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IMPACT OF BLACK T SUPRA ON HAEMATOLOGY OF ALBINO RATS

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ABSTRACT

The toxic haematological effects of carpet dye Black T Supra on various hematological parameters were evaluated in *Rattus norvegicus* (Berkenhout) following oral exposure at sublethal dose of this dye (20 mg/kg of body weight per day) for different periods. Exposure to this dye decreased haemoglobin content from 17 to 12.6 percentage, RBC from 6.5 to 2.88 x 10^6 per cmm., PCV from 54 to 34.5 percentage at the end of 90 days, HCV from 83.07 to76.10 c.µ. at the end of 30 days and MCHC after 90 days from 31.48 to 23.33 as compared to control. However, an increase was observed in MCV from 83.07 to 187.5 c.µ. after 30 days up to 120 days, MCHC from 31.48 to 37.10 percentage up to 90 days and MCH from 26.15 to 43.75 pg. PCV become constant after 90 days. Leukocyte count increases up to 90 days from 10.0 x 10^3 per cmm to 16.28 x 10^3 per cmm and decreased to 6.65 x 10^3 per cmm after 120 days. This caused haemocytic anemia, leucopenia, monocytosis, neutropenia showing declined immunity. The hypoglycemia, hypocholesteremia and increase in blood urea indicating renal failure. The stress inhibits erythropoiesis due to destruction of erythrocytes, haemopoietic tissue and haemodilution causing reduction of O_2 consumption leading to the death of animals. Hypoglycemia is an indicator of physiological stress and impaired liver function and may be due to rapid utilization of blood glucose during hyperexcitability. The hypocholesteremia may inhibit conversion of esterified cholesterol to the free form.

KEYWORDS: Black T Supra, carpet dye, *Rattus norvegicus* (Berkenhout), haematology, TLC, DLC, cholesterol, blood urea

The carpet industry of Eastern Uttar-Pradesh is spread in Sant Ravidas Nagar Bhadohi, Mirzapur, Varanasi, Allahabad and Jaunpur districts. Nearly 600 metric tons of carpet dyes, dye supporting chemicals, washing chemicals and insecticides are used in this industry every year. This has cumulative toxic environmental effects because effluents are poured in the terraquatic environment without proper denaturation.

Kumar and Sharma (1984), Tripathi and Sriwastwa (1985) indicated chromium pollution due to carpet industry effluents. Chromium compound is a carpet dye which is used in this industry. Sriwastwa et al. (1991) studied the biogeochemical cycle of chromium in carpet belt of Eastern Uttar Pradesh in view of hazardous effect of this dye. Sriwastwa et al. (1990) and Awasthi et al. (2008) studied the toxicity assay of carpet dyes following exposure to fish. The effect of carpet dyes on blood of fish and rat (Srivastav, 2004 and Gupta et al., 2006) was observed. However the study on the blood of mammals are very scanty. Hence this work was undertaken to observe the impact of toxic effects of Black T Supra on haematological parameters of *Rattus norvegicus* (Berkenhout).

MATERIALS AND METHODS

120 mature albino rats *Rattus norvegicus* (Berkenhout) weighing between 480-490 g were used. The animals were maintained on rat gram pellet diet and water *ad libitum.* They were acclimatized for 15 days prior to experimentation according to institutional ethical committee. The adult rats were divided in 10 groups of 12 each.

LD_{50} Studies

Out of 10 groups, seven groups were taken for the estimation of LD_{50}. Animals of each group were administered a single oral dose mixed with gram pellet diet of 2000 mg/kg (VII group), 1500 mg/kg (VI group), 500 mg/kg (V group), 200 mg/kg (IV group), 100 mg/kg (III group), 50 mg/kg (II group), 20 mg/kg (I group) respectively of Black T Supra carpet dye. The mortality was observed in percentage in all groups at the end of 24 hours and LD_{50} was calculated according to graphical method of Miller and Tainter (1944).

Hematological Studies

After the determination of $LD_{50,}$ 20 living albino rats were taken for experiment by dividing them in two

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groups of 10 each. First group of 10 albino rats including both male and female were used as control. They were fed normal gram pellet soaked in water. The second group of 10 albino rats was used as experimental animals. They were fed orally with gram pellet mixed with 20 mg/kg of body wt/day of Black T Supra carpet dye for 4 months and observation were recorded.

Every month blood samples were taken for haematological study after anesthatisation of animal by chloroform. The blood was collected directly from cardiac puncture by sterilized disposable syringe and stored in vials having anticoagulant (EDTA). All haematological parameters viz., haemoglobin concentration (Hb), red blood corpuscle (RBC) count, white blood corpuscle (WBC) count, packed cell volume (PCV) with their indices mean corpuscle volume (MCV), mean corpuscle haemoglobin (MCH), mean corpuscle haemoglobin concentration (MCHC) and erythrocyte sedimentation rate (ESR) were estimated by standard methods as prescribed by Dacie and Lewis (1977). The data of treated and control animals for haematological studies were taken after 0, 30, 60, 90 and 120 days. Sugar was estimated by furfural method using Anthrone reagent. Optical density was measured at 620 nm, blood cholesterol by acetic anhydride method using optical density at 640 nm and the blood urea was estimated by Phenol red method using optical density at 620 nm.

Statistical Analysis

The data were subjected to statistical analysis for the calculation of standard error of mean (SEM). The significance of the differences was assessed by one-way of analysis of variance (ANOVA) and are presented in table-IV and V.

RESULTS AND DISCUSSION

The estimated acute oral LD_{50} of Black T Supra carpet dye in white rats are found 500 mg/kg of body weight. Dose and mortality of rats in percentage are given in table-I. Toxic symptoms were salivation, bleeding through nose and unbalanced movement found in high dose treated rats viz. 500 mg/kg, 1000 mg/kg, 2000 mg/kg of body weight (table-I).

Table I: Percentage of mortality in *Rattus norvegicus* (Berkenhout) with a single oral dose of Black T Supra Carpet dye

Group	dose mg/kg	number of died animals	number of survived animals	% of died animals	correct %	LD_{50}
1	20	0	12	0	2	
2	50	0	12	0	2	
3	100	0	12	0	2	
4	200	5	7	42	42	
5	500	6	6	50	50	500mg/kg
6	1000	10	2	83	83	
7	2000	12	0	100	98	

The results of present investigation indicate profound changes in the haematological profile of the test animals treated with this dye. Table II and table III show the effect of Black T Supra carpet dye on different haematological parameters in albino rat. Table IV and V show comparison of difference in *p* between control and experimental values. From the data we have found differences were significant in most of the parameters except BUN suggesting that Black T Supra carpet dye have profound effect on haematological parameters of *R. norvegicus* (Berkenhout).

Haematological parameters are a valuable tool for assessing the injuries caused by certain substances. Decrease in blood cholesterol was observed by Lata (1985) in fish under Chrome Black T stress and she suggested it to compensate the effect of stress. Ahmed et al. (1987) found hypocholesteremia in blood of rat. Srivastav (2004) found hypercholesteremia in blood cholesterol under chromium stress in albino rat. In the present study under stress of Black T Supra carpet dye in *Rattus norvegicus* (Berkenhout) hypercholesteremia was found up to 90 days and thereafter hypocholesteremia was observed after 120 days. The result is highly significant.

Singh et al. (1993) reported significant decrease of cholesterol (hypocholesteremia) due to inhibited conversion of esterified cholesterol to the free form.

In the present study under Black T Supra stress hypoglycemia is observed. Hypoglycemia is an indicator of physiological stress and impaired liver function. This may be due to rapid utilization of blood glucose during hyperexcitability. Srivastav (2004) observed hypoglycemia under chromium stress in mammal. Tremor and convulsion are the characteristics of metal toxicosis reported in behaviour of fish and mammal (Singh et al.,1993, Rahman et al.,1996).

The Cu rich haemocyanin protein involved maximum toxic nature to blood parameter (Eaton 1973), this causes blood cell injury and disturbs total leucocyte count and haemoglobin synthesis. Srivastava and Kumar (1990) and Srivastav (2004) observed decrease in TLC under stress of basic chromium sulphate and hexavalent chromium stress. But under Black T Supra stress increase in TLC up to 90 days was observed and after 120 days it decreased showing leucopenia in albino rat.

Under Black T Supra stress the lymphocytes and monocytes first increase up to 90 days and decrease after 120 days where as Polymorph (Neutrophils) decrease during 120 days spell. Monocytosis and Neutropenia are seen indicating the decrease in immunity. Such type of observation was also reported by Srivastava and Kumar (1990) and Srivastav (2004) in same species under the stress of dye chromium.

Increased blood urea under Black T Supra stress was found which indicates renal failure in albino rat in the present study and the result is highly significant. A similar report was made by Srivastav (2004). The decrease in urea and serum creatinine under high dose of treatment is also a similar observation as reported by Al-Shinnawy (2009).

The decreased level of serum protein is indicative of metal toxicity. The heavy metals alter the protein concentration thus impairing the metabolism of protein. The reports are available on toxicants showing decrease in protein concentration in fish (Ram and Sathyanesan, 1987; Singh et al., 1993). It is possible that metal influences the conversion of tissue protein into soluble fractions reaching in the blood for utilization in cell repair. Increase in Calcium (Ca) is reported to be associated with hyperparathyroidism, multiple myeloma and rapid demineralization of the bone (Varley et al., 1980, Rahman et al.,1996).

Srivastava and Kumar (1990) and Srivastav (2004) observed decrease in haemoglobin under chromium stress. Chakravarty et al., (2005) found decrease in haemoglobin and total erythrocyte count under Malenchite green stress and the same was found under Black T Supra stress also which is highly significant in the present study. Decrease in haemoglobin may be a cause of haemocytic anemia.

Larsson et al., (1985) reported that metal stress inhibits erythropoiesis due to enhancement in destruction of erythrocytes, haemopoietic tissue and haemodilution. This causes reduction of O_2 consumption and thus may be the cause of fish death.

In the present study Hb percentage, RBC count and PCV percentage decreases significantly after 30, 60, 90 and 120 days which indicates inhibition of erythropoiesis in bone marrow but PCV becomes constant after 90 days, MCV decreases at the end of 30 days and MCHC decreases after 90 days whereas MCV increases after 30 days up to 120 days, MCHC increases up to 90 days and MCH increased up to 120 days under the stress of Black T Supra carpet dye. The results are highly significant.

Decrease Hb percentage, RBC count and PCV percentage values leads to anaemia. Anemia might have led to a fall in the red blood cell count, haemoglobin concentration and haematocrit volume. Which may also be due to blood cell injury and disrupted haemoglobin synthesis. According to Pamila et al., (1991), the reduction in haemoglobin content may be due to inhibiting effect of toxic substances on the enzyme system responsible for synthesis of haemoglobin. Joshi et al., (2002) suggested that heavy metal exposure decreases the RBC, Hb percentage and PCV percentage due to impaired intestinal absorption of iron. Increase in MCV value and total leucocytes count suggested that the anaemia was of macrocytic type (Sinha et al., 2000).

CONCLUSION

Carpet dye Black T Supra is found to exert toxic haematological effects under laboratory conditions. The stress of Black T Supra suppressed haemopoietic system, which decrease RBC count, WBC count, Hb Concentration and PCV. Further reduction of MCHC indicated poor haemoglobin carrying capacity of erythrocytes. Decreased PCV is an indicater of effect of stress on animal health and oxygen carrying capacity of blood. Increase MCH is compatible with haemocytic anaemia which shows that Hb has been replaced by erythrocytic stomal materials. The exposure caused leucopenia, monocytosis and neutropenia showing loss of immunity. Hypocholesteremia is due to inhibited conversion of esterified cholesterol to the free form. Hypoglycemia might be due to rapid utilization of blood glucose during hyperexcitability. Increased blood urea suggests renal insufficiency. The inhibited erythropoiesis due to destruction of erythrocytes, haemopoietic tissue and haemodilution in turn causing reduction of oxygen consumption causing death of animals.

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Table II: Changes in blood of *Rattus norvegicus* (Berkenhout) under the stress of Black T Supra Carpet dye

Groups	Days	Leucocytic Cell Count (Differential)					Serum Cholesterol (mg%)	Blood Sugar (mg%)	Blood Urea (mg%)	BUN (mg%)	Serum Uric acid (mg%)	ESR (mm/hr)
		Lymphocytes (%)	Polymorphs/ Neutrophils (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)						
Control		40.0 ± 1.5	70.0 ± 0.28	----	2.0 ± 0.1	2.0 ± 0.13	63.0 ± 2.3	150.35 ± 2.2	36.50 ± 2.8	20.15 ± 1.1	----	5.0 ± 0.2
Experimental	30	45±1.8 (+12.5%)	65.0±1.1 (-7.14%)	----	1.0±0.05 (-50%)	2.5±0.5 (+25.0%)	72.0±25 (+14.3%)	125.25±2.6 (-16.7%)	38.25±3.1 (+4.8%)	21.72±1.21 (+7.8%)	----	4.0±0.1 (-20.0%)
	60	55.0±1.94 (+37.5%)	55.0±2.1 (-21.43%)	----	0.0	3.0±0.67 (+50.0%)	78.0±2.68 (+23.8%)	106.8±2.8 (-28.96%)	40.20±3.6 (+10.13%)	22.25±1.32 (+10.42%)	----	3.0±0.4 (-40.0%)
	90	60±1.7 (+50.0%)	38.0±1.6 (-45.71%)	----	0.0	3.5±0.68 (+75.0%)	83.0±3.0 (+31.74%)	100.2±3.2 (-33.35%)	45.63±3.9 (+25.01%)	23.12±1.26 (+14.73%)	----	2.0±0.3 (-60.0%)
	120	85±1.77 (+112.5%)	13.0±1.9 (-81.42%)	----	0.0	2.0±1.1 (0%)	48.0±2.7 (-23.80%)	90.4±2.73(39.87%)	50.0±4.1 (+36.98%)	23.36±1.2 (+15.93%)	7.5± 1.68	0.0

Data in parenthesis indicate % reduction, Values in comparison to Control ± SEM, BUN = Blood Urea Nitrogen, ESR = Erythrocyte Sedimentation rate.

Table III : Haematological changes in *Rattus norvegicus* (Berkenhout) under the toxicity of Black T Supra Carpet dye

Groups	Exposed Period in Days	RBC ($x10^6$/cmm)	WBC ($x10^3$/cmm)	Hb (g%)	PCV (%)	MCV (C.µ)	MCH (p.g.)	MCHC (%)
Control		6.5±0.23	10.0±0.1	17.0±0.22	54.0±1.8	83.07±1.8	26.15±1.76	31.48±2.4
Experimental	30	5.65±0.26 (+13.07%)	12.0±0.22 +20.0%)	15.0±0.53 (-11.76%)	43.0±2.3 (-20.3%)	76.10±2.7 (-8.39%)	26.54±2.8 (+1.49%)	34.88±0.93 (+10.80%)
	60	4.32±0.30 (-33.53%)	14.0±0.31 (+40.0%)	13.2±1.6 (-22.35%)	36.4±2.5 (-32.59%)	84.25±2.65 (+1.42%)	30.55±2.76 (+16.82%)	36.26±1.45 (+15.18%)
	90	3.75±0.7 (-42.30%)	16.28±0.48 (+62.8%)	12.8±2.0 (-24.70%)	34.5±2.1 (-36.11%)	92.0±2.26 (+10.74%)	34.13±2.92 (+30.51%)	37.10±1.47 (+17.85%)
	120	2.88±0.9 (-55.69%)	6.65±0.43 (-33.5%)	12.6±2.1 (-25.88%)	54.0±0.9 (0)	187.5±2.4 (+125.71%)	43.75±3.2 (+67.30%)	23.33±2.3 (-25.89%)

Data in parenthesis indicate % reduction, Values in comparison to Control ± SEM, RBC red blood Corpuscles (Total Erythrocytes Counts), WBC white blood corpuscles (Total Leucocytes Counts), Hb = haemoglobin concentration, PCV = Packed Cell Volume, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin concentration.

Table IV: One way ANOVA of the differences in blood of *Rattus norvegicus* (Berkenhout) under the stress of Black T Supra Carpet dye

Parameters	DF	SS	MS	F
Lymphocytes				
Between experimental groups	4	3690.00	922.50	107.292**
Within experimental groups	10	85.980	8.598	
Total	14	3775.980		
Neutrophils				
Between experimental groups	4	6440.400	1610.100	163.096**
Within experimental groups	10	98.721	9.872	
Total	14	6539.121		
Eosinophils				
Between experimental groups	1	1.500	1.500	9.375*
Within experimental groups	4	0.640	0.160	
Total	5	2.140		
Monocytes				
Between experimental groups	4	5.100	1.275	19.922**
Within experimental groups	10	0.640	0.0640	
Total	14	5.740		
Serum Cholesterol				
Between experimental groups	4	2317.067	579.267	92.260**
Within experimental groups	10	62.787	6.279	
Total	14	2379.853		
Blood Sugar				
Between experimental groups	4	6739.106	1684.776	182.965**
Within experimental groups	10	92.082	9.208	
Total	14	6831.188		
Blood urea				
Between experimental groups	4	380.857	95.214	11.171**
Within experimental groups	10	85.235	8.523	
Total	14	466.092		
Bun				
Between experimental groups	4	19.806	4.952	3.206 NS
Within experimental groups	10	15.443	1.544	
Total	14	35.249		
ESR				
Between experimental groups	3	15.000	5.000	6.346*
Within experimental groups	8	6.304	0.788	
Total	11	21.304		

*Significant at $p < 0.05$ and Highly **Significant at $P < 0.001$

NS = Non Significant

Table V :One way ANOVA of the haematological change s of *Rattus norvegicus* (Berkenhout) under the stress of Black T Supra Carpet dye

Parameters	DF	SS	MS	F
RBC				
Between experimental groups	4	25.409	6.352	17.279**
Within experimental groups	10	3.676	0.368	
Total	14	29.086		
WBC				
Between experimental groups	4	164.136	41.034	36.181**
Within experimental groups	10	11.341	1.134	
Total	14	175.477		
Hb				
Between experimental groups	4	41.904	10.476	26.508**
Within experimental groups	10	3.952	0.395	
Total	14	45.856		
PCV				
Between experimental groups	4	833.400	208.35	69.218**
Within experimental groups	10	30.101	3.010	
Total	14	863.501		
MCV				
Between experimental groups	4	25877.215	6469.304	72.973**
Within experimental groups	10	886.536	88.654	
Total	14	26763.752		
MCH				
Between experimental groups	4	616.438	154.10	10.417**
Within experimental groups	10	147.940	14.794	
Total	14	764.377		
MCHC				
Between experimental groups	4	378.074	94.519	41.097**
Within experimental groups	10	22.999	2.300	
Total	14	401.073		

*Significant at $p < 0.05$ and **Highly Significant at $P < 0.001$

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