Bull. Environ. Contam. Toxicol. (2003) 71:453–457

DOI: 10.1007/s00128-003-8781-5

Bulletin of Environmental Contamination and Toxicology

Toxic Effects of Textile Printing Industry Effluents on Liver and Testes of Albino Rats

N. Mathur,[1] R. Krishnatrey,[1] S. Sharma,[1] K. P. Sharma[2]

[1] Department of Zoology, University of Rajasthan, Jaipur 302004, India
[2] Department of Botany, University of Rajasthan, Jaipur 302004, India

Received: 2 November 2002/Accepted: 12 May 2003

The famous Sanganer textile printing industry began during the 18th century and flourished after the 19th century. Mineral colors like copper sulphate ($CuSO_4$) and tin chloride ($SnCl_2$) or vegetable and animal dyes were widely used at that time. Such natural dyes have now been almost completely replaced by synthetic ones. Reasons for this shift include the slow and expensive process of traditional dyeing, and the fact that synthetic dyes have relatively stronger colors. Many of the synthetic dyes are coal-tar derivatives and can be chemically classified as azo dyes, indigo and aniline dyes.

The textile printing industry requires large volumes of freshwater at various stages. These printing plants then discharge the untreated wastewater into several drains and ditches on ground, which causes serious pollution. The color of the wastewater is the most apparent indicator of pollutants. The accumulation of wastewater in the pools and ponds adversely affects the groundwater due to the highly porous sandy soil of Sanganer. Heavy metals in the effluents of Sanganer (Sharma et al. 1999) and sewage and sludge waters have been contaminating the environment. Fish mortality has been recorded in a pond receiving textile effluents, due to the presence of H_2S, NH_3 and Cl_2 (Mishra et al.1990). Textile effluents were also found to be responsible for the decrease in the RBC count and the Hb content in male Wister rats (Kurde and Singh 1995; Mathur et al. in press). Due to the mutagenicity of some of the synthetic dyes, disposal of textile printing wastewater has become a matter of great concern for human and animal health. In light of the above, a study was conducted to assess the impact of dye wastewater from Sanganer on albino rats. During this process, histopathological and biochemical investigations were performed to determine the effect of toxic effluents on the liver and testes of Wistar albino rats. The results and analysis of the aforementioned experiments are reported in this paper.

MATERIALS AND METHODS

Textile dye wastewater was collected from three pools outside factories situated close to Rajashri Resort near Sanganer Railway Crossing in Jaipur. This water was chemically analyzed by AAS (Varian Spectra 30) (Table 1).

Correspondence to: N. Mathur, A-25, Malviya Nagar, Jaipur 302017, India

Table 1. Chemical analysis of textile wastewater effluent pools near Sanganer, Jaipur District, Rajasthan (ppm).

Water Source	Cu	Pb	Zn	Fe	Mn	Na	K	Cd	Ca	Mg
Pool 1	1.03	0.06	0.33	0.79	0.17	155	6.6	0.005	-	-
Pool 2	0.33	0.09	0.56	1.50	0.15	185	6.00	0.005	200	153
Pool 3	1.00	0.27	0.23	1.60	0.27	147	7.50	-	-	-

After finding the LD_{50} value using Finney's method (Finney 1971), the concentration of the wastewater sample was diluted to 5% for study purposes. Adult male Wister albino rats weighing 140-160g were selected for the study. The animals were maintained under uniform laboratory conditions and were equally divided into two groups of 10 animals each. The animals in Group 1 served as control and were provided a pellet diet and water *ad libitum*, while those in Group 2 received the 5% solution of dye waste effluent and a normal pellet diet. The animals were sacrificed in batches after 30, 45 and 60 days. Their livers and testes were quickly excised, washed with cold physiological saline and used for estimation of total lipid (Folch et al. 1957), cholesterol (Zak et al. 1954), protein (Lowry et al. 1951) and glycogen (Montgomery 1957) content. Light microscopic observations of liver and testes were made on 5μm thick paraffin sections stained with hematoxylin-eosin. The data was statistically analyzed using the Student 't' test (Ipsen and Feigl 1970).

RESULTS AND DISCUSSION

Histologically, in the liver, treatment was observed to cause focal necrosis, cytoplasmic vacuolation, pycnotic nuclei with cellular degeneration, disarray of hepatic cords, and congestion of sinusoids. With increase in treatment time, degenerative changes of the hepatocyte, severe cellular vacuolation and neuclear karyolysis increased. Binucleate condition was also seen in a few hepatocytes.

The testes after treatment showed presence of a few spermatogonia, primary spermatocytes and spermatids. The interstitial area showed marked damages and the Leydig cell number decreased with increase in experimental time. The tubules appeared normal in size but they showed varying degrees of degenerative changes with the increase in time. Arrest of the spermatogenesis at secondary spermatocytes level, followed by the absence of spermatids and spermatozoa, cytoplasmic vacuolation and accumulation of cellular debris were seen.

For all the experimental intervals after treatment with effluent, there was a significant increase in the total lipid and the total cholesterol contents in both the testes and the liver, as compared to controls (Table 2). On the other hand, the total protein and glycogen showed reduced levels with increase in experimental time as compared to controls.

Table 2. Effect of textile dye effluents on liver and testicular biochemistry in albino rats.

Exposure Days		Control	30 Days	45 Days	60 Days
Total Lipid (mg/g)	Liver	17.23 ±0.61	21.02 ±1.06**	24.53 ±1.33**	25.69 ±1.00**
	Testes	12.25 ±0.43	17.87 ±1.45**	18.20 ±0.47**	19.21 ±0.66**
Total Cholesterol (mg/g)	Liver	9.62 ± 0.25	14.02 ±0.51**	16.36 ±0.61**	19.54 ±0.88**
	Testes	6.27 ±0.55	10.14 ±0.46**	11.11 ±0.83**	12.10 ±0.48**
Total Protein (mg/g)	Liver	43.09 ±2.15	121.35 ± 4.63**	118.77 ± 2.17**	110.22 ± 4.32**
	Testes	205.17 ± 3.83	190.02 ± 4.99*	187.72 ± 4.00*	184.00 ± 2.99**
Glycogen (mg/g)	Liver	6.17 ±1.02	5.83 $\pm 0.75^{ns}$	5.45 $\pm 0.56^{ns}$	4.01 ±0.10*
	Testes	3.65 ± 0.25	2.90 $\pm 0.43^{ns}$	2.81 $\pm 0.85^{ns}$	2.74 ±0.15**

* Values significant at P<0.05; ** Values significant at P<0.01;
^{ns} Values not significant

The liver is known to be the first organ to face any foreign molecule that is carried through portal circulation and exposed to damage. It is the chief detoxifying organ and hence is adversely affected by presence of toxicants. The histopathological characteristics observed here are in agreement with those noted by researchers in the past (Karasasi 1975; Devi and Singh 1988). A significant rise in cholesterol and lipid levels showing liver dysfunction was observed. Hypercholesterolaemia due to liver damage and dysfunction, leading to inhibition of cholesterol conversion to bile acids and sex steroids was also detected. Similar results were also recorded by Murray et al. (1990).

Liver glycogen levels decreased as the duration of the experiment was increased. It is known that approximately two-thirds of free glucose entering liver is phosphorylated to glucose-6-phosphate and converted into glycogen, fatty acids or blood glucose. Glycogen conversion process may have been affected due to the fact that the hypatocytes were necrotic, as has been reported by Hurkat (1978). Another possibility is the destruction of G-6-phosphatase located in the membrane of ER, as a result of toxics in the effluent.

The liver is responsible for the synthesis of most of the plasma proteins. In this investigation, decrease in the liver protein may have resulted from necrotic action

of dye effluent. This is supported by the morphological damages seen in the liver cells. In progressive liver damage, there is a continued fall in the level of total protein. Hence it can be inferred that liver-insufficiency which is the most significant causative factor in the genesis of dysproteinemia, was evoked by toxic chemicals present in the effluent.

In this study, histopathological changes in the testes revealed signs of toxicity. Similar degenerative changes have been reported after the use of polluted water (*Gupta 1996 (unpublished); Yadav 2001(unpublished)*. The degree of damage was observed to increase with time. The damages in the seminiferous tubule were shown to result in the rise of cholesterol and lipid contents. The testes of dogs and rats, when treated with cadmium chloride and selenium respectively, have been shown to have similar affects (Dixit et al. 1975; Kaur et al. 1999). The total lipid and the total cholesterol contents of the testes are indicative of decrease in testicular functions. The decreased utilization for steroidogenesis, which may be due to pituitary inhibition or a direct inhibitory action on the target tissue, results in the rise of cholesterol. Leydig cells of the testes can continuously utilize cholesterol from circulating blood for the synthesis of hormones, but the dyes and other toxicants present in the effluent destroy these cells, and the unutilized cholesterol raises the cholesterol content.

It is well known that testicular damages impair protein secretions, because the Sertoli cells synthesize proteins under the control of FSH. Depletion in glycogen and protein correspond to the inhibition of spermatogenesis and suppressed Leydig cell functions in treated rats. It is evident that protein synthesis in the spermatogenic cells is dependent on glucose, and a marked decrease in the glycogen content can affect protein synthesis, thus inhibiting spermatogenesis. Protein depletion is known to cause a decreased androgen production in the adult rat testes. Studies indicate a close relationship between protein and steroid hormone synthesis, which is sensitive to protein synthesis inhibition (Stocco 2001). This can be one of the factors in inhibiting steroidogenesis, as is also evident from the present study.

Acknowledgments. We would like to thank the Head, Department of Zoology and Coordinator, SAP for providing various facilities during the study. Two of the authors (SS and KPS) would also like to thank the Department of Biotechnology, India for financial support.

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