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Research Article

EFFECT OF α-TOCOPHEROL ON HAEMATOLOGICAL ALTERATION INDUCED BY CHLORPYRIFOS IN ALBINO RATS

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ABSTRACT

The study evaluated the ameliorative effects of vitamin E on chlorpyrifos-induced haematological alterations in albino rats. Adult male rats (n=18) were divided into three groups (groups I, II and III) of six animals each and were given the following treatments: group I (control) was administered soya oil (2 mL/kg b.wt.), group II was given chlorpyrifos (CPF; 10.6mg/kg b.wt.) and group III was pretreated with vitamin E (150 mg/kg) and then exposed to CPF (10.6mg/kg b.wt.) 30 minutes later. The treatments were administered by oral gavage once daily for a period of 15 days. Blood samples collected at the end of the study revealed reduction in packed cell volume, hemoglobin, total red blood cells, leukocytes (attributed to lymphopenia and monocytopenia) in the CPF treated group when compared to controls. Pretreatment of rats with vitamin E ameliorated the adverse effects on these haematological parameters. Treatment of rats with CPF adversely affected the body weight of rats. The study revealed that CPF-induced adversity on body weight and haematological parameters of albino rats may be mitigated by pretreatment with vitamin E.

Key words: Albino rats, chlorpyrifos, α- tocopherol

Chlorpyrifos (O-O-diethyl-O-{3, 5, 6 trichloro-2-pyridyl}-phosphorothioate) is one of the most widely used organophosphate pesticide for domestic and agricultural purposes throughout the world. In India, chlorpyrifos (CPF) is classified as an extremely hazardous pesticide (Akhtar *et al.,* 2009). Blood parameters have rapid and detectable variations under stress and are important in assessing the health condition. There may be used as valuable indicators of disease or stress in animals. The present investigation was aimed to study the effect of oral administration of vitamin E on haematological alterations and growth depression induced by CPF in albino rats.

MATERIALS AND METHODS

Chemicals and Substances: Technical grade chlorpyrifos (20%) was procured from local market. Evion® caps of 600 mg (Merck Limited, Worli, Mumbai) were used as a source of α-tocopherol (vitamin E).

Experimental Animals: Healthy male albino Wistar rats (*Rattus norvegicus)*, 6-8 weeks of age, weighing 100–120 g were procured locally. They were housed in poly propylene cages and maintained in a 12/12 hours light/dark cycle with good hygienic conditions. The rats were maintained on a standard feed and water *ad libitum* and were acclimatized for a period of one week prior to the start of the experiment. The experimental work on rats was performed with approval of the Institutional Animal Ethics Committee of the college and all the protocols were followed according to the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Experimental Design: The rats were randomly divided into three groups (groups I, II and III) with six rats in each. Rats of group I were fed with standard feed and water throughout the experiment and were given soya oil (2 ml); this group acted as a control group. Group II rats were treated with CPF (@10.6mg/kg b.wt.) reconstituted with 2 ml soya oil orally daily for 15 days. Group III was treated with CPF with same dose as in group II, however, vitamin E @150mg/kg b.wt. was administered orally 30 minutes before the administration of CPF daily till the end of the experiment.

Body Weight: Rats were weighed individually at the start of the experiment (0 day) and on day of sacrifice (day 15).

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Haematological Parameters: Blood samples were collected from all rats on day 15 prior to sacrifice. Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) were estimated as per the protocol of Benjamin (2001).

Statistical Analysis: One way analysis of variance was used to test the significant effect in different groups (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

There was a progressive increase in the body weight of rats in group I with advancing age (Table 1). Oral gavage of CPF (group II) resulted in significant reduction in body weights as compared to control group (group I) at the end of the experiment (Table 1). Further, administration of α- tocopherol countered the growth depressing effect of the CPF and showed a significant improvement at day 15 of experiment when compared with group I. Earlier, Mansour and Mossa (2010b) and Yano *et al*. (2000) reported similar findings on body weights of rats orally administered with CPF. The adverse effect may be due to induction of lipid peroxidation by CPF, which may reduce the body tissue mass possibly via deteriorative changes in the fat and protein contents and also due to the combined effect of oxidative, toxic and cholinergic stress. Anorexia and general weakness of animals may also be the reasons for weight loss (Yano *et al*., 2000) despite the fact that we did not observe appreciable differences in the feed consumption among the groups in this study. Akhtar *et al*. (2009) also reported the similar findings. Rats receiving vitamin E along with CPF showed a significant increase in body weight as compared to CPF alone indicating that the vitamin E supplementation may have boosted the body's reserve and antistress effect. The improvement in body weights following vitamin E supplementation is in agreement with the previous finding of Mansour and Mossa (2010a).

Administration of CPF (group II) led to a significant decrease in all haematological parameters such as Hb, PCV, TEC, TLC and DLC when compared to control group (group I). Administration of vitamin E in rats simultaneously given CPF (group III) led to a significant improvement in all parameters except TLC when compared to group administered CPF alone (group II) (Table 2). A significant ($p<0.05$) decrease in Hb, PCV and TEC (Table 2) indicated that CPF caused microcytic-hypochromic anemia in rats. The decrease in these parameters might be due to the effect of chlorpyrifos on blood forming organs or may be due to the inhibition of erythropoiesis and haemosynthesis. Previous studies have shown that repeated chlorpyrifos exposure causes anaemia in rats (Ambali *et al*., 2007, 2009; Ambali *et al*., 2010a; Savithri *et al*., 2010). This may be due to the ability of the organophosphate compounds to decrease tissue iron concentration (Goel *et al*., 2006), interference with haemoglobin biosynthesis and induced RBC life span shortening or even increase in erythrocyte fragility (Ambali *et al*., 2010a, 2010b). Significant decrease in erythrocytic indices as observed in the present study is in conformity with the findings of Patel *et al*. (2006). Supplementation of vitamin E improved the erythrocyte parameters depressed by CPF. This may be due to the ability of the antioxidant property of the vitamin to improve the absorption of iron from the gut

Table 1
Mean body weight (g) in different groups of rats

Treatment	Mean body weights (g)	
	0 day	15th day
Control	$119.50^{a}\pm0.93$	$131.83^{a}\pm0.93$
CPF	$120.00^{a}\pm0.00$	$125.80^{c}\pm0.88$
CPF+ VE	$119.16^{a}\pm0.91$	$129.60^{b}\pm0.93$

Number of rats in each group was six; CPF = chlorpyrifos; VE = vitamin E; Values with different superscript within a column indicate significant difference for a parameter ($p\leq0.05$)

Table 2
Effect of oral administration of vitamin E on chlorpyrifos induced alterations in haematological parameters in rats

Parameters	Control	CPF	CPF + VE
Hb (g%)	$14.44^{a}\pm0.20$	$12.12^{e}\pm0.04$	$13.05^{b}\pm0.16$
PCV (%)	$40.81^{a}\pm0.90$	$38.23^{c}\pm0.91$	$39.36^{b}\pm0.84$
TEC ($x10^{6}$ µl)	$7.16^{a}\pm0.66$	$6.58^{c}\pm0.76$	$6.85^{b}\pm0.77$
TLC ($x10^{3}$ µl)	$7.83^{c}\pm0.71$	$8.53^{b}\pm0.90$	$8.10^{bc}\pm0.84$
Neutrophil (%)	$18.80^{f}\pm0.91$	$29.00^{b}\pm0.00$	$24.22^{d}\pm0.86$
Lymphocyte (%)	$74.66^{a}\pm0.33$	$62.20^{f}\pm0.22$	$68.50^{c}\pm0.40$
Monocyte (%)	$3.10^{c}\pm0.30$	$4.50^{a}\pm0.33$	$4.10^{b}\pm0.33$

Number of rats in each group was six; CPF = chlorpyrifos; VE = vitamin E; Values with different superscript within a row indicate significant difference for a parameter ($p\leq0.05$)

(Ambali *et al*., 2011).

The present investigation also revealed an increase in TLC with relative increase in neutrophil count in group II in comparison to group I (Table 2) which may be due to the stress caused by CPF. These findings corroborated with the result of Ambali *et al*. (2010a). In contrast, Goel *et al*. (2006) and Ambali *et al*. (2007, 2010b) reported leucopenia on repeated exposure to CPF in rats. The reason for the apparent leucocytosis is not known. Rabideau (2001) reported that pesticides may to be toxic to immune cells via induction of necrosis and apoptosis. Das and Mukherjee (2000) reported increased TLC due to stimulated lymphopoiesis and/or enhanced release of lymphocyte from lymph myeloid tissue.

In the present study, there was a significant ($p<0.05$) increase in percent neutrophils in group II as compared to group I (Table 2). This finding is in accordance with the observation of Goel *et al*. (2006) and the stress may be the reason for neutrophilia (Benjamin, 2001). However, percent lymphocyte count was significantly lower in CPF treated group (group II) as compared to control group. This might be due to the immunosuppressant effect of CPF and an increase in percent neutrophils in peripheral blood in group II was indicative of a decrease in percent lymphocytes. Deficits in percent lymphocyte in the present study in CPF treated group may be due to the slower rate of production or release of leucocyte to the blood circulating and relative increase in the neutrophil count due to stress as well as inflammatory response exhibited towards various organ damage caused by the CPF. Pretreatment with vitamin E did ameliorate the CPF-induced leukocytosis which may be due to its modulatory role on the immune cells.

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