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Haemato-pathological studies of subchronic profenofos toxicity in broiler birds

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Abstract
The study was conducted to assess the toxic effects of Profenofos, an organophosphate pesticide on haematological and histopathological picture of broiler birds following subchronic exposure. A total of 20 day old chicks, 10 in each group were taken for the study. Group I served as control while Group II as treatment group. One tenth of LD_{50} dose, was orally administered daily for 60 days to test group (Group II). The treated birds showed significant escalation of all the haematological parameters with the exception of Lymphocyte count. The histopathological examination showed various alterations in different organs in group II, manifested by periportal fibrosis in liver, congested blood vessels of the lung and brain and coagulative necrosis with hydropic degeneration in the Kidney. From the present study it can be concluded that subchronic exposure of profenofos cause significant changes in haematology as well as histopathology in broiler birds.

Keywords: Profenofos, broiler birds, subchronic, haematology, histopathology

Introduction
Environmental pollution is the presence of a pollutant in environment such as air, water, soil and consequently in food which may be poisonous or toxic and will cause harm to living things in the polluted environment [1]. The pollution of the environment plays a crucial role in the occurrence of many diseases affecting plants, animals and man. A variety of pesticides have reached sufficient concentration in the environment to constitute potential hazarded to man and animals. One of the main factors causing pollution of the environment is the irrational use of organophosphorus insecticides [2]. Profenofos is a broad spectrum organophosphate which is one of the most extensively used pesticide for protecting agricultural crops from pests [3]. In recent years, the use of profenofos in agriculture has taken its peak because it is less persistence and readily decomposed in the environment. Like other commonly used pesticides profenfos may find its way into the food chain and can adversely effect the agents involved in it [4]. Profenofos is extensively used in maize plants and the latter constitute a major feed ingredient for poultry ration. Therefore, the purpose of the present study was to evaluate the toxic effects of Profenofos on Broiler Birds with regards to their effects on haematological parameters and histopathological changes.

Materials and Methods
Subjects (Birds)
Twenty unsexed day old chicks were procured from the poultry corner, Khanapara, Guwahati, Assam. The birds were wing banded, weighed, and reared in Instructional Poultry Farm (IPF), CVSc with *ad libitum* supply of feed and water. The experimental trials were approved by the Institutional Animal Ethics Committee (No.770/ac/CPCSEA/FVSc/AAU/IAEC/15-16/356).

Chemical (Insecticide)
Profenofos 50% EC (Current®), procured from Plant remedies, Hazipur, India was used for this study.

Experimental protocol
The birds were divided into two group, ten in each group. To make the birds acclimatised in the new environment the birds were kept in the farm for 7 days prior to experiment. Group I served as control which were fed with distilled water p.o. orally daily with gavage neddle for 60 days (excluding the acclimatisation period of 7 days). Profenofos was administered daily to birds of group II @ 1.6 mg/kg i.e, $1/10^{th}$ LD_{50} dose which was obtained after conducting pilot

study. Doses were calculated on body weight basis and administered accordingly. Blood was collected from jugular and wing vein at 0 week, 1st week, 2nd week, 3rd week, 4th week, 5th week, 6th week, 7th week, and at 8th week. The collected blood was used for estimation of haematological parameters (Haemoglobin, Total Erythrocyte Count, Total Leucocyte Count, Lymphocyte Count and Heterophil Count) using Automated Haematology Cell counter (Model: MS4S, Sartorius).The birds were sacrificed at the end of the experiment and the representative samples of the liver, kidney, brain and lungs were collected in 10% formal saline for histopathological study. After washing in running tap water and dehydration in alcohol, tissues were embedded in liquid paraffin, 5 µm paraffin sections cut and stained with hematoxylin and eosin as per standard protocol [5].

Statistical analysis

Statistical analysis for the experimental data was performed by one way ANOVA using SPSS 16.0. Values were expressed as mean ± SE. P value less than 0.05 was considered statistically significant.

Result

The mean ± SE plasma concentration of Haemoglobin (Hb) in control and treated group at 0 week was 9.67 ± 0.85 g/dl and 10.05 ± 0.24 g/dl, at 1st week was 10.22 ± 0.04 g/dl and 10.87 ± 0.34 g/dl, at 2nd week was 10.88 ± 0.25 g/dl and 10.52 ± 0.26 g/dl, at 3rd week was 10.11 ± 0.20 g/dl and 11.75 ± 0.27 g/dl, at 4th week was 10.89 ± 0.18 g/dl and 10.53 ± 0.15 g/dl, at 5th week was 10.08 ± 0.23 g/dl and 11.85 ± 0.39 g/dl, at 6th week was 10.98 ± 0.03 g/dl and 11.45 ± 0.13, at 7th week was 10.37±0.09 g/dl and 11.84 ± 0.23 g/dl , and finally at 8th week was 10.35 ± 0.14 g/dl and 12.56 ± 0.27 g/dl respectively. The Hb concentration was significantly (P<0.05) increased from 3rd week to 4th week and the increase was found to be highly significant (P<0.01) from 5th week till the end of experiment in treated group compared to control. (Table 1)

The mean ± SE plasma concentration of Total Erythrocyte Count (TEC) at 0 week was 2.65 ± 0.06 (10^6 /µL) and 2.41 ± 0.29 (10^6 /µL), at 1st week was 2.91 ± 0.13 (10^6 /µL) and 2.82 ± 0.69 (10^6 /µL), at 2nd week was 2.09 ± 0.09 (10^6 /µL) and 3.51 ± 0.15 (10^6 /µL), at 3rd week was 3.12 ± 0.20 (10^6 /µL) and 3.43 ± 0.09 (10^6 /µL), at 4th week was 2.89 ± 0.03 (10^6 /µL) and 3.31 ± 0.07 (10^6 /µL), at 5th week was 2.45 ± 0.54 (10^6 /µL) and 3.75 ± 0.18 (10^6 /µL), at 6th week was 2.74 ± 0.17 (10^6 /µL) and 3.06 ± 0.01 (10^6 /µL), at 7th week was 2.39 ± 0.14 (10^6 /µL) and 3.83 ± 0.25 (10^6 /µL) , and finally at 8th week was 2.27 ± 0.02 (10^6 /µL) and 3.25 ± 0.06 (10^6 /µL) in control and treated group respectively. The TEC level was found to be significantly increased (P<0.01) from 4th week onwards till the end of the experiment in group II as compared to group I. (Table 2)

The mean ± SE plasma concentration of Total Leucocyte Count (TLC) in control and treated group at 0 week was 26.16 ± 1.99 (10^3 /µL) and 26.87 ± 0.63 (10^3 /µL), at 1st week was 26.35 ± 1.26 (10^3 /µL) and 27.51 ± 0.61 (10^3 /µL), at 2nd week was 25.36 ± 1.25 (10^3 /µL) and 27.99 ± 1.75 (10^3 /µL), at 3rd week was 26.96 ± 1.13 (10^3 /µL) and 28.44 ± 1.83 (10^3 /µL), at 4th week was 26.67 ± 2.74 (10^3 /µL) and 28.63 ± 0.58 (10^3 /µL), at 5th week was 28.63 ± 0.58 (10^3 /µL) and 25.17 ± 0.90 (10^3 /µL), at 6th week was 25.44 ± 1.78 (10^3 /µL) and 28.17 ± 0.65 (10^3 /µL), at 7th week was 24.42 ± 1.56 (10^3 /µL) and 28.82 ± 0.82 (10^3 /µL), and finally at 8th week was 23.01 ± 0.99 (10^3 /µL) and 27.11 ± 0.97 (10^3 /µL) respectively. The TLC level was significantly (P<0.05) increased from 7th week onwards in treated group compared to control. (Table 3)

The mean ± SE plasma concentration of lymphocyte at 0 week was 92.89 ±1.53% and 94.42 ± 0.13%, at 1st week was 91.74 ± 1.73% and 91.87 ± 0.19%, at 2nd week was 93.08 ± 1.12% and 95.32 ± 0.81%, at 3rd week was 91.93 ± 0.83% and 91.11 ± 2.62%, at 4th week was 91. 24 ± 0.89% and 90.61 ± 2.09%, at 5th week was 92.55± 1.16% and 89.84 ± 2.60%, at 6th week was 93.22 ± 0.81% and 89.07 ± 1.13% ,at 7th week was 94.54 ± 0.83% and 87.70 ± 0.88%, and finally at 8th week was 93.28 ± 1.44% and 86.41 ± 0.78%. There was gradual decreased in lymphocyte per cent from 2nd week onwards. The result was statistically significant (P<0.05) at 6 weeks and it was found to be highly significant at 7th and 8th weeks in treated group as compared to that of control. (Table 4)

The mean ± SE plasma concentration of Heterophil at 0 week was 4.53 ± 0.10% and 4.24 ± 0.25%, at 1st week was 4.25 ± 0.34% and 4.96 ± 0.75%, at 2nd week was 4.87 ± 0.26% and 5.96 ± 0.81%, at 3rd week was 4.24 ± 0.24% and 6.90 ± 0.80%, at 4th week was 4.21 ± 0.22% and 7.68 ± 0.94%, at 5th week was 3.91 ± 0.63% and 10.58 ± 0.62%, at 6th week was 3.92 ± 0.18% and 13.41 ± 0.55%, at 7th week was 3.61 ± 0.17% and 15.49 ± 0.36%, and finally at 8th week was 3.51 ± 0.32% and 17.78 ± 0.30%. The heterophil percent show increasing trend from the beginning of experiment till the end in treated group. (Table 5)

The heterophil percent was significantly (P<0.05, P<0.01) increased from 3rd week onwards in treated group compared to the control.

Histopathological Lessions

The section of liver (Fig 1) showed distinct periportal fibrosis along with mild proliferation of bile duct epithelium. Congestion of blood vessels and vacuolar degeneration of hepatocytes, with focal infilration and mononuclear cells were observed. Kidney revealed areas of necrosis where the parenchyma of the kidney was distorted and marked infiltration of inflammatory cells was observed. Tubules show dilatation with necrosis of the epithelial lining (Fig.2). Extensive congestion was observed in the lung. Focal haemorrhage was seen in some of the areas. Marked thicknening of the interalverolar septa was evident (Fig.3). Brain section revealed mild vascular congestion, neuronophagia and satellitosis (Fig.4).

Table 1: haemoglobin level (mean ± se) in control and tretment group

Time of blood Collection (wks)	Haemoglobin (g %)	
	Control	Treatment
0	9.67± 0.85	10.05± 0.24
1	10.22±0.04	10.87±0.34
2	10.88±0.25	10.52±0.26
3	10.11±0.20	11.75±0.27*
4	10.89±0.18	10.53±0.15*
5	10.08±0.23	11.85±0.39**
6	10.98±0.03	11.45±0.13**
7	10.37±0.09	11.84±0.23**
8	10.35±0.14	12.56±0.27**

*P<0.05, **P<0.01

Table 2: total erythrocyte count (mean ± se) in control and treatment group

Time of blood Collection (wks)	TEC (10^6/µl)	
	Control	Treatment
0	2.65±0.06	2.41±0.29
1	2.91±0.13	2.82±0.69
2	2.09±0.09	3.51±0.15**
3	3.12±0.20	3.43±0.09
4	2.89±0.03	3.31±0.07**
5	2.45±0.54	3.75±0.18**
6	2.74±0.17	3.06±0.01
7	2.39±0.14	3.83±0.25**
8	2.27±0.02	3.25±0.06**

***P*<0.01

Table 3: Total leucocyte count (mean ± se) in control and treatment group

Time of blood Collection (wks)	TLC (10^3/ul)	
	Control	Treatment
0	26.16 ± 1.99	26.87 ± 0.63
1	26.35 ± 1.26	27.51 ± 0.61
2	25.36 ± 1.25	27.99 ± 1.75
3	26.96 ± 1.13	28.44 ± 1.83
4	26.67 ± 2.74	28.63 ± 0.58
5	22.73 ± 1.50	25.17 ± 0.90
6	25.44 ± 1.78	28.17 ± 0.65
7	24.42 ± 1.56	28.82 ± 0.82*
8	23.01 ± 0.99	27.11 ± 0.97*

**P*<0.05

Table 4: Lymphocyte percent (mean ± se) in control and treatment group

Time of blood Collection (wks)	Lymphocyte (%)	
	Control	Treatment
0	92.89±1.53	94.42±0.13
1	91.74±1.73	91.87±0.19
2	93.08±1.12	95.32±0.81
3	91.93±0.83	91.11±2.62
4	91. 24±0.89	90.61±2.09
5	92.55±1.16	89.84±2.60
6	93.22±0.81	89.07±1.13*
7	94.54±0.83	87.70±0.88**
8	93.28±1.44	86.41±0.78**

**P*<0.05, ***P*<0.01

Table 5: Heterophil percent (mean ± se) in control and treatment group

Time of blood Collection (wks)	Heterophil (%)	
	Control	Treatment
0	4.53±0.10	4.24±0.25
1	4.25±0.34	4.96±0.75
2	4.87±0.26	5.96±0.81
3	4.24±0.24	6.90±0.80*
4	4.21±0.22	7.68±0.94*
5	3.91±0.63	10.58±0.62**
6	3.92±0.18	13.41±0.55**
7	3.61±0.17	15.49±0.36**
8	3.51±0.32	17.78±0.30**

**P*<0.05, ***P*<0.01

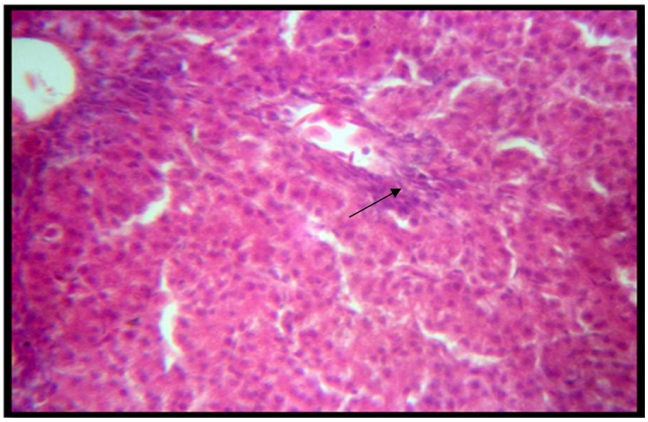

Fig 1: Liver showing periportal fibrosis with infiltrating cells (H & EX400)

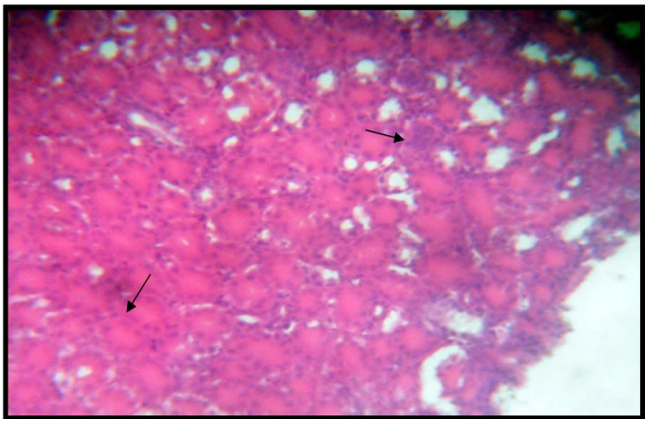

Fig 2: Kidney showing coagulative necrosis and hydropic degeneration (H&EX400)

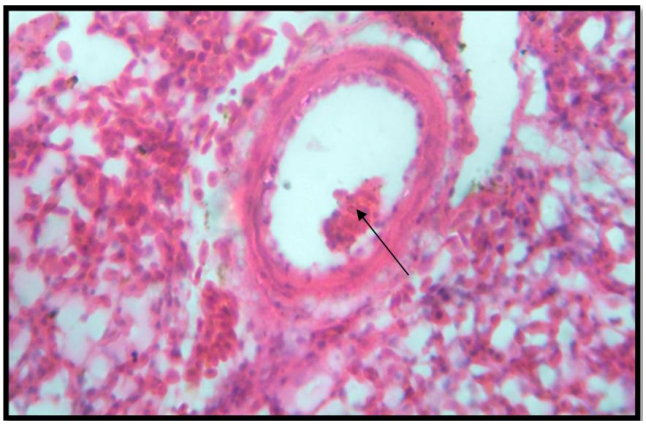

Fig 3: Lung showing congestion (H&EX400)

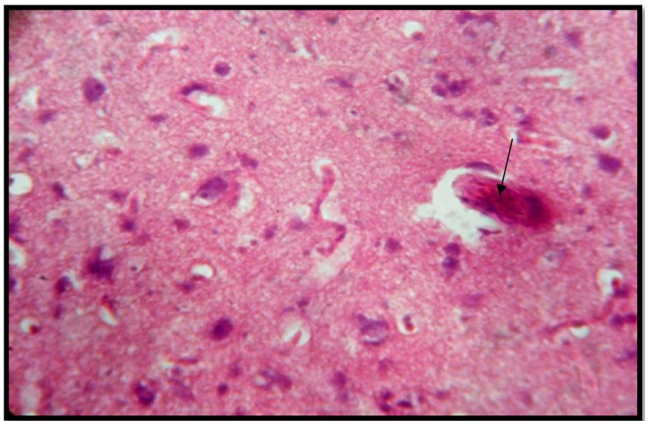

Fig 4: Brain showing congestion (H&EX400)

Result and Discussion

From the present study it can be said that Liver is the prime organ to be affected in Profenofos toxicity. Haematological parameters can be considered as a tool in the monitoring and management of health status. Profenofos administration at the rate $1/10^{th}$ of LD_{50} caused marked changes in the haematological parameters under study. the Hb concentration was found to be significantly ($P<0.05$, $P<0.01$) increased from 3th week onwards in treated chickens compared to control. On the contrary, Some authors [6] reported the Hb concentration to be reduced (due to anaemia) from 6.442 ± 0.052 to 3.386 ± 0.069 g/dl at 96 hrs while studying the toxicity of mixture of heavy metal (lead nitrate) and profenofos against the Estuarine fish *Lates calcarifer*. The TEC level showed variation from 2 week onwards till the end of the experiment. In contrast the TEC level was reported to be reduced from 6.80 ± 0.10 in control to 5.87 ± 0.115 10^6/µl in treatment group while studying the combined effect of Imidocloprid, Profenofos and Carbosulfan in albino rats [7]. The difference in result of the present study could be due to species variation. Moreover, Severe dehydration due to excessive salivation and diarrhoea was observed in the present study which might be the reason for haemoconcentration and hence erythrocytosis. Similar findings were reported in previous studies [8]. The mean values of TLC was significantly ($P<0.05$) increased in treated chickens from 7th week onwards as compared to control. This could be due to severe dehydration leading to haemoconcentration. While studying the impact of Profenofos in fishes studied for duration of 96 hrs the Total Leucocyte Count was found to increased from 682.1 ± 5.1 in control to 937.4 ± 3.7 10^3/mm^3 in treatment group [8]. The lymphocyte count showed gradual decreasing trend compared to the control which was statistically significant ($P<0.05$, $P<0.01$) from 6th week onwards in Group II. Lymphopenia was also observed while studying cytogenetic studies on the ameliorative effect of propolis against profenofos toxicity in rats for 60 days The lymphocyte percent was reported to be reduced from 9.75 ± 0.50 in control to 7.10 ± 0.50 percent in profenofos treated rats [10]. The statistical analysis revealed significant ($P<0.05$, $P<0.01$) increase of heterophil from 3rd week onwards in Group II as compared to Group I. The heterophil percent was reported to be increase from 4.03 ± 1.28 in control to 7.84 ± 0.61 percent in profenofos treated rats [10]. Acute degenerative changes and stress might be the reason for increase heterophil and lymphocytopenia respectively. Similar observations were also reported by other workers [8, 10].

Liver plays a very important role in the mechanism of detoxification and elimination of toxic substances from the body so it is very true that continuous exposure and intoxication of liver to different types of exogenous compounds on a daily basis may lead to hepatic dysfunction. Following oral administration, Profenofos reached the liver via the gastro intestinal tract blood supply, therefore, the necrosed areas mainly appeared around portal tract. Also, inflammatory cells were aggregated in portal tracts and present as differential foci in the liver parenchyma [11]. In the present study liver was found to be affected to a greater extent which can be well established with the haematological and histopathological results. Liver of mice treated with $1/10^{th}$ LD_{50} showed congestion of the blood vessels and vacuolar degeneration of hepatocytes, with focal infilration of mononuclear cells and periportal fibrosis [9, 10, 11]. Profenofos while excreting through kidney gets deposited in the cells adjacent to lumen hence renal toxicity is obvious. Kidney showed tubular degeneration when treated with $1/10^{th}$ LD_{50} Profenofos. Necrosis of tubular epithelium, cloudy swelling of epithelial cells of renal tubules, narrowing of the tubular lumen, contraction of the glomerulus and expansion of space inside the Bowman's capsule were observed in the kidney tissues of fish after exposure with Profenofos [11]. Lung revealed congestion and haemorrhage, the inflammatory response were observed in the septal wall. The effect of Profenofos in brain was mild and mainly involved congestion. Pathological finding in Brain showed menengial hemorrhages and congestion of blood vessels, with neuronophagia and satelletosis. Similar findings were stated by other workers [12, 13] in rats and chickens respectively.

Conclusion

From the analysis of the parameters studied, it can be inferred that profenofos has the potential to produce significant toxic changes in the haematology as well as histology in exposed birds. So, public awareness regarding irrational use of profenofos should be raised in order to prevent such accidents.

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Conflict of interest

Nil

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