

# Toxic Effects of Levafix Blue CA and Levafix Amber CA Reactive Dyes on Liver and Kidney in Mice

Mohammad Farhadur Rahman<sup>1</sup>, Nargish Jahan Ara<sup>1</sup>, Mohammad Mesbah Uddin<sup>2</sup>,  
Mohammad Zakir Sultan<sup>3</sup>

<sup>1</sup>Department of Chemistry, Bangladesh University of Textiles, Dhaka, Bangladesh

<sup>2</sup>Department of Clinical Pathology, Dhaka Medical College, Dhaka, Bangladesh

<sup>3</sup>Centre for Advanced Research in Sciences, University of Dhaka, Dhaka, Bangladesh

## Email address:

zakir.sultan@du.ac.bd (M. Z. Sultan), chemistry.butex2010@gmail.com (M. Z. Sultan)

## To cite this article:

Mohammad Farhadur Rahman, Nargish Jahan Ara, Mohammad Mesbah Uddin, Mohammad Zakir Sultan. Toxic Effects of Levafix Blue CA and Levafix Amber CA Reactive Dyes on Liver and Kidney in Mice. *International Journal of Nutrition and Food Sciences*.

Vol. 6, No. 5, 2017 pp. 187-193. doi: 10.11648/j.ijnfs.20170605.11

**Received:** June 18, 2017; **Accepted:** July 5, 2017; **Published:** July 31, 2017

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**Abstract:** The healthy life of human depends on healthy foods as well as food safety. But unethical practice of textile dyes as adulterant damages the safety of food chain. Textile industries also uses textile dyes to full-fill increase demand of textile products, which produce large waste dye as effluent in ground water due to inefficiency of dying process and causes serious environmental pollution. In this study, we investigated the toxic effects of two textile reactive dyes Levafix Blue CA and Levafix Amber CA by in vivo experiments in mice. Mice were administered Levafix Blue CA and Levafix Amber CA textile dyes at a single oral dose of 0.04 g/kg daily for 21 days to observe any toxic effect of those dyes in mice. The toxic effects were evaluated by measuring the serum activity of aspartate amino-transferase (AST), glutamate pyruvate transaminase (ALT), serum total bilirubin (STBI), serum creatinine (SCR), serum urea (SBUN) and also histopathology of liver and kidney. The levels of AST, ALT, STBI, SBUN and SCR were found to be increased by both Levafix Blue CA and Levafix Amber CA. Histopathological examination of liver and kidney revealed inflammation in mice.

**Keywords:** In Vivo, Reactive Dyes, Levafix Blue CA, Levafix Amber CA, Toxicity

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## 1. Introduction

Food adulteration is a national issue. It began a couple of decades ago and this practice is increasing day by day. The problem of adulteration persists at every level of foods from preparation to consumption. Textile dyes use as coloring agents instead of food grades colorant due to cheapness of textile dyes. Consumption of adulterated food items severely affects the human health by producing many acute and chronic diseases. [1]

In various kinds of sweets and fast-foods like jilapi (local name of a sweetmeat), piaju (local name of one kind of fast food), chop, beguni (local name of one kind of fast food) textile dyes used in indiscriminate manner in Bangladesh. [2] A report by the 'Poribesh Bachao Andolan' disclosed that about 7.9 million (79 lakh) people in South Asia die every year by non-contagious diseases, and food adulteration is

marked as one of the most important reasons. The report also warned if this food adulteration cannot be prevented now, it will affect the mental growth in next generation. [1, 3]

The supply of unsafe foods is negatively contributing to the public health seriously with numerous acute and chronic diseases. Yet, little work on the toxicity was published before. [4]

A study in Dhaka city in 2003 found that 96% of sweetmeats, 24% of biscuits, 54% of bread and 59% of ice-creams were seriously adulterated by poisonous chemicals. [5] Use of poisonous chemicals in perishable foods is endangering the lives of people. [6]

Moreover, the textile industry consumes a substantial amount of water in its manufacturing processes used mainly in the dyeing and finishing operations of the plants. The wastewater from textile plants is classified as the most polluting of all the industrial sectors, considering the volume

generated as well as the effluent composition. [7], [8] It is estimated that over 10,000 different dyes and pigments are used industrially. [9] In the textile industry, up to 200,000 tons of these dyes are lost to effluents every year during the dyeing and finishing operations, due to the inefficiency of the dyeing process. [10] Unfortunately, most of these dyes escape conventional wastewater treatment processes and persist in the environment as a result of their high stability to light, temperature, water, detergents, chemicals, soap and other parameters such as bleach and perspiration. [11] In addition, anti-microbial agents resistant to biological degradation are frequently used in the manufacture of textiles, particularly for natural fibers such as cotton. [11, 12]

It has been reported that during the dyeing process 10% of the dye stuffs used remain unfixed from the fibers. [13] Another report published that up to 30% of the used dyestuff remains in the spent dye-bath after the process due to inefficiency of dying process. [14]

The unused dyes in the effluent mix with water of river, pond or ground, and leading to serious environmental pollution which may ultimately serious health hazard. [7] In river, pond or ground water fishes are administered those textile dyes and we are taking those fishes as one of the main item of daily foods. Many dyes are carcinogenic and affect the life of aquatic organisms. [15] Olukanni *et al.* (2005) reported that the serious environmental problems for rapid growth of textile industry leads to harmful causes of agriculture issues, rise of heavy metals in ground water, drastic effects on flora and fauna in the surrounding area. [16]

Therefore, to evaluate the impact of textile dyes on human health, we conducted toxicity studies of some textile dyes in mice model. This paper describes the effects of the two textile dyes on the activity of some organs (liver and kidney) of mice.

## 2. Materials and Methods

### 2.1. Materials

Dye used: (a). Levafix Blue CA and (b). Levafix Amber CA

Levafix Blue CA and Levafix Amber CA are reactive dye, widely used in textile industry. Both reactive dyes were supplied by Dye Star Ltd., Dhaka, Bangladesh.

### 2.2. Instruments

Aspartate aminotransferase (AST) [AST formerly was called serum glutamic oxaloacetic transaminase (SGOT)], alanine aminotransferase (ALT) [ALT used to be called serum glutamic-pyruvic transaminase, or SGPT], serum urea and serum creatinine were determined by biochemical auto analyzer (Dimension Xpand Plus, Siemens, Germany). Histopathological observations were carried out under a light microscope (Olympus BX 53, Olympus, Japan).

### 2.3. Experimentations

Nine adult male mice, weighing 40-50 mg, were used in

this experiment. All the rats were acclimatized to the new environment for a period of one week. During the experiment period the rats were kept in a well ventilated animal house at 25°C. They were supplied with standard pellets and fresh drinking water. All the rats were kept in cage and maintained with natural 12 h light and dark cycle in the animal house of Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. The mice were divided into three groups consisted of three mice each.

Group A and Group B were administered Levafix Blue CA dye and Levafix Amber CA, respectively at a single oral dose of 0.04 g/kg daily (half dose of Mahmoud N. H.) for 21 days to observe any toxic effect of those dyes in mice. [17] 100.0 mL solution for both dyes was prepared at 0.04 g/kg concentration. For each mouse oral dose was administered twice per day at 0.5 mL/time for 21 days besides normal foods. Group C was left as control and administered normal foods.

### 2.4. Histopathological Examinations

At the end of the experiment the control and treated mice were sacrificed. Their livers and kidneys were removed and fixed in 10% formalin solution, processed and embedded in paraffin wax. Sections of 3-6  $\mu$ m thickness were stained with hematoxylin and eosin.

### 2.5. Biochemical Analysis

By decapitation of each mouse, blood was collected in centrifuge tube and kept 1 hour at room temperature for coagulation. Then centrifuged at 4000 rpm for 20 minutes and serum was separated by decantation and placed at -20°C until biochemical analyses were done.

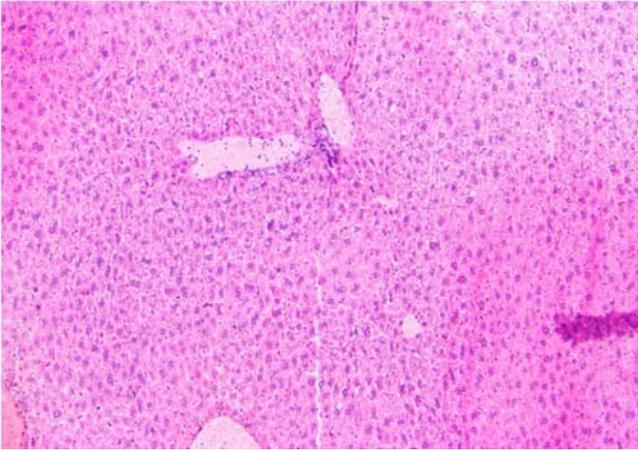
AST and ALT were measured according to Wroblewski and LaDue [18] (recommended by IFCC as described by Bergmeyer [19, 20]). Serum TBI and serum urea were measured by the method of Doumas [21] (modification of diazo method described by Jendrassik and Grof [22]). Measurement of serum creatinine was determined by Larsen. [23]

## 3. Results

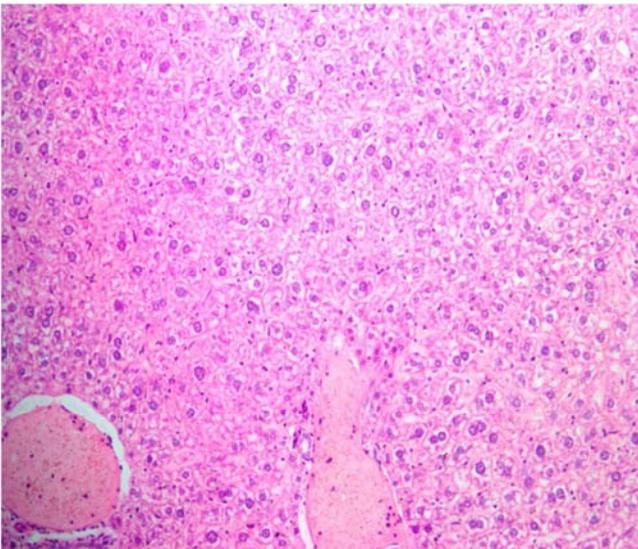
### 3.1. Histopathological Results

#### 3.1.1. Liver

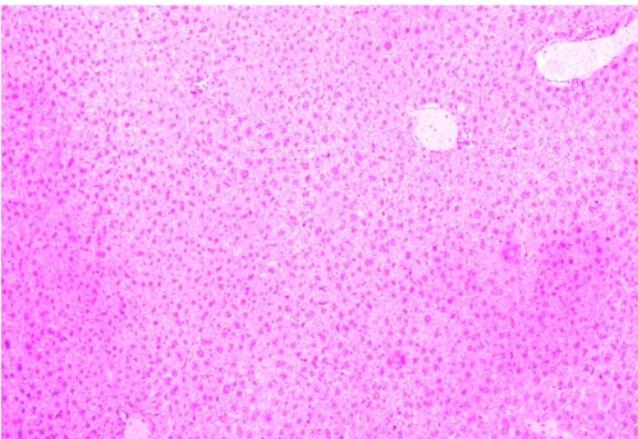
An unremarkable change was observed in liver of control mice (Figure 1 and 2). After treating with Levafix Blue CA reactive dye a mild inflammation of liver was observed in two mice (No. 1 and 3) but there was no significant effect on third one (No. 2) (Figure 3 and 4, respectively). Figure 5 shows unremarkable changes in liver which was obtained from Group B, mice No. 1 treated with Levafix Amber CA reactive dye but Figure 6 revealed mild inflammations of livers of mice No. 2 and 3 for the induced effect of the same dye.



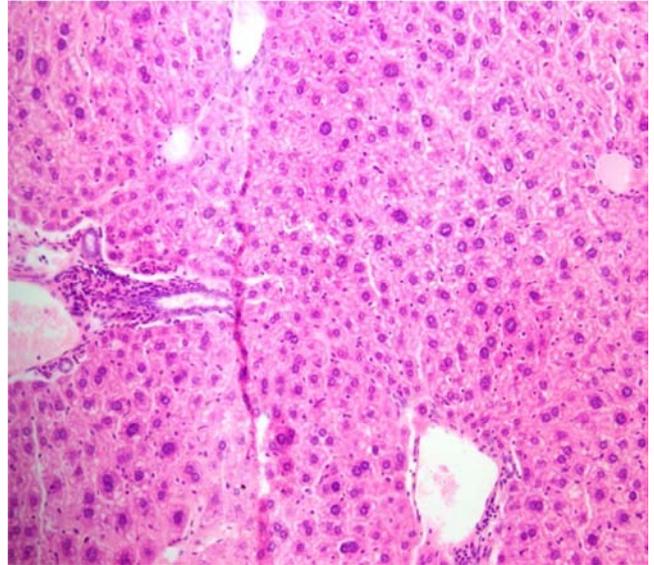
**Figure 1.** Liver section of control 1<sup>st</sup> mice showing unremarkable liver tissue (100 X).



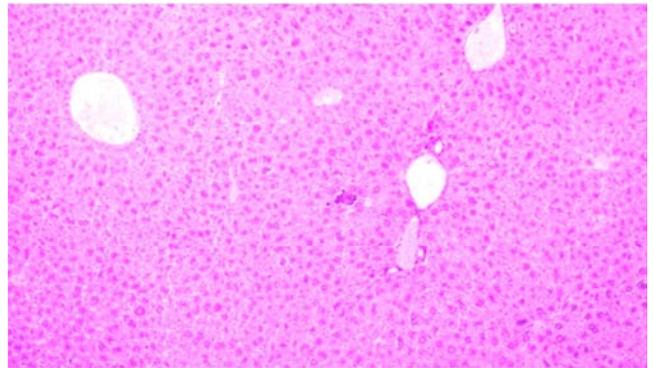
**Figure 2.** Liver section of control 2<sup>nd</sup> mice showing unremarkable liver tissue (200 X).



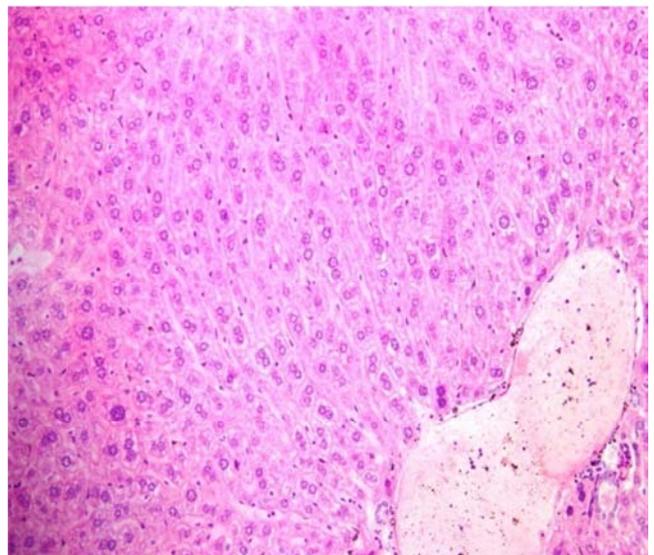
**Figure 3.** Liver section of Levafix Blue CA groups 1<sup>st</sup> mice showing unremarkable liver tissue (100 X).



**Figure 4.** Liver section of Levafix Blue CA groups 2<sup>nd</sup> mice showing mild inflammation of liver tissue (200 X).



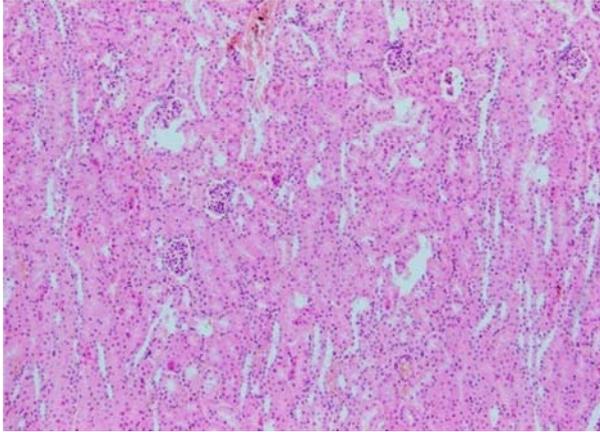
**Figure 5.** Liver section of Levafix Amber CA groups 1<sup>st</sup> mice showing unremarkable liver tissue (100 X).



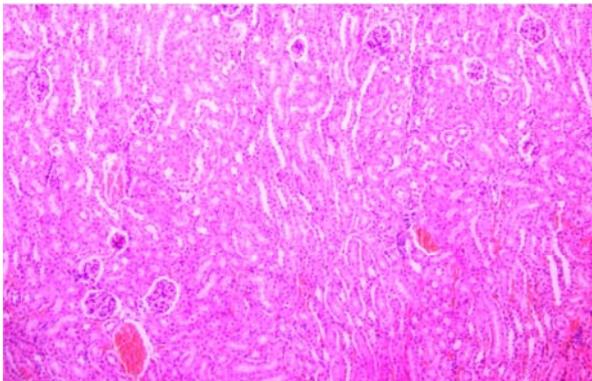
**Figure 6.** Liver section of Levafix Amber CA groups 2<sup>nd</sup> mice showing mild inflammation of liver tissue (200 X).

### 3.1.2. Kidney

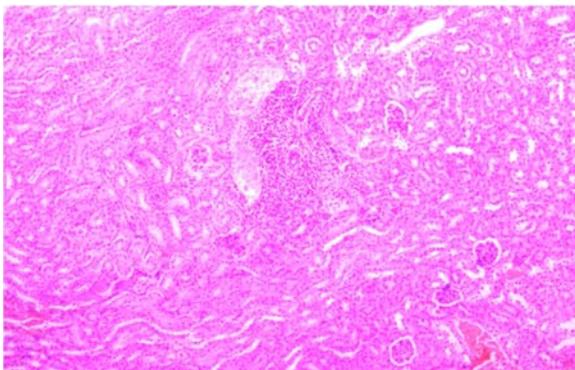
An unremarkable change showed in kidney for control mice (Figure 7 & Figure 8). The observation for Group A after treating Levafix Blue CA reactive dye showed moderate inflammations in kidney of first and third mice (Figure 9). But for same dye there was mild inflammation in kidney of second mouse (Figure 10). A mild inflammation was observed in kidney in mouse No. 1 and 2 showed in Figure 12 for Group B after administered Levafix Amber CA reactive dye but unremarkable changes showed in Figure 11 for mouse No. 3 in kidney for same dye.



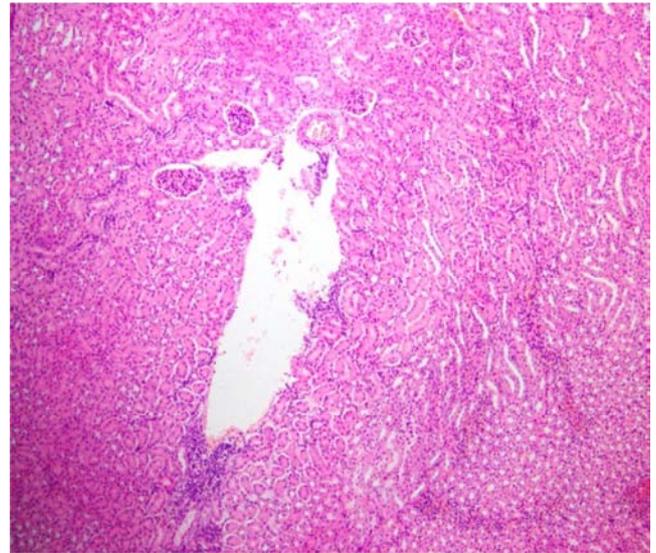
**Figure 7.** Section in kidney of 1<sup>st</sup> control mice showing unremarkable kidney tissue (100 X).



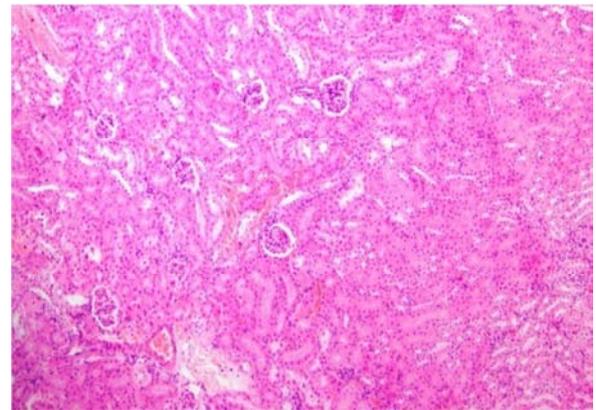
**Figure 8.** Section in kidney of 2<sup>nd</sup> control mice showing unremarkable kidney tissue (100 X).



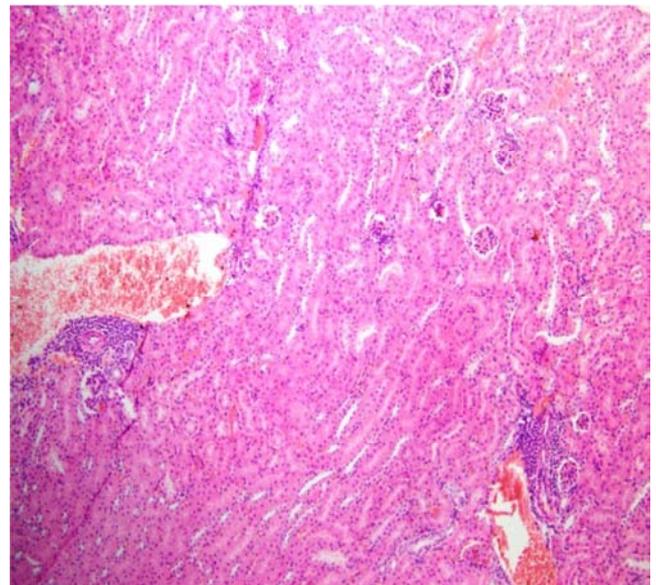
**Figure 9.** Section in kidney of Levafix Blue CA groups 1<sup>st</sup> mice showing moderate inflammation kidney tissue (100 X).



**Figure 10.** Section in kidney of Levafix Blue CA groups 2<sup>nd</sup> mice showing mild inflammation kidney tissue (100X).



**Figure 11.** Section in kidney of Levafix Amber CA groups 1<sup>st</sup> mice showing unremarkable kidney tissue (100 X).



**Figure 12.** Section in kidney of Levafix Amber CA groups 2<sup>nd</sup> mice showing mild inflammation kidney tissue (100 X).

### 3.2. Biochemical Results (Serum Activity)

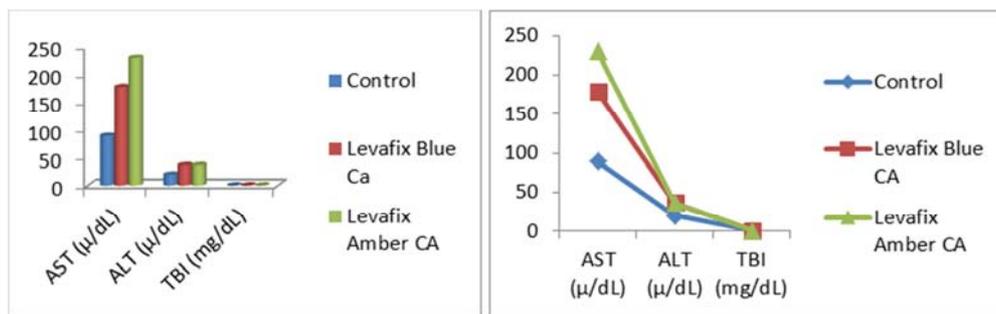
#### 3.2.1. Liver Function Study

Table 1 shows AST, ALT and TBI level of serum obtained from Group A (Levafix Blue CA), Group B (Levafix Amber

CA) and Group C (control). The values of AST, ALT and TBI level were changed significantly after the administration of Levafix Blue CA and Levafix Amber CA reactive dyes (Figure 13).

**Table 1.** Effects of Lever reactive textile dye on serum activity in liver function in male mice.

Parameters	AST ( $\mu$ /dL)		ALT ( $\mu$ /dL)		TBI (mg/dL)	
	Individual	Average	Individual	Average	Individual	Average
Control	85.0	89.3	20.0	19.7	0.50	0.40
	96.0		18.0		0.30	
	87.0		21.0		0.50	
Levafix Blue CA	155.0	178.3	30.0	36.7	0.50	0.50
	225.0		45.0		0.50	
	155.0		35.0		0.50	
	425.0		60.0		0.50	
Levafix Amber CA	130.0	230.0	15.0	36.7	0.50	0.50
	135.0		35.0		0.50	



**Figure 13.** Comparison the effect of textile dyes on serum activity of liver function in male mice.

#### 3.2.2. Kidney Function Study

Table 2 shows creatinine and SBUN level of serum obtained from Group A (Levafix Blue CA), Group B (Levafix Amber CA) and Group C (control). The values of creatinine and SBUN level were changed less significantly after the administration of Levafix Blue CA and Levafix Amber CA dyes (Figure 14).

**Table 2.** Effects of reactive textile dye on serum activity on kidney function in male mice.

Parameters	Creatinine (mg/dL)		SBUN (mg/dL)	
	Individual	Average	Individual	Average
Control	0.15	0.14	10.0	8.3
	0.24		6.0	
	0.03		9.0	
Levafix Blue CA	0.25	0.22	15.0	11.7
	0.25		10.0	
	0.15		10.0	
Levafix Amber CA	0.60	0.45	25.0	18.3
	0.40		20.0	
	0.35		10.0	

### 3.3. Discussion

Textile dyes represent a large group of organic compounds that could have undesirable effects on the environment, and in addition, some of them can pose risks to humans. The increased demand for textile products and the proportional increase in their production, and the use of textile dyes have together contributed to dye wastewater becoming one of the

substantial sources of severe pollution problems in current times. Besides, consumption of adulterated food items severely affects the human health by producing many acute and chronic diseases.

This study was measuring the toxic effect of daily supply foods adulterated with textile dyes. To observe any effects of textile dyes mice were administered Levafix Blue CA and Levafix Amber CA daily at oral dose of 0.04 g/kg for 21 days.

Results obtained in the present work indicated that treating mice with Levafix Blue CA and Levafix Amber CA caused significant increase of AST, ALT levels in mice serum. Previous study suggested that damaged organs showed increase in enzyme activity and chronic intoxication was accompanied by continuous increase in serum levels in both AST and ALT activities. [17] It seems that the changes of AST and ALT activities are due to cellular degradation by Coralene Red XF and Remazol Red RR.

Treating mice with Levafix Blue CA and Levafix Amber CA induced less significant increase in serum TBI which may be due to the defect in liver function produced from hepato cellular damage. [24]

This study also found less significant changes in serum creatinine and serum urea for administering Levafix Blue CA reactive dye. But treating with Levafix Amber CA reactive dye showed significant increase in serum creatinine and serum urea. Mackenzie et al. (1992) found that the significant elevation in creatinine and urea levels related to impairment of renal or post renal function. [25]

Histopathological examination of both liver and kidney revealed inflammation on mice and some times unremarkable after treating with Levafix Blue CA and Levafix Amber CA. Levafix Blue CA reactive dye showed moderate changes in kidney but mild inflammation in liver and some cases unchanged in liver. Levafix Amber CA reactive dye showed mild inflammation in both liver and kidney. There was also an unremarkable changes of liver and kidney for this dye. The unremarkable change might be due to the body immunity of mice. The protection capability varies mouse to

mouse. The duration of study time also needs to extend for more accurate observation.

Through this work, the changes on serum activity of blood and histopathological impact on liver and kidney for textile dyes show the alarming about textile dyes for human health. Study at cellular and molecular level with this dye is necessary to get more insight about mechanism of impacts on liver and kidney. The increasing complexity and difficulty in treating textile wastes has led to a constant search for new methods that are effective and economically viable.

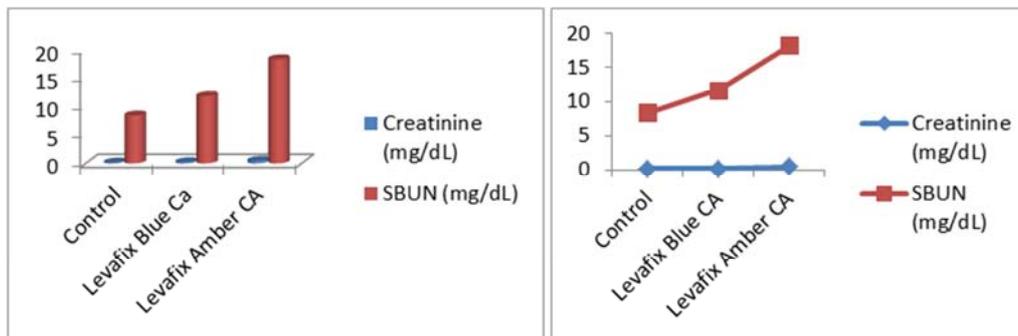


Figure 14. Comparison the effect of textile dyes on serum activity of kidney function in male mice.

## 4. Conclusion

Many people add textile dyes in foods to increase attraction or careless use of these dyes makes environmental pollution. From our study it was obviously found that the textile dyes are not safe for health. There are profound impacts on blood, liver and kidneys. The results of this study will aware people for not to use textile dyes in food. It will also aware the people working in textile industries for careful handling the dyes to prevent environmental pollution or food contamination.

## Acknowledgement

The authors would to thanks Md. Bayejid Hosen, Divisional Forensic DNA Screening Laboratory, Khulna, Bangladesh and Bishnu Pada Dey, Department of Pathology, Bangladesh Sheikh Mujib Medical University, Bangladesh, for kind cooperation during research work. The authors also pay special thanks to Syeda Saba-E-Zannat, Mymensing Agriculture University, Bangladesh, for helping throughout the research period.

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