperative myocardial infarction by detecting heart injury [11]. AST was the first biomarker used ever since 1950s to assess acute myocardial infarctions which was later replaced by cardiac troponins due to its non-specificity [12]. However, ALT is more concentrated in liver cells which concludes that ALT is more elevated in response to liver injury than AST [13].

Serum biochemical parameters were assessed on days 0, 7, 14 and 21 days of the experiment and it was observed that AST, ALT, ALP, BUN and creatinine concentrations were altered on a weekly basis. The enzyme concentrations were increased on day 7 and returned to normal range on day 14 onwards in AST, BUN and creatinine. ALT and ALP concentrations returned to normal level in a slow manner compared to the other enzymes and ALT concentration remained higher on day 14 in the highest dose groups (IND 2000).

The significant rise on day 7 followed by decrease in enzyme concentrations may be due to withdrawal of indoxacarb incorporated feed after five days of dosing. A similar increase in ALT and AST was observed in male Japanese quails[14] and in rats exposed to atrazine [15].

Similarly, an experiment to study indoxacarb induced subchronic toxicity in Wister albino male rats administered 12 mg/kg and 24 mg/kg orally for a period of 28 days [16]. In the study they found an increase in kidney biomarkers like creatinine and BUN in rats at 24 mg/kg, indicated toxic effect of indoxacarb on kidney. In the present study BUN level of birds were elevated on day 7 after treatment with indoxacarb at high doses.

Serum creatinine was also significantly elevated in subacute study which is in line with the findings in quails [17] induced by sub-chronic exposure to triazophos. The elevated concentrations of ALT and AST enzymes in the study are in agreement with studies which reported a significant elevation of these enzymes in indoxacarb treated male albino rats [18] and broiler chicken [19] respectively.

Conclusion

From the results, it is clear that the biomarkers of hepatic and renal function tests are altered which surely is an indication that the insecticide indoxacarb is hepatotoxic as well as nephrotoxic to the birds.

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