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ORAL DOSE EFFECTS OF REACTIVE DYE ORANGE ME2R2 ON BLOOD PROFILES AND ORGANS OF RABBIT

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ABSTRACT

The present work has been undertaken to investigate the effects of active dye Orange ME2R2 on body weight, biochemical and histopathological changes in rabbit organs. The body weight changes of all the treated rabbits and control group were in normal ranges. Significant changes on WBC, RBC, platelet count, hemoglobin concentration and ESR were observed in the treated rabbits in comparison to control group. Amazing changes on creatinine, SGPT, SGOT and uric acid of treated groups were found with respect to untreated rabbits. The different values of TC, HDL, LDL and TG of dye treated rabbits were obtained compared to the control rabbits. Remarkable abnormalities on histopathology of liver, kidney, heart, lung, brain and spleen of the active dye treated rabbits were observed as compared to untreated rabbits.

1. INTRODUCTION

Synthetic dyes are present in our everyday life and their usage is continuously growing. Dyes have been widely used as colorants in different industries such as textile, paper, pharmaceutical, food, cosmetic, etc described by Saeed *et al.* (2010). Producers and users of dyes are interested in stability and consequently, are producing dyes which are more difficult to degrade after application (Hassan, 2009). Color additives exempt from certification are used for a wide variety of purposes in foods, drugs and cosmetics (Van Bever *et al.,* 1989). Large quantities of colored wastewater are discharged from the dyeing process with strong persistent color that is aesthetically and environmentally unacceptable (Madsen, 1997). Food colors are added in various stages of food production with two main purposes, one is to make food safe by preventing bacteria growth, oxidation formation and other chemical changes and the other is to improve consumer state by enhancing the organoleptic properties such as color, appearance, flavor and smell of the food reported by Hallagan *et al*. (1995). Some of the dyes are toxic and even carcinogenic to aquatic organisms (Adrade *et al*., 2014). Food colors are substances which added to food, food products and drink to changes its color to improve the visual quality of food for attraction of the consumers (Tripathi *et al*., 2007). During the reactive dyeing of cotton by salts including sodium chloride are placed in a dye bath to aid the exhaustion of various dyes onto the fabric, while bases are added to raise the pH from 7 to 11 to achieve fixation. Consequently, many raw materials are lost in the waste stream ending up in the environment as pollutants (deMan, 1990). When any dye is added to food or drink, then that pigment or substance is called food coloring. They come in many forms consisting of liquids, powders, gels, and pastes. Food coloring is used both in commercial food production and in domestic cooking (Kobylewski and Jacobson, 2012*)*. It has been concluded that food colors affect and alter bioelements levels in vital organs e.g. liver, kidney and brain (Cemek *et al.,* 2014). The main routes of human exposure to azo dyes identified are i) oral ingestion, mainly referring to the sucking of textiles by babies and young children, ii) dermal absorption, the route of primary concern for consumers wearing azo compound-dyed products, as well as for workers in dye production and use plants and iii) inhalation, a route of concern for workers in dye production and use industries as well as those handling newly dyed products. Dyes containing anthraquinone or azo structures are known to cause contact dermatitis presented by Helal *et al*. (2000). The result of a clinical and immunological investigation of respiratory disease indicated that about 15% of 400 workers handling reactive dyes experienced work-related respiratory and nasal symptoms (Sirianuntapiboon and Srisornak, 2007). Many studies have also found statistically significant relationships between reactive dyes and increasing immunoglobulin blood values in workers who have been contact with these dyes. The high concentration and longtime administration of active dye has adverse effects on children's immunity (Sahar and Manal, 2012). In Bangladesh, maximum restaurants and street food corners use active dyes in several ways. Therefore, this present study has been conducted to investigate the effects of active dye orange ME2R2 on physiology of rabbits.

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2. MATERIALS AND METHODS

2.1 Materials

Active dye (Orange ME2R2) was collected from local market. All chemicals and reagents were of analytical grade and obtained from local suppliers. Eighteen (18) healthy and mature rabbits (530–600g) were taken from the local market, Khulna, Bangladesh.

2.1.1 Experimental rabbit grouping

18 rabbits were divided equally into three groups having six rabbits per group and given treatment as follows: (i) control group, (ii) Diet with dose 0.5 mg Orange ME2R2 dye solution (S_1D_1) per day and (iii) Diet with dose 3.0 mg orange ME2R2 dye solution (S_1D_2) per day.

2.1.2 Maintenance of the rabbit and diet

Rabbits were kept in stainless steel cages at room temperature. They had been used to feed diet and water. Control group was fed only diet and fresh water. On the other hand S_1D_1 and S_1D_2 groups were fed diet and water with active dye.

2.1.3 Study design of the rabbit

The rabbits were maintained for 1 week before treatment and the duration of treatment was 14 days. Body weight was checked in everyday using the weight measuring machine. After 14 days of study, the rabbits were sacrificed under chloroform anesthesia, blood was collected and the heart, lung, liver, spleen, kidney and brain were taken out. Biochemical and hematological profiles of blood were measured and histopathology of organs was executed in the course of study.

2.1.4 Monitoring of the hematological profiles

The hematological profiles of the experimental rabbit were done to check the hematological abnormalities after administration of the treated active dye (Orange ME2R2). For this purpose, the following parameters were observed: total RBC count, total WBC count, differential count of WBC, Platelet count, Hemoglobin estimation and ESR (Erythrocytes Sedimentation Rate). The hematological parameters were performed.

2.1.5 Monitoring of the biochemical profiles

The biochemical profiles of the experimental rabbit were done to check the biochemical abnormalities after administration of the treated oral dose of active dye. For this purpose, the following parameters were observed: Serum glutamate-oxalo-acetate transaminase (SGOT), Serum glutamate-pyruvate transaminase (SGPT), Serum alkaline phosphatase (SALP), Serum Creatinine, Random plasma glucose (RBS), lipid profile [triglyceride, total cholesterol (TC), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL)], Creatinine and Uric acid.

2.1.6 Collection of serum

Blood samples were collected from the Jugular vein at fasting state. The blood was collected in plastic centrifuge tubes. These were then allowed to clot at 40°C for 4 hours. After clotting, the blood samples were centrifuged at 4000 rpm for 15 minutes using a WIFUNG centrifuge LABO-50M. The clear straw color serum was then collected in vials with Pasteur pipette and stored at -20°C.

2.1.7 Histopathological procedure

The treated rabbits were sacrificed, dissected and their liver, kidney, heart, lung, brain and spleen were removed and sliced into pieces with few millimeters of thickness. The sliced tissues were then immersed in 10% buffered formalin, processed and stained by haematoxylin and eosin stain for light microscopic examination.

3. RESULTS AND DISCUSSION

The body weight (Table 1) of each rabbit of the control group (C), S_1D_1 group (dose 0.5 mg dye/rabbit/day) and S_1D_2 group (dose 3.0 mg dye/rabbit/day) were measured before and after administration of the dye treatment. Subsequently, all the rabbits were given mixed diet and oral dose of orange ME2R2 dye. The body weight changes of all the rabbits were recorded in every day. During the study the weight gain of the rabbit was

calculated day by day. The body weight change (%) of all treated rabbit was almost unchanged compared to control group.

Table 1: Body weight of rabbit after active dye treatment

Day	Body weight(g) C n = 6 $M_1 \pm SD_1$	Body weight (g) S_1D_1 n = 6 $M_2 \pm SD_2$	Body weight (g) S_1D_2 n = 6 $M_3 \pm SD_3$
Fresh	531.43±1.14	645.17± 0.39	581.78±1.14
1^{st}	531.74±1.23	645.27±0.95	582.16±1.64
7^{th}	533.49±1.13	647.72±0.99	583.51±1.64
14^{th}	536.42±1.11	649.44±0.74	585.94±0.38

n = Number of rabbits, M = Mean value and SD = Standard deviation

This study, long term administration of oral dose of active dye (Orange ME2R2) treated rabbits have been observed to cause alterations in count white blood cell (WBC), count red blood cell (RBC), hemoglobin (Hb), erythrocyte sedimentation rate (ESR) and the platelet count (PC). The results (Table 2) showed that the total white blood cell count (WBC) of S_1D_1 group was increased with respect to control. Total WBC of S_1D_1 group ($6.5\times10^3\pm 0.16\times10^3$ blood cell/µl) was significantly higher than that of untreated rabbits. White blood cells (WBCs) also called leukocytes, are the cells of the immune system that are involved in protecting the body against infectious disease. The present study showed that the WBC of all S_1D_1 and S_1D_2 treated groups was increased compared with control group. The RBC of control group (5.48±0.03 m/µl) was higher than that of treated S_1D_1 (4.94±0.03 /µl) and S_1D_2 (4.12±0.34 m/µl) groups. The hemoglobin concentration of treated S_1D_1 (10.0±0.08g/dl) and S_1D_2 (9.4±0.10 g/dl) groups were decreased compared to control rabbits. The hemoglobin concentration of control group (11.5±0.08 g/dl) was higher than that of treated groups. ESR of all groups were same (10±00).The erythrocyte sedimentation rate (ESR) is the rate at which red blood cells sediment in a period of one hour. It is a common hematology test and is a non-specific measure of inflammation. It may be an indication of sickle cell anemia and leukemia. The platelet count (PC) was increased in S_1D_1 ($489\times10^3\pm0.14\times10^3$ blood cell/µl) and S_1D_2 ($489\times10^3\pm0.14\times10^3$ blood cell/µl) groups compared with control group ($241\times10^3\pm0.81\times10^3$ blood cell/µl). Abnormalities in platelet number are an indication of a defect in primary hemostasis. An increase in platelet number above normal serves as a marker of vascular disease.

Table 2: Effect of treated active dye on Hematological profiles

Parameters		Units	C n = 6 $M_5 \pm SD_5$	S_1D_1 n = 6 $M_6 \pm SD_6$	S_1D_2 n = 6 $M_7 \pm SD_7$
Total WBC		thousand/µl	2.8±0.16	6.5±0.14	4.7±0.14
Differential count of WBC	Neutrophils	%	50±0.82	60±00	68±00
	Lymphocytes	%	49±0.82	37±0.12	27±0.30
	Monocytes	%	01±00	02±00	02±00
	Eosinophils	%	00±00	01±00	02±00
Total RBC		m/ µl	5.48±0.01	4.94±0.03	4.12±0.34
Hemoglobin		g/dl	11.5±0.08	10.0±0.08	9.4±0.10
ESR		g/dl	10±00	10±00	10±0.0
PC		thousand/µl	241±0.81	514±1.41	489±0.14

n = Number of rabbits, M = Mean value, SD = Standard deviation, WBC = White Blood Cell, RBC = Red Blood Cell, ESR = Erythrocyte Sedimentation Rate and PC = Platelet Count.

Biochemical profiles such as creatinine, SGPT, SGOT, ALP and uric acid of dye treated rabbits have been observed (Table 3). Creatinine was decreased in feeding oral dose treated groups. On the other hand the serum creatinine of control group (0.8±0.00 mg/dl) was higher than that of S_1D_1 (0.7±0.08 mg/dl) and S_1D_2 (0.7±0.08 mg/dl) groups. The present study showed that serum creatinine in the untreated rabbit group was significantly increased compared with treated groups. Creatinine levels in plasma are usually measured to determine acute or chronic renal insufficiency. They are usually raised in renal disease. The serum glutamic pyruvic transaminase (SGPT) of dye treated groups was decreased compared with control group. The SGPT of control group (33±0.82U/L) was higher than that of treated rabbits S_1D_1 (27±2.16U/L) and S_1D_2 (13±2.12) groups. Serum glutamic pyruvic transaminase (SGPT), an enzyme that is normally present in liver and heart cells. SGPT is

released into blood when the liver or heart is damaged. The blood SGPT levels are thus elevated with liver damage or with an insult to the heart. The present study showed that the SGPT of all active dyetreated groups were significantly decreased compared with control group. So this indicates that the liver or heart of active dye treated rabbits may be damaged. The serum glutamic oxaloacetic transaminase (SGOT) was increased in all dyetreated groups with respect to control. The SGOT of S_1D_1 group (67±0.82 U/L) and S_1D_2 group (54±0.95 U/L) were higher than that of untreated group (37±1.41 U/L). The blood SGOT is a liver enzyme which is produced in liver cell. When liver cells are damaged SGOT leaks out into the bloodstream and the level of SGOT in the blood becomes higher than normal value. SGOT is found in parts of the body other than the liver including the heart, kidney, muscles and brain. When cells in any of those parts of the body are damaged, SGOT can be elevated. In addition, this study showed that the SGOT of all treated dye groups were significantly increased compared with control group. So it indicates that the liver, heart, kidneys, muscles and brain of active dyetreated rabbits may be damaged. Alkaline phosphatase (ALP) of dyetreated groups was decreased compared with control rabbit. The blood ALP of S_1D_1 group (146±1.24U/L) was lower than that of control (260±2.16 U/L). Low levels of ALP are occasionally an indication of liver disease at a cellular level such as cirrhosis or chronic hepatitis. But in present study, the ALP of all active dye treated groups was significantly decreased which indicates the cellular damage in liver. Uric acid of control group (1.4±00mg/dl) was significantly higher than that of S_1D_2 group (1.0±0.08mg/dl) whereas the similar uric acid is found in S_1D_1 group (1.4±00 mg/dl).

Table 3: Effect of active dye on biochemical profiles

Parameters	Unit	C n = 6 $M_9 \pm SD_9$	S_1D_1 n = 6 $M_{10} \pm SD_{10}$	S_1D_2 n = 6 $M_{11} \pm SD_{11}$
Creatinine	mg/dl	0.8±00	0.7±0.08	0.7±0.08
SGPT	U/L	33±0.82	27±2.16	13±2.12
SGOT	U/L	37±1.41	67±0.82	54±0.95
ALP	U/L	260±2.16	103±1.63	146±1.24
Uric acid	mg/dl	1.4±00	1.4±00	1.0±0.08

n = Number of rabbits, M = Mean value, SD = Standard deviation, SGPT = Serum glutamic pyruvic transaminase, SGOT = Serum glutamic oxaloacetic transaminase and ALP = Alkaline phosphatase

Biochemical profiles of serum lipid as total cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Triglyceride (TG) in blood of active dye treated rabbit group has been observed (Table 4). The total cholesterol (TC) of dye treated groups was decreased compared with control rabbit. The TC of control group (96±00mg/dl) differs from all other groups. The blood High Density Lipoprotein (HDL) level after supplementation of active dye in different groups of rabbits had significant differences from that of control group. The HDL level of rabbits of group S_1D_2 was found 37±1.67 mg/dl whereas it was 26±0.82 mg/dl for control group. The blood Low Density Lipoprotein (LDL) of dye treated S_1D_1 group (81±1.41 mg/dl) was increased but S_1D_2 group was decreased compared with control group (61±2.45 mg/dl).The effects of active dye on triglyceride (TG) level of treated rabbits were increased compared with control group. The blood TG of S_1D_1 (176±0.82 mg/dl) and S_1D_2 (176±0.82 mg/dl) groups were significantly higher than that of control group (44±00 mg/dl).

Table 4: Effect of active dye on Lipid profiles

Parameters	Unit	C n = 6 $M_{13} \pm SD_{13}$	S_1D_1 n = 6 $M_{14} \pm SD_{14}$	S_1D_2 n = 6 $M_{15} \pm SD_{15}$
TC	mg/dl	96±00	56±2.16	37±1.63
HDL	mg/dl	26±0.82	13±0.82	37±1.67
LDL	mg/dl	61±2.45	81±1.41	10±0.63
TG	mg/dl	44±00	176±0.82	176±0.82

n = Number of rabbits, M = Mean value, SD = Standard deviations, TC = Total Cholesterol,
HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, TG = Triglyceride

In the present study, the histopathology of liver, kidney, heart, lung, brain and spleen of rabbits were examined for the detection of pathological lesions if any. In histopathology, some specific lesions were found in the liver, kidney, heart, lung, brain and spleen of dyed diet groups as compared with the control rabbits.

In Figure1 histological structures of liver of control group showed that the liver is divided into hepatic lobules formed of radically arranged strands of hepatocytes that extend from the central vein to periphery of the lobule.

The hepatocytes strands are separated from each other by blood sinusoids that are lined with the endothelial cells and kupffer cells (a) (image i).The S_1D_1 showed fatty degenerative change and the portal area showing severe dilatation (b) vacuolation group of central vein and hepatocytes (c) (image ii). The group S_1D_2 showed abnormal shape of central vein (d) swollen cell with chronic inflammatory cell infiltration (e) tissue lost its attachment and vacuolation (f) (image iii).

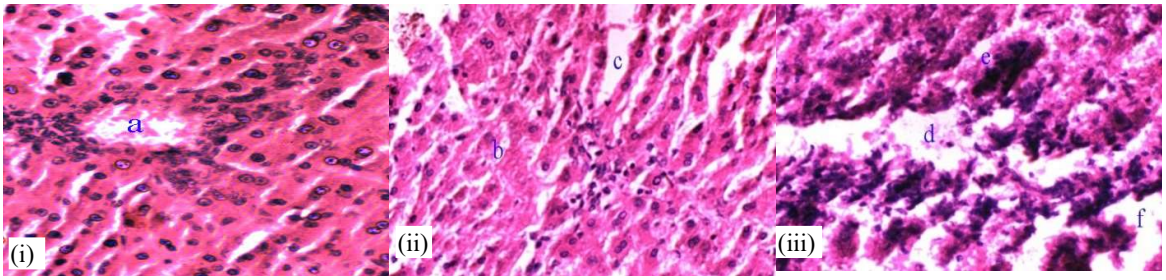

Figure 1: Histopathological section of liver of rabbits (Hematoxylin & Eosinx 200) (i) Control group, (ii) S_1D_1 group and (iii) S_1D_2 group.

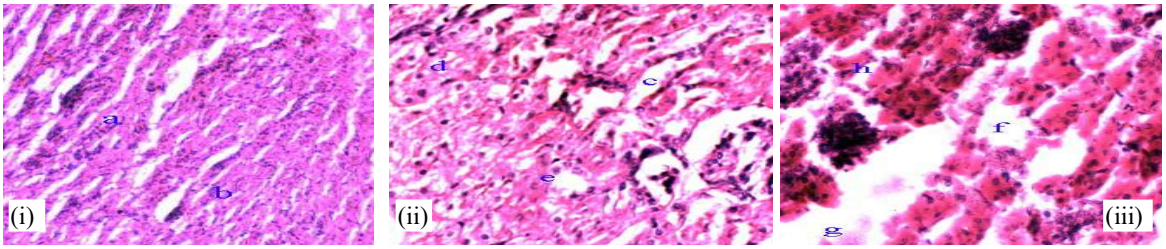

Figure 2: Histopathological section of kidney of rabbits (Hematoxylin & Eosinx 200) (i) Control group, (ii) S_1D_1 group and (iii) S_1D_2 group.

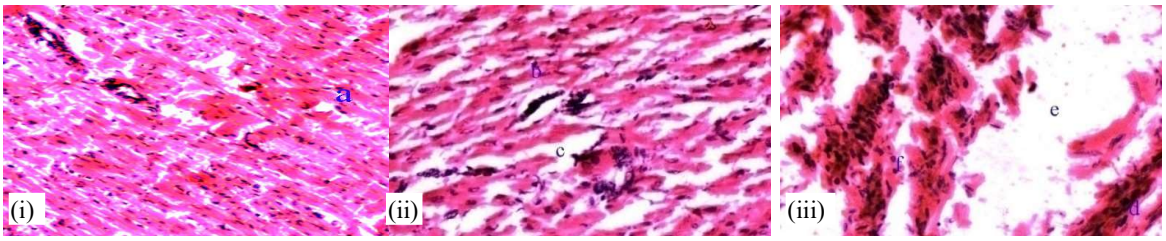

Figure 3: Histopathological section of heart of rabbits (Hematoxylin & Eosinx 200) (i) Control group, (ii) S_1D_1 group and (iii) S_1D_2 group.

Figure 2 is showing the histological examination of the kidney of control group of rabbits which revealed the normal histological features. Control group showed normal histological structure of glomeruli (a) and renal tubules of kidneys in rabbits (b), (image i). Treated 0.5 mg oral dose diet group S_1D_1 showed cells in the medullary region vacuoles (c), focal inflammatory cells infiltration in between the tubules associated with dilatation in the blood vessels (d) and the glomerular tuft showed vacuolization in the lining endothelium (e), (image ii).Treated 3.0 mg oral dose diet group S_1D_2 showed abnormal shape of glomeruli (f), vacuolation (g), atrophy of a glomerulus with degeneration in the lining epithelial cells of renal tubules and edema of tissue (h) (image iii).

Histological examination of the heart of control group of rabbits showed normal structure (Figure 3). Control group showed normal arrangement of cardiac muscular layer (a), (image i). Treated 0.5 mg oral dose diet group S_1D_1 showed congested myocardial (b) few vacuolation in papillary muscle and splitting of longitudinal muscles (c) (image ii).Treated 3.0 mg oral dose diet group S_1D_2 showed hemorrhage (d) vacuolation (e) myocardial degeneration and necrosis (f) (image iii).

Histological structures of lung of rabbits are shown in Figure 4. Untreated rabbits showed the lung pulmonary tissues compact configuration with airway, interalveolar septa, regular alveolar sacs and capillaries (image i). Bronchiolar (a) and alveolar (b) structures in the control group in their normal structures. Treated 0.5 mg oral dose diet group S_1D_1 showed lung tissue containing collagen fiber accumulation along with distinctive cell proliferation (c) and mononuclear cell invasion in the alveolar septa (d) (image ii).Treated 3.0 mg oral dose diet

group S_1D_2 showed a bronchus lined with pseudo stratified epithelium and containing lymphocytes in their lamina propria and surrounding these structures the saccusalveolar is (e) alveoli with regular walls, interalveolar septa (f) and interalveolar connections, connecting the alveoli to each other were observed in the lung parenchyma (g) (image iii).

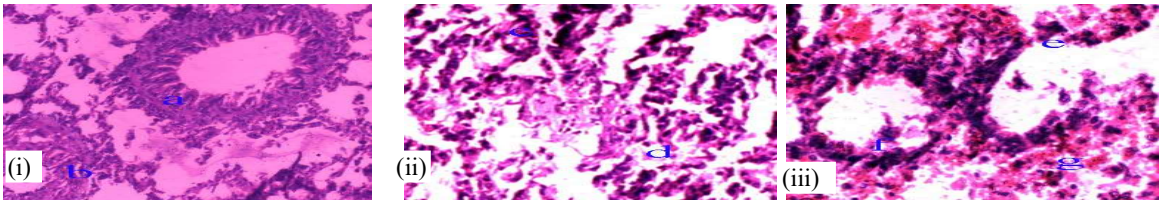

Figure 4: Histopathological section of Lung of rabbits (Hematoxylin & Eosinx 200) (i) Control group, (ii) S_1D_1 group and (iii) S_1D_2 group.

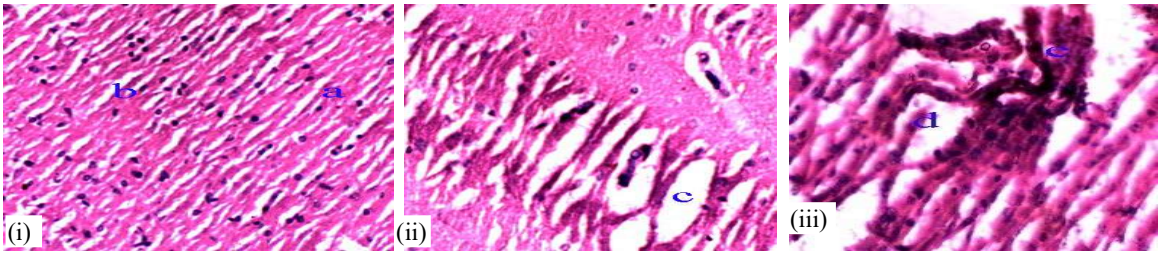

Figure 5: Histopathological section of brain of rabbits (Hematoxylin & Eosinx 200) (i) Control group, (ii) S_1D_1 group and (iii) S_1D_2 group.

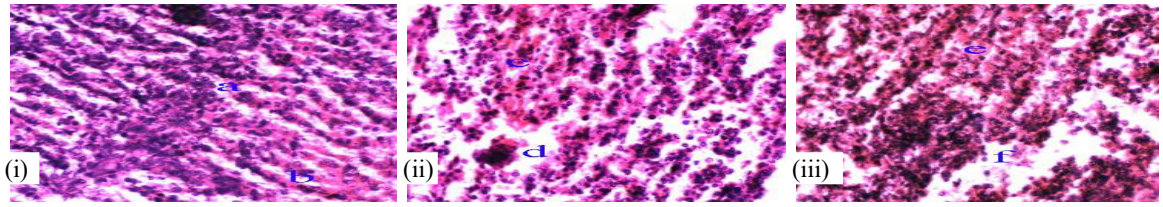

Figure 6: Histopathological section of spleen of rabbits (Hematoxylin & Eosinx 200) (i) Control group, (ii) S_1D_1 group and (iii) S_1D_2 group.

Untreated and dye treated rabbits histological structures of brain are presented in Figure 5. Image (i) showed the histological structure of brain of control rabbits. Normal histological structure of the meanings (a) and cerebral cortex (b) were observed in brain of control rabbits. Treated 0.5 mg oral dose diet group S_1D_1 showed the medulla oblongata vacuolation (c) in the matrix (image ii). Treated 3.0 mg oral dose diet group S_1D_2 showed the deep cerebrum had fat vacuoles in the matrix (d) as well as focal gliosis (e) (image iii).

In Figure 6, image (i) has shown the normal spleen structure of control rabbits. The histopathological examination of spleen of the control group showed normal structure which composed of normal white and red pulp (a), normal capsule and blood vessels (b). Treated 0.5 mg oral dose diet group S_1D_1 showed atrophy in white pulp and edematous in red pulp (c) vaculation in matrix (d) (image ii). Treated 3.0 mg oral dose diet group S_1D_2 showed atrophy of lymphoid tissue of white pulp (e) while the red pulp showed foamy vacuolated macrophages (f) (image iii).

4. CONCLUSIONS

Consumption of active dye with diet had deleterious effects on body weights, hematological parameters, biochemical profiles, lipid profiles and organs of rabbits. The results of this study indicate that active dye may be hazardous to the consumer's health. Long time treated dye consumption may occur certain diseases such as infection, stress, inflammation, allergy, anemia, liver cirrhosis, hepatocellular disease, renal failure, pulmonary infarction, atherosclerosis (coronary artery disease) that means myocardial infarction of the consumers. Liver, heart, kidney, lung, brain, spleen and muscles of users may be damaged by chronic consumption of active dye. Moreover, consumption of colored food items should also be controlled by making the society aware of the hazardous effects of food colors.

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