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Original Article

Effect of Fytolan on Haematology and Serum Parameters of Male Albino Rats

BL Mehra¹, Priyanka Sharma², Utkarsh Kaushik², SC Joshi^{2,3,*}

¹ Government Lohiya PG College, Churu, Rajasthan, India

² Reproductive Toxicology Unit, Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India

³ Centre for Converging Technologies, University of Rajasthan, Jaipur, India.

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The present study was designed to evaluate the possible adverse effects of fytolan on the haematology and serum parameters of male albino rat. The tested doses were given orally to the wistar rats for 30 days at the dose level 5 mg, 7.5 mg and 10 mg/kg. b.wt./day. In the present investigation a significant decrease in total erythrocyte count, haemoglobin content and haematocrit value were observed, however increase in the total leukocyte count was noticed. The level of blood sugar and blood urea was increased significantly when exposed to fytolan. Protein content was found to be increased significantly after oral administration of fytolan. Significant increases in triglyceride and phospholipid level were noticed in higher doses exposed rats. In serum, total cholesterol, HDL, LDL and VLDL cholesterol were found to be significantly elevated compared to control.

Key words: Fytolan, haematology, serum, rat

Corresponding author *

Prof. S. C. Joshi

Centre for Advanced Studies

Department of Zoology,

University of Rajasthan, Jaipur

E-mail: s_c_joshi2003@rediffmail.com

1. INTRODUCTION

Fungicides will remain a keystone component of crop protection for several decades to come. These fungicides are found to be teratogenic, carcinogenic, mutagenic, reproductive toxicant as well as show harmful effects on ecology including non target plants and animals.^{1,2}

Fytolan is one of the broad spectrum copper based fungicide. It controls wide range of fungal and bacterial diseases on fruits, vegetables and ornamental

plants, so being used widely for crop protection. But copper released from fytolan are found to be very toxic for crops¹. Unfortunately the rampant and indiscriminate usage, further lack of safe handling, illiteracy and insufficient scientific knowledge of these chemicals makes them potential environmental contaminants, which cause severe harmful effects in many organisms, including man. Of all the toxicological hazards arising from these chemical least is known about its risks on blood and its components.

Blood plays a very important role in the maintenance of life and is the good bio-indicator to know about the health status of an individual. Any physio-morphological change in blood disturbs the homeostasis of body. Most fungicides are absorbed through gastrointestinal tract, so their appearance along with their metabolites can easily be detected in blood³ from where they reach to various organs and show their toxic effects⁴. Therefore present study attempted with aim to assess the risk associated with use of fytolan and its effects on haematology and serum constituents.

2. MATERIALS AND METHODS

2.1 Chemical used : Fytolan (Chemical name- copper oxychloride; Trade name- Blitox, Blue copper, Agrizan; Molecular formula- $\text{CuCl}_2 \cdot 3\text{Cu}(\text{OH})_2$). Technical grade fytolan (98% pure) obtained from Kisaan Chemicals Pvt. Ltd., Jaipur was used as test fungicide for experimentation.

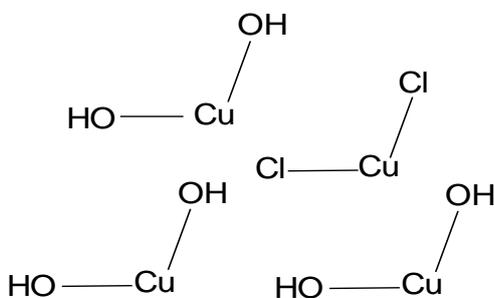


Fig.1: Fytolan Structure

2.2 Animal model

Twenty four healthy adult male rats of wistar strain weighing 150-200 gms were used for experimentation. They were housed in polypropylene cages at room temperature with natural light and dark cycles (12 h dark, 12 h light) and relative humidity 55 ± 5 %. They were fed on standard commercial pallet feed procured from Ashirwad food industries ltd., Chandigarh, India and water *ad libitum*.

2.3 Experimental Design

Male rats were divided into four groups of six animals in each group. The group I served as control, received only the vehicle (olive oil) whereas the animals of group II, III and IV received fytolan dissolved in olive oil by pearl point needle orally at the dose level of 5, 7.5 and 10 mg/kg b.wt./day for 30 days. At the end of the experiment all treated animals along with control were weighed and sacrificed under light ether anaesthesia. Blood was collected by cardiac puncture and serum was then separated.

2.4 Parameters studied:

2.4.1 Haematological parameters: haemoglobin⁵ haematocrit⁶ total erythrocyte count⁷ total leukocyte count⁷ blood sugar⁸ blood urea⁹ was performed from collected blood sample.

2.4.2 Serum Analysis: Serum separated from clotted blood was used to total cholesterol¹⁰, total protein¹¹, phospholipid¹², triglyceride¹³, HDL-cholesterol¹⁴, LDL-cholesterol¹⁵, VLDL-cholesterol¹⁵.

2.4.3 Statistical analysis: The data were analyzed statistically using Student's t-test (Gad and Weil, 1982) and the significance difference was set as $P < 0.01$ and $P < 0.001$.

3. RESULTS

Male rats exposed to different dose level of fytolan for 30 days showed sign of haematological and serum toxicity. In the present study, the treated rats showed remarkable reduction in the total erythrocyte count,

haemoglobin and haematocrit value ($P < 0.001$) (Table 1) after administration of fytolan. Whereas, total leukocyte count, blood sugar and blood urea ($P < 0.001$) has been significantly increased ($P < 0.001$) (Table 1). Total protein, phospholipid, triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol in serum were increased significantly ($P < 0.001$) (Table 2). However, Haematological indices (MCH, MCV and MCHC) showed no significant change in all treated groups (Table 3).

4. DISCUSSION

Haematopoietic and leukocytic systems are the two dynamic systems which react quickly to environmental changes and maintain the homeostasis¹⁵. Haematological parameters show conspicuous and significant changes in response to any kind of toxic stress. Blood is a sensitive index of the physiological changes in animal to any environmental contaminant. Hence, for evaluating the intoxication by fungicides, haematology is used as a clinical tool to know physiological and metabolic status. Number of report showed that fungicides may cause mobilization of the haematopoietic system by alterations in the number of erythrocytes, haemoglobin, haematocrit as well as the differential leukocytic count in rats^{16, 17}.

In present investigation the decrease in erythrocyte count may be due to either inhibition of erythrocyte production (Erythropoiesis in bone marrow) or destruction of erythrocyte due to poisoning. Two factors, vitamin B₁₂ and folic acid are essential for the proper maturation and production of nucleated red cells in bone marrow. Vitamin B₁₂ is stored in the liver and its deficiency is characterized by disturbances in erythropoiesis. Further deficiency of vitamin B₁₂ leads to impaired synthesis of nucleic acid resulting into defective maturation of erythrocytes and their nuclei^{7, 18}. The histopathological destruction of liver and

kidney which leads to reduce availability of erythropoietin, which is produced in the juxta glomerular apparatus in the kidney and is secreted in the plasma for the utilization by stem cells of bone marrow further supported the present result. The destruction of erythrocytes is indicated by haemolysis¹⁹.

The increased in leukocyte count may have resulted from the mobilization of the immunological system and/or shift of the leukocytic pool from the spleen to peripheral blood as fungicide act as chemical stressor leading to leukocytosis^{19, 20, 21}.

A decline in the haemoglobin content of treated group indicates that it may be due to either decrease in the rate of haemoglobin synthesis or increase in the rate at which the haemoglobin is destroyed (Mass and Hathway, 1964). Haemoglobin synthesis begins in the polychromatic normoblast stage. This synthesis requires iron which is generally obtained from stored ferritin and from dietary sources. The reduction in general food intake by experimental rats and no supplementary supply of extra iron might be the reason for the iron deficiency, which are essential for haemoglobin synthesis^{22, 23, 24}. A decline in the rate of haemoglobin synthesis occurs during all the stages of maturation of erythrocytes when the supply of iron is inadequate²⁵.

Diminished Hb content can also be correlated to the reduction in size of red blood cells or to the impede biosynthesis of heme in bone marrow²⁶. Such red blood cells with impaired integrity are fragile which then were easily ruptured and destroyed in the circulation. Similar results have been quoted by various workers in their studies^{27, 28, 29, 30, 31}.

The reduction in the size or number of erythrocytes may have resulted due to decrease in hematocrit count in the animals treated with benomyl and fytolan. The reduced Hb synthesis may be responsible for decreased

erythrocytic size resulting in decline of haematocrit values. And as there is a decrease in the rate of erythrocyte production with increase in its destruction rate, it could be the possible reason for decline in the number of erythrocytes³¹.

Hyperglycemia was observed in the rats in the present study due to fytalon. Pesticides increase glucose concentration in blood and also impair its uptake and utilization by body tissues^{19,32}. Further glycogenolysis in muscle and liver cause significant increase in blood glucose³³. Another possible reason for increased blood glucose may be due disrupted carbohydrate metabolism, due to enhanced break down of liver glycogen possible mediated by increase in adrenocorticotrophic and glucagon hormones and /or reduced insulin activity³⁴.

Whereas the elevation of plasma levels of urea is considered as significant marker of renal dysfunction³⁵. Elevated blood urea is correlated with an increased protein metabolism in mammalian body or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production³⁶. Additionally, the increase in blood urea was correlated closely with histopathological changes in the kidney which were degenerative and caused disturbances in the transport system of biochemical constituents³⁷. Fungicides induced increase in urea level observed in the present study may be due to the effect of fungicide on liver function, as urea is the end product of protein catabolism³⁸. Cases of acute renal failure followed by exposure to EBDC fungicide has also been reported³⁹.

Decline in the erythrocyte count and haemoglobin can be correlated with the alteration in the values of Mean Corpuscular Haemoglobin, Mean Corpuscular Volume and Mean Corpuscular Haemoglobin Concentration^{17, 29, 40}. The changes may be due to physiological dysfunctioning of the haemopoietic system^{41, 42}.

Triglyceride is an energy source of spermatozoa⁴³. In the present investigation the intoxication of rats with the fytolan showed a significant increase in the triglyceride level thus, indicating that its increase or decrease is suggestive of imbalanced triglyceride synthesis⁴⁴.

Significant increase in the phospholipid level may be due to the change in anabolism or catabolism of very low-density lipoproteins⁴⁵.

An increase in the serum total cholesterol following exposure to fytolan has been observed in present study. Elevation in the cholesterol level indicates the non-utilization of this precursor for steroidogenesis^{46, 47}. Also the accumulation of fungicides in the liver was associated with the disturbance of lipid metabolism and an elevation of serum cholesterol. Therefore, pesticide induced increase in serum cholesterol can be attributed to the effect of pesticides on the permeability of liver cell membrane and liver dysfunction. Similar hypercholesterolemia has been observed in other studies of pesticide intoxication^{17, 31, 32}. Therefore, it is concluded that fytolan produces significant toxic changes in the haematology and serum parameters of male rats.

Table 1: Blood Analysis

Treatment	Total erythrocyte count (TEC) million/mm ³	Total leukocyte count (TLC) Th/mm ³	Haemoglobin gm. %	Haematocrit %	Blood Sugar mg/dl	Blood Urea
Group I	6.98	4981.91	14.97	43.02	97.98	46.59
Control	±0.21	±241.01	±0.33	±3.11	±2.87	±4.01
Group II	5.81 ^{ns}	5117.65 ^{ns}	13.24 ^{ns}	41.18 ^{ns}	101.84 ^{ns}	49.29 ^{ns}
5 mg	±0.15	±365.40	±0.67	±1.02	±1.16	±7.45
Group III	5.01 *	6323.45 *	11.86 *	32.01 *	115.84 *	71.64 *
7.5 mg	±0.49	±349.82	±0.71	±1.10	±1.16	±3.94
Group IV	4.84 **	7425.63**	10.45 **	29.37 **	124.62 **	77.48 **

10 mg	±0.21	±376.97	±0.46	±0.16	±3.31	±5.68
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(Mean ± SEM of 6 Animals)

Group II, III and IV Compared with group I

ns = non-significant

* = significant (p<0.01)

** = highly significant (p<0.001)

Table 2: Serum Analysis

Treatment	Total protein	Phospholipid	Triglyceride	Total Cholesterol	LDL-Cholesterol	LDL-Cholesterol	VLDL-Cholesterol
Group I Control	1710 4.12 ±	153.21 ±7.29	98.97 ±5.92	95.61 ±4.45	41.5 8 ±4.5	38.4 6 ±2.9	21.2 7 ±2.6
Group II 5 mg	1814 4.01 ns ±727.18	172.07 ^{ns} ± 10.26	115.62 * ± 18.37	125.68 * ±5.58	160. 85 * ±12. 65	45.6 1 ^{ns} ±3.4 7	45.5 6 * ±2.2 8
Group III 7.5 mg	2693 3.17 * ±610.31	225.46 ** ±17.42	145.38 * ±10.15	140.31 * ±8.81	197. 99 ** ±14. 66	54.0 9* ±3.4 7	97.8 1 * ± 22.0 1
Group IV 10 mg	3213 6.39 ** ± 437.51	271.39 ** ± 7.32	198.35 ** ±14.74	201.38 ** ±10.34	202. 34 ** ±12. 32	78.4 8** ±2.6 2	105. 89 * ± 18.4 1

(Mean ± SEM of 6 Animals)

Group II, III and IV Compared with group I

ns = non-significant

* = significant (p<0.01)

** = highly significant (p<0.001)

Table 3: Hamatological Indices

Treatment	Mean Corpuscular Haemoglobin (MCH)	Mean Corpuscular Volume (MCV)	Mean Haemoglobin Concentration (MCHC)	Corpuscular Concentration
Group I Control	21.25 ^{ns} ±0.59	63.25 ^{ns} ±2.87	34.17 ^{ns} ±0.51	
Group II 5 mg	23.66 ^{ns} ±0.27	57.50 ^{ns} ±0.34	35.63 ^{ns} ±0.83	
Group III 7.5 mg	22.68 ^{ns} ±2.67	56.61 ^{ns} ±2.24	37.53 ^{ns} ±0.63	
Group IV 10 mg	18.93*± 3.27	58.74*± 1.19	29.41*± 0.55	

(Mean ± SEM of 6 Animals)

Group II, III and IV Compared with group I

ns = non-significant

* = significant (p<0.01)

** = highly significant (p<0.001)

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