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RESEARCH ARTICLE

# Toxicity impact of Silk dye effluent on blood profile of Swiss albino mice *Mus musculus*

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## Abstract

Toxicity due to long term (15 , 30 , 45 and 60 d) exposure of untreated silk dye effluent (50 and 75%) of small scale cottage industry at Bhagalpur was studied on various haematological parameters (RBC, WBC, Hb%, PCV, MCV, MCHC) and total cholesterol on swiss albino mice *Mus musculus*. A significant decrease in RBC count from 5.9 ± 0.05 to 2.7 ± 0.06 million/cubic mm was noted whereas, WBC count increased from 6.1 ± 0.02 to 9.4 ± 0.13 thousand/cubic mm. Hb% decreased significantly from 11.6 ± 0.12 to 6.0 ± 0.17 gm/dL and PCV% also significantly decreased from 31.09 ± 0.12 to 21.0 ± 0.69 cubic micron. The MCHC% also decreased from 36.8 ± 0.06 to 21.9 ± 0.59 and MCV% increased from 52.6 ± 0.19 to 71.3 ± 0.68 cubic micron in 50 and 70% from 15-60 d treatment. The total cholesterol had a decreasing trend throughout the treatment period (81.13 ± 0.29 to 51.1 ± 3.08 mg/dL). The findings indicated that silk dye effluent causes a marked alteration in blood profile and decreases the total cholesterol level. The present investigation may be a valuable step in the toxicity assessment of silk dye effluent in swiss albino mice *Mus musculus*.

**Keywords:** Silk dye effluent, *Mus musculus*, blood profile, total cholesterol, toxicity assessment**.**

## Introduction

As a consequence of rapid industrialization after the independence, the problem of waste materials in the form of solid or liquid has grown significantly. Industrial effluents are more toxic to environment than the sewage. Bhagalpur ($25^0$15'N latitude and $87^0$E longitude), a commissionaire headquarters is situated on the Southern Bank of river Ganga about 200 km faraway from Patna. In Bhagalpur, several silk manufacturing industries are situated and the production of silk fiber and fabric is followed by their dyeing process. Different dyestuffs, used in this process are synthetic dyes mostly of them azo and vat dyes. These dyes are one of the largest chemical classes of dyes with varieties of colors. Analysis of azo dyes based on structural activity relationship suggests that these dyes are likely to have a carcinogenic potential (Boeniger, 1980).

Meyer (1981) reported the chemical structure of azo benzene and azo napthol derivatives. Boeniger (1980) reported a higher incidence of urinary bladder tumors among dye industry workers than in the general population. Against these backdrops, toxicity due to long term (15 , 30 , 45 and 60 d) exposure of untreated silk dye effluent (50 and 75%) of small scale cottage industry at Bhagalpur was studied on various haematological parameters (RBC, WBC, Hb%, PCV, MCV, MCHC) and total cholesterol levels on swiss albino mice *Mus musculus*.

## Materials and methods

*Experimental animals:* Three month old female Swiss Albino mice (Body weight: 25 ± 5 g) were obtained from CDRI Lucknow were maintained at the animal house of University Dept. of Zoology, Bhagalpur, Bihar. They were separated and kept in stainless steel cages in a temperature and humidity controlled with 12 h light/dark cycle. Food and water were given to the animal according to their need. All the animals were kept as accepted principles for laboratory animal use and care as per the guidelines of CPCSEA. The mice were acclimatized for one week before the experiment and then used in experiment at about 12 weeks of age.

*Collection of chemicals:* Silk dye effluent is collected directly from the silk dyeing mills situated in the Nath Nagar area Bhagalpur and these effluents were untreated and the different concentrations were made by adding distilled water.

*Experimental design:* The mice were randomly dived into 9 groups of 10 animals each. The first group served as control and was given normal food and water without silk dye effluent. Silk dye effluent (1 mL) (50 and 70% conc.) per mice was administered orally to the second to ninth groups of animals for 15, 30, 45 and 60 d exposure. Treated and control groups were used for haematological and blood cholesterol parameters. Blood samples were collected from heart of anaesthetized mice after 15, 30, 45 and 60 d treatment.

 

*Biological assays:* Blood from mice was collected by cardiac puncture every time after the completion of treatment period. Blood was collected in the vancutainer tubes containing no anticoagulant. Blood (1.5 mL) were centrifuged for 15 min at 1500 rpm speed and supernatant (serum) was carefully aspirated at room temperature and used for the further experiments (Henry, 1979). Serum was used for the estimation of total cholesterol and Neubauer chamber haemocytometer is used in the calculation of RBC (Red Blood Cells) and WBC (White Blood Cells). Heamoglobin concentration (g/100 mL) was determined by Sahils method with Sahils haemoglobinometer (Germany) (D'amour *et al.,* 1965). The packed cell volume (PCV) or haematocrit value was estimated by the method of Wintrob (1974). MCV (Mean Corpuscular Volume) and MCHC (Mean Corpuscular Haemoglobin Concentration) (%) was determined by using formula suggested by Lee *et al*. (1999).

MCV (micron $mm^3$) = PCV (%)/total RBC million/$mm^3$ X 10.

MCHC = HB (g %) /PCV% X 100.

*Statistical analysis:* The data are expressed as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) is used for the calculation of significant value and all the values are significant at 1 and 5% of significance level.

## Results and discussion

Silk dye effluent affected the blood profile and cholesterol profile of test animals markedly caused toxicity which is resulted into altered body physiology and metabolism of mice. Different concentrations of these effluents administration changes the blood parameters which over all affects the metabolism and may cause invasive diseases and development of tumor reported in mice. A significant decrease in RBC count from 5.9 ± 0.05 to 2.7 ± 0.06 million/cubic mm was noted (Table 1) whereas, the WBC count increased from 6.1 ± 0.02 to 9.4 ± 0.13 thousand/cubic mm (Table 1). Hb% decreased significantly from 11.6 ± 0.12 to 6.0 ± 0.17 gm/dL) (Table 1) and PCV% also significantly decreased from 31.09 ± 0.12 to 21.0 ± 0.69 cubic micron (Table 1). In the present investigation, significant decrease in RBC, Hb% and PCV value was observed by treatment at different concentrations (50 and 75%) of silk dye effluent with $15^{th}$, $30^{th}$, $45^{th}$ and $60^{th}$ d exposure while WBC has shown opposite trend. Sharma *et al*. (2007) reported decrease in RBC, Hb% and PCV of rat treated with textile dye and the decrease in RBC may be described to decline in their survival period when treated with dyes or toxic effects on haemopoietic cells in the bone marrow (Lee *et al.*, 1999). Eaton and Klassen (1996) reported the toxic substance present in wastewater interacts with RBCs and may cause metabolic disorders decreasing their Hb carrying capacity.

Vitamin B12 and folic acid deficiency causes maturation of erythrocytes ceases in the process of erythropoiesis and the non-availability of Vitamin B12 results in decreased RBC count. The PCV value indicates oxygen carrying capacity of blood and the degree of stress on animal health (Larson *et al*., 1985). Reduction in PCV values of male Wistar rats exposed to dye effluent and the low PCV also indicated anaemia (Wepener and Vuren, 1992). The present output was also supported by Sharma and Goel (2005) and the similar observation was also reported by Devi and Singh (1988) who worked with Yellow and Organce-II also reported the decrease in the haemoglobin content which might be due to decrease rate of haemoglobin synthesis due to dye poisoning. The rate of haemoglobin synthesis decreases during all stages of maturation of erythrocytes when the supply of iron was not sufficient. Normally the globin portion of haemoglobin is broken down into amino acids which return to the protein port while; porphyrin is metabolized and accredited as bile pigment. The iron released from breaking of haemoglobin is carried by transferrin either to bone marrow for production of new red blood cells or to the liver for storage in the form of ferritin. The synthesis of haemoglobin requires iron, which is generally supplied from the stored ferritin. Therefore, it seems that the dye may have prevented the iron supply for the synthesis for haemoglobin by inhibiting the absorption by developing erythrocytes, which resulted in the fall of haemoglobin content.

Significant decrease in haemoglobin content and packed cell volume was also observed in mice when treated with Fellon (Webner, 2003), sodium benzoate (Sinha and D'souza, 2008), fluoride (Choudhary *et al*., 2008), distillery effluent (Varma and Pratap, 2008). The MCHC% decreased from 36.8 ± 0.06 to 21.9 ± 0.59 (Table 1) and MCV% increased from 52.6 ± 0.19 to 71.3 ± 0.68 cubic micron in 50 and 70% from 15 to 60 d treatment (Table 1). A decreased MCHC value was observed at all exposure periods and effluent concentration when compared to control group (Table 1). The MCHC expresses the concentration of haemoglobin in the cytoplasm of the erythrocytes. MCV count and MCHC is dependent on the RBC count, Hb content and PCV value. Due to effluent toxicity, the bone marrow lacks the capacity to manufacture haemoglobin at the required rate, so the haemoglobin content of each cell has diminished the MCHC value. Significant decrease in MCHC was observed in laboratory mammal when exposed to tannery effluent (Breathy *et al*., 2003). In the present investigation, there was an increase in the total WBC count recorded at all the concentration level and incubation period i.e. because of the leucocytes are the mobile units of the body's defensive mechanism (Table 1). Leucopenia might be the cause of blood poisoning in which the blood completely runs out WBCs.

Table 1. Effect of silk dye effluent on haematological parameters and total cholesterol levels.

| Blood profile | Silk dye effluent (conc. %) | Exposure period | | | |
|---|---|---|---|---|---|
| | | 15 d | 30 d | 45 d | 60 d |
| RBC | Control | 5.9 ± 0.05 | 5.9 ± 0.49 | 5.9 ± 0.05 | 5.9 ± 0.05 |
| | 50% | 5.3 ± 0.04 | 4.6 ± 0.07 | 4.2 ± 0.03 | 3.4 ± 0.09 |
| | 75% | 4.4 ± 0.07 | 3.5 ± 0.9 | 3.2 ± 0.04 | 2.7 ± 0.06 |
| WBC | Control | 6.1 ± 0.02 | 6.1 ± 0.02 | 6.1 ± 0.01 | 6.2 ± 0.01 |
| | 50% | 6.4 ± 0.07 | 6.8 ± 0.05 | 7.3 ± 0.09 | 8.4 ± 0.10 |
| | 75% | 6.7 ± 0.06 | 7.3 ± 0.09 | 8.2 ± 0.11 | 9.4 ± 0.13 |
| Hb% | Control | 11.6 ± 0.12 | 11.5 ± 0.12 | 11.4 ± 0.12 | 11.3 ± 0.10 |
| | 50% | 10.8 ± 0.13 | 9.7 ± 0.14 | 8.9 ± 0.13 | 7.9 ± 0.11 |
| | 75% | 9.9 ± 0.12 | 8.9 ± 0.13 | 8.2 ± 0.05 | 6.0 ± 0.17 |
| PCV | Control | 31.9 ± 0.12 | 31.4 ± 0.19 | 31.4 ± 0.190 | 31.5 ± 0.22 |
| | 50% | 31.4 ± 0.35 | 30.7 ± 0.06 | 28.5 ± 0.273 | 25.8 ± 0.27 |
| | 75% | 30.6 ± 0.06 | 28.7 ± 0.13 | 26.8 ± 0.231 | 21.0 ± 0.69 |
| MCV | Control | 52.6 ± 0.19 | 52.6 ± 0.19 | 52.5 ± 0.18 | 52.7 ± 0.21 |
| | 50% | 61.9 ± 0.34 | 67.2 ± 0.06 | 64.9 ± 0.27 | 63.9 ± 0.26 |
| | 75% | 70.2 ± 0.06 | 81.7 ± 0.13 | 83.1 ± 0.23 | 71.3 ± 0.68 |
| MCHC | Control | 36.8 ± 0.45 | 36.7 ± 0.55 | 36.3 ± 0.46 | 35.8 ± 0.46 |
| | 50% | 33.0 ± 0.42 | 31.5 ± 0.49 | 31.3 ± 0.66 | 28.9 ± 0.45 |
| | 75% | 32.6 ± 0.39 | 31.1 ± 0.47 | 30.5 ± 0.36 | 21.9 ± 0.59 |
| Total cholesterol | Control | 81.13 ± 0.29 | 81.7 ± 0.21 | 82.4 ± 0.35 | 82.7 ± 0.30 |
| | 50% | 73.1 ± 2.07 | 71.5 ± 2.97 | 68.2 ± 3.33 | 62.3 ± 4.05 |
| | 75% | 65.8 ± 2.29 | 61.8 ± 3.35 | 55.6 ± 2.81 | 51.1 ± 3.08 |

The cause of enhancement of WBC content may affect the defensive mechanism against the silk dye induced toxic condition. Lymphocytes response could be the result of direct stimulation of the immunological protection against toxic substances which may be present in effluent or may associated with different effluent indeed tissue disruption. It may be due to stimulated lymphopoiesis or accelerated release of lymphocyte from lymphomyeloid tissue. In the differential count, the percentage of lymphocytes was increased accompanied by decreased level of neutrophil among wastewater exposed groups of mice. Mathur and Krishnatrey (2005) reported the albino mice exposed to 5% concentration solution of textile waste water for 60 d and noticed signs of stomatocytes, schistocytes, poikilocytes, anulocytes, acanthocytes and Heinz bodies. He also noticed large number of basophilic inclusions in the cytoplasm of erythrocytes. Anaemia is a condition in which the average life span of red blood cells is greatly reduced due to destruction of blood. In leucocyte hypersegmentation in neutrophils and granulation in lymphocytes were found in treated rats. Besides this, the nuclei of most of the leucocytes showed disorganization.

Marked increase in the average diameter observed (data not shown). It is thus, evident from the foregoing account that silk dye effluent exposed mice had severe haematological disorder. The total cholesterol had a decreasing trend through the treatment period (81.13 ± to 51.1 ± 3.08 mg/dL) (Table 1). Total cholesterol decreased throughout the period of treatment i.e. may be because of renal toxicity which may increase serum urea and creatinine levels (Eaton and Klaassen, 1996; Pathak *et al*., 2002).

The reduction in food intake of influent (29%) and effluent rats (12%) possibly decreased cholesterol, total protein, albumin, globulin and glucose contents. Sharma *et al*. (2007) have reported that the cholesterol level gradually decrease according to the increase in concentration and day dependent manner.

## Conclusion

Toxicity due to long term exposure of untreated silk dye effluent (50 and 75%) of small scale cottage industry at Bhagalpur was studied on various haematological parameters (RBC, WBC, Hb%, PCV, MCV, MCHC) and total cholesterol on swiss albino mice *Mus musculus*. From the findings, it was found that silk dye effluent has a deleterious effect upon the blood profile and lipid profile of mice, which can lead to many metabolic and physiological disorders. The present investigation may be a valuable step in the toxicity assessment of silk dye effluent in swiss albino mice *Mus musculus*.

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