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Textile dyeing wastewater negatively influences the hematological profile and reproductive health of male Swiss albino mice

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ABSTRACT

Objective: To determine the effects of textile dyeing industrial wastewater on the hematological parameters and reproductive health including histoarchitecture of male gonad (testes) of mice.**Methods:** Twenty-four Swiss albino mice at 4-weeks old were divided into four groups ($n=6$ per group). Mice of group 1 supplied with normal drinking water were served as the control group. Mice of group 2, 3 and 4 were supplied normal drinking water mixed with textile dyeing wastewater at 5%, 10% and 20% concentration, respectively. After completing 24 weeks of treatment, different hematological profile, weight of testes, gonadosomatic index (GSI), sperm concentration and morphology were measured. Moreover, histopathological changes in testes were examined.**Results:** Hematocrit value and hemoglobin concentrations were decreased in all groups of wastewater-treated mice compared to the control group. Likewise, weight of testes, GSI and sperm concentration were decreased significantly in wastewater-treated mice in comparison to the control group. The percentage of morphologically healthy epididymal sperm was significantly reduced in wastewater-treated mice. Histopathological examination revealed degenerative changes in seminiferous tubules, a smaller number of spermatogenic cells, elongation of seminiferous tubules and degenerative changes of seminiferous tubules in wastewater-treated mice.**Conclusions:** Textile dyeing wastewater has harmful effects on hematological profile and reproductive health of male mice.**KEYWORDS:** Textile dyeing wastewater; Gonads; Hematology; Histopathology; Mice

1. Introduction

Industrial sector of Bangladesh is growing these days, despite

the fact that Bangladesh is still developing. An emerging nation like Bangladesh, industrialization is crucial to their economic progress. However, it is arguable that environmental contamination of the air, water, and soil is always a result of industrial progress. The main industries that create pollution are those that deal with textiles, tanneries, fertilizer plants, medicines, cement, pulp and paper, etc. Bangladesh is the world's second-largest manufacturer of ready-made garments, and the country's textile industry is one

Significance

Contamination of textile dyeing wastewater is a worrying issue in Bangladesh. These contaminants harm vital systems of animal body including human and have carcinogenic effect. There are few published studies looking into how textile dyeing wastewater affects the reproductive system. However, no studies have been conducted yet to look into the impacts of textile dyeing industrial wastewater that is dispersed throughout agriculture fields in Bangladesh on sperm morphology and hematology. The findings of this study revealed that textile dyeing wastewater caused reductions in hematocrit value, hemoglobin concentrations, testicular weight, gonadosomatic index, sperm concentration and number of healthy sperms.

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of its two primary water consumers. These industries produce a variety of acidic and alkaline pollutants, including benzidine, beta-naphthylamine, sulfide, lime, cadmium, copper, chromium, and several hazardous dyes[1–3]. Among textile industries, textile dyeing wastewater is the main source of water pollution in surface and river water since most textile farms do not want to discharge wastewater once it has undergone adequate treatment[4,5]. The agricultural fields, which are a major source of food for the people of Bangladesh, an agrarian nation, are spread with this wastewater. Many different kinds of animals, such as cattle, goats, dogs and cats, consume the tainted water, and people also use it due to a lack of access to clean water sources. One of the reasons of calf mortality is accidental consumption of wastewater[6].

This textile dyeing wastewater contains a number of dangerous substances, some of which may even have an impact on reproductive health[7]. Exposure to substances that interfere with ovulation or spermatogenesis can also have an impact on humans[8,9]. Neoplasm in the human urinary bladder is caused by the toxicity of azo dyes, which are used in the paper, leather, and textile industries and are based on benzidine and its congeners, dimethoxy and dimethyl benzidine[10]. Alkali from the paper and textile industries frequently results in skin problems including eczema, rashes, irritation, etc. Hydrogen sulfide is responsible for a number of respiratory issues, as well as headaches, eye irritation, sore throats, etc. Additionally, certain metals have a carcinogenic impact[11,12].

The term "pollution" is commonly used in everyday speech in Bangladesh, where industrial pollution is the worst kind and one of the primary causes of pollution. There is growing concern about the possibility of soil, sediment, and water system pollution in several industrial zones in the country[13]. The already severely polluted Buriganga, Turag, Balu, and Shitalakshya rivers, as well as neighboring canals, lakes, and streams, are frequently inundated with copious amounts of untreated sewage, solid waste from riverside settlements, petroleum products from ships, launches, cargoes, and boats, and industrial wastewater[14]. The surface water along these remote rivers, canals, lakes, and streams in Dhaka city and the neighboring districts of Narayanganj and Gazipur is known to be extremely polluted because untreated municipal and industrial specially textile waste fluids are discharged into these water sources[15,16]. This wastewater is dumped into agriculture fields, which provide a significant portion of the food supply to a densely populated country like Bangladesh. Furthermore, both humans and animals that are exposed to this wastewater develop acute and chronic diseases as well as several carcinogenic problems. Therefore, this study was to delineate how textile dyeing wastewater from textile industry affects hematological parameters, reproductive health, and the histoarchitecture of the gonad of male Swiss albino mice.

2. Materials and methods

2.1. Collection and analysis of wastewater

Textile dyeing wastewater was collected from wastewater treatment plant of a textile industry located in Gazipur district of Bangladesh and was stored at 4°C during the study period. Collected wastewater was analyzed for pH, level of biological oxygen demand (BOD) and chemical oxygen demand (COD). The heavy metal concentrations in diluted samples were analyzed by atomic absorption spectrophotometer (AAS, Perkin Elmer, the PinAAcle™ 900H, USA) according to the manufacturer's instructions.

2.2. Collection of mice and experimental design

Three weeks old twenty-four specific pathogen free (SPF) Swiss albino male mice (*Mus musculus*) were purchased. Completely randomized design was applied to perform this experiment where mice at the age of week four were divided into four groups ($n=6$ per group) and ears were coded. Power analysis was performed to determine the sample size for one-way analysis of variance (ANOVA) test using G*Power version 3.1, while the number of groups was set at 4 with a significance criterion of $\alpha=0.05$ and $\text{power}=0.8$.

The mice were housed in compartmentalized rectangular plastic cages wrapped with wire mesh. The cages were kept in well ventilated room at $(26\pm 2)^{\circ}\text{C}$ and a relative humidity of 70%-80% with natural day and light. The mice were cared in proper hygienic conditions.

Standard mice pellets were used as normal feed. Normal water was supplied to group 1 (the control group) of mice. Mice of group 2, 3 and 4 were supplied normal water mixed with textile dyeing wastewater at 5%, 10% and 20% concentration[17,18], respectively orally *ad libitum* up to study week 24. There was no difference in the amount of food and water consumed by the experimental groups. Initial body weight of each mouse was measured with the help of a digital balance. Body weight was taken at first day and at the end of experiments.

2.3. Collection of blood for hematological studies

After completing study period, mice were anaesthetized with ether during sacrifice and thoracotomy was performed. Heart bleeds were carried out into heparinised capillary tubes (Seltzer Labortechnik, Germany) for the determination of various hematological parameters. Total erythrocyte count, hemoglobin (Hb) concentration, hematocrit value, erythrocyte sedimentation rate, total leukocyte count and differential leukocyte count were determined by calculating mean $\text{value}\pm\text{SD}$. Hematological parameters were measured using veterinary auto hematology analyzer (Guangzhou Medsinglong Medical Equipment Co., Ltd., Guangzhou, China).

2.4. Effects of textile dyeing wastewater on weight of testes and gonadosomatic index (GSI)

At terminal sacrifice, the testes of the experimental and control mice were removed in study week 24 and were weighed. GSI was calculated by dividing the testis weight by the body weight of each mouse and expressed as the percent[19].

2.5. Effects of textile dyeing wastewater on epididymal sperm count and sperm morphology

Following terminal sacrifice, the left cauda epididymis of the treated and control mice were excised, weighed and minced with sterile and fine scissors in 10 mL Dulbecco's Modified Eagle medium (DMEM) containing 10% fetal calf serum and incubated for 6 h at 37°C. Sperm morphology was evaluated using a differential interference contrast (DIC) microscopy of wet-mount semen fixed in isotonic formol saline solution at approximately 20× magnification. The total number of sperms per 10 mg cauda epididymis were counted under a light microscope. All types of sperm morphological abnormalities were recorded, classified, and documented in record sheet. A total of 100 spermatozoa/mouse will be examined to explore the morphological defects in the head (normal, amorphous, pin-head and banana shaped), hook (knobbed, bent, short, no hook) and tail pieces (tail folded overhead, two tails and coiled tails).

2.6. Sample collection for histopathological study

After completing 24 weeks of watering of mice, systemic dissection was made at necropsy and gross changes in different tissues were recorded. Following necropsy, testes of treated and control mice were collected. For histopathological studies, all the collected samples were cut into pieces, fixed in the 10% neutral buffered formalin[20], dehydrated in a series of ascending grades of alcohol, cleared in several changes of xylene and infiltrated with different grades of melted paraffin in the oven at 56°C. The tissues were subsequently embedded in paraffin, and a rotatory microtome was used to cut the sections at a thickness of 5 µm. The sections were then stained using the hematoxylin and eosin (H & E) staining technique[21].

2.7. Statistical analysis

Statistical analyses were performed using SPSS software (ver. 27; SPSS Inc., Chicago, IL, USA). Differences between the control and experimental groups were calculated by one-way ANOVA followed by Duncan's multiple range *post-hoc* test. Data were expressed as mean±standard deviation (mean±SD). Differences were considered to be statistically significant at $P<0.05$.

2.8. Ethics statement

The study on mouse experimentation was carried out under the

Table 1. Characteristics of textile dyeing wastewater in comparison to normal water (control).

Characteristics	Textile dyeing wastewater	Normal water (control)
pH	8.2	7.0
Biological oxygen demand (BOD), mg/L	84	nil
Chemical oxygen demand (COD), mg/L	155	nil
*Chemicals/ Metal, mg/L		
Lead (Pb)	0.69	nil
Copper (Cu)	0.42	nil
Nickel (Ni)	0.23	nil
Sulphates	596	nil
Color	Light black	Transparent

*Permissible limit in drinking water: Pb 0.5, Cu 0.2, Ni 0.2, sulphates 200.

guidance of the Animal Research Ethics Committee, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh (FVMAS/AREC/2023/14).

3. Results

3.1. Analysis of textile dyeing wastewater

Wastewater sample collected from the textile industry were light black in color, and had foul odor. Water sample had pH values 8.2 (Table 1), indicating an alkaline state. The following features of the wastewater used in this investigation were compared to those of regular water (Table 1). The levels of COD and BOD exceeded the limitations established by Department of Environment, Bangladesh. The value of BOD of the collected water was 84 mg/L. The value of the COD of collected water was 155 mg/L (Table 1). Among the heavy metals, the concentrations of lead (Pb), copper (Cu), nickel (Ni) and sulphates were 0.69, 0.42, 0.23 and 596 mg/L (Table 1), respectively in collected wastewater. All these metal in wastewater exceeded the permissible limit in drinking water.

3.2. Body weight of mice

Body weight was decreased in all wastewater-treated mice in comparison to the control group after 24 study weeks. A moderate reduction of body weight of the mice in group 2 compared to the control group was observed. Significant reduction of body weight of mice was observed in mice of group 3 [(46.96±0.34) g] and group 4 [(45.86±0.57) g] compared to the control group [(50.03±2.71) g] ($P<0.05$) (Figure 1).

3.3. Hematological findings

After completing 24 study weeks, the main hematological alterations were observed in hematocrit value and Hb concentration. The

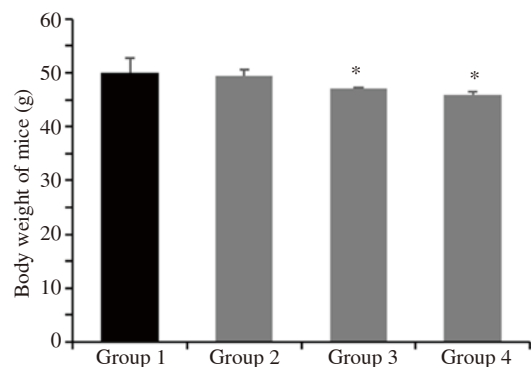


Figure 1. Effects of watering of mice with textile dyeing wastewater on body weight of male mice. Normal water is supplied to the control group (group 1) of mice. Mice of group 2, 3 and 4 are supplied normal water mixed with textile dyeing wastewater at 5%, 10% and 20% concentration, respectively, orally up to study week 24. * $P < 0.05$: compared to the control group.

mean of hematocrit value (Figure 2A) were significantly decreased in group 4 $[(37.03 \pm 0.67)\%]$ in comparison to the control group $[(41.23 \pm 1.11)\%]$ ($P < 0.05$). Moreover, the mean of Hb concentration (Figure 2B) were significantly decreased in group 4 $[(11.82 \pm 0.39) \text{ g/dL}]$ in comparison to the control group $(13.0 \pm 0.54 \text{ g/dL})$ ($P < 0.05$). In both parameters, insignificant reduction was observed in group 2 and group 3. Insignificant difference was also observed in total erythrocyte count, total leukocyte count and differential leukocyte count.

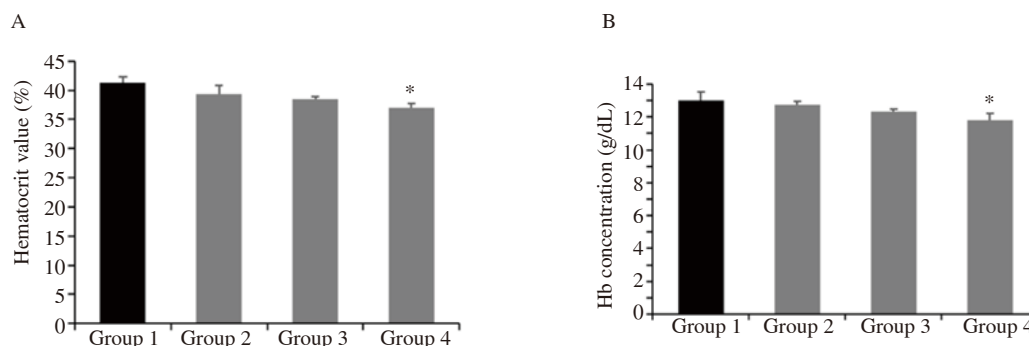


Figure 2. Effects of textile dyeing wastewater on hematocrit value (A) and hemoglobin (Hb) concentration (B) of male mice. * $P < 0.05$: compared to the control group (group 1).

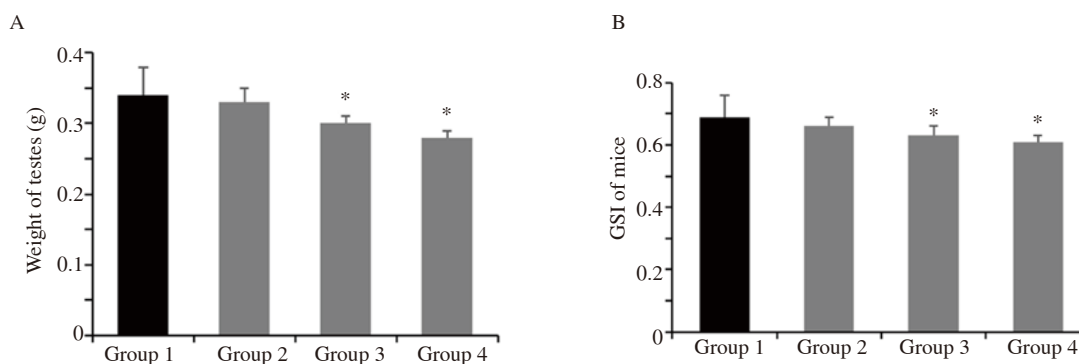


Figure 3. Effects of textile dyeing wastewater on weight of testes (A) and gonadosomatic index (GSI) (B) of male mice. * $P < 0.05$: compared to the control group (group 1).

3.4. Weight of testes and GSI

The weight of testes was significantly decreased in the mice of group 3 $[(0.30 \pm 0.01) \text{ g}]$ and group 4 $[(0.28 \pm 0.01) \text{ g}]$ in comparison to the control group $[(0.34 \pm 0.04) \text{ g}]$ ($P < 0.05$). The insignificant reduction of weight of testes was seen in the mice of group 2 (Figure 3A).

Again, after 24 study weeks, GSI was decreased in all the wastewater-treated mice in comparison to the control group. Moreover, significant reduction of GSI was observed in the mice of group 3 (0.63 ± 0.03) and group 4 (0.61 ± 0.02) compared to the control group (0.69 ± 0.07) ($P < 0.05$). The insignificant reduction of GSI was recorded in group 2 (0.66 ± 0.03) (Figure 3B).

3.5. Total sperm count

The epididymal sperm count in all wastewater-treated groups was decreased compared to the control group after completing 24 study weeks. Sperm obtained from the left cauda epididymis (10 mg) of treated mice showed significant decrease in total sperm counts in group 4 $[(6.43 \pm 0.04) \times 10^6]$ in comparison to the control group $[(7.00 \pm 0.27) \times 10^6]$ ($P < 0.05$) (Figure 4).

3.6. Morphology of sperm

The morphologically healthy epididymal sperm count (Figure 5A)

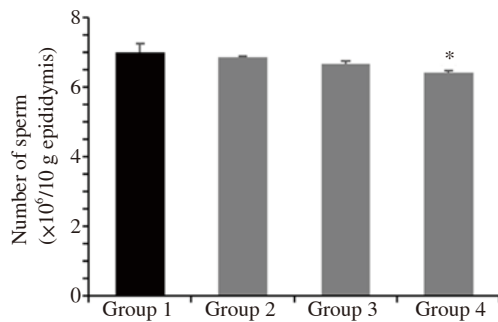


Figure 4. Effects of textile dyeing wastewater on sperm count of male mice. * $P < 0.05$: compared to the control group (group 1).

was reduced following 24 weeks of study in mice treated with textile dyeing wastewater in comparison to the control group (Table 2).

Table 2. Effects of textile dyeing wastewater on the morphology of sperm in the wastewater-treated mice and the control mice following 24 experimental weeks.

Characteristics	Group 1	Group 2	Group 3	Group 4
Healthy sperm	71.33±3.20	68.16±1.14	63.50±2.07*	57.00±3.74*
Coiled tail	3.25±1.26	4.25±0.96	5.25±0.96	5.75±0.96
Broken neck	1.75±0.50	2.75±1.26	3.50±0.58	4.75±1.26
Filamentous tail	3.75±1.26	3.25±0.50	4.00±0.82	5.00±0.82
Folded tail	2.75±0.10	3.50±1.00	4.50±0.58	4.75±1.26
Stump tail	3.75±0.10	3.00±1.15	3.75±1.26	5.00±0.82
Microcephaly	1.50±0.58	2.25±1.26	2.75±0.50	3.50±0.58
Decapitated	2.25±0.50	2.75±1.26	3.00±1.41	3.75±2.22
Supercoiled midpiece	1.50±0.29	2.75±1.26	3.50±0.58	3.25±1.50
Bent tail	2.75±1.50	2.50±1.29	2.00±1.15	3.00±1.63
Others	4.50±0.58	3.25±1.50	4.25±0.50	5.00±0.82

Data are expressed as mean±SD. Differences between the control and experimental groups are calculated by one-way ANOVA followed by Duncan's multiple range *post-hoc* test. * $P < 0.05$: compared to the control group. Normal water is supplied to group 1 (the control group) of mice. Mice of group 2, 3 and 4 are supplied normal water mixed with textile dyeing wastewater at 5%, 10% and 20% concentration, respectively orally up to study week 24.

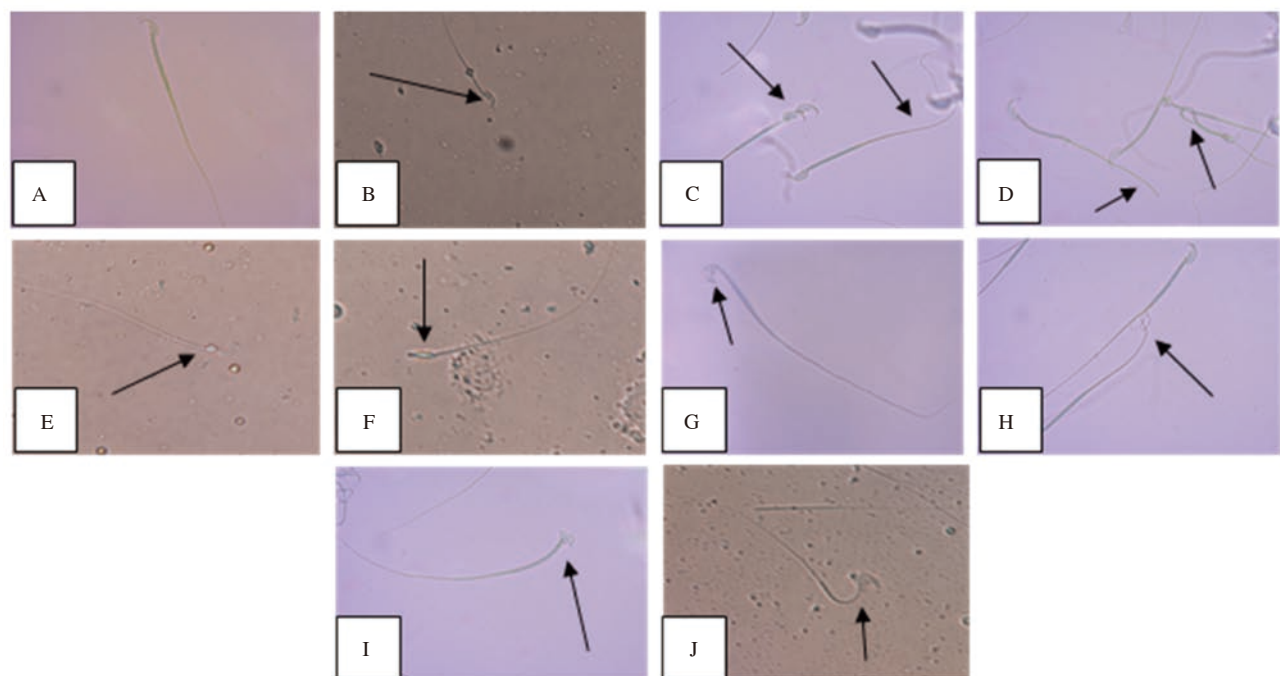


Figure 5. Morphologically defective sperm present in the cauda epididymis of the wastewater-treated and control male mice following 24 experimental weeks (magnification 20×). This examination reveals healthy sperm (A), distal cytoplasmic droplet (B), decapitated and filamentous tail (C), stump tail, folded tail and bent tail (D), proximal cytoplasmic droplet (E), microcephaly (F), broken neck (G), coiled tail (H), distorted head with ruffled acrosome (I), and super coiled mid-piece (J). Morphologic deviations are indicated by black arrows.

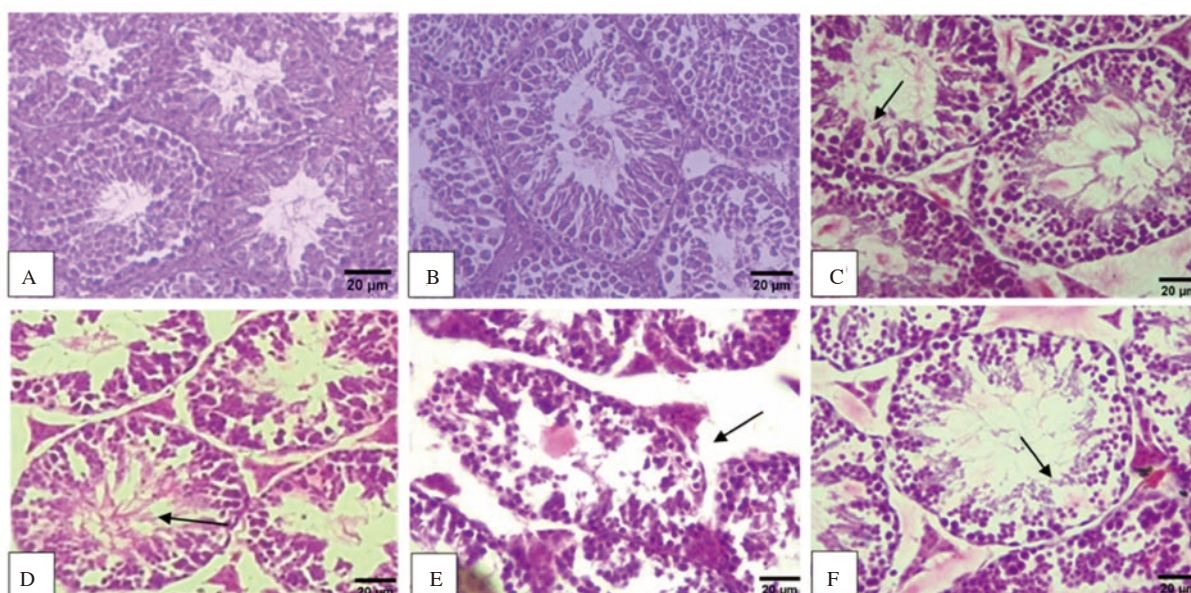


Figure 6. Histopathological alterations in the testes of wastewater-treated mice following 24 experimental weeks (H & E; scale bar 20 μ m). (A, B) No significant difference in the section of testes is seen in the mice of the control (A) and group 2 (B); (C) less number of spermatogenic cells are observed; (D) accumulation of spermatozoa with exudates in tubular lumen are evident; (E) elongation of seminiferous tubules are found and (F) degenerative changes of seminiferous tubules are noticed. Histopathologic changes are indicated by black arrows.

3.7. Gross and histopathological changes

Grossly, reduction of size of testes in the wastewater-treated group of mice was observed in contrast to the control group. The highest reduction of the size of testes was seen in the mice of group 4 compared to other groups. Mice of this group were treated with the highest concentration (20%) of wastewater.

Histopathologically, insignificant difference in the section of testes was seen in the control (Figure 6A) and group 2 mice (Figure 6B). Histopathological alterations of testes were observed in the mice of groups 3 and 4. Less number of spermatogenic cells (Figure 6C), accumulation of spermatozoa with exudates in tubular lumen (Figure 6D) and elongation of seminiferous tubules (Figure 6E) were noticed in groups 3 and 4. Moreover, there were degenerative changes in the spermatogenic cells of seminiferous tubules (Figure 6F).

4. Discussion

The lack of hygienic disposal facilities and plants that meet the required standards causes textile wastes to be scattered haphazardly over the environment. When the wastewater suspended in the water, or settles on the bed, it can release infectious agents, chemicals, and trace elements into lakes, streams, rivers, the ocean, and other bodies of water[22]. The ecosystems of the environment are negatively impacted by it[23]. Pollution of the environment by industrial waste is a major issue in Bangladesh. The high quantities of heavy metals, alkalis, dyes, and other contaminants in this waste make it very toxic. The current investigation examined the impact of textile dyeing

wastewater on the hematological parameters and reproductive health of male Swiss albino mice. We have first collected wastewater from the wastewater treatment plant of a textile industry situated in the Gazipur area. The collected wastewater exhibited a hue of light black. The investigation focused on the awful smell that comes from the water. Water quality suffers greatly when industrial waste is dumped into rivers and other water sources[24,25].

Collected wastewater had pH value 8.2, indicating an alkaline threshold of wastewater. Different industrial wastewaters in Bangladesh have pH values ranging from 7.2 to 11.9[26–29]. BOD is the quantity of dissolved oxygen required for aerobic biological organisms to break down biodegradable organic material in a water sample at a particular temperature over a specific time period[30]. The measurement of the impact of pollution using BOD measurements has gained widespread acceptance. A high BOD concentration is a sign of contaminated water, whereas a low BOD value denotes high-quality water[30]. In the current experiment, the established BOD level was 84 mg/L. Conversely, COD is the amount of dissolved oxygen in water required for a strong chemical oxidant to oxidize chemical organic molecules[30]. Levels of BOD and COD are frequently thought of as markers of the relative oxygen-depletion impact of a waste pollutant. The COD value in this investigation was 155 mg/L, which is far higher than the usual permitted levels. Reports stated that the industrial wastewater in this research location had higher BOD and COD levels[27,28,31,32]. Higher quantities of heavy metals, including Pb, Cu, Ni, and sulphates, were found in these collected wastewater samples.

This study demonstrated the detrimental impacts of textile dyeing wastewater on the hematological profile and reproductive health of

male Swiss albino mice. In this investigation, body weight of the treated mice was declined after the study period in comparison to the control mice. The presence of dissolved heavy metals in the water may have caused these consequences. Rats and mice treated with industrial effluent showed comparable outcomes[7,33].

When compared to the control group, all wastewater-treated mice had lower testicular weights, GSI, and epididymal sperm counts. The mice treated with the highest concentration of wastewater from the textile dyeing industry showed the largest reduction rate. Furthermore, after 24 weeks of watering the mice with wastewater, there were less morphologically healthy epididymal sperm than there were in the control group. Numerous harmful metals, including Pb, Cu, Ni, and sulphates, were found in wastewater drainage through textile dyeing industry. These compounds were found in wastewater at concentrations higher than those permitted in drinking water[27,31]. According to a number of studies, Pb has a significant negative impact on human male reproduction. It reduces libido[34], lowers semen quality, sperm count, motility, viability, and integrity, and increases the frequency of morphological defects in sperm[35–37]. Additionally, it has been shown that Ni causes reproductive toxicity and has detrimental effects on it. Ni alters testicular anatomy and spermatogenesis, which harms mice's reproductive system[38]. Conversely, sperm morphology decreases significantly in response to high concentrations of Cu exposure[39,40]. Furthermore, sperm quality and ultrastructure of the blood-testis barrier was significantly reduced in testis of mice when exposed to high sulfur dioxide levels in drinking water[41].

The hematocrit value and Hb concentration were seen to be altered in the hematological profile of mice treated with wastewater. When comparing the experimental groups to the control group, the hematocrit value and Hb concentration were lower in the former. According to a report, Pb has decreased the estimation of PCV, Hb, and red blood cell (RBC) in Lohi sheep[42]. Once more, eating more Cu in milk lowered the mean corpuscular hemoglobin (MCH) levels in dairy calves[43].

Our findings are consistent with earlier research on the effects of industrial wastewaters on histopathological abnormalities of reproductive organs of animals, which reported multiple alterations in mice and rats following exposure[44]. Previous reports[7,45] have revealed similar results. According to the current study, the textile dyeing wastewater seriously harms the male reproductive system. Due to the greater quantity of heavy metals in the wastewater from the textile dyeing sector, the toxic consequences were severe.

The limitations of this study lie in its focus on the serum testosterone level and the level of testicular spermatogenesis. Further investigation is required to clarify any potential correlation with the female reproductive system of mice and *in vivo* fertility following exposure to textile dyeing wastewater.

The rapid industrialization of Bangladesh without enough consideration for the environment has made the country's environmental pollution situation more complex. In Bangladesh,

industrial waste contamination is a concerning problem. Due to the high concentration of dyes, alkalis, heavy metals, *etc.*, this waste is extremely poisonous. According to a World Health Organization research, cancer patient population in Bangladesh is growing gradually, and by 2050, the nation may have more than twice as many new instances of the disease as it did in 2022. Moreover, the prevalence of infertility is rising in Bangladesh. One of the primary reasons for the rise in cancer patients and infertility in Bangladesh could have linked with industrial pollution. These findings determined how textile dyeing industrial wastewater affects reproductive health, hematology, and the histoarchitecture of male gonad. Wastewater-treated animals showed significant reductions in hematocrit value, Hb concentrations, testicular weight, GSI, and sperm concentration. In addition, the proportion of morphologically healthy epididymal sperm in the mice-treated with wastewater was considerably lower. By raising public awareness about the detrimental and lethal impacts of industrial effluent, these results may help to improve both human and animal health.

Conflict of interest statement

Declared conflicts of interest do not exist for the authors. There are no financial conflicts to disclose, and all co-authors have reviewed and approved the manuscript's content.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Md. Taimur Islam conceived the idea, designed the experiments, performed laboratory experiment and drafted the first version of the

manuscript; Nusrat Binte Rafique, Minhaz Ahmed, Md. Mizanur Rahman and Mohosina Mou performed the sample collection and laboratory experiment; Dipu Roy and Robius Sani Sadi edited the manuscript; Anup Kumar Talukder participated in the idea development, data analysis and edited the manuscript; Ziban Chandra Das and Md. Golam Haider reviewed the manuscript. All authors have read and approved the final version of the manuscript.

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