Research Article

Individual and Combined Effect of Indoxacarb and Glyphosate on Biochemical Alterations in Japanese Quails (*Cuturnix Cuturnix Japonica*)

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Abstract

The experiment was undertaken to study the biochemical alteration due to indoxacarb and glyphosate or combination of both induced toxicity in quails from 4th week to 12th week of age. The study was conducted on 252 Japanese quails. The birds were equally divided in to seven groups of 36 birds in each group. Control C group maintained on commercial feed with adlibitum water for up to 12th week age of quails. The indoxacarb was mixed in the feed @30mg/kg and 60mg/kg and given to T1 and T2 groups, respectively. Glyphosate was mixed in feed @250mg/kg and 500mg/kg and allotted to T3 and T4 groups respectively. Combination of both indoxacarb and glyphosate has been given to T5 (indoxacarb@ 30mg/kg + glyphosate @ 500 mg/kg) and T6 (indoxacarb@ 60 mg/kg + glyphosate @ 250 mg/kg). The toxins were given up to 11th week age. After 8th week 15 birds were separated from all treatment groups to see the residual (withdrawal) effect for 7 and 14 days viz.9th and 10th week of age. Withdrawal effect for 7 days after 11th week of age also seen. The blood was collected at the time of scarification of bird in 8th and 11th week in indoxacarb and glyphosate treated quails. Also blood was collected after post treatment in 9th and 11th week, 10th week to study the biochemical parameters such as total protein, albumin, ALP, AST, ALT, creatinine, cholesterol, GGT and AChE levels. From the results it is observed that values of total protein, albumin and acetylcholine esterase were decreased. However, values of ALP, AST, ALT, GGT, cholesterol and creatinine were increased in indoxacarb and glyphosate treated groups quails as compared to control groups. After withdrawal of both the toxin from feed for 14 days biochemical values in post treatment were nearer to the values of control groups.

Keywords: Indoxacarb, Glyphosate, quails, biochemical alterations, withdrawal effect

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Introduction

Indoxacarb is a representative of the new pesticide class Oxadiazine. Indoxacarb [(S) methyl 7 chloro 2, 5 dihydro 2{(methoxy carbonyl) {4 (trifluoromethoxy) phenyl} amino]–carbonyl] indenol {1,2 e} {1,3,4} oxadiazine 4a pH) carboxylate] is used widely in agriculture and horticulture as an insecticide and exhibits strong activity against lepidopteron pests of vegetables, tree fruits, cotton, corn, peanut, soybean, alfalfa and other crops [1]. Activity of this compound has also been shown against some homopteran and coleopteran species [2]. However, the biological activity of indoxacarb is not just limited to insects, but its extensive use poses inadvertent serious health hazards in livestock, aquatic organism and man [3]. It is a proinsecticide, must undergo bioactivation to the N decarbomethoxyllated metabolite [4] which is much more toxic than parent compound. The activated metabolite of indoxacarb has a unique mode of action involving the blocking of sodium channels of nerve cells, resulting in paralysis and death of the target pest species [5]. The neuronal nicotinic AChR could be one of the primary target sites of the insecticide in mammals [6].Glyphosate (N-[phosphonomethyl]-glycine, H203P-CH2-NH-CH2-C02H) is a unique broad-spectrum post emergence herbicide [7]. Glyphosate and its combined product have the genotoxic and endocrine disrupting effect [8]. Glyphosate and glyphosate based product caused endocrine disruption that reported a decrease in mRNA synthesis following its exposure [9, 10] experimental studies, opined that the toxicity of the surfactant, polyoxyethyleneamine (POEA), was greater than the toxicity of glyphosate alone and also commercial formulations alone. There was insufficient evidence to conclude that glyphosate preparations containing POEA were more toxic than those containing alternative surfactants. There was a paucity [11] of data on the toxicity of the

formulated products. Little information is available showing effect of indoxacarb and glyphosate in quails. Therefore, the present study was conducted to evaluate the toxic effect of indoxacarb and glyphosate on various biochemical parameters in quails.

Material and Methods

Experiment was conducted on poultry research center, Post Graduate Institute of Veterinary and Animal Sciences, Akola. The experiment on Japanese quail was for a period of twelve weeks. 252 two week old Japanese quails were procured from M/S Venkateshwara Hatcheries Pvt. Ltd. Pune and were acclimatized for one week prior to experiment. These were divided into seven groups, each group comprising of 36 quails. The details of experimental groups are given below.

Details of experimental groups

S.N.	Group	Number of birds	Levels of indoxacarb/ glyphosate in diet	Period
1	C	36	control	Up to 12th week
2	T1	36	Commercial feed + @ 30mg/kg indoxacarb	From 4th to 11th week
3	T2	36	Commercial feed + @ 60 mg/kg indoxacarb	From 4th to 11th week
4	T3	36	Commercial feed + @ 250 mg/kg glyphosate	From 4th to 11th week
5	T4	36	Commercial feed + @ 500 mg/kg glyphosate	From 4th to 11th week
6	T5	36	Commercial feed + @ 30mg/kg indoxacarb +500 mg/kg glyphosate	From 4th to 11th week
7	T6	36	Commercial feed + @ 60 mg/kg indoxacarb + 250 mg/kg glyphosate	From 4th to 11th week

Initial body weight of individual quail chick was recorded before the start of experiment and birds were maintained group wise under standard identical managemental conditions. All groups were given ad-lib of water, feed containing pesticide (Indoxacarb, technical grade, purity 67% procured from Gharda Chemicals Ltd Khed Dist. Ratnagiri) and herbicide (Glyphosate, Technical Grade purity 95.30% procured from Krishi Rasayan Export Pvt. Ltd. New Delhi) for 11th week period of experiment. 15 birds were separated after 8th week and offered Pesticide and herbicide free feed from 9th to 10th week for these birds and for other 15 birds treatment was continued for 11th week. Last week i.e. 12th week also kept for withdrawal group.Serum samples collected from 6 birds of each group on the basis selection at the end of 8thand 11th (indoxacarb and glyphosate treated quails in feed) where as 9th and 12th week (withdrawal of indoxacarb and glyphosate from feed for 7 days), 10th (withdrawal of indoxacarb and glyphosate from feed for 14 days). Biochemical parameters included were estimated by using autospan diagnostic kits (Span diagnostic Ltd, Surat) on serum autoanalyser (Autochem, 2011).

- **Serum Total Proteins (g/dl):-** Total proteins levels in each group were estimated by modified Biuret and Doumas method [12].
- **Serum Albumin (g/dl):-** Albumin levels were estimated by Bromocresol Green method [13].
- **Serum Creatinine (mg/dl):-** Creatinine values were estimated by modified Jaffes method [14].
- **Serum Alanine aminotransferase (ALT) (IU/L):-** ALT values were estimated as per standard procedure [15].
- **Serum Aspartate Aminotransferase (AST)(IU/L):-**AST values were estimated as per standard procedure [15].
- **Serum alkaline Phosphatase (ALP) (IU/L):-**Serum alkaline phosphatase estimated by the method of pNPP-AMP (IFCC), Kinetic Assay [16].
- **Serum Acetyl choline esterase (AChE) (IU/L):-**Serum acetyl choline esterase was estimated by DGKC method [17].
- **Serum Gamma Glutamyltransferase (GGT) (U/L):-**Serum Gamma Glutamyltransferase estimated by gamma glutamyl-p-nitroanalide to glycylglycine by gamma GT kinetic assay [18].
- **Serum Cholesterol:-**Serum cholesterol estimated by enzymatic cholesterol oxidase – peroxidase method [19].

Result and Discussion

Various biochemical parameters such as total protein, total albumin, ALP, AST, ALT, cholesterol, cholinesterase, creatinine and GGT were estimated on 8th, 9th 10th 11th and 12th week age of quails.

Total serum Protein (g/dl)

Average total protein levels in different groups were estimated on 8th week (toxin treated up to 4th to 8th week age), 9th and 10th (toxin withdrawal feed after 8th week age for 7 and 14 day), 11th (toxin treated up to 4th to 11th week age), 12th (toxin withdrawal feed for 7 days) and depicted in **Table 1**.

Table 1 Serum total protein (g/dl) levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	6.18±0.844	5.21±0.661	6.18±0.412	6.32±0.991[a]	6.358±0.552
T1	5.52±0.720	4.78±0.347	5.537±1.047	4.61±0.202[b]	5.975±0.565
T2	4.74±0.325	3.65±0.538	6.662 ±1.264	4.31 ±0.277[bc]	5.503±0.473
T3	5.79±0.872	4.53±0.365	5.414±0.802	4.34±0.238[bc]	5.429±0.325
T4	5.34±0.641	4.30±0.774	3.235±1.006	4.501±0.403[b]	5.931±0.281
T5	5.47±0.715	3.89±0.470	6.615±0.901	3.05±0.282[c]	5.594±0.315
T6	4.66±1.191	4.16±0.617	6.415±0.557	3.06±0.325[c]	6.115±0.291
CD	NS	NS	NS	1.336	NS

Average mean values of protein were decreased as compared to control group in toxin fed birds up to 8th and 11th week. Values of total protein in 8th week were non-significant. However, in 11th week total protein values were found significant at 1% and 5% level of significance. The total protein were reduced by (27 to 31%) in indoxacarb fed birds i.e.T1 and T2, 31 to 28% in glyphosate fed birds i.e T3, T4 groups and 51% in combination of indoxacarb and glyphosate i.e. in T5 and T6 birds. This fall in total protein in birds might be due stressogenic effect or general toxic effect of Indoxacarb and Glyphosate in quails. The decrease in protein values might be due to increased catabolism [20].

In withdrawal period i.e. in 9th and 10th week (7th and 14th day of withdrawal of toxin) protein values were numerically decreased as compare to control groups. Statistically all these groups of withdrawal period is nonsignificant. It indicates that in the recovery period birds were tried to compensate the protein values. By feeding racemicindoxacarb (DPX-JW062; purity, 94.7%) at a concentration of 0, 15 (females only), 30, 60, 125 or 250 (males only) ppm for 90 days [21]. Clinical chemistry evaluations were performed on days 48 and 90. Female rats at 125 ppm found decreased total protein and globulin concentrations at both sampling times.

Indoxacarb did not cause any change in total proteins, albumin and globulin levels in plasma at both the dose levels, suggesting no effect on protein metabolism at 24mg/kg for 14 and 28days dose levels [22]. Increase total plasma proteins (10.8%) by repeated oral administration of Indoxacarb, an oxadiazine insecticide, at the dose rate of 1 mg/kg/day for 90 consecutive days produced mild signs of toxicity in buffalo calves[23].Anorexia, inadequate indigestion or absorption due to glyphosate induced damage in gastrointestinal tract might have contributed to hypoproteinaemia to some extent reduced serum total protein might be caused by damage to liver in present study [24].

Serum albumin (g/dl)

Serum albumin were estimated in 8th, 9th, 10th, 11th and 12th week and depicted in **Table 2**. In 8th week (toxin fed quails) values of serum albumin were drastically reduced in T4 group by 125% followed by T6 (86%),T3 and T2 (75%) as compared to control group. 8th week treatment groups was statistically highly significant at 1% and 5% level.

On 11th week (toxin fed quails) of treatment values of serum albumin differ highly significant among groups. Significant reduction is found in T4 group (45%), followed by T6 (42%), T1, T2 (41%), T5 and T3 (32%) as compared to control group.

In withdrawal period i.e. 9th, 10th week (14 days) and 12th week (7 days), there were numerical decreases in serum albumin. All serum values on 9th, 10th and 12th week did not differ significantly. In first week of withdrawal i.e. 9th

week, there were marked reduction in serum albumin as compare to control groups. These values in 9th week were also reduced as compared to 10th and 12th weeks. 10th and 12th week values of albumin were not comparable within groups or within weeks. In 10th weeks (14 days of toxin withdrawal) serum albumin shows normal values nearer to control groups.

Table 2 Serum Albumin (g/dl) levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	3.243±0.443[a]	4.009±0.899	3.909±0.412	3.195±0.092[a]	2.064±0.243
T1	2.180±0.178[bc]	2.055±0.636	3.875±0.714	1.863±0.447[b]	1.802±0.340
T2	1.852±0.319[bc]	1.635±0.542	3.578±0.627	1.863±0.447[bc]	1.788±0.310
T3	1.852±0.319[bc]	2.105±0.724	4.567±0.805	2.159±0.182[bc]	1.121±0.218
T4	1.435±0.106[c]	1.806±0.310	3.184±0.544	1.744±0.272[b]	1.364±0.355
T5	2.696±0.467[ab]	2.225±0.588	4.107±0.286	2.156±0.337[c]	1.444±0.134
T6	1.741±0.300[c]	1.747±0.203	3.898±0.714	1.833±0.158[c]	1.659±0.267
CD	0.945	NS	NS	0.876	NS

Albumin and globulins are the constitutive fraction of total serum protein and among them albumin constituents the major bulk. Decrease in total serum albumin values is observed in liver, kidney and gastrointestinal disease, malnutrition, blood and plasma loss [25]. Decreased values of serum albumin in all the treated quails observed in present study might be due to liver and kidney dysfunction caused by indoxacarb and glyphosate which is evident by the increased ALP levels in all the treatment groups of the present study. Indoxacarb did not cause any change in total proteins, albumin and globulin levels in plasma at both the dose levels, suggesting no effect on protein metabolism at 24mg/kg for 14 and 28 days dose levels [22]. Albumin was significantly increase in 50% lethal dose of indoxacarb but no significant changes in 25% and 15% lethal dose of Indoxacarb as compare to control group [26].

Serum Alkaline Phosphatase (ALP) (IU/L)

Values of serum alkaline phosphatase on 8th, 9th, 10th 11th and 12th week were recorded and depicted in **Table 3**. On 8th week average mean values of ALP were highest in T4 (543.450 ± 70.568) group birds and lowest (313.200 ± 14.325) in control group. Whereas, these ALP values numerically increased in dose dependent Indoxacarb and glyphosate treated birds and statistically were nonsignificant.

Table 3 ALP (IU/L) levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	313.200±14.325	316.517±11.557[a]	311.333±7.482	303.993±55.193[c]	221.700±9.400
T1	386.917±12.111	266.253±9.775[ab]	295.624±19.565	348.160±115.969[bc]	368.233±70.432
T2	425.650±19.534	206.468±56.899[b]	315.432±23.709	667.550±135.398[a]	379.037±84.661
T3	408.567±27.564	281.340±30.630[ab]	301.503±17.894	345.217±72.570[bc]	294.950±57.396
T4	543.450±70.568	291.940±21.757[a]	319.954±21.017	564.950±108.514[ab]	510.517±109.635
T5	471.633±61.859	307.988±27.472[a]	327.183±24.641	432.200±56.558 [abc]	416.643±75.700
T6	483.300±81.207	347.212±4.401[a]	314.725±4.122	448.167±50.500[abc]	376.783±78.690
CD	NS	81.924	NS	242.836	NS

On 11th week (toxin fed birds) ALP values were highest in T2, followed by T4, T6, T5 and T3 as compared to control. All these groups were differ significantly. In withdrawal groups i.e. 9th, 10th and 12th week found nonsignificant except 9th week. In 9t week highest ALP values was in T5 (347.212± 4.401) and lowest was in T2 (206.468 ± 56.66). Statistically all these groups were highly significant. Other withdrawal groups values were near to the normal values. Data depicted that there was dose dependent elevation of serum alkaline phosphatase levels in insecticide and herbicide treated quails.Pathological elevation in serum ALP are commonest in osteoclastic and liver diseases [27].It is also increased in case of the damage of the hepatic cells and obstruction of the bile ducts due to proliferation [28]. Increase in serum ALP activity is probably the consequence of indoxacarb and glyphosate induced pathological changes in the liver. Increase plasma value of ALP in repeated oral administration of Indoxacarb, an

oxadiazine insecticide, at the dose rate of 1 mg/kg/day for 90 consecutive days produced mild signs of toxicity in buffalo calves [23]. Significant increase of Alkaline Phosphatase (ALP) level in broilers fed with 50%, 25 % and 15% lethal dose of indoxacarb as compare to control group [26].

Serum aspartate amininotransferase (AST)(IU/L)

Average mean values of serum aspartate aminotransferase (AST) calculated at the end of 8th, 9th, 10th,11th and 12th week in the quails and depicted in **Table 4**.

Table 4 AST (IU/L) levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	122.770±6.622	122.997±2.437	124.663±3.835	134.923±8.029^{e}	126.747±3.952
T1	142.292±14.301	131.955±14.707	135.288±9.490	152.046±3.868^{de}	137.622±26.066
T2	155.180±12.991	143.120±9.493	139.786±8.311	176.121±6.091^{bcd}	144.846±12.188
T3	127.419±6.097	122.373±2.564	130.707±3.133	155.473±11.189^{cde}	165.707±11.739
T4	169.009±44.465	144.848±6.448	141.515±3.969	178.298±9.956^{bc}	136.515±9.184
T5	176.198±13.299	146.932±12.537	146.932±8.597	195.228±8.439^{ab}	124.432±29.209
T6	191.348±17.905	166.529±14.203	146.529±5.616	211.204±10.799^{a}	133.196±19.895
CD	NS	NS	NS	25.054	NS

On 8th week (toxin fed quails) AST values were numerically highest in combined toxicity groups T6 and T5 (191.348 ± 17.905, 176.198 ± 13.299) followed by higher glyphosate dose group T4 (169.001 ± 44.465), higher dose of indoxacarb group T2 (155.180 ± 12.991) and mild increase in ALT were in low dose of indoxacarb and glyphosate dose as compared to control group. Statistically all these groups were nonsignificant.

On 11th week (toxin fed up to 11th week) significantly highest values were recorded in combine toxicity of groups T6 and T5 (211.204 ± 10.790, 195.228 ± 8.439), followed by higher doses of indoxacarb and glyphosate, T4, T2 (178.298 ± 9.956, 176.121 ± 6.091). Mild increase in AST values were recorded in low dose of indoxacarb and glyphosate and significantly lower (134.923 ± 8.029) was recorded in control group. As dose and duration of toxicity increased the level of AST in the serum were increased.

In treatment withdrawal weeks 9th, 12th (7 days of withdrawal of toxins) there were numerical increase level of AST recorded. Highest numerical values were recorded in combined toxicity groups and higher doses of indoxacab and Glyphosate withdrawal groups. All the groups on the 9th week and 12th week were statistically non significant.

On 14th day (10th week) of withdrawal serum AST levels were nearer to the control group and not comparable to each other. Statistically all groups differ nonsignificantly. On administration of Indoxacarb at a dose rate of 10mg/kg/day for 24 consecutive days produced significant increase in plasma aspartate aminotransferase levels and alanine aminotransferase levels from 0 day to 24th day of treatment, respectively [29]. However, the aminotransferases concentration started decreasing after 7th day of withdrawal of indoxacarb treatment. The increase in aspartate aminotransferse and alanine aminotransferse levels due to subacute and subchronic oral exposure of indoxacarb in broilers and buffalo calves, respectively [26, 30].

Repeated oral administration of Indoxacarb, an oxadiazine insecticide, at the dose rate of 1 mg/kg/day for 90 consecutive days produced significant increase in plasma aspartate aminotransferase (AST) [23]. Activity of AST is usually high in acute and chronic liver injury and muscular damage [31, 32].Alanine aminotransferase is employed as a marker of hepatocellular damage and in general is considered a more sensitive indicator of liver cell injury than AST [33, 34]. Though AST and ALT are not known to have any function in the plasma, yet their increased level in blood indicates cellular damage, increase membrane permeability and their altered metabolism [35, 36]. Although it is difficult to suggest the damage to any particular organ by indoxacarb and Glyphosate, but increase level of aminotransferase in quails is attributed to liver damage, as it is primary organ of biotransformation of insecticide [37].

Serum Alanine Aminotransferase (ALT) (IU/L)

Serum alkaline aminotransferase were calculated on 8th, 9th, 10th, 11th and 12th weeks and depicted in **Table 5**.

On 8th week (toxin treated) quails were showed significant increase in level of ALT in insecticide and herbicide toxicity. The highest level of ALT were observed in combined toxicity groups T5, T6 (52.628 ± 5.292, 50.428 ± 3.072) followed by high dose treated quails T4, T2 (49.693 ± 3.769, 44.289 ± 5.657). Mild elevated levels of ALT

were observed in low dose of toxin either indoxacarb or glyphosate. Lowest level was in control group quails. On 11th week (toxin treated) also similar pattern of 8th week were found. There was significant increase in values of combined toxicity followed by high dose of indoxacarb and glyphosate and mild increase in level of ALT in low doses of toxin as compared to control group. On withdrawal of toxin in 9th week and 12th week (for 7 days withdrawal), 9th week birds shows significant increase in level of ALT. Highest level in T5 (40.128 ± 1.583) and lowest was in control group birds. However, in 12th week (days withdrawal of toxin) statistically all groups were not comparable. Numerical increase in the level of ALT in post withdrawal treatment of high dose groups. Otherwise other group's values were near to normal values.

Table 5 ALT (IU/L) levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	20.536±1.475^{d}	20.036±0.519^{d}	19.270±0.529^{d}	19.536±2.440^{c}	25.195±0.519
T1	27.909±2.104^{cd}	23.626±0.973^{cd}	20.960±0.425^{cd}	27.576±2.292^{c}	24.844±1.030
T2	44.289±5.657^{ab}	34.122±3.554^{ab}	26.955±1.908^{ab}	43.289±7.680^{ab}	25.889±3.554
T3	34.679±6.311^{bc}	29.679±4.750b^{c}	23.179±2.059^{bcd}	32.012±5.742^{bc}	23.106±4.750
T4	49.693±3.769^{a}	39.027±2.290^{a}	28.860±2.948^{a}	48.360±5.150^{a}	40.979±2.290
T5	52.628±5.292^{a}	40.128±1.583^{a}	24.795±1.495^{abc}	52.128±7.268^{a}	30.389±1.583
T6	50.428±3.072^{a}	37.428±1.199^{a}	25.094±1.941^{abc}	45.594±2.870^{ab}	30.158±1.199
CD	12.429	7.358	5.229	15.049	NS

Significant elevation of alanine aminotransferase (ALT) when commercial glyphosate was administered orally at dose level of 4.87, 48.7, 487mg/kg after every two days for 75 days in rats [38].Following an acute injury resulting in moderate to severe zonal necrosis by a liver toxicant, there is generally a moderate to marked increase in the serum ALT and AST activities; suggesting inflammatory process in the liver [39].

In present study also there was significant increase in levels of ALT that correlated with the liver damage as observed during the histopathological examination of liver from the experimental groups.

Serum Cholesterol (mg/dl)

Serum cholesterol were estimated on 8th, 9th, 10th, 11th and 12th weeks and depicted in **Table 6**.

Table 6 Serum cholesterol (mg/dl) levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	113.441±39.457^{b}	191.592±22.495	232.775±11.311	180.752±17.313^{ab}	147.155±12.357
T1	341.418±69.446^{a}	136.405±16.630	196.150±17.352	185.168±20.305^{ab}	147.693±9.568
T2	175.527±29.832^{b}	154.217±14.401	246.333±82.825	195.560±4.285^{a}	150.073±29.467
T3	228.783±37.692^{ab}	149.617±12.161	280.083±8.907	122.857±12.337^{c}	125.460±18.435
T4	203.083±31.661^{b}	140.792±18.243	232.905±50.299	128.380±12.536c	155.985±31.358
T5	190.080±53.965^{b}	145.572±8.496	236.213±36.951	113.727±11.000^{c}	196.107±45.221
T6	199.583±9.236^{b}	147.233±9.914	197.013±24.470	144.702±22.661^{bc}	163.667±18.479
CD	122.753	NS	NS	44.587	NS

On 8th week (toxin fed) serum cholesterol level was highly significant in all treated groups. Highest cholesterol level were found in low toxic groups i.e. T1, T3 (341.418 ± 69.45, 228.78 ± 37.69), moderate in combined toxicity T6, T5 (199.58 ± 9.24, 190.80 ± 53.97), mild increase in T4, T2 (203.08 ± 31.66, 175.53 ± 29.83) and lowest in control group C.

On 11th week (toxin fed) serum cholesterol level is also highly significant in all treated groups. Highest level of cholesterol were found in indoxacarb treated groups T2, T1 (195.56± 4.28, 185.17 ± 20.31), moderate in combined toxicity T6, T5 (144.70 ± 22.66, 113.73 ± 11) and decreased in glyphosate treated groups T3 and T4 (122.86 ± 12.34 & 128.38 ± 12.536) as compared to control group. After withdrawal of toxin on 9th, 12th (for 7days) weeks, all groups there were numerical decrease in serum cholesterol and these values were not comparable. However, in 12th week T5,

T6 withdrawal treatment showed higher cholesterol and lower in T3 post treatment level as compared to normal. All these values were statistically non significant.

10th week (14day) withdrawal serum cholesterol level increased in T3 followed by T2 and T5 and lowered in T1 and T6 as compared to control group. Statistically all these group is nonsignificant.

Data indicated that there was dose dependent elevation and decreases of serum cholesterol levels in insecticide and herbicide treated quails.Depletion of adrenal cholesterol was seen in various stressogenic stimuli (insecticide) [40]. No significant change in cholesterol level of Indoxacarb exposed rat [22]. Increased serum cholesterol activity found in the present study are not in accordance with findings of this could be due to high dose level in the present experiment [22]. Accumulation of pesticide in liver was associated with disturbance of lipid metabolism and elevation of serum cholesterol [41]. Therefore, pesticide induced increase in serum cholesterol can be attributed to the effect of pesticides on the permeability of liver cell membrane also, increase in serum cholesterol level may be attributed to the blockage of liver bile ducts causing reduction of cessation of its secretion to the duodenum[42-44]. Increase in cholesterol level may be a sign of liver damage. Significant increase in serum cholesterol level in mice fed on 7.5mg/kg B.wt per day deltamethrin through feed on 15 day of study [45]. Histopathological alterations in liver found in the present study support the elevation of serum cholesterol level in this experiment.

Serum acetyl cholinesterase (AChE) (IU/L)

Serum acetyl cholinesterase were calculated on 8th, 9th, 10th, 11th and 12th week of experiment and depicted in **Table 7**.

Table 7 Serum acetyl cholinesterase (AChE) (IU/L) levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	2,341.817±123.613	2,040.017±240.450	2,206.683±120.828	2,492.167±288.361[a]	2163.767±41.830[a]
T1	1,904.853±341.410	2,081.217±588.465	2,267.700±741.937	1,771.083±222.921[b]	1905.558±288.017[ab]
T2	2,181.286±724.423	1,971.000±488.860	1,973.833±198.292	1,574.033±218.701[b]	1686.467±223.864[abc]
T3	1,634.972±139.663	2,023.883±358.257	2,049.050±226.584	1,728.162±88.107[b]	1864.200±234.590[ab]
T4	1,938.255±317.305	1,829.778±73.158	1,982.333±536.875	1,267.300±66.451[bc]	1542.050±35.619[bc]
T5	2,023.928±349.955	1,906.850±508.161	1,850.283±161.406	858.938±200.879[c]	1522.133±102.839[bc]
T6	1,781.533±198.638	1,983.288±708.418	1,929.133±476.291	974.742±211.031[c]	1292.700±63.892[c]
CD	NS	NS	NS	574.272	494.308

On 8th week serum acetyl cholinesterase did not differ significantly. There was numerical decrease in cholinesterase in all toxin given quails. Lowest level was found in T3 group quails (1634.97± 139.66) followed by T6 group birds (1781.53 ± 198.64). Highest AchE value was found in control group birds (2341.82 ± 123.61).On 11th week serum cholinesterase was significantly lower in indoxacarb and glyphosate given birds. Lowest AchE found in combine toxicity groups T5 and T6 (858.94 ± 200.88, 974.74 ± 211), followed by high dose of indoxacarb and glyphosate treated groups i.e. T4, T2 (1267.3 ± 66.45 & 1574 ± 218.7) and mild lowest in T1 and T3 as compared to control group C.

In toxin withdrawal weeks i.e. 9th and 12th week (for 7days), almost all groups were shows nonsignificant difference on 9th week. Only combine toxicity groups showed numerical decrease in AchE values. These values were not comparable. In 12th weeks all post treatment groups showed significant decrease in AchE values, even though the toxins were withdrawal. It indicates that effect of toxin was still persisting in the body.

10th week (14 days of toxin withdrawal) quails almost all AchE values were near to normal except combine toxicity groups T5 and T6.Data indicate that there was dose dependent reduction of AchE level in insecticide treated quails. Elevation in plasma cholinesterase levels [22, 23] by 11.8%. 21.9% elevation in erythrocyte cholinesterase levels and 13.8% elevation in whole blood cholinesterase level.

Elevation in plasma cholinesterase levels [29]from 0 day to 24th which returned to normal after 7 days of withdrawal of insecticide but there was no change in erythrocyte cholinesterase level and whole blood cholinesterase levels suggesting slight inhibitory effect of indoxacarb on neurological system of insects [46].

Indoxacarb insecticide significantly reduced the activity ofAChE with 33.4, 29.6, 15.3 μMATChI mg-1 protein compared to 41.3 for the control [47]. Abamectin and indoxacarb showed similar trend of the reduction of AChE activity, where they were not significantly different. Sumithion was the most potent inhibitor of AChE with 62% compared to ABM and IND with 19 and 28% reduction in AChE activity of control, respectively. Current results were in agreement with [48, 49] who reported that abamectin decreased the AChE activity. Acetyl cholinesterase is a

biomarker in the toxicological examinations of exposure to cholinesterase-inhibiting pesticides (organophosphates and carbamates) [50, 51].

Serum Creatinine (mg/dl)

Serum creatinine were estimated on 8th, 9th, 10th, 11th, and 12th week and depicted in **Table 8**. On 8th week (toxin fed quails) numerical increase in serum creatinine level were found. Statistically all groups did not differ significantly. Highest creatinine level were found combine toxicity groups T5, T6 (1.123 ± 0.13, 122 ± 0.28) and in low dose of glyphosate (1.243 ± 0.165) and lowest in control in control group (0.739 ± 0.131). There were dose dependent increases in level of serum cratinine in the quails.

Creatinine level in 11th week (toxin fed quails) was significantly increased in all groups. Highest level of creatinine was in high dose of indoxacarb i.e T2 (3.53 ± 0.24) followed by T3 (3.16 ± 29), T5, T4 (2.81 ± 0.33, 2.66 ± 0.38) and lowest in control group (1.49 ± 0.08). As compared to 8th week values of serum creatinine was higher indicating duration effect of toxin on quails. There were dose and duration dependent increase in level of serum creatinine observed.

Table 8 Serum Creatinine (mg/dl)levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	0.739±0.131	0.847±0.047	0.961±0.067^{ab}	1.493±0.080^{d}	1.833±0.134^{b}
T1	0.918±0.181	2.005±0.541	0.864±0.023^{bc}	1.745±0.597^{cd}	2.388±0.327^{b}
T2	0.787±0.110	1.878±0.394	0.990±0.008^{ab}	3.534±0.236^{a}	1.695±0.411^{b}
T3	1.243±0.165	1.532±0.250	0.860±0.025^{bc}	3.162±0.288^{a}	2.693±0.483^{ab}
T4	0.943±0.198	1.712±0.229	1.038±0.093^{a}	2.662±0.379^{abc}	1.817±0.243^{b}
T5	1.226±0.131	1.544±0.402	0.853±0.031^{bc}	2.809±0.325^{ab}	1.852±0.432^{b}
T6	1.220±0.284	1.895±0.274	0.798±0.043^{c}	2.196±0.187^{bcd}	3.614±0.398^{a}
CD	NS	NS	0.143	0.966	1.051

Withdrawal of toxin for 7days on 9th and 12th week levels of creatinine noted. On 9th week numerical increase in the level of creatinine was observed. Highest level of creatinine was noted in indoxacarb post treatment group T1 and T2 (2.05 ± 0.54, 1.88 ± 0.39) followed by combination of both toxin T6 (1.8 ± 0.27), high dose of glyphosate T4 (1.71 ± 0.22), T5 and T3 as compared to control group. On 12th week post treatment groups showed significantly reduction as well as increased serum creatinine level was found. In low doses of Indoxacarb, glyphosate and combination of both doses showed higher creatinine level. Other values were nearer to the control group.

Post treatment of 14th day i.e. 10th week showed significantly decrease in creatinine levels except high dose of either indoxacrb or gyphosate. It indicates that toxic effect of high dose was persisting up to 14th day of post treatment. All values other than high dose of toxin were normal on 14th day of treatment. Creatinine is derived from phosphocreatine in muscle. The levels of creatinine remain constant in the blood. Traumatic or infectious damage to the kidney leads to its elevation. Creatinine level in blood is important indication of renal functional status [34] [24]. Further presence of this substance in blood is indication of marked impairment of kidney function. Exposing broilers to indoxacarb at 50%, 25% and 12% lethal dose and found significant increase in serum creatinine level in indoxacarb treated groups as compare to control group and it is observed that it damages to kidney [26]. 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 and found increase plasma creatinine level in rats at 24 mg/kg indicated some toxic effect of indoxacarb on kidney [22].

Present findings are in agreement with [23, 29], who reported significant elevation in levels of blood creatinine from 0 days to 24th day by exposing 10mg/ kg B.wt. indoxacarb of the study However, the values became normal after the withdrawal of insecticide on 7th day.

Gamma-glutamyltranspeptidase (GGT) (IU/L)

GGT were estimated on 8th, 9th, 10th, 11th and 12th week and depicted in **Table 9**. GGT was significantly higher in all indoxacarb and glyphosate treated groups either 8th week or in 11th week as compared to control group. There was dose dependent increase in value of GGT was observed. In 8th week highest GGT level was found in T5 (29.40 ±

0.89) followed by T6 (28 ± 1.58), T4 (28.14 ± 0.74), moderate increase in T2 (23.27 ± 1.57) and mild increase in T1 and T3 groups as compared to control group. Similar pattern were found in 11th weeks quails.

After withdrawal of toxin up to 7 days i.e. in 9th week and 12th week both the weeks shows significant increase in level of GGT. In 9th week serum GGT level were reduced as compare to 8th week. Highest reduction was observed in T6 (27%) followed by T5g (26%), T4 (21%), T1 (16%), and T3, T2 (15%) as compared to 8th week values of GGT. In 12th week (7 day withdrawal of toxin) highest reduction was observed in T5 (37%) followed by T6 (32%), T4 (27%), T2 (18%), T1 (16%) and lowest in T3 (10%) as compared to 11th week. After withdrawal of toxin up to 14 day GGT values significantly reduced as compared to 8th week and increased as compared to control. Highest reduction in GGT value was observed in T4 (52%) followed by T6 (45%), T5 and T3 (43%), T2 (37%) and lowest in T1 (33%) as compared to 8th week.

Data indicate that there was dose dependent increase level of GGT. As withdrawal days increases value of GGT also reduced as compared to toxin treated group.

Gamma - glutamyltranspeptidase (GGT) is a membrane bound glycoprotein which catalyses the transfer of Gamma - glutamyl group to other peptides and amino acids. It is present in large amounts in the kidneys, pancreas, liver, intestine and prostate glands.

Table 10 Gamma-glutamyltranspeptidase (GGT) (IU/L) levels in Indoxacarb and Glyphosate treated groups quails.

Table 9 Serum GGT (IU/L)levels in Indoxacarb and Glyphosate treated groups quails

Weeks / Group	8th week (Toxin treated)	9th week (7days toxin withdrawal after 8th week)	10th week (14days toxin withdrawal after 8th week)	11th week (Toxin treated)	12th week (7days toxin withdrawal after 11th week)
C	11.893±0.431^{d}	10.393±0.333^{c}	10.060±0.316^{e}	11.393±0.566^{d}	11.252±2.078^{c}
T1	18.744±1.461^{c}	15.744±1.255^{b}	12.411±0.411^{cd}	17.744±1.297^{c}	14.778±1.161^{abc}
T2	23.278±1.568^{b}	19.778±0.684^{a}	14.611±1.149^{abc}	21.945±1.964^{b}	17.878±1.446^{ab}
T3	18.173±0.825^{c}	15.339±0.417^{b}	10.339±0.436^{de}	15.839±0.854^{c}	14.100±1.662^{bc}
T4	28.140±0.748^{a}	21.973±0.410^{a}	13.473±0.727^{bc}	25.473±0.672^{ab}	18.440±0.332^{a}
T5	29.402±0.894^{a}	21.735±1.261^{a}	16.568±1.180^{a}	28.235±1.521^{a}	17.702±0.949^{ab}
T6	28.000±1.580^{a}	20.333±0.841^{a}	15.333±0.793^{ab}	27.500±1.970^{a}	18.555±1.163^{a}
CD	3.326	2.386	2.267	3.964	3.911

Indoxacarb and glyphosate induced elevation in GGT might have occurred due to hepatic damage is supported in birds, high levels of serum γ-GT are commonly used as an index of liver disease, as well as damages in biliary ducts and renal epithelium, but it also increases in female birds, apparently reflecting increased liver metabolism due to egg laying [52, 53].

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