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Clinical and haematological changes induced by chlorpyrifos and amelioration by cow urine distillate in broilers

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Abstract
Day old broiler chicks (n=60) were divided into four groups comprising 15 birds in each. Group I served as control, group II was treated with chlorpyrifos @ 50 ppm in feed, group III was treated with cow urine distillate @ 10 ml/litre of drinking water, group IV was treated with chlorpyrifos @ 50 ppm and cow urine distillate @ 10 ml/litre of drinking water for a period of 28 days. Few birds from group II exhibited closed eyes and diarrhoea. Significant decrease was noted in body weight gain of group II whereas improved body weight gain was noted in group IV. Differences of TEC, MCV and MCH were found to be significant between different treatment group birds. The significant decrease in TLC values was recorded in groups II however; progressive increase was recorded in group IV. The significant decrease in lymphocyte count and increase in heterophil count was observed in group II. Co-administration of cow urine distillate against chlorpyrifos restored these changes in group IV. This study indicated that, subacute exposure of chlorpyrifos @ 50 ppm in feed has adverse effect on clinical and haematological observations and co-administration of cow urine distillate ameliorated these changes.

Keywords: Broiler, chlorpyrifos, cow urine distillate

Introduction
Chlorpyriphos (O, O-diethyl O-3,5, 6-trichloro- 2-pyridyl phosphorothioate**)** is broad spectrum insecticide, widely used for domestic and agricultural applications throughout the world. It is used as insecticide on grain, fruit nut, and vegetable crops. It is also applied to the soil surrounding or beneath buildings as protection against termites including chicken houses [1]. Chickens are more commonly affected with pesticide toxicity because poultry houses are frequently dusted with pesticides. Studies have shown that chlorpyrifos has a very narrow margin of safety in birds and accumulates in their system [2]. Chlorpyrifos induces toxicities by generating free radicals and by altering the levels of enzymatic and non-enzymatic antioxidant defenses [3]. Therefore, supplementation of antioxidants can be considered as the alternative method.

In poultry, cow urine enhances the immune-competence status of birds and has positive effect on their body weight gain, haematological profiles [4] and immunomodulatory effect on both the humorol and cellular immune response [5]. Cow urine is able to clear the toxins from the system and act as an anti-toxin that protects the body from various types of poisons [6]. Though, there are numerous claims of the efficiency of cow urine, its efficiency against pesticide-induced toxicity has been rarely explored. The present study is aimed to evaluate subacute chlorpyrifos toxicity in broilers and to determine protective role of cow urine distillate on chlorpyrifos-induced clinical and haematological alterations.

Materials and Methods
60, day old broiler chicks procured from Amruta Hatcheries Pvt. Ltd., Pune were acclimatized for one week and divided into four groups comprising 15 birds in each group kept on *ad libitum* supply of feed and water. The experimental trial was approved by the Institutional Animal Ethics Committee (Reg. No. 312/CPCSEA).

Chemical (Insecticide)
Chlorpyrifos, Technical grade (C9H11Cl3NO3 PS; 98.06% by mass, 0.1% moisture content by mass and 0.1% acidity as H_2SO_4% by mass) was procured from Krishi Rasayan Export

Pvt. Ltd., Samba, Jammu, India. All other chemicals used in the study were of analytical grade.

Cow urine Distillate
The cow urine was collected from indigenous cows raised under standard feeding and managemental conditions from Aadarsh Goseva Evam Anusandhan Prakalp, Akola. Fresh cow urine at morning time was collected in sterile containers and then urine was filtered through Whatman filter paper no.1. Distillate was prepared from filtered cow urine using distillation apparatus. The distilled cow urine was stored in air tight bottle until use.

Experimental Design
Sixty, day old broiler chicks were randomly allocated to four equal groups, fifteen birds in each. Group I served as control, group II was treated with chlorpyrifos @ 50 ppm in feed, group III was treated with cow urine distillate @ 10 ml/litre of drinking water, group IV was treated with both chlorpyrifos @ 50 ppm and cow urine distillate @ 10 ml/litre of drinking water for a period of 28 days.

Clinical Observations
Birds of all the groups were closely observed throughout the period of experiment (28 days) twice a day for development of clinical signs. Body weights of all the birds, body weight gain, feed consumption, feed conversion ratio and water consumption were recorded at weekly intervals.

Haematological Investigations
At the end of 28th days of experimental period, blood samples from six birds of each group were collected into dry sterilized vials containing anticoagulant, EDTA (1mg/ml). The haematological estimations, haemoglobin concentrations (Hb), packed cell volume (PCV), total erythrocyte counts (TEC), total leucocyte counts (TLC) and differential leucocyte counts (DLC) were carried out. The values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived from the values of Hb, PCV and TEC [7].

Statistical Analysis
The data was subjected to statistical analysis by applying simple CRD [8].

Results and Discussion
In chlorpyrifos group, diarrhoea was observed from 2nd week post intoxication and nervous symptoms of closed eyes, dropped neck, dropping of wings and inability to stand were exhibited after 2 weeks post intoxication. These findings were supported with the observations of earlier workers in layers [9]. These signs were absent in group IV supplemented with cow urine distillate @ 10 ml/liter of drinking water along with chlorpyrifos indicating its ameliorative action. Cow urine enhances immune competence and improves general health of individual [10]. Cow urine distillate possesses bioenhancing ability by facilitating absorption of drugs across the cell membrane [11].

Body Weight
The body weight difference was not-significant between the birds of various treatment groups as compared to control group (Table 1). At the end of 3rd and 4th week, group II showed significant reduction whereas group IV revealed significant increase in body weight gain.

Feed consumption and feed conversion ratio (FCR)
At the end of 4th week of experiment, no significant difference was evident in average feed consumption within different groups. However, higher feed consumption was recorded in group II as compared to control. The cumulative means for FCR were non-significant but higher value of FCR was recorded in group II and the lowest cumulative mean value was recorded in group IV. Decrease in feed consumption during chlorpyrifos toxicity is in accordance with the findings of earlier worker in quails [12]. Increased FCR in chlorpyrifos treated broilers is supported by earlier worker [13]. No significant difference in feed consumption and higher FCR in Panchagavya supplemented broiler chickens as compared to control were also reported [14].

Water Consumption: At the end of 5th week of experiment, no significant difference was evident in average water consumption within different groups (Table 1).

Haematological Investigations
The haemoglobin and PCV levels showed non-significant variations in treated groups as compared to control group (Table 2). The decrease in mean values of TEC were recorded in groups II and IV whereas group I and III recorded significant ($P\leq 0.05$) increase in these values. These results indicated that chlorpyrifos might be exhibiting suppressive action on erythropoiesis. This observation is in accordance with the opinion of earlier worker who expressed decreased count of erythrocytes in broiler chicks [15]. The lower mean value of MCV was recorded in group III and higher mean value was observed in group II. Birds received chlorpyrifos in combination with cow urine distillate showed decrease in MCV value as compared to group II. Higher value was recorded in group II when compared with control. The birds received chlorpyrifos in combination with cow urine distillate showed numerical decrease in MCH value as compared to group II birds fed with chlorpyrifos alone. The values showed non-significant difference in average MCHC within different groups. Amelioration with cow urine distillate resulted in improved blood parameters.
In the present study, the findings of haematological alteration might be due to chlorpyrifos induced stress, decreased erythropoiesis or toxic damage to vital organs particularly spleen, liver and kidney which directly or indirectly related with haematopoiesis [16].
In cow urine distillate treatment group, significant increase in TEC and non-significant increase in Hb and PCV values were recorded in birds of III and IV groups as compared to birds of II group. The presence of hormone erythropoietin and traces of iron in cow urine which stimulates the bone marrow of long bone indicating the utility of cow urine in treatment of anemia [17]. The significant difference ($P\leq 0.05$) in TLC values was recorded in groups II and IV as compared to control group. The highest values were recorded in groups I and III where as the lowest value in group II. However, it was observed that birds received chlorpyrifos along with cow urine distillate @ 10 ml/liter of drinking water showed progressive increase in TLC values suggesting beneficial effect of cow urine distillate. Present findings were supported by various workers in birds [15, 18]. Beneficial role of cow urine distillate in restoring TLC values were also reported earlier [19].
The significant difference was recorded in lymphocyte count

in group II birds when compared to control and other treatment groups. The highest count was recorded in group IV whereas it was lowest in group II birds. Group IV showed increase in the average lymphocyte count when compared to chlorpyrifos treated group II indicative of beneficial effect of cow urine distillate against chlorpyrifos toxicity. Decreased lymphocyte count during chlorpyrifos toxicity in present study is in agreement with earlier report in broilers [13]. Decreased percent of lymphocyte and TLC in chlorpyrifos treated group indicated a risk of lymphopenia and immunomodulation. Birds received cow urine distillate @ 10 ml/ liter of drinking water along with chlorpyrifos showed significantly higher lymphocyte count indicating beneficial effect of cow urine distillate at this dose.

Average values of heterophil showed significant difference between control and treatment groups. Birds treated with chlorpyrifos alone showed higher mean values of heterophil as compared to I, III and IV groups. However, birds received chlorpyrifos along with cow urine distillate @ 10 ml/ liter of drinking water showed decrease in mean values of heterophil than birds treated with chlorpyrifos alone suggesting ameliorative effect of cow urine distillate. Differences in mean values of monocyte, eosinophill and basophill counts were found to be statistically non-significant between control and treatment group birds. No variation in monocyte, eosinophill and basophill percentage in chlorpyrifos treated indigenous chicken was also reported earlier [20].

Conclusion

This study indicated that, subacute exposure of chlorpyrifos @ 50 ppm in feed has adverse effect on clinical and haematological observations and co-administration of cow urine distillate ameliorated these changes.

Table 1: Body weight (g), body weight gain (g), feed consumption (g), feed conversion ratio (FCR) and water consumption (litre) in different experimental groups (Mean ± S.E., n=15)

Parameter/Intervals	Group I	Group II	Group III	Group IV
Body weight				
2nd week	295.73± 10.224	312.40± 11.383	301.46± 6.591	308.53± 13.021
3rd week	624.20±19.782	603.66±13.453	645.73±15.947	616.53± 26.159
4th week	966.13± 30.587	949.46± 21.749	1021.33± 22.793	1019.60± 4.354
5th week	1306.26±26.488	1283.80±35.652	1319.40±31.856	1357.93±36.583
Body weight gain				
2nd week	164.80±7.612	181.06±6.794	171.33±3.200	179.06±9.605
3rd week	328.46^{ab}±12.868	291.26^{c}±4.522	344.26^{a}±10.001	308.00^{bc}±14.599
4th week	341.93^{c}±14.492	345.80^{bc}±9.468	375.60^{ab}±8.926	403.06^{a}±8.766
5th week	340.13±12.701	334.33±16.835	298.06±11.883	338.33±14.813
Feed consumption				
2nd week	5038	5832	5484	5560
3rd week	6942	8198	7518	7419
4th week	7863	10373	9892	8050
5th week	8081	9966	9546	7725
Feed conversion ratio (FCR)				
2nd week	2.04	2.15	2.14	2.07
3rd week	1.41	1.88	1.46	1.61
4th week	1.53	2.00	1.75	1.33
5th week	1.58	1.99	2.14	1.52
Water consumption				
2nd week	9.64	10.38	20.02	40.04
3rd week	18.17	24.58	42.75	85.5
4th week	19.22	25.68	44.9	89.8
5th week	28.46	36.98	65.44	130.88

Table 2: Haematological parameters (Mean ± S.E.) in birds of different experimental groups (n=6).

Parameter	Group I	Group II	Group III	Group IV
Hb (g/dl)	10.16 ± 0.601	9.33 ± 0.760	10.00 ± 0.563	9.83 ± 0.703
PCV (%)	30.00 ± 3.307	26.33 ± 1.174	28.33 ± 1.801	28.50 ± 2.218
TEC (x 10^6/µL)	6.11^{ab} ± 0.507	4.75^{c} ± 0.313	6.61^{a} ± 0.345	5.33^{bc} ± 0.469
MCV (fl)	48.52^{bc} ± 2.202	55.96^{a} ± 2.003	42.70^{c} ± 0.898	54.27^{ab} ± 3.386
MCH (pg)	16.81^{bc} ± 0.543	19.60^{a} ± 0.703	15.11^{c} ± 0.246	18.77^{ab} ± 1.198
MCHC (%)	35.33 ± 3.376	35.19 ± 1.488	35.48 ± 1.096	34.65 ± 1.132
TLC (x 10^3/µL)	13.63^{a}±0.383	11.20^{b}±0.517	13.60^{a}±0.495	11.25^{b}±0.400
Heterophil (%)	43.66^{b}±0.760	49.66^{a}±1.564	44.66^{ab}±1.430	42.50^{b}±2.579
Lymphocyte (%)	52.50^{a}±0.563	45.16^{b}±1.621	51.16^{a}±1.778	53.66^{a}±2.907
Monocyte (%)	0.83±0.307	2.00±0.447	1.50±0.428	1.83±0.307
Eosinophil (%)	2.66±0.333	2.66±0.333	2.00±0.258	1.50±0.428
Basophil (%)	0.33±0.211	0.50±0.342	0.66±0.333	0.50±0.224

Means bearing common superscript do not differ significantly between groups (P≤0.05)

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