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Haematological Studies on Atrazine Induced Toxicity in Broilers

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Abstract: *The present study was undertaken to elucidate the haematological alteration in the atrazine toxicity in broilers. For this study, 120 day old broiler chicks were randomly divided into four groups comprising 30 birds in each group. The dose of atrazine was selected on the basis of lethal dose of the herbicide in birds. The herbicide was given to 2nd, 3rd and 4th group of the experimental birds in the broiler starter ration for the study period of 28 days respectively.*

The results revealed that hemoglobin, PCV and TEC mean values were decreased in atrazine birds. The erythrocyte indices in the birds of group 2nd and 3rd indicated microcytic hypochromic anemia while group 4th showed macrocytic hypochromic anemia.

Leucocytopenia in the birds of group 4th at every intervals and leucocytosis in the birds of group 2nd and 3rd on day 28 was observed. Lymphocytopenia was a constant feature in all the toxicity groups as compared to value of control group. Significant heterophilia in birds of group 3rd and 4th and monocytosis in the birds of group 2nd, 3rd and 4th were observed.

I. Introduction

The herbicide atrazine is widely used for selective control of broadleaf and grassy weeds in corn and sorghum in addition to other crops. Atrazine is prepared from cyamuric chloride, which is treated sequentially with ethylamine and isopropyl amine. The primary target of Atrazine in animals and humans is the endocrine system as it is an endocrine disruptor which alters the natural hormonal system in animals. Atrazine has been linked to limb deformities, abnormal sexual changes, weakened immune systems, and declining populations of frogs and amphibians. Atrazine persists in the soil from 7 to 18 months depending upon soil properties, influence of weather, and the soil environment (Burnside et al. 1971). Atrazine breaks down in soil and water as a result, it is frequently detected as a contaminant in streams, rivers, lakes and drinking water resulted in the health hazards.

Atrazine caused suppression of haemoglobin, erythrocyte count and packed cell volume which was early observed by Wei and McMuirety (1996) in layers after subjected to atrazine exposure. Kim and Larson (1999) observed that atrazine exposure in Japanese quail cause reduction in circulating erythrocytes. Cho et al. (1989) noticed that there was Neutrophilia and lymphocytopenia in rats after being exposed with atrrazine. The lethal dose of atrazine is 500 mg/kg b.wt for birds.

Keeping in view the paucity of information regarding the toxicity of the chemical, the present study shall be undertaken in broiler chickens in order to study the hematological alterations in atrazine induced toxicity in broiler chickens .

II. Material And Methods

A total number of 120 healthy broiler chicks was procured from the market and used for experimental purpose. All the chicks were vaccinated against Marek's disease on the first day of hatching. The chicks were given Furasol @ ½ gram per liter of water for 5 days in the first week of experiment used to prevent coccidiosis in chicks. The chicks were given broiler starter ration for two weeks followed by broiler finisher ration as per standards.

Experimental design

The birds were randomly divided into five groups containing 30 birds in each group . The control group received fungicide free diet, where as acute and subacute groups receive the fungicide at different dose rate based on the LD 50, which is given in the table.

Group	No. of Birds	Drug concentration
1st	30	No herbicide(Control)
2nd	30	375mg (3/4 of lethal dose)
3rd	30	250mg(1/2 of lethal dose)
4th	30	125mg(1/4 of lethal dose)

For hematological investigations, blood samples were collected from wing vein of chicks. Roughly 3ml blood from each bird was collected in sterile vials. The blood was collected at 24 hours interval in acute case and in subacute case after 28 days interval for the estimation of Haemoglobin, Packed Cell Volume, Total Erythrocyte Count, Erythrocyte indices, Total Leucocyte Count and Differential Leucocyte Count .

Statistical Analysis

The data was analyzed by applying two way analysis of variance in the program CRD.

Hematological Observations

Treatment Group II (3/4th of Lethal Dose)

The analysis of variance indicated significant (P<0.05) decrease in the mean values of PCV, Hb, MCV ,MCH and MCHC values during the study period. The analysis of variance indicated non significant change in TLC and TEC in between groups

Treatment Group III (1/2nd of Lethal Dose)

The analysis of variance indicated significant (P<0.05) increase in level of PCV in between groups (group 3rd vs. control). The analysis of variance indicated significant (P<0.05) decrease in the mean values of Hb, MCV ,MCH and MCHC values during the study period

The analysis of variance mean values of TLC indicated significant (P<0.05) decrease in between groups (group 3rd vs. control) on interval 21 and 28 days post treatment as shown in fig

Treatment Group IV (1/4th of Lethal Dose)

The analysis of variance indicated significant (P<0.05) decrease in the mean values of Hb, MCV ,MCH , MCHC and TLC values during the study period. The analysis of variance indicated significant (P<0.05) increase in level of PCV in between groups (group 4th vs. control.

Evaluation of DLC revealed a significant (P≤0.05) alteration in mean heterophil and lymphocyte count when compared with control birds.

Table 01: Mean Values ± Se Of Pcv, Hb, Tec, Mcv, Mch And Mchc In The Birds Of Control And Group 2ND At Different Time Interval

GROUP 2nd		7 DAYS	14 DAYS	21 DAYS	28 DAYS
PCV(%)	Control	33.427± 0.03_{b}	$30.340\pm0.680_{a}$	$32.522\pm0.290_{b}$	$34.522\pm0.290_{a}$
	Intoxicated	$33.26\pm0.04^{a}{}_{A}$	$34.0\pm0.10_{C}{}^{b}$	$33.90\pm0.50_{B}{}^{a}$	$32.90\pm0.3^{a}{}_{B}$
Hb(g/dl)	Control	9.3 ± 0.50^{a}	8.72±0.49	8.6 ± 0.8^{b}	8.5 ± 0.5^{b}
	Intoxicated	$9.6\pm0.8^{b}{}_{C}$	$8.52\pm0.49_{B}$	$8.28\pm0.8_{A}{}^{a}$	$8.10\pm0.5_{A}{}^{a}$
TEC(million/mm3)	Control	2.12±0.23	2.08±0.47	2.06±0.40	2.02±0.43
	Intoxicated	$2.12\pm0.34_{B}$	$2.08\pm0.23_{B}$	$2.07\pm0.40_{A}$	$2.06\pm0.43_{A}$
MCV (FI)	Control	159.47 ± 1.87^{b}	163.44±1.86	165.20 ± 1.60^{b}	170.53 ± 1.32^{b}
	Intoxicated	$154.01\pm2.13_{A}{}^{a}$	$164.30\pm3.63_{B}$	$164.30\pm3.0_{B}{}^{a}$	$164.52\pm3.38_{B}{}^{a}$
MCH (Pg)	Control	44.39±0.47	41.86±0.51	41.70 ± 0.40^{a}	42.84 ± 0.44^{a}
	Intoxicated	$45.70_{A}\pm0.72_{A}$	$41.13\pm1.02_{B}$	$41.20\pm1.00_{B}{}^{b}$	$40.10\pm0.80_{B}{}^{b}$
MCHC(gm/dl)	Control	$27.85\pm0.51_{a}$	25.61±0.12	25.50±0.60	25.12±0.21
	Intoxicated	$29.10\pm0.26_{A}{}^{b}$	$25.02\pm0.10_{b}$	$24.80\pm0.10_{C}$	$24.67\pm0.11_{C}$

- The value with different superscripts / subscripts are different significantly in between groups.
- The value with no superscripts / subscripts are having no significant relationship.

Table 02: Mean Values ± Se Of Pcv, Hb, Tec, Mcv, Mch And Mchc In The Birds Of Control And Group 3RD At Different Time Interval

GROUP-3rd		7 DAYS	14 DAYS	21 DAYS	28 DAYS
PCV(%)	Control	33.47 ± 0.35^{a}	30.34 ± 0.68^{a}	32.52 ± 0.29^{b}	34.52 ± 0.29^{b}
	Intoxicated	$35.08\pm0.24_{B}{}^{b}$	$32.77\pm0.40_{A}{}^{b}$	$32.50\pm0.50_{A}{}^{a}$	$32.33\pm0.41_{A}{}^{a}$
Hb(g/dl)	Control	9.32 ± 0.49^{b}	8.72 ± 0.49^{b}	8.68 ± 0.80^{b}	8.58 ± 0.50^{b}
	Intoxicated	$8.97\pm0.44_{C}{}^{a}$	$8.12\pm0.49_{B}{}^{a}$	$8.00\pm0.80_{A}{}^{a}$	$7.52\pm0.49_{A}{}^{a}$
TEC(million/mm3)	Control	2.10 ± 0.23^{a}	2.08 ± 0.23^{b}	2.06 ± 0.20^{b}	2.02 ± 0.19^{b}
	Intoxicated	$2.28\pm0.27_{C}{}^{b}$	$1.69\pm0.18_{B}{}^{a}$	$1.68\pm0.10_{A}{}^{a}$	$1.16\pm0.20_{A}{}^{a}$
MCV (FI)	Control	159.47±1.87	163.44±1.86	165.20±1.60	170.53±1.32
	Intoxicated	153.69±1.79	193.73±2.16	200.10±1.15	177.24±0.39
MCH (Pg)	Control	44.39 ± 0.47^{a}	41.86 ± 0.51^{a}	41.70 ± 0.40^{a}	42.84 ± 0.44^{a}
	Intoxicated	$39.06\pm0.27_{A}{}^{b}$	$47.96\pm0.49_{B}{}^{b}$	$50.40\pm0.25_{C}{}^{b}$	$64.45\pm0.34_{C}{}^{b}$
MCHC(gm/dl)	Control	27.85 ± 0.51^{a}	25.61 ± 0.12^{a}	25.50 ± 0.60^{a}	25.12 ± 0.21^{a}
	Intoxicated	$25.56\pm0.12_{C}{}^{b}$	$24.66\pm0.17_{B}{}^{b}$	$24.0\pm0.10_{A}{}^{b}$	$23.24\pm0.16_{A}{}^{b}$

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Table 03: Mean Values ± Se Of Pcv, Hb, Tec, Mcv, Mch And Mchc In The Birds Of Control And Group 4TH At Different Time Interval

GROUP- 4th		7 DAYS	14 DAYS	21 DAYS	28 DAYS
PCV(%)	Control	33.47 ± 0.35^{a}	30.34 ± 0.68^{a}	32.52 ± 0.29^{a}	34.52 ± 0.29^{a}
	Intoxicated	$34.94\pm0.38_{C}{}^{b}$	$33.47\pm0.35_{B}{}^{b}$	$33.40\pm0.50_{A}{}^{b}$	$33.52\pm0.19_{A}{}^{b}$
Hb(g/dl)	Control	9.32±0.49	8.72±0.49	8.68±0.80	8.58±0.50
	Intoxicated	$8.68\pm0.80_{C}$	$8.12\pm0.80_{B}$	$8.00\pm0.80_{A}$	$7.52\pm0.75_{A}$
TEC(million/mm^3)	Control	2.1±0.23	2.08±0.23	2.06 ± 0.20^{b}	2.02 ± 0.19^{b}
	Intoxicated	$2.19\pm0.40_{BC}$	$2.06\pm0.30_{B}$	$1.90\pm0.10_{A}{}^{a}$	$1.61\pm0.30_{A}{}^{a}$
MCV (FI)	Control	159.47±1.87	163.44 ± 1.86^{b}	165.20±1.60	170.53±1.32
	Intoxicated	$159.06\pm2.78_{BC}$	$162.29\pm0.33_{B}{}^{a}$	$190.10\pm1.15_{A}$	$207.83\pm0.39_{A}$
MCH (Pg)	Control	44.39 ± 0.47^{b}	41.86 ± 0.51^{b}	41.70 ± 0.40^{a}	42.84 ± 0.44^{a}
	Intoxicated	$39.50\pm0.66_{C}{}^{a}$	$39.37\pm0.37_{B}{}^{a}$	$40.40\pm0.25_{A}{}^{b}$	$44.88\pm0.41_{A}{}^{b}$
MCHC(gm/dl)	Control	27.85 ± 0.51^{a}	25.61 ± 0.12^{b}	25.50 ± 0.60^{b}	25.12 ± 0.21^{b}
	Intoxicated	$24.84\pm0.24_{C}{}^{b}$	$24.25\pm0.24_{B}{}^{a}$	$24.0\pm0.10_{A}{}^{a}$	$21.59\pm0.21_{A}{}^{a}$

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Table 04: Mean Values ± Se Of Tlc & Dlc In The Birds Of Control And Group 2ND At Different Time Interval

GROUP 2nd		7 DAYS	14 DAYS	21 DAYS	28 DAYS
TLC(10/mm^3)	Control	22.50 ± 0.16^{a}	19.65 ± 0.13^{a}	19.70 ± 0.10^{a}	19.90 ± 0.36^{a}
	Intoxicated	$22.29\pm0.10_{B}{}^{b}$	$20.20\pm0.11_{B}{}^{b}$	$20.10\pm0.10_{A}{}^{b}$	$19.18\pm0.19_{A}{}^{b}$
LC(%)	Control	73.8±1.15	69.2±2.39	70.3±0.40	70.4±1.07
	Intoxicated	$71.4\pm0.87_{B}$	$69.8\pm0.58_{A}$	$70.2\pm0.50_{A}$	$72.8\pm1.68_{A}$
H(%)	Control	22.74±0.47	24.93±0.54	25.00 ± 0.10^{a}	25.23±0.49
	Intoxicated	$25.07\pm0.67_{A}$	$25.83\pm0.23_{B}{}^{b}$	$25.0\pm0.20_{A}$	$24.11\pm1.12_{A}$
L/H	Control	4.95 ± 0.22^{b}	3.89 ± 1.98^{a}	3.70±0.10	3.89±0.20
	Intoxicated	4.01 ± 0.26^{a}	3.67 ± 0.08^{b}	3.90±0.10	4.49±0.45
M(%)	Control	4.80±0.37	4.40 ± 0.51^{b}	4.60 ± 0.40^{a}	4.80 ± 0.37^{a}
	Intoxicated	$4.4\pm0.51_{B}$	$4.8\pm0.37_{A}{}^{a}$	$4.5\pm0.30_{C}{}^{b}$	$4.4\pm0.24_{C}{}^{b}$
E(%)	Control	5.00 ± 0.54^{b}	5.80 ± 0.37^{b}	5.20±0.40	4.80±0.37
	Intoxicated	4.40 ± 0.51^{a}	4.80 ± 0.37^{a}	4.50±0.20	4.40±0.24
B(%)	Control	1.60±0.40	1.20±0.20	1.20±0.20	1.60±0.24
	Intoxicated	1.40±0.24	1.00±0.05	1.10±0.02	1.6±0.40

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- The value with no superscripts / subscripts are having no significant relationship

Table 05: Mean Values ± Se Of Tlc & Dlc In The Birds Of Control And Group 3RD At Different Time Interval

GROUP 3rd		7 DAYS	14 DAYS	21 DAYS	28 DAYS
TLC(10/mm^3)	Control	22.50±0.16	19.65±0.13	19.70±0.10	19.90±0.36
	Intoxicated	$24.70_{c}\pm0.52$	$13.85\pm0.93_{A}$	$12.50\pm0.40_{B}$	$11.45\pm0.98_{B}$
LC(%)	Control	73.8±1.15	69.2±2.39	70.3±0.40	70.4±1.07
	Intoxicated	54.4±1.96	53.6±2.15	55.5±1.15	64.2±0.86
H(%)	Control	22.74±0.47	24.93±0.54	25.00±0.10	25.23±0.49
	Intoxicated	36.61±1.04	32.87±1.19	30.10±0.20	27.95±0.61
L/H	Control	4.95±0.22	3.89±1.98	3.70±0.10	3.89±0.20
	Intoxicated	$1.55\pm0.12_{A}$	$1.87\pm0.17_{A}$	$2.00\pm0.10_{B}$	$2.94\pm0.15_{B}$
M(%)	Control	4.80±0.37	4.40±0.51	4.60±0.40	4.80±0.37
	Intoxicated	3.60±0.40	12.20±1.24	5.20±0.10	6.20±0.49
E(%)	Control	5.00±0.54	5.80±0.37	5.20±0.40	4.80±0.37
	Intoxicated	$5.00\pm0.31_{A}$	$4.20\pm0.58_{B}$	$4.80\pm0.20_{A}$	$5.40\pm0.24_{A}$
B(%)	Control	1.60±0.40	1.20±0.20	1.20±0.20	1.60±0.24
	Intoxicated	1.40±0.24	2.20±0.37	2.00±0.40	2.20±0.37

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- The value with no superscripts / subscripts are having no significant relationship

Table 06: Mean Values ± Se Of Tlc & Dlc In The Birds Of Control And Group 4TH At Different Time Interval

GROUP 4th		7 DAYS	14 DAYS	21 DAYS	28 DAYS
TLC(10/mm3)	Control	22.50±0.16	19.65±0.13a	19.70±0.10	19.90±0.36b
	Intoxicated	21.03±0.26C	22.62±0.14A	14.50±0.40B	15.40±0.14B
LC(%)	Control	73.8±1.15b	69.2±2.39b	70.3±0.40b	70.4±1.07b
	Intoxicated	60.60±1.16aA	67.4±1.12Aa	60.50±1.15Ba	62.60±2.24Ba
H(%)	Control	22.74±0.47a	24.93±0.54a	25.00±0.10	25.23±0.49
	Intoxicated	30.93±0.75Ab	25.53±0.37Bb	25.50±0.47C	28.18±1.18C
L/H	Control	4.95±0.22b	3.89±1.98b	3.70±0.10b	3.89±0.20b
	Intoxicated	2.18±0.12Aa	3.63±0.32Ba	2.00±0.10Ca	2.88±0.30Ca
M(%)	Control	4.80±0.37	4.40±0.51a	4.60±0.40	4.80±0.37
	Intoxicated	4.80±0.37A	7.40±0.51Cb	6.40±1.10B	8.80±1.86B
E(%)	Control	5.00±0.54	5.80±0.37b	5.20±0.40	4.80±0.37
	Intoxicated	4.60±0.40	4.60±0.24a	4.80±0.30	5.00±0.31
B(%)	Control	1.60±0.40	1.20±0.20	1.20±0.20	1.60±0.24
	Intoxicated	2.00±0.31	2.00±0.31	2.05±0.20	1.20±0.20

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- The value with no superscripts / subscripts are having no significant relationship

III. Discussion

The PCV value significantly increased up to day 21 and then decreased in group 2nd and 3rd where as in group 4th it significantly decreased at every interval. Low PCV together with decline in Hb and TEC revealed liver damage accompanied with anemia (Benzamin, 1989). In group 4th the TEC decreased till 7 day and then increased significantly till 28 day. These values revealed development of variable degree of anemia due to atrazine toxicity and confirmed with the observation of American society worker who reported anemia in birds having atrazine toxicity on the basis of decrease of Hb and TEC values (Coles, 1967). Contrary to our observations, Blood, (1989) reported increase in the value of Hb in experimental rats intoxicated with atrazine herbicide.

The mean values of MCV and MCH in the birds of group 2nd and 4th showed significant decrease presented a picture of microcytic hypochromic anemia in this study. This type of the anemia in human having atrazine toxicity are normocytic hypochromic in nature. In the birds of group 2nd and 3rd, the mean value of MCH and MCV showing significant increase at most of intervals of experiment revealed macrocytic and hypochromic anemia and suggested a recovery phase and a responsive condition of bone marrow to produce large number of erythrocytes (Coles,1967).

The values of TLC in the birds of group 2nd, 3rd and 4th showed a tendency of increase on day 7 and 21 and marked decrease on day 28 suggested leucocytosis and thereafter toxic suppression of bone marrow later.

Significant lymphocytopenia observed in birds of all the toxicity groups in acute condition on indicated depletion of lymphoid reserve of the body. The observation of lymphocytopenia has been postulated in stress condition (Benzamin, 1989)

IV. Conclusion

Haematological examination showed anaemia together with haemoconcentration and decreased TEC count. The number of heterophills was considerably increased after the exposure of herbicide in birds.

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