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Haemotoxic Effects of Chocolate Brown, A Commonly Used Blend of Permitted Food Colour on Swiss Albino Mice

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Abstraet : The present study was conducted to evaluate the haemotoxic effects of Chocolate Brown, a commonly used food color on Swiss albino mice *(Mus musculus)* fed with a diet containing 2 gm/kg of body weight and 6 gm/kg of body weight of the dye for 21 days and 42 days as short term and long term, respectively. The blood parameter data showed a decrease in RBC count, WBC count, haemoglobin content, haematocrit, MCH and MCHC values and slight increase in the values of MCV. Significant increase in the levels of alkaline phosphatase, triglycerides and proteins and decrease in the levels of cholesterol and glucose shows abnormal functioning of the liver.

Key words : Chocolate Brown, *Mus musculus*, haemotoxic

Introduction :

Literature has revealed that the human beings have been using colour additives for a very long time approximately since 1500 B.C. to hide the poor quality of spoiled food products. Originally, the colour dyes of natural origin were used such as Caramel, Saffron, Curcumin etc, however, the use of natural color dyes has largely been suppressed by the use of synthetic coal tar dyes which are much cheaper and provide better coloration. According to the Food Adulteration Act ,1954 (PFAA, 1954) eight synthetic colors namely-indigo, carmoisine, erythrosine, brilliant blue, tartrazine, fast green, sunset yellow and ponceau 4R are permitted to be added in the food items. These dyes are widely encountered in a variety of eatables from both urban and rural markets (Khanna *et al.*, 1973) and are used in the form of blends of one or more dyes. The food processing industry in India comprises the organised and unorganised sectors, the latter being much larger, consisting of small cottage or household type manufacturing units. Monitoring the quality of the processed food prepared in this sector is especially difficult. It is the poor community which is more exposed to food produced in the unorganised sector, the quality of which is suspect, as borne

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out by several independent surveys. Studies conducted by the Industrial Toxicology Research Centre (ITRC) in the state of Uttar Pradesh have revealed that nearly 62 per cent of the artificially coloured eatables in the rural markets have non-permitted colours that are hazardous to health (Dixit *et al* 1995).

Another study in the central and suburban areas of Calcutta also revealed the use of non-permitted colours including some textile dyes, by itinerant vendors, the unorganised sectors as well as small and cottage scale industries. In 6.6 per cent of the cases where permitted colours were used, the statutory limit of 200 ppm was exceeded with some eatables containing as much as 730 ppm of colour (Biswas *et al*, 1994). Studies conducted by the National Institute of Nutrition (NIN), Hyderabad, have shown the use of adulterants such as *Lathyrus sativus* and non-permitted colours in urban street foods (Bhatt *et al*, 1994).

Thus, in the Indian diet, non-permitted colours, permitted colours added in excess and a large number of other adulterants are likely to be present. These have been shown to induce a wide range of adverse reactions in humans. Metanil yellow, the frequently used non-permitted colour in foods, was found to cause toxic methaemoglobinaemia (Sachdeva *et al*., 1992) and cyanosis (Chandra and Nagaraja, 1987) in two incidences of human food poisoning. Among the permitted food colours, tartrazine has been most frequently reported to be associated with hypersensitivity responses (Miller, 1982).

One such commonly used blend is Chocolate Brown which is a blend of tartrazine, carmoisine and brilliant blue FCF, which is widely used in soft drinks, candies, ice-creams and beverages etc.

However, no systematic studies have so far been made to evaluate the toxicity of blends of food dyes. Therefore in the present study, an attempt has been made to evaluate the haemotoxic effects of Chocolate Brown on Swiss albino mice.

Materials and Methods :

The dye blend Chocolate Brown used in the present study was procured from Mallaya Fine – Chem Pvt. Ltd., Bangalore. Other chemicals

used were of analytical grade. Healthy and active Swiss albino mice, weighing 25±2 gms, and of 4-5 weeks were used for the present study. The mice were grouped into five batches. The animals of Group I (control) were divided into two sub groups *viz*. IA and IB having 10 mice each group. Animals of Group IA were fed standard mice feed mixed with 1.65 gm/kg of body weight of NaCl for 21 and 42, days respectively and this group serves as a control for experimental Groups II and III. Animals of Group IB were fed standard mice feed mixed with 4.95 gm/kg of body weight of NaCl for 21 and 42 days respectively, and this group serves as a control for experimental Groups IV and V. Group II and Group III animals were fed with standard diet containing 2 gm/kg body weight of Chocolate Brown for 21 and 42 days as short term and long term experiments, respectively. Group IV and Group V animals were fed with standard diet containing 6 gm/kg body weight of Chocolate Brown for 21 and 42 days as short term and long term experiments, respectively. RBC count, water was given *ad libitum*. Ideal husbandry conditions were maintained throughout the experimental period. Fresh diet for each treatment group was prepared at weekly intervals. A weekly record of body weight was maintained. After 21 days and 42 days of experiment, 5 animals each of various groups were weighed, sacrificed and blood samples were collected to study the impact of the blend of dye on the haematological indices like total erythrocyte count (RBC count), haematocrit, haemoglobin content, total leucocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular haemaoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH). Serological parameters studied include alkaline phosphatase, triglycerides, protein, glucose and cholesterol.

Results and Discussions :

Table 1 shows the mean body weight (in gms) of the mice of control and treated groups. No significant change has been observed in the body weight of experimental mice when compared with control. The effects of feeding different doses of the blend (Chocolate Brown) on the serological and haematological indices have been appended in Tables 2 and 3. The serological studies revealed an increase in the alkaline phosphatase. It is a hydrolase enzyme found in all tissues but in high concentrations in the liver,

Table – 1 : The mean body weight (in gm) of mice fed with Chocolate Brown

Exposure period (days)	Group I (Control)		Group II	Group III	Group IV	Group V
	IA	IB				
1	24.60±0.40	24.50±0.31	25.80±0.37	24.80±0.20	23.80±0.37	24.20±0.20
7	25.00±0.20	25.80± 0.37	26.20±0.28	26.00±0.50	25.80±0.37	25.80±0.37
14	26.20±0.20	26.20± 0.41	27.40±0.41	28.20±0.64	26.20±0.20	27.40±0.41
21	26.80±0.20	27.20 ±0.26	27.60±0.64	27.00±0.50	28.00±0.31 [a]	28.60±0.64 [a]
28	28.00±0.31	28.80±0.37		29.60±0.64		29.00±0.31 [a]
35	29.60±0.64	28.90±0.24		29.00±0.31		29.70±0.20
42	30.40±0.26	30.10±0.15		30.20±0.20		30.80±0.37 [c]

Table – 2 : Showing serological effects in mice fed with Chocolate Brown

Dosage (gm/kg of body weight)	Exposure period (in days)	Groups	Alkaline phosphatase (U/L)	Triglycerides (gm/dl)	Protein (gm/dl)	Glucose (gm/dl)	Cholesterol (gm/dl)
	21	Control	150.78±0.05	110.03±0.16	7.96±0.07	129.69±0.04	128.54±0.05
	21	Experimental	228.01±0.11 [a]	115.03±0.16 [a]	8.87±0.08 [a]	121.63±0.06 [a]	105.52±0.04 [a]
2	42	Control	150.78±0.05	110.03±0.16	7.96±0.07	129.69±0.04	128.54±0.05
	42	Experimental	231.06±0.14 [a]	122.70±0.22 [a]	9.26±0.10 [a]	116.24±0.06 [a]	95.80±0.09 [a]
	21	Control	150.78±0.05	110.03±0.16	7.96±0.07	131.08±0.08	130.14±0.01
	21	Experimental	253.74±1.17 [a]	132.05±0.19 [a]	9.46±0.14 [a]	105.71±0.07 [a]	99.89±0.03 [a]
6	42	Control	150.78±0.05	110.03±0.16	7.96±0.07	131.08±0.08	130.14±0.01
	42	Experimental	263.80±0.56 [a]	145.03±0.29 [a]	10.86±0.10 [a]	92.35±0.10 [a]	79.48±0.12 [a]

Table – 3 : Showing haematological effects in mice fed with Chocolate Brown

Dosage (gm/kg body weight)	Exposure period (days)	Groups	Haemoglobin (gm%)	Haematocrit (%)	TLC (1000cells/ cmm)	RBC (X$10^{6/}$ cmm)	MCV (μ^3)	MCH (µg)	MCHC (%)
	21	Control	12.86±0.06	37.96±0.07	5.63±0.02	7.27±0.94	52.21±0.11	17.68±0.02	33.87±0.06
	21	Experimental	11.84±0.05 [a]	34.90±0.10 [a]	4.52±0.08 [a]	6.94±0.05 [c]	50.28±0.20 [a]	17.05±0.06 [a]	33.92±0.05 [a]
2	42	Control	12.86±0.06	37.96±0.07	5.63±0.02	7.27±0.94	52.21±0.11	17.68±0.02	33.87±0.06
	42	Experimental	10.80±0.07 [a]	32.11±0.34 [a]	3.81±0.02 [a]	6.57±0.08 [a]	48.88±0.57 [a]	16.43±0.18 [a]	33.46±0.22 [a]
	21	Control	12.86±0.06	37.96±0.07	6.26±0.12	7.27±0.94	52.21±0.11	17.68±0.02	33.87±0.06
	21	Experimental	9.25±0.11 [a]	34.32±0.12 [a]	3.69±0.02 [a]	6.01±0.03 [c]	57.11±0.55 [a]	15.39±0.15 [a]	26.94±0.03 [a]
6	42	Control	12.86±0.06	37.96±0.07	6.26±0.12	7.27±0.94	52.21±0.11	17.68±0.02	33.87±0.06
	42	Experimental	6.73±0.03 [a]	30.02±0.16 [a]	2.93±0.03 [a]	5.56±0.03 [a]	54.03±0.69 [a]	12.10±0.18 [a]	22.41±0.17 [a]

Values are mean ± S.E. of 5 individual observations

c = $p \leq 0.05$ almost significant

b = $p \leq 0.01$ significant

a = $p \leq 0.001$ highly significant

kidneys, bile ducts, placenta and bone. In one study the ingestion of colourant C (brown HT and indigocarmine) significantly decreased rat body weight, serum cholesterol and HDL-cholesterol fraction, while, T4 hormone, liver RNA content, liver enzymes (S. GOT, S. GPT and alkaline phosphatase), total protein and globulin fractions were significantly elevated. Significant increases were observed in serum total lipids, cholesterol, triglycerides, total protein, globulin and serum transaminases in rats whose diets were supplemented with chocolate colours A and B (sunset yellow, tartrazine, carmoisine and brilliant blue in varying concentrations). Haematological investigations demonstrated selective neutropenia and lymphocytosis with no significant alterations of total white blood cell counts in all rat groups, while haemoglobin concentrations and red blood cell counts were significantly decreased in the rats who were administered food additives A and B. Eosinophilia was noted in rats fed on colourant A only. No changes were recorded for blood glucose, growth hormone and kidney function tests. Histopathological studies showed brown pigment deposition in the portal tracts and Van Kupffer cells of the liver as well as in the interstitial tissue and renal tubular cells of the kidney mainly induced by colourant A. Congested blood vessels and areas of haemorrhage in both liver and renal sections were revealed in those rats who were given colourants B and C. There were no-untoward-effects recorded in the stomach tissue (Aboel-Zahab *et al*., 1997).

Webner (2003) reported that the damaged or diseased tissues release enzymes into the blood, so serum alkaline phosphatase measurements can be abnormal in many conditions including bone diseases and liver diseases. Moreover, serum alkaline phosphatase is also increased in response to a variety of drugs. In the present investigation, the increased alkaline phosphatase level indicates liver damage due to dye toxicity.

Results also revealed an increase in the triglyceride and protein levels in the serum. Triglycerides are a form of fat carried through the blood stream. Most of the body's fat is in the form of triglycerides stored in fat tissues. They are also present in blood, plasma and in association with cholesterol forming the plasma lipids (Heit, 2001). The present study, however, fails to explain the increasing concentration of triglycerides, as an increase in the body weight of the experimental animals is not significant when

compared with the control. Proteins are the fundamental components of all living cells and include many substances such as enzymes, hormones and antibodies that are necessary for the proper functioning of an organism. Increased release of enzymes by the damaged tissues and the antibodies to counter act the dye might be the cause of increase in serum protein.

A decreased level of glucose was observed at all the dose levels of the dye, with the effect being more pronounced in Group V. Glucose, formed by the digestion of carbohydrates and the conversion of glycogen in the liver is the primary source of energy for most of the cells. It is regulated by insulin, glucagons, thyroid hormones, liver enzymes and adrenal hormones. So the hypoglycaemia in the present investigation might be due to disturbance in the enzymatic function of the liver caused by the dye blend. Similar findings have also been reported by (Scarpelli *et al.*, 1960; Capen *et al.*, 1965).

The level of cholesterol was also decreased at all the dose levels. Cholesterol is a soft waxy substance found among the lipids in the blood stream and in the body's cells. It is an important part of healthy body because it is used to form cell membranes and to produce certain hormones. The total body content of cholesterol depends on the balance between the amount of cholesterol formed in the body plus that absorbed from diet (Cook, 1958). The deviation from normal values of cholesterol, in the blood serum is considered as symptoms of liver diseases (Singh *et al.*, 1988). In the present study the decreased cholesterol level implies liver damage which is in accordance with increased alkaline phosphatase level discussed earlier (Rauiller, 1964).

The present study revealed a significant decrease in the contents of haemoglobin in all the dose levels of the blend. The decrease in the haemoglobin content might be due to decrease rate of haemoglobin synthesis due to dye poisoning. Lewis (1970) reported a fall in the rate of haemoglobin synthesis during all the stages of maturation of erythrocytes when the supply of iron is not adequate. Normally, the globin portion of haemoglobin is broken down into amino acids, which return to the protein pool, while porphyrin is metabolized and excreted 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 on the form

of ferritin (Guyton, 1986). The synthesis of haemoglobin requires iron, which is generally obtained from the stored ferrtin. Therefore, it seems that the dye blend prevented the supply of iron for synthesis of haemoglobin by inhibiting the absorption by developing erythrocytes which resulted in the fall of haemoglobin content in the blood. (Chakravarty *et al*., 2005).

The present study further reveals a marked decrease in the RBC count at all the dose level which is in accordance with the reduced haemoglobin content, as the deficiency of haemoglobin results in inhibition of erythropoiesis in bone marrow. Vitamin B_{12} and folic acid deficiency causes maturation failure in the process of erythropoiesis. Lynch *et al*., 1969 reported that the non availability of the vitamin results in decreased RBC count. The present investigation reveals that the dyes interfere with the absorption of these vitamins and resulted in erythrocytopenia.

The present study also revealed a decrease in haematocrit per cent at all the dose levels. The decrease in the haematocrit may be due to decrease in the number of RBC by way of reduction in haemoglobin synthesis (Wintrobe, 1970). Since, the dye blend does affect haemoglobin synthesis as discussed earlier, the reason appears to be responsible for the reduction in the haematocrit percent in the present investigation.

The MCV was found to decrease in groups II and III and an increase was observed in groups IV and V when compared with the control groups. Reduction in MCH was recorded at all dose levels, and the decrease can be explained on the basis of iron deficiency anaemia. Within the developing red cell, the iron acquired is present as ferritin until it is delivered to the mitochondria, incorporated into protoporphyrin, and finally attached to the globin molecules formed at the ribosomes. Each of these combinations involves energy consuming activity by enzymes systems. Interference with these enzyme actions through toxic substances forming a metabolic bottleneck; erythrocytes are then seen which are inadequately filled with haemoglobin even though loaded with iron (Lewis, 1970). It appears that the dye blend interferes with the enzyme action regulating the incorporation of iron to form haemoglobin.

A decreased MCHC values was observed at all doses when compared to control groups. The MCHC expresses the concentration of

haemoglobin in the cytoplasm of the erythrocytes. Due to dye toxicity, the bone marrow lacks the capacity to manufacture haemoglobin at the required rate and since the haemoglobin content of each cell is diminished the MCHC also decreases (Lewis, 1970).

A decrease in the total WBC count was recorded at all the dose levels. The leucocytes are the mobile units of the body's protective system. Leucocytopenia might be the cause of (blood poisoning) in which the body completely runs out of WBCs. Similar results has been reported in rats fed with sunset yellow (Mannell *et al.*, 1958).

Conclusion :

In conclusion, the present study demonstrates that intake of chocolate brown has deleterious effects on both the haematological and serological parameters and the prolonged consumption of the blend appears to be of greater severity. Therefore it is necessary to create consumer awareness regarding the ill effects of this blend of dye.

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