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Toxicity of Textile Dye Wastewater on Liver of Mice

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Abstract: *Health Hazards due to environmental pollution by textile dye waste water is an alarming issue in Bangladesh. It is very toxic as it contains large quantities of dyes (azoic, indigo and aniline), bleaching agents, salts, acids/alkalies and heavy metals in high concentration. However, no comprehensive study has yet been undertaken in Bangladesh knowing the systemic effects of textile dye wastewater in animal and human being. Therefore, we want to investigate the effects of textile dye wastewater. For this purpose we collected 15 Swiss albino mice from iccddr'b which were divided into three equal groups control influent and effluent group. After intraperitoneal injection for 7 consecutive days, samples (blood and liver) were collected from the mice of each group. Blood is analyzed for haematological, serum biochemical studies and liver for pathological studies. Our haematological studies revealed that the values of TEC (total erythrocyte count), ESR (erythrocyte sedimentation rate), Hb (hemoglobin) and PCV (packed cell volume) were decreased significantly ($p<0.05$) in addition with the serum biochemical parameters, SGOT (serum glutamic oxaloacetic transaminase) and SGPT (serum glutamic pyruvate transaminase) were also increased significantly ($p<0.05$) in wastewater exposed animals. However, more pronounced alteration were recorded in influent group than the other groups. Morphological study showed that congestion, pin-point hemorrhages, hepatomegally with fragility of liver were found as a gross lesion. In histopathology, we found marked congestion in central vein with loss of cellular architecture of liver. These findings noticed that both untreated and treated textile dye wastewater has toxic effects on the liver of mice.*

Keywords: *Effluent, Haematology, Hepatomegally, influent, Toxicity.*

I. Introduction

The textile industry is one such source that grew out of the industrial revolution in the 18^{th} 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.

That's why industrialization is believed to cause inevitable problems, such as pollution of air, water and soil as well as health problems. Textile mill operations consist of weaving, dyeing, printing and finishing. Many processes involve several steps each contributing particular type of waste, which may invite many diseases: both occupational and general [1,2] and consequently escalating the economic cost. 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 wastewater a major challenge [3]. Most of the time wastewater 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 wastewater (2.4±0.9 g/L). Accidental drinking of pool wastewater resulted in calf mortality [5]. 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 causes cancer of the bladder in humans [6]. 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 [7-9]. Their urinary detection has been reported in several exposed species, including humans [10], monkeys [11], rodents and dogs [12]. The amine derivatives may cause mutagenic effects [13-14], which may lead to cancer as observed in animals repeatedly exposed to aniline through diet [15].

There is an incomplete knowledge regarding the potential toxicity of textile dye wastewater in mammals. As liver is primary organ of detoxification, an attempt has been made to study the effects of textile dye wastewater on liver of Swiss albino mice with its hematological and biochemical parameters.

II. 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) as per guidelines of the 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 including: control group, influent group (untreated textile dye wastewater) and effluent group (treated textile dye wastewater). Each group had five mice (three female and two male). These animals are treated with intraperitoneal injection of distilled water (control group), influent (influent group) and effluent (effluent group) for seven days.

2.2 Dye wastewater: The textile dye wastewater 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 wastewater 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)	- Hydrogen peroxide	Absence of such chemicals
	- Calcium carbonate	- Ferrous sulphate	
	- Chloride	- Sodium hydroxide	
	- Arsenic	- Sulfuric acid	
	- Oil and grease	- Alum	
Colour	Dark black	Light black to greyish	No
Turbidity	Very turbid	turbid	No turibidity

2.3 Assay of Liver Functions: The sera were separated from blood samples using cooling centrifugation and stored at 20°C until analysis. The above collected serum was used for the assay of marker enzymes of liver function; serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) [16].

2.4 Haematological Examination: Blood samples with anti coagulant EDTA (Ethelene-diamine tetra acetic acid) were analyzed for haematological parameters; total erythrocyte count (TEC), packed cell volume (PCV), hemoglobin (Hb) (g/dl) and erythrocyte sedimentation rate (ESR) in mm according to standard techniques [17].

2.5. Histopathological Examination: Specimens of liver 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].

2.6. 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 between 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,

III. Results

3.1. Clinical sign and mortality rate: In the present study, it was found that mice became aggressive immediately after intraperitoneal injection of waste water and then looked weak and showed fearrness compared to control group. Although mice lost their appetite in both influent & effluent group but total body weight was not significantly affected owing to short duration of waste water administration. Swollen thorax & abdomen as well as partial paralysis followed by staggering gait were observed only in influent group. It is very important to note that mortality (60%) was also found in influent group. There was no mortality found in effluent & control group.

3.2. Serum Biochemical Parameters: The biochemical indices showed that the effect of the wastewater was remarkable in all the parameters observed (SGOT and SGPT) (Fig. 1). There were significant ($p<0.05$) increase in SGOT only in the influent group whereas SGPT is increased significantly ($p< 0.05$) in both influent & effluent group ($p<0.01$). The SGOT increased for 63.35±0 in influent group and SGPT increased for 34.75±t' 34 to 19.95±1.63

at influent and effluent group respectively when compared to control group.

3.3. Haematological Parameters: The results of the present study (Fig. 2) revealed that textile dye wastewater affected the hematological profile of the test animals markedly. In comparison to control mice, the values of Hb (Haemoglobin) and PCV (Packed Cell Volume) ($p < 0.05$) decreased significantly both in influent and effluent group, while reduction in ESR (Erythrocyte Sedimentation Rate) was not significant. But in case of TEC (Total Erythocyte Count), the value is decreased ($P < 0.01$) only in influent but not in other groups.

3.4. Pathomorphology:

3.4.1. Gross study: The present study showed that, hepatomegally (table 2) with dark colouration & pin point hemorrhage (Fig.3) in the liver was found both in influent & effluent group in comparison to control group. But these alterations were more pronounced in influent group.

Table 2: Hepatomegally of mice

Diameter	Control		Influent		Effluent	
Length	Mean	SD	Mean	SD	Mean	SD
Width	1.75	0.21	2.85**	0.07	2.15*	0.21
weight	1.62	0.01	2.20**	0.02	1.90	0.13

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

3.4.2. Histopathological Examination: Histopathological examination of liver tissue sections from the control mice showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and central vein (Fig 4A). Liver tissue sections from the influent group showed lymphocytic infiltration with severe vascular congestion in central vein (Fig 4B&C) which were not founded in control group. Dilatation of main blood vessels and blood sinusoids and karryolysis (4D) also found in influent group. In effluent group, there were no lymphocytic infiltration but congestion in central vein (Fig.4E) was founded.

IV. Discussion

This study was designed to evaluate the toxicity of textile dye wastewater in normal Swiss albino mice. 60% mortality observed in the influent group showed that the influent contained deleterious constituents that may be harmful to organisms that utilize such water. Similar findings were also reported by Oloyede et al., 2014 [19].

As for the biochemical parameters, data present here showed significant biochemical changes in liver functions in mice injected by textile dye wastewater. Very great elevation in serum SGOT and SGPT activity was observed as a result of the wastewater treatment. The activity of SGOT and SGPT is a sensitive indicator of acute hepatic necrosis and hepatobiliary disease and increase in SGOT and SGPT activity indicates initial hepatocellular damage [20,21]. Similar to our results, on the effect of water contaminated with phenol on liver and kidney functions of rats, it was found that the enzyme activity of the serum is significantly ($p<0.05$) higher than control. Such increases can be attributed to cell necrosis, changes in cell membrane permeability or impairment of biliary excretion [22]. Also, significant elevations in SGOT and SGPT were observed in rats treated with carbon tetrachloride [23]. Haematopoetic indices have been reported to be very sensitive to toxic compounds and serve as important index of physiologic and pathologic status for both animals and humans [24]. Hematological parameters may be considered useful as health indicators of animals during changing environmental conditions. The obtained results on this case clearly depict that the animal exposed to wastewater treatment showed a tendency to adapt to changing environmental condition by a highly significant augmentation in TEC, TLC and hemoglobin content. The increase in the number of circulating RBC in mice in the present work is comparable to observation made on fish by Mishra and Niyogi [25], It may be hypothesized that increased RBC count probably reflects hypoxic stress exposure of the animals resulting in secondary polycythaemia. The reduction in RBC counts may be ascribed to a decline in their survival period, as reported by Shukla [26] in dyes treatment, or toxic effects on haemopoietic cells in the bone marrow [27].The PCV value indicating oxygen carrying capacity of the blood measures the degree of stress on animal health [28]. Similar to present study, Kurde and Singh reported fall in PCV values of male Wistar rats exposed to dyes and dye wastewater [29]. The low PCV also indicated anaemia [30]. Similar changes were also seen in case of hemoglobin (Hb) content. Our results are in agreement with studies on fish by Mishra and Niyogi [25]. They reported that the increase in Hb content reflects an adaptive response by animals attempting to increase oxygen transport in the face of hypoxic stress,Concerning, the histopathological changes, the results of the present study revealed that wastewater caused in hepatic cells and, dilatation with congestion of blood vessels and interstitial hemorrhage, which can be explained by Michalowicz and Duda [20]. The noxious influence of phenols and their derivatives, causes acute toxicity and histopathological changes [31, 32]. These effects may be due to the accumulation of phenols or phenol compounds in liver, kidneys, brain and muscles leading to pathological changes [20]

V. Conclusion

The present study has thus established that textile dye wastewater was highly toxic to the test animals. But untreated wastewater was more harmful than treated Wastewater. From the data presented here, it can be concluded that the application of textile dye wastewater of Valuka in irrigation may be potential threat to the health of cattle and human beings in view of heavy metal biomagnification. Regular monitoring of water quality is therefore essential. The present investigation may be a valuable step in the toxicity assessment production area as it seeps into their wells and rivers.

Acknowledgements

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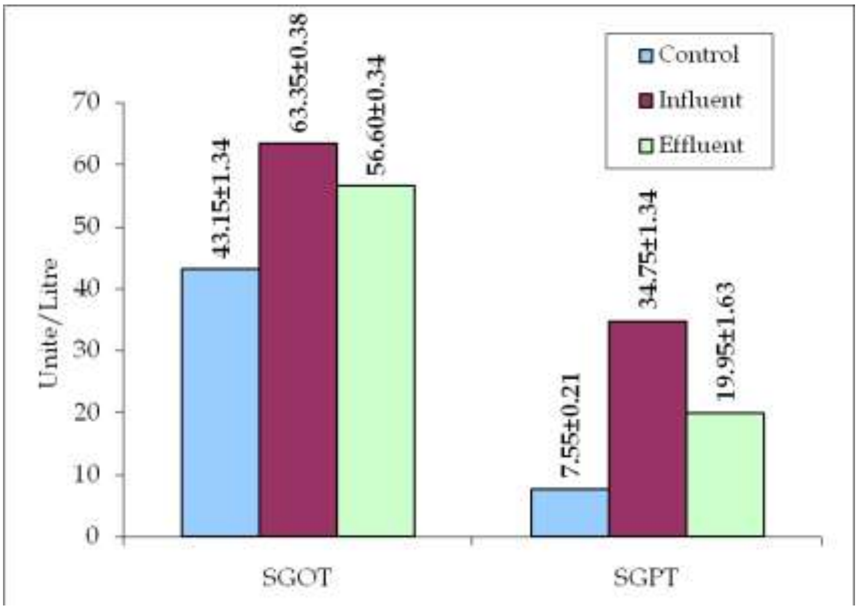
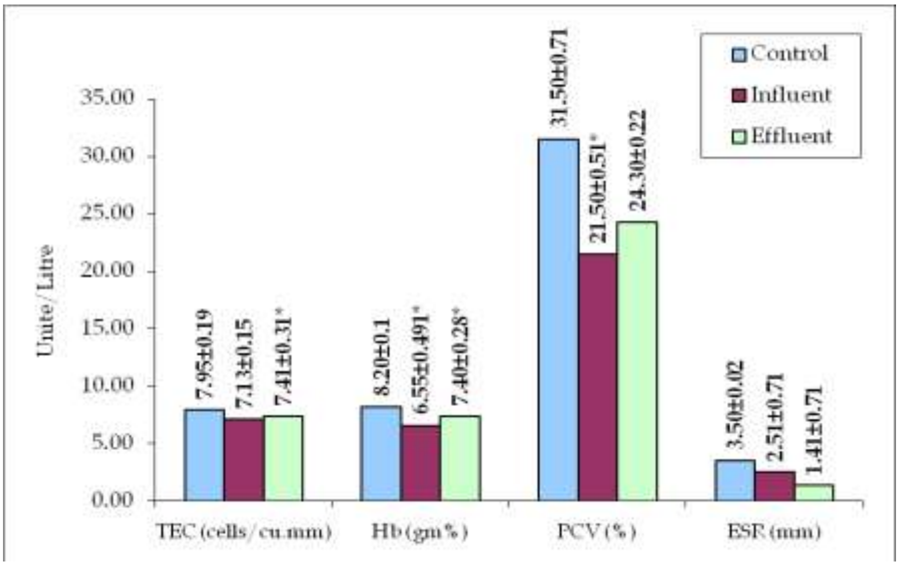


Mean±Standard deviation; * 5% level of significance (p<0.05)
Fig. 1: Changes of biochemical parameters in the control & treated male mice



Mean±Standard deviation; * 5% level of significance (p<0.05)
Fig. 2: Changes of hematological parameters in the control & treated male mice

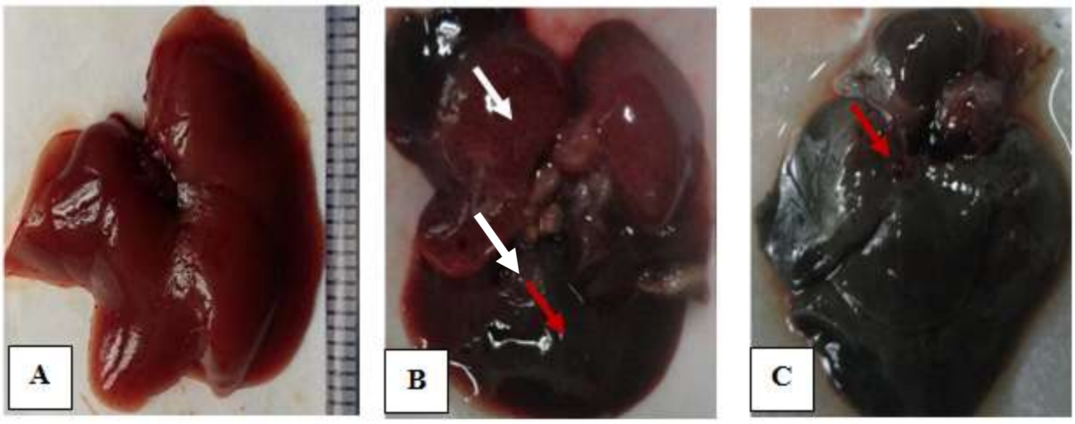


Fig. 3. (A-C) Gross study of liver in both control and treated mice.

(A) Normal appearance of liver in control group. No congestion and hemorrhage found in this group. (B) Liver showing congestion (red arrow) and pinpoint hemarrhage (white arrow) in influent group (C) Liver showing congestion (red arrow) and dark coloration in mice of effluent group.

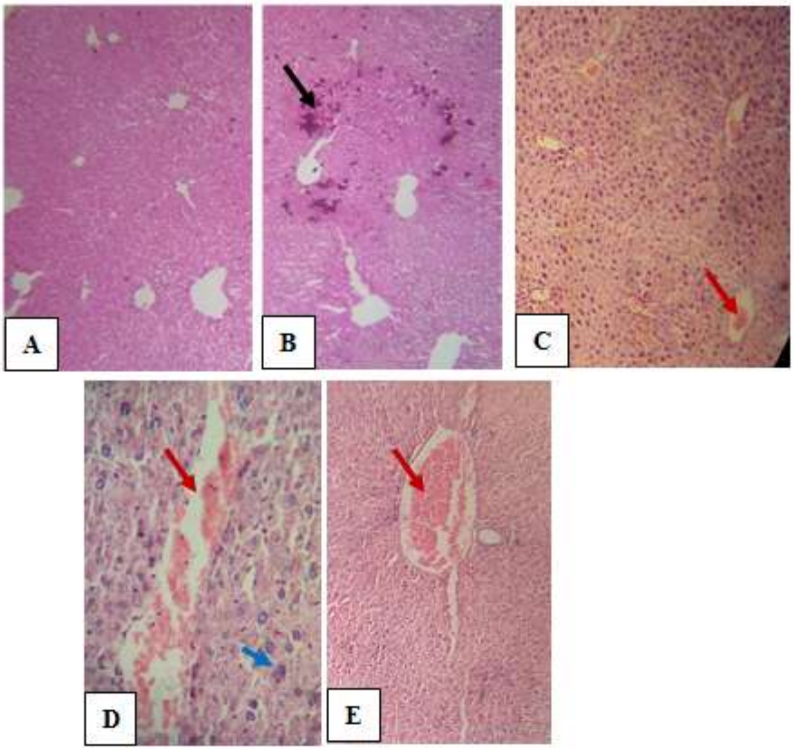


Fig. 4 Photomicrograph of a section of liver tissue from control and treated mice stained with H & E (A-C 10X, D-E 40X).

(A) Normal appearance of liver in control group No. congestion and lymphocytic infiltration found in this group (B) Liver showing lymphocytic infiltration (black arrow) (C) congestion in central vein (red arrow) (D) Karyolysis (blue arrow) with congestion (red arrow) in mice of influent group (E) Liver showing congestion in central vein (red arrow) in mice of effluent group.