Effects of Textile Dye Waste water on Reproductive System of Mice and their Progeny

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ABSTRACT: Environmental pollution by textile dye waste water is an alarming issue in Bangladesh. This water is very toxic as it contains dyes, alkalis, heavy metals etc. in high concentration. However, no comprehensive study has yet been undertaken in Bangladesh knowing the effects of textile dye waste water in living animal. This study investigated the effects on the reproductive system of swiss albino mice exposed to waste water collected from a textile dye industry. For this purpose, 24 sexually matured mice were divided into three equal groups- control, influent (untreated water) and effluent (biologically treated water) group. Each group contains six female and two male. The mice were exposed to waste water for 30 days through oral route and were kept in the cages in the ratio of 3:1(female : male). Gross study revealed that, no abnormality found in ovary and testis but there was significant ($p<0.05$) weight reduction in testes while no reduction in weight of ovary. Histologically, lymphocytic infiltration and displacement of seminiferous tubule from basement membrane were found. The magnitude of these alterations was stronger in the influent group compared to effluent group. In case of progeny, from control and effluent group, dams gave birth 4-5 liters whereas no liter from influent group. From this study, it is observed that significant histopathological changes were found in the testes of influent group which may be one of the causes of infertility of that group. In the progeny of effluent group, no abnormalities were observed. So, from these findings it is concluded that, water from textile dye industry specially influent has a toxic effect on reproductive system particularly on male mice.

Keyword: histomorphology, influent, infertility, mice, progeny, toxic

1. INTRODUCTION

INDUSTRIALIZATION is believed to cause inevitable problems, such as pollution of air, water and soil. The textile industry is one such source that grew out of the industrial revolution in the 18th century as mass production of clothing became a mainstream industry. Workers of textile industry are mainly exposed to a variety of toxic dyes, bleaching agents, salts, acids, alkalis and heavy metals like cadmium, copper, zinc, chromium, iron etc and possibly carcinogenic compounds such as dyes, organic solvents and fixatives throughout the printing process [1]. Textile mill operations consist of weaving, dyeing, printing and finishing [2]. Many processes involve several steps, each contributing a particular type of waste, which may invite many diseases: both occupational and general [2] and consequently escalating the economic cost [3]. The voluminous amount, toxic nature and restricted land area for disposal makes environment management of chemical sludge generated from Common Effluent Treatment Plants (CETPs) for textile dyeing and printing process waste water a major challenge [3]. Most of the time, waste water from textile dye industry of which <10% is treated (effluent); the remainder is discharged untreated (influent) in drains and shallow pools adjoining printing industries, causing a serious pollution problem [4]. A wide range of animals including cattle drink the contaminated water, either because of the lack of access to safe water or because of the high salt content of the waste water (2.45±0.9 g/L). Accidental drinking of pool waste water resulted in calf mortality [5].

However, potentially hazardous agents and situations are encountered in this industry, some of which might even influence the reproductive health [6]. Humans may also be affected from exposure to agents that interfere with ovulation or spermatogenesis [7]. The toxicity of azo dyes based on benzidine and its congeners, dimethyl and dimethoxybenzidine, has been extensively studied in so far as

textile, leather and paper industries use a large number of dyes derived from these chemicals. Benzidine csuses cancer of the bladder in humans [8]. In mammals, the azo dyes are metabolized to their parent amines by intestinal microflora. These amine derivatives unlike their parent compounds, are readily absorbed by the gut [9], [10], [11]. Their urinary detection has been reported in several exposed species, including humans [12], monkeys [13], rodents and dogs [14]. The amine derivatives may cause mutagenic effects [15],[16] which may lead to cancer, as observed in animals repeatedly exposed to aniline through diet [17].

There is an incomplete knowledge regarding the potential reproductive toxicity of textile dye waste water in mammals. Therefore, in the present study, an attempt has been made to study the effects of textile dye waste water on reproductive system of Swiss albino mice and their progeny.

2. MATERIALS AND METHODS

2.1 Animals: Healthy, mature Swiss albino mice (*Mus musculus*) (age: 50-55 days), weighing 30-35g were acclimated 1 week prior to entry into the experimental protocol. Animals were housed in a well ventilated facility (temperature = 25±3 °C; humidity = 40-60%; 12 h light: dark cycle) maintaining the guidelines of institutional ethical committee and fed a standard diet provided by International Centre of Diarrheal Disease Research, Bangladesh (icddr,b) and tap water ad libitum. Thereafter, animals were divided into three groups supplied different water treatments orally for 30 days, including: control group (university tap water), influent group (untreated textile dye waste water) and effluent group (treated textile dye waste water). Each group had eight mice (six female and two male). Female and male mice were kept in the cages in the ratio of 3:1 (female: male). The females were checked for the presence of vaginal plug every morning. The day vaginal plug was seen was taken as day 0 of gestation and the female was presumed to be pregnant. Such females were caged singly.

2.2 Dye waste water: The textile dye waste water samples used during the present study were collected from an Effluent Treatment Plant (ETP) of a textile dye industry located at Valuka, Mymensingh and stored at 4°C during the study period. The waste water was analyzed in the laboratory of Department of Environment, Dhaka. The characteristics of the influent (untreated) and effluent (biologically treated) were as follows which were compared with normal water (Table 1). In case of influent, level of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were higher than the limits set by Department of Environment, Bangladesh.

Table 1: Characteristics of waste water in comparison to normal water

Characteristics	Influent	Effluent	Normal water
pH	9.68	7.99	7.00
BOD (mg/L)	180	26	Nil
COD (mg/L)	481	72	Nil
TDS (mg/L)	2555	2210	Nil
Chemicals	- Different dyes (azoic) - Calcium carbonate - Chloride - Arsenic - Oil and grease	- Hydrogen peroxide - Ferrous sulfate - Sodium hydroxide - Sulfuric acid - Alum	Absence of such chemicals
Colour	Dark black	Light black to greyish	No
Turbidity	Very turbid	turbid	No turibidity

2.3 Necropsy: The initial and final body weights were recorded on the 1st and 31th days, respectively. After 24 hour of the experimental period (31th day), all animals (except pregnant animals and progeny) were sacrificed to expose their

reproductive systems (ovary and testis). These organs were carefully removed, washed in normal saline solution (0.9%), blotted and weighed. Total procedures were done with the recommendation of the Institutional Ethical committee for research.

2.4 Histopathology of testes and ovary: Specimens of testes and ovary were dissected from all animals immediately after killing, washed thoroughly with formal saline and then fixed in 10% neutral-buffered formal saline for 72 hours at least. All the specimens were washed in tap water for half an hour, dehydrated in ascending grades of alcohol (70-90-95% absolute), cleared in xylene and then embedded in paraffin wax. Serial sections of 6 um thick were cut and stained with Haematoxylin and eosin [18] for histopathological investigation.

2.5 Statistics: The data are expressed as mean±SEM. Statistical tests (student's `t'-test; one and two way ANOVA) were applied to find significant difference be tween values of various parameters recorded for control and treated animals. The differences will considered to statistically significant When the p value obtained will less than 0.05 or 0.01

3. RESULTS

3.1 Body weight and weight of individual reproductive organs: Body weight was significantly lower in treated animals in relation to the control group especially for those animals receiving influent (Table 2). The percent reduction in body weight was greater in influent group (26±0.16) than in effluent group (27±0.52). Similar trends were observed in the weight of testis (Table 2) but there was no significant weight variation in the weight of ovary.

Table 2: Weight (total final body weight & organ weight) in control and treated water

Criteria	Control	Influent	Effluent
(gm)	Mean	SD	Mean	SD	Mean	SD
Total weight	34	0.14	26*	0.16	27*	0.52
Ovary	0.071	0.02	0.069	0.01	0.062	0.02
Testis	0.164	0.36	0.101**	0.51	0.153*	0.43

SD= standard deviation; significant at 5%* and 1%** level

3.2 Pathological features of testes and ovary: There were no gross abnormalities in both testes and ovary. There was also no histopathological lesion in ovary. But histopathological alterations of testes were observed in waste water treated mice (Fig 1). These abnormalies were greatest in influent group and included the following features: accumulation of spermatozoa with exudates in tubular lumen and displacement of seminiferous tubule from basement membrane in influent and vascular congestion with marked atrophied spermatozoa in effluent group.

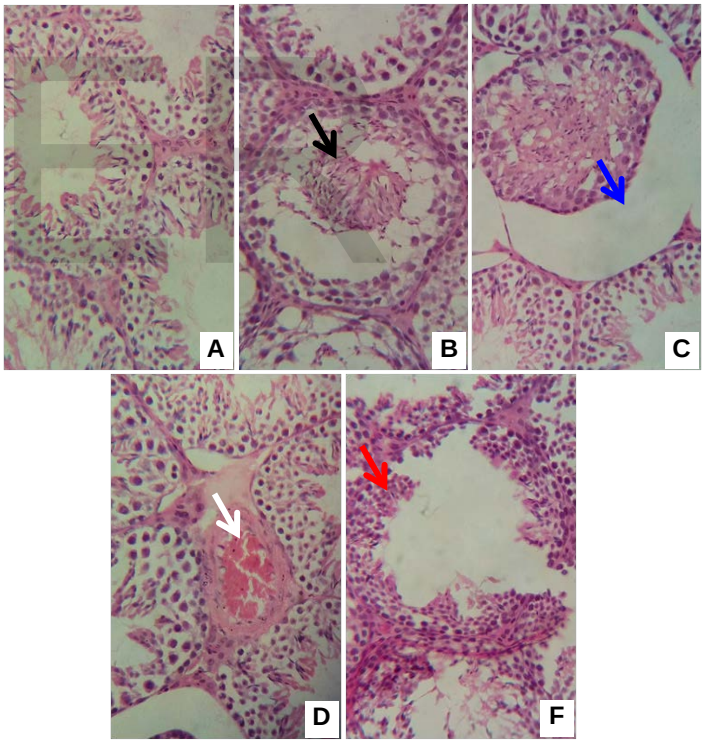

Fig. 1: Photomicrograph of a section of testis from control and treated mice stained with H&E (40X). (A) Normal appearance of testis in control group. (B, C) The sections showing accumulation of spermatozoa with exudate in tubular lumen (black arrow and displacement of seminiferous tubule from basement membrane (blue arrow) in influent & vascular congestion (white arrow) with marked atrophied spermatozoa (red arrow) in effluent group (oral group).

3.3 Fertility: Mice exposed to the influent (untreated waste water) failed to sire a litter whereas mice of effluent and

control group gave birth 4-5 and 5-6 litters from each dam respectively.

3.5 Progeny: The progeny were observed for 15 days for investigating any behavioural changes. But there were no behavioural changes were found. After 15 days they were sacrificed for observing any gross or histopathological changes. There was no gross (Fig 2) and histopathological changes were found in the effluent group in comparison with control group.

Fig. 2 : Progeny from control (left) and effluent (right) group (5 days old)

4. DISCUSSION

Findings in the present study with acute (30 days) exposure of male mice to drinking water from treated (Effluent) and untreated (Influent) textile dye wastewater of Valuka, Bangladesh showed adverse effect on reproductive parameters. These findings are generally consistent with the studies by Gray and Ostby [19], who reported a reduction in testicular weight of mice and rats exposed directly to dyes such as congo red, diamine bule and chlorazol Black E, which may be ascribed to a widespread testicular damage [20]. In the present study, body weight of treated animals decreased. This may be due to the heavy metals in the wastewater. Similar results were observed by Ravibau [21] in rats after treated with industrial effluent collected from the common effluent treatment plant located near Hyderabad. This result also found in rats and mice after treated with textile dye waste water [6, 22]. The histopathological alterations were also found in testis in the present study. Similar results were also observed by Chowdhury and Naha [23]. It is important to note that there was no gross and histopathological changes in ovary.

Himani [24] described that maternal toxicity to certain extent were produced in mice including muscular tremors, ataxia, convulsions, hypersalivations, lacrimation and restlessness. But in the present study, such type of alterations were not found. Again another study revealed a reduction of litter size [25] which is not similar with our study. Our study observed that there was no litter from influent treated animal. So the reduced ability of mice of influent group to sire a litter would reasonably be explained by the alterations observed in the testes. Histopathological changes may affect the capacitation of male mice for fertilization. But the sire from effluent treated animal were normal in gross appearance which vary the results from another study [24] that showed some external malformations in mice.

In conclusion, the present study indicates that textile dye waste waters of Valuka have severe toxic effects on male reproductive system. The toxicity is, however, relatively mild amongst those animals receiving treated (effluent) waste water versus those exposed to the untreated (influent) waste water. The untreated waste water (influent) caused complete sterility in male mice which was not found in treated waste water (effluent). Although other studies suggest some alterations in ovary of mice and their progeny due to exposure of waste water of textile industry, we did not find convincing results to support the results of other studies. Further studies are needed to better establish the possible association between waste water and female reproductive system of mice and their progeny.

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REFERENCES

[1] International Agency for Research on Cancer (IARC). Monographs on the evaluation of carcinogenic risk to humans. Lyon 1997; 69: 545-51.

[2] Pratima S, Sharma S, Sharma S, Kumar S and Sharma KP. A comparative study on the toxic effects of textile dye waste waters (untreated and treated) on mortality and RBC of a freshwater fish Gambusiaaffinis (Baired and Gerard). J Environ Biol 2006; 27: 623-628.

[3] Tiwari H. Reproductive Performance of Swiss Albino Mice Treated with Leachate From CETP Pali, Administered During Various Stages of Reproductive Cycle. Res J Chem Env Sc 2013; 1(3): 03-10.

[4] Sharma KP, Sharma K, Bhardwaj SM, Chaturvedi RK, Sharma S. Environment impact assessment of textile printing industries in Sanganer, Jaipur: a case study. J Indian Bot Soc 1999; 78: 71-85.

[5] Sharma K. Environmental impact assessment of textile industry waste waters in Sanganer environment. Ph.D. Thesis. University of Rajasthan, Jaipur, India; 2000.

[6] Suryavathi V, Sharma S, Sharma S, Saxena P, Pandey S, Grover R, et al. Acute toxicity of textile dye wastewaters (untreated and treated) of albino rats and mice. Reproductive Toxicology 2005; 19: 547-556.

[7] Olshan AF and Faustman E. Male medicated developmental toxicity. Ann Rev Public Hlth 1993; 14: 159-181.

[8] Haley TJ. Benzidine revisited: a review of the literature and problems associated with the *use* of benzidine and its congeners. Clin Toxicol 1975; 1:13-42.

[9] Bowman MC, Oller WL, Nony CR, Rowland KL, Billedeau SM, Lowry LK. Metabolism and distribution of two 14C benzidine congener-based dyes in rats as determined by GC, HPLC and radioassays. J Anal Toxicol 1982; 6: 164-74.

[10] Bowman MC, Nony CR, Billedeau SM, Martin JL, Thompson Jr HC, Lowry LK. Metabolism of nine benzidine-congener-based azo dyes in rats based on gas chromatographic assays of the urine for carcinogenic metabolites. J Anal Toxicol 1983; 7: 55-60.

[11] Bos RP, Krieken W, Seijsters L, Koopman JP, Dejonge HR, Theuws JLG, et al. Internal exposure of rats to benzidine-based dyes after intestinal azo reduction. Toxicology 1986; 40: 207-13.

[12] Lowry LK, Tools WP, Boeniger MF, Nony CR, Bowman MC. Chemical monitoring of urine from workers potentially exposed to benzidine-derived azo dyes. Toxicol Lett 1980; 7: 29-36.

[13] Rinde E, Troll W. Metabolic reduction of benzidine azo dyes to benzidine in the rhesus monkey. J Natl Cancer Inst 1975; 55: 181-2.

[14] bisazobiphenyl dyes derived from benzidine, 3, 3-dimethylbenzidine or 3,3-Lynn RK, Danielson DW, Ilias AM, Wong Y, Kennish JM, Mathews HB. Metabolism of dimethoxybenzdine to carcinogenic aromatic amines in dog and rat. Toxicol Appl Pharmacol 1980; 56: 248-59.

[15] Miller JA, Miller EC. The carcinogenic *azo* dye. Adv Cancer Res 1953; 1: 340-90.

[16] Chung KT, Fulk GE, Andrews AW. Mutagenicity testing of some commonly used dyes. App Environ Microbiol 1981; 42: 641-8.

[17] USEPA. Aniline 'act sheet- pollution prevenrion and toxics. 1985; 749: F-95-002.

[18] Drury RAB and Wallington EA. Carleton's Histological Technique, 5th ed. London: Oxford University Press;

1980.

[19] Gray Jr LE, Ostby JS. The effects of prenatal administration of azo dyes on testicular development in the mouse: a structure activity profile of dyes derived from benzidine, dimethyl benzidine or & Imethoxybenzidine. Fund Appl Toxicol 1993; 20: 177-83.

[20] Keel AB, Abhey TO. Influence of bilateral cryptorchidism in the mature rat:alteration in testicular function and serum hormonal levels. Endocrinology 1980; 107: 1226-33.

[21] Ravibau M, Nagaveni C, Jamil K. Toxic effects of industrial effluents on rats: analysis and remediation methods. The international Journal of Toxicology 2007; 3: 1-9.

[22] Sharma S, Sharma S, Sharma A, Kumar P, Suryavati V, Sharma KP. Textile dye wastewater (untreated and treated) exposed albino rats-model for testing steritility role of pollutents in mammals. Proceedings of National Academy Science India 2007; 77(B).

[23] Chowdhury AR, and Naha N. Heavy metels induced in male reproductive system. Indian Journal of Toxicology 2002; 9: 61-67.

[24] Himani I, Soni P, Bakre PP and Bhatnagar P: Assessment of teratogenicity and embryotoxicity from textile industries at Pali (India) in Swiss albino mice exposed during organogenetic period, Journal of Environmental Biology 2008; 29: 965-969. 25. Kavlock RJ, Chemoff N, Hanisch RC, Gray LE. Perinatal toxicity of endrin in rodents. II Fetotoxic effects of prenatal exposure in rats and mice. Toxicology 1981; 21: 141-150.