

DDT-INDUCED HAEMOTOXICITY IN SPRAGUE DAWLEY RATS***SYED SHAHID ALI AND ABDUL RAUF SHAKOORI***Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore- 54590, Pakistan.*

Abstract: The various haematological parameters in albino rats were prominently altered after oral administration of DDT (100mg, 20mg and 10mg/kg body wt/day) alongwith the feed for a total period of 48 hours, 15 days and 18 months respectively. Total erythrocytic count (TEC) and haemoglobin (Hb) content was reduced upto 16 and 11% (48 hours), 17 and 9% (15 days) and 14 and 8% in case of 18 month treatment, respectively. The decrease in PCV was 7 and 8% after 15 days and 18 months of DDT feeding. Among the slight but various indices values, the mean corpuscular haemoglobin concentration (MCHC) exhibited significant decline at 48 hours (10%) and 15 days (5%). Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) increased up to 19 and 12% during 48 hours, 26 and 9% during 15 days and 13 and 11% in 18 months experiments respectively. Significant increase (48%, 64%, and 29% was also observed in total leukocytic count (TLC) in all three treatments, respectively. The study suggests that DDT is extremely toxic compound which involves variety of pathology in the blood components of non-target systems.

Key words: DDT toxicity, blood morphology haematology, rats.

INTRODUCTION

H aematological characteristics have been widely used in the diagnosis of variety of diseases and pathologies, induced by industrial components, drugs, dyes, heavy metals, pesticides and several others (Morgan and Stockdale, 1980; Hermanowicz *et al.*, 1982; Szubartowska, 1983; Ali *et al.*, 1988; Ali and Shakoori, 1988; Pain and Rattner, 1988; Haniffa and Vijayarani, 1989).

The measurement of haemoglobin, erythrocytic and leukocytic counts, erythrocyte sedimentation rate, haematocrit estimation and various haematological indices values are considered sensitive and valuable indicators of mild and sublethal exposure of animals and humans to variety of toxic pollutants (Ali and Shakoori, 1981, 1990, 1993; Nishihara and Utsumi, 1983; Shakoori *et al.*, 1988, 1990, 1992; Jabbar *et al.*, 1991). Organochlorine pesticides constitute a very significant proportion of these environmental pollutants which have been used quite extensively and indiscriminately in the past several decades for control of agricultural and public health insect pests (Artemev *et al.*, 1992; Douthwaite, 1992). Although the use and manufacture of these pesticides has been banned or restricted in many parts of the world, they are still being used in several third world countries including India and Pakistan (Metcalf, 1973; Suzuki *et al.*, 1976; Parveen and Masud, 1983; Jabbar *et al.*, 1991; Lodha and Saxena, 1991; Bhatnagar *et al.*, 1992; Calero *et al.*, 1992; Chandra *et al.*, 1992).

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Highly stable and persistent nature of these compounds and their metabolites, which may be equally toxic as the parent compound, in plants, animals, soil, water and air, is the real cause of concern for toxicologists and public health scientists and engineers (Suzuki *et al.*, 1976; Mugambi *et al.*, 1989; Radulescu *et al.*, 1990; Bhatnagar *et al.*, 1992; Chandra *et al.*, 1992; Hitch and Day, 1992; Sahu *et al.*, 1992). Because of this property these organochlorine pesticides enter and move in the food chains (Atuma, 1985; Sitarska *et al.*, 1991; Hargrave *et al.*, 1992; Hovinga *et al.*, 1993), and inflict variety of toxic effects in the animal systems (Mukharjee *et al.*, 1980; Saigal *et al.*, 1982; Ali *et al.*, 1988; Ali and Shakoori, 1981, 1988; Shakoori *et al.*, 1988, 1992).

This study was undertaken to investigate the short and long term effects of strong and weak doses of DDT on some haematological parameters of laboratory rats.

MATERIALS AND METHODS

A colony of Sprague Dawley albino rats, raised in the animal house of the Department of Zoology, University of the Punjab was used in the present study. For short term experiments two groups of female rats, about 6 months of age were used. One group with 180 g average weight was used for feeding insecticide for 48 hours, while the second with average weight 196 g was used for feeding insecticide for 15 days. For long term experiments, the rats weighing about 90-105 g and four months of age were used. They were provided with feed (see Shakoori *et al.*, 1988) and water *ad libitum*.

Insecticide used and its administration

A chlorinated insecticide DDT; 1,1,1,- trichloro-2,2-bis (4-chlorophenyl) ethane, was obtained from the Plant Protection Division of Punjab Agriculture Department, in the form of 75% powder which was administered to animals alongwith feed. DDT was administered to rats as strong and weak doses. For short term (ST) experiments, two levels of strong doses of DDT were administered. In one group of rats a strong dose of 100 mg/kg body weight/day (0.4 LD₅₀) was administered for a total period of 48 hours (ST-I). In the second group 20 mg/kg body weight/day (0.08 LD₅₀) was administered for a total period of 15 days (ST-II).

A weak dose at a rate of 10mg/kg body weight/day (0.04 LD₅₀) was administered to another group of rats for 18 months. For ST-I experiment, DDT mixed diet was prepared by mixing 800 mg of 75% DDT in 1 kg of dry feed. Since each experimental rat, on the average, consumed 30 g of feed daily, it will take 100 mg/kg body weight/day. For ST-II, the insecticide mixed diet was prepared by mixing 525 mg of 75% DDT powder in about one liter molasses-mixed water and added to 3 kg of feed mixture. Each rat of 196 g average weight consumed about 30 g of this feed daily. In this way rats got a DDT dose of 20 mg/kg body weight/day.

For long term experiment, 87.5 mg of 75% DDT powder was mixed/kg of ingredient-mixed feed. That way the rats consumed 10mg DDT/kg body weight/day.

HAEMATOLOGICAL EFFECTS OF DDT

Procedure adopted

For short (ST-I and ST-II) and long term experiments three groups of rats, with 8, 20 and 9 animals in each, respectively were fed on three different doses of insecticide mixed diet for various durations as mentioned above. A group of four rats was anaesthetized and slaughtered after 24 and 48 hours in ST-I experiment. Similarly 4 animals each were slaughtered at 3, 6, 9, 12, and 15 day intervals in case of ST-II experiment. In long term experiment a group of three DDT fed animals was slaughtered at 6, 12, and 18 month durations. After stipulated periods the blood samples were collected from inferior vena cava with 10 ml disposable syringe and used for various types of analyses. About 1 ml of blood was quickly collected in tubes containing EDTA as an anticoagulant and mixed gently. This sample was used for study of various haematological parameters which involved the estimation of haemoglobin (Hb) content according to Van-Kampan and Zijlstra (1961), packed cell volume (PCV) according to microhaematocrit method of Strumia *et al.*, (1954) and total erythrocytic count (TEC) and total leukocytic count (TLC) according to routine clinical methods. The data obtained was then utilized for calculating different haematological indices *i.e.* mean corpuscular haemoglobin (MHC) mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) according to Dacie and Lewis (1977).

RESULTS

Administration of DDT alongwith feed as three different concentrations and durations induced significant changes in rat blood. Tables I, II, and III show the effects of DDT feeding at 100, 20 and 10 mg/kg body weight/day for total durations of 48 hours, 15 days and 18 months, respectively on various haematological parameters.

The Hb content, TEC and PCV exhibited significant reduction. The Hb content decreased upto 11% in 48 hours (Table I), 9% in 15 days (Table II) and 8% in case of 18 months (Table III) DDT feeding experiment. The decrease in TEC was 16% (48 hours), upto 17% (15 days) and 15, 13 and 14% in case of 6, 12 and 18 months DDT intoxication, respectively. The PCV content did not show any significant change in 48 hour experiment at 100 mg/kg dose level but reduced upto 7% in 15 days and upto 8% in 18 months toxicant feeding.

Corresponding changes were also observed in various haematological indices. Statistically significant but slight decrease was also found in MCHC which was 10% in 48 hours and upto 5% in 15 days DDT treatment and remained unchanged in long term experiment (Table III). DDT at all doses produced significant increase in MCV and MCH. The rise in MCV in ST-I experiment was 19%, in ST-II experiment it was upto 26%, while 13% in case of 18 months of un-interrupted DDT feeding.

Another important parameter which showed maximum and consistent increase was TLC. The rise in this case was upto 48% in 48 hours treatment, from 13-64% in 15 days treatment and upto 19% in case of 18 months experiment (Table I-III).

Table I. Effect of feeding DDT mixed diet (100mg/kg body weight/day) for 48 hours on the various haematological parameters of albino rats

Parameters	Control (n=7)	DDT - fed animals	
		24 hours (n=4)	48 hours (n=4)
Hb (g/dl)	13.27±0.14 ^a	12.47±0.25*	11.87±0.22***
TEC (X10 ⁶ /μl)	6.84±0.25	5.76±0.17**	5.74±0.13**
TLC (X10 ³ /μl)	6.36±0.20	9.00±0.44***	9.44±0.20***
PCV (%)	42.09±0.34	41.94±0.29	41.81±0.20
MCV (fl)	61.56±0.17	72.95±1.66***	72.93±1.34***
MCH (pg)	19.38±0.08	21.67±0.53**	20.69±0.42**
MCHC (g/dl)	31.52±0.09	29.72±0.39**	28.39±0.39***

^aMean±SEM, student's 't' test; *P<0.05; **P<0.01; ***P,0.001

^bAbbreviations used; TEC, total erythrocytic count; TLC, total leukocytic count; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; dl, deci=100ml; fl, femtolitre=10⁻¹⁵ litres; pg, picogram=10⁻¹²g.

Table II. Effect of feeding DDT mixed diet (20mg/kg body weight/day) for 15 days on the haematological parameters of albino rats.

Parameters	Control (n=6)	DDT - fed animals				
		3days (n=4)	6days (n=4)	9 days (n=4)	12 days (n=4)	15days (n=4)
Hb (g/dl)	13.04±0.16 ^a	12.37±0.39	12.28±0.36	11.98±0.37*	11.84±0.41*	11.85±0.36*
TEC (X10 ⁶ cells/μl)	6.91±0.12	6.36±0.12*	6.29±0.11**	5.9±0.09***	5.88±0.14***	5.76±0.14***
TLC (X10 ³ cells/μl)	6.55±0.52	7.70±0.56	8.67±0.51*	9.50±0.72**	9.01±0.66*	9.57±0.23*
PCV (%)	43.28±0.40	42.17±0.93	41.76±1.09	40.85±0.82*	40.50±0.97*	40.48±0.06***
MCV (fl)	62.64±0.46	66.26±0.36***	66.34±0.67**	79.21±0.89***	68.82±0.25***	70.41±0.68***
MCH (pg)	18.88±0.28	19.42±0.27	19.50±0.26	20.29±0.17**	19.75±0.16*	20.56±0.24*
MCHC (g/dl)	30.15±0.36	29.31±0.32	29.39±0.14	29.31±0.05*	28.70±0.23**	29.22±0.19*

^aMean ± SEM, Student's 't' test; *P<0.05; **P<0.01; ***P<0.001
For other details, see Table I.

HAEMATOLOGICAL EFFECTS OF DDT

Table III. Effect of feeding DDT mixed diet (10mg/kg body weight/day) for 6-18 months on the various haematological parameters of albino rats.

Parameters	DDT feeding experiment (months)					
	6		12		18	
	Control (n=6)	DDT fed (n=3)	Control (n=4)	DDT fed (n=3)	Control (n=6)	DDT fed (n=3)
Hb (g/dl)	13.79±0.32 ^a	12.79±0.51	13.14±0.23	12.64±0.26	13.04±0.16	12.02±0.19 ^{**}
TEC (X10 ⁶ cells/ μ l)	7.09±0.12	6.08±0.11 ^{***}	7.05±0.13	6.14±0.15 ^{**}	6.91±0.12	5.99±0.13 ^{**}
TLC (X10 ³ cells/ μ l)	6.55±0.40	7.20±0.11	6.24±0.13	7.43±0.32 [*]	6.55±0.52	8.43±0.28 [*]
PCV (%)	45.92±0.44	42.45±0.22 ^{***}	42.90±0.20	40.37±0.39 ^{***}	43.28±0.40	40.75±0.63 [*]
MCV (fl)	64.38±0.65	69.81±0.81 ^{**}	60.90±0.86	69.06±1.10 ^{***}	62.64±0.46	68.01±0.92 ^{**}
MCH (pg)	19.45±0.32	21.01±0.55 [*]	18.64±0.06	20.60±0.22 ^{***}	18.88±0.28	20.05±0.21 [*]
MCHC (g/dl)	30.24±0.65	30.12±1.05	30.62±0.41	29.84±0.33	30.15±0.36	29.48±0.09

^aMean \pm SEM, Student's 't' test; *P<0.05; **P<0.01; ***P<0.001
For other details, see Table I.

DISCUSSION

The Hb, TEC, PCV and MCHC decreased in almost all treatments after DDT treatment, while TLC, MCV and MCH increased at the same time. The TEC decreased 16%, 17% and 14% after DDT treatment for 48 hours, 15 days and 18 months administration at their respective doses. On analysing the data, it is clear that strong doses of DDT induced immediate reduction in Hb and TEC while in other two experiments the effect was delayed and induced gradually, which was indicated by absence of any change at 3 and 6 day treatments in 15 days experiment and at 6 and 12 months in 18 month DDT feeding experiments. No change was recorded in PCV in 48 hours experiment and during early part (up to 6 days) of 15 days experiment. Similarly MCH and MCHC remained resistant to DDT toxicity up to day 6 in 15 day experiment.

The parameters, most sensitive to DDT intoxication were TEC and TLC which showed alterations in all treatments. The study indicates that DDT administration at all above doses and durations is responsible for the induction of anemia and leukocytosis in rats. Effects of DDT and various other pesticides on blood and its main cellular constituents were reported by different laboratories (Vrochinskii *et al.*, 1976; Traczyk *et al.*, 1978; Raalte and Jansen, 1981; Nisihara and Utsumi, 1983).

The decrease in Hb, PCV and TEC was also observed with another organochlorine insecticide, aldrin (Ali and Shakoori, 1990). Similar changes in haematological parameters were reported in birds with other pesticides (Ali and Shakoori, 1988; Szubartowska, 1983; Mandal and Lahiti, 1985; Qadri *et al.*, 1987; Ali *et al.*, 1988, 1992). The decrease in TEC and Hb content could be attributed to breakdown of erythrocytes due to DDT treatment (O'Brien and Hamilton, 1979) or it may be ascribed to changes in haemopoietic tissue which according to Nohara (1986) may be caused by the disturbances in the metabolisms of nucleic acids which persist in this tissue. These chlorinated insecticides including DDT have also been shown to be responsible for the appearance of aplastic anemia and bone marrow atrophy in human body (Vrochinski *et al.*, 1976; Sternberg, 1979). Kamarova (1976) showed that prolonged contact with pesticides produce leukemia and hypoplastic anemia in pesticide workers. He further reported that amount of DDT and its main metabolite DDE was greater in the haemopoietic organs of persons who died of hypoplastic anemia than in subcutaneous tissue.

The TLC showed drastic increase after DDT feeding at different durations and doses. In 48 hours feeding experiment, the TLC increased 48%, while in 15 day experiment it was increased 64% at the end of experimental period. In long term experiment the TLC showed gradual increase which was maximum (29%) at 18 months DDT intoxication. The increase in TLC in the present study indicated the induction of body's defence against the foreign chemicals. The TLC may also increased in response to abnormal or subnormal leukocytic functioning due to toxic effects of DDT (Hermanowicz, 1982). DDT also stimulate the lymphocyte mitogenesis under certain condition (McCable and Nowak, 1986). The MCV and MCH also showed significant increase after DDT treatment, while MCHC either exhibited decrease as in both short term experiments or remained unchanged as in long term DDT treatment. The increased values of MCV and MCH and decreased MCHC revealed that anemia produced was of macrocytic type.

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