Tartrazine Induced Haematological and Serological Changes in Female Swiss albino Mice, *Mus musculus*

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Summary

Tartrazine, being cheapest, is the commonly used synthetic dye to impart lemon yellow colour to the food items but there is paucity of literature concerning to the toxicity of this dye. The present study was aimed to evaluate toxic effects of tartrazine on haematological and serological parameters of female Swiss albino mice. The experimental animals were feed with 0.2 gm/kg b.wt and 0.4 gm/kg b.wt of tartrazine as low dose (LD) and high dose (HD) respectively for 35 days. A highly significant decrease in the body weight was recorded at both the dose levels. The dye at both the dose levels caused almost significant decrease in parameters like Hb, haematocrit % and TEC, and highly significant decrease in TLC and polymorph count. A highly significant increase was observed in lymphocytes count, MCV and MCH at both the dose levels however, no significant change was seen in eosinophils and monocytes counts. Serologically, the dye caused a highly significant decrease in the level of glucose but a highly significant increase was observe in the levels of triglycerides, alkaline phosphates and total cholesterol at both the dose levels. The increase in the total serum protein was found to be highly significant at low dose but non-significant at high dose.

Key words: common food dye; tartrazine; *Mus musculus*; haemotoxicity; serotoxicity

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Introduction

Colour is one of the first and most important sensory qualities which enables the identification and selection of food. Hence colours are added to eatables to give them an attractive appearance, which has been lost during processing and storage, to overcome natural colour variation and to ensure a consistent product. Sometimes the aim is to simulate a colour that is perceived by consumer as natural.

Food colours are generally classified as natural and synthetic [1] (Harris, 1986) and the synthetic colours are further divided into permitted and non-permitted. In India, according to the Prevention of Food Adulteration Act (1954) [2], eight synthetic colours are permitted to be used in the eatables and that too in a limited quantity. It was found that the foods manufactured by unorganized private sectors and small vendors did contain colours in much higher concentration than permitted range. (Biswas *et al*., 1994) [3]

Tartrazine (otherwise known as **E102** or **FD&C Yellow 5 or CI 19140**) is a synthetic lemon yellow azo dye and it is one of the commonly used food colourant. Many researchers [4, 5. 6] (Reyes et al., 1996; Tanaka, 2005 and Zraly et al., 2006) studied the metabolic and toxicological disorders induced by the administration of specific food colourant additives to rats and other mammals. Many azo compounds are genotoxic in short-term tests and carcinogenic in laboratory animals [7, 8] (Combes and Haveland-Smith, 1982 and Sasaki et al., 2002). However, there is paucity of literature concerning to the toxicity of this dye. The present attempt is a part of the programme which is planned to evaluate the toxic effects of various food dyes and to make the public aware regarding the ill effects of dye consumption.

Materials and Methods

Animal's Model

Adult female Swiss albino mice of B-6 strain, 4-5 weeks old, weighing 25±3 gms were used for the present study. They were maintained at standard laboratory conditions. Animals were housed individually in the polypropylene cages and maintained under standard conditions (12-h light/dark cycle; 25±3°C temperature; 35–60 relative humidity), and were fed on standard mice feed procured from Aashirwad Food Ltd., Chandigarh (India). The water was given *ad libitum*.

Chemicals Used

The dye tartrazine used in present study was manufactured and packed by Mallaya Fine- Chem Pvt. Ltd, Banglore, India and it is sold in the Indian markets with the trade name "lemon yellow IH 6597." The other chemicals used in the experimentation were of analytical grade.

Chemical Characterization

IUPAC Nomenculature: Trisodium (E)-5-oxo-1-(4-sulfonatophenyl)-4-((4-sulfonatophenyl) diazenyl)-4,5-dihydro-1H-pyrazole-3-carboxylate

Chemical Formula : $C_{16}\ H_9\ N_4\ Na_3\ O_9\ S_2$

Treatment Protocol

Animals were divided in to 4 groups having 8 animals in each groups, The animals of groups II and IV mice were fed with standard mice feed mixed 0.2 gm and 0.4 gm of tartrazine per kg/b.wt/day as low dose (LD) and high dose (HD) respectively for 35 days. The animals of groups I and III were served as control for experimental

groups II and IV and they were fed with only standard mice feed (daily food consumption data are given in Table 1). The experimental doses of tartrazine were decided after calculating the LD_{50} value.

Table 1. Showing consumption of food in both control and experimental mice

Groups	No. of mice in a group (kept individually)	Amount of food/mice/day (gm)	Dye added/ mice/ day (gm/kg/b.wt)	Food intake/mice/day
Group I Control (LD)	8	5	nil	all food consumed
Group II (LD)	8	5	0.2	all food consumed
Group III Control (HD)	8	5	nil	all food consumed
Group IV (HD)	8	5	0.4	all food consumed

A daily record of body weight was maintained and 24 hours after the last doses, the animals were weighed and sacrificed by cervical dislocation. The body organs and the blood samples were collected to analyze the toxicity caused by the tartrazine.

Clinical Toxicity Studies

Blood Analysis

Blood samples were collected and the values of haemoglobin content, total erythrocyte count (TEC), Haematocrit, TLC (Total Leucocyte Count) and DLC (Differential Leucocyte Count) were estimated using the methods described by Schalm *et al*. (1975) [9]. MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated according to Natelson (1951) [10].

Serum Analysis

The serum glucose was determined following the method described by Asatoor and King (1954) [11]. Alkaline phosphates (ALP) activity was measured according to the method given by Kind and King (1954) [12]. Triglycerides were determined by the method given by Gottfried & Rosenberg (1973) [13]. Serum total protein was determined according to the method given by Lowry *et al.* (1951) [14]. Total Cholesterol was estimated using the method described by Wybenga and Pileggi's (1970) [15]. LDL (Low Density Lipoprotein) cholesterol was estimated by Schriewer et al. (1984) [16] and HDL (High Density Lipoprotein) cholesterol was estimated by Assman et al. (1983) [17].

Ethical Aspects

The study was approved by the ethical committee, Center for Advance Studies, Department of Zoology, University of Rajasthan, Jaipur (India). The Indian National Sciences Academy, New Delhi (INSA, 2000) [18] guidelines were followed for maintenance and use of experimental animals. Statistical significance between the control and experimental data were subjected to one way analysis of variance (ANOVA).

Results

During the whole tenure of the experiment, no apparent sign of toxicity was observed in any experimental animal. However, a highly significant decrease in the body weight was observed at both the dose levels when compared with the respective control (Table 2).

Table 2. Showing changes in the body weight of mice fed with tartrazine

Groups	Body weight (g) (mean ± S.E.M)		% changes in body weight
	Initial	Final	
Group I Control (LD)	25.5±0.53	25.62±0.37	0.48% ↑
Group II (LD)	25.87±0.48 (P<0.6)	22.75±0.49*** (P<0.0004)	12.06% ↓
Group III Control (HD)	25.37±0.42	27.00±0.37	6.42% ↑
Group IV (HD)	24.5±0.37 (P<0.25)	19.00±0.37*** (P<0)	22.45% ↓

*** Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ns Non-significant (P<0.1) compare to control.

Haematologically, a significant decrease in the haemoglobin (Hb) content, TEC count, TLC count and haematocrit percentage was observed at both the dose levels which was found to be significant when compared with the respective controls but the decrease in TEC at high dose and TLC at both the dose levels were found to be highly significant. On the contrary, a highly significant increase was recorded in MCV and MCH values at both the dose levels when compared with the respective controls (Table 3).

Table 3. Showing changes in haematological parameters in mice fed with tartrazine

Groups	Haemoglobin (gms/dl)	TEC (mil/cu-mm)	Haematocrit (%)	MCV (µ3)	MCH (µg)	MCHC (%)
Group I Control (LD)	13.9 ± 0.37	6.37 ± 0.36	39.0 ± 0.73	61.22± 0.76	21.82 ± 0.77	35.64 ± 0.88
Group II (LD)	12.9 ± 0.24* (P<0.040)	5.27± 0.31* (P<0.037)	36.0 ± 0.77* (P<0.014)	68.31± 0.44*** (P<0)	24.47 ± 0.51* (P<0.01)	35.83 ± 0.92 ns (P<0.88)
Group III Control (HD)	13.31± 0.21	6.47 ± 0.23	40.0 ± 0.74	61.82 ± 0.60	20.57 ± 0.59	33.25 ± 0.74
Group IV (HD)	12.5 ± 0.22* (P<0.0219)	4.12 ± 0.14*** (P<0)	37.0 ± 0.80 * (P<0.01)	89.80 ± 2.25*** (P<0)	30.33 ± 0.27*** (P<0)	33.78 ± 0.94 ns (P<0.665)

***Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ns Non-significant (P<0.1) compare to control.

The dye at both the dose levels caused a significant to highly significant increase in polymorphs and lymphocytes counts. However, the changes observed in eosinophils and monocytes counts were fond to be non-significant when compared to the respective controls (Table 4).

Table 4. Showing Changes in TLC and DLC in mice fed with tartrazine

Groups	TLC Th/mm^3	DLC			
		Polymorphs (%)	Eosinophils (%)	Lymphocytes (%)	Monocytes (%)
Group I Control (LD)	9.81± 0.27	34 ± 0.56	2 ± 0.36	63 ± 0.91	1 ± 0.37
Group II (LD)	8.31±0.36*** (P<0.005)	30 ± 0.82** (P< 0.06)	2 ± 0.27 ^{ns} (P<1)	69 ± 0.73*** (P<0)	1 ± 0.32 ^{ns} (P<1)
Group III Control (HD)	11.20 ± 0.36	38 ± 1.11	1 ± 0.37	60 ± 2.44	1 ± 0.21
Group IV (HD)	5.7 ± 0.09*** (P<0)	26 ± 1.26*** (P<0.000)	2 ± 0.46 ^{ns} (P<0.11)	71 ± 1.03*** (P<0.000)	1 ± 0.21 ^{ns} (P<1)

***Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ^{ns} Non-significant (P<0.1) compare to control.

The serological studies revealed a highly significant decreased in glucose and HDL-cholesterol levels whereas a highly significant increased was recorded in the levels of alkaline phosphates, triglycerides, total cholesterol and LDL-cholesterol in both the experimental groups (Group II and IV). The dye caused a highly significant increase in serum protein at low dose but the increase was found to be non-significant at high dose when compared with the respective controls (Table 5).

Table 5. Showing serological changes in mice fed with tartrazine

Groups	Glucose mg/dl	Alkaline Phosphatase IU/L	Triglycerides mg%	Protein gm%	Total Cholesterol mg/dl	LDL Cholesterol mg%	HDL cholesterol mg%
Group I Control (LD)	235 ± 1.00	288 ± 1.34	92 ± 0.96	4.1 ± 0.21	110 ± 0.77	92 ± 0.92	41 ± 0.77
Group II (LD)	161 ± 1.21*** (P<0)	370 ± 1.25*** (P<0)	122.1 ± 1.11*** (P<0)	7.2 ± 0.11*** (P<0)	130 ± 1.26*** (P<0)	102 ± 0.88*** (P<0)	29.6 ± 0.78*** (P<0)
Group III Control (HD)	233 ± 0.53	340 ± 1.18	92 ± 0.92	4.8 ± 0.11	125 ± 1.28	90 ± 0.73	36 ± 0.92
Group IV (HD)	170 ± 0.94*** (P<0)	414 ± 1.11*** (P<0)	112 ± 1.03*** (P<0)	5.1 ± 0.15 ns (P<0.122)	162 ± 1.03*** (P<0)	117 ± 1.18*** (P<0)	28 ± 0.59*** (P<0)

***Highly significant (P<0), ** Significant (P<0.05), * Almost significant (P<0.01-0.04), ns Non-significant (P<0.1) compare to control.

Discussion

The toxic effects of dye can be analyzed by monitoring alterations in body weights of animals. The present study showed a highly significant decrease in the body weight of mice fed with tartrazine. When a substance reduces the body weights of animals submitted to treatment, it is considered that some kind of toxic effect has occurred by them (Hiremath *et* al., 1997) [19]. A similar finding was also reported by decreased in the body weight of rats fed with some synthetic food colourants was reported by Aboel-Zahab *et al.* (1997) [20] and Helal *et al.* (2000) [21] in albino rats fed with synthetic food colours.

The present study reveals a marked decrease in the TEC count, haemoglobin content and haematocrit percentage at both the dose levels. Similar result have been reported in albino mice fed with chocolate brown (a blend of tartrazine, carmoisine and brilliant blue) by Sharma *et al.* (2005a) [22]; in albino mice fed with orange red (a blend of

carmoisine and sunset yellow) by Sharma *et al.* (2005b) [23]; in mice fed with malachite green by Chakravarti *et al.* (2005) [24] and in albino mice fed with apple green (a blend of tartrazine and brilliant blue) by Sharma *et al.* (2006) [25]. When erythrocytes are damaged, the globin portion of the haemoglobin is broken down and the iron released is carried by transfer in either to the bone marrow for production of new red cells or to the liver for storage in the form of ferritin (Khanna *et al*., 1973) [26]. The synthesis of haemoglobin requires iron, which is obtained from the stored ferritin and from the dietary sources. In the present study, no significant observation of less diet consumption by the experimental animals is available. Therefore, it seems that the dye prevented the supply of iron for haemoglobin synthesis by inhibiting the absorption of iron by developing erythrocytes which resulted in the fall of haemoglobin content in the blood. The decrease in haematocrit percent might be attributed to decrease in the size of erythrocyte by way of reduction in haemoglobin synthesis which in turn regulates the maturation of erythrocytes by the accumulation in the cells. Since the dye blends affects haemoglobin synthesis as discussed earlier, the reason dose appear to be responsible for the reduction in haematocrit percent in the present investigation.

The present study further revealed decrease in TLC count at both the dose levels of the tartrazine. Similar results have also been reported in rats fed with sunset yellow by Mannel *et al*. (1958) [27]; in albino mice fed with orange red by Sharma *et al.* (2005b) [23]; in albino mice fed with chocolate brown by Sharma *et al.* (2005a) [22]. The decrease in TLC indicates that the dye toxicity might have caused blood poisoning (septicaemia) in which blood literary run out of WBC's.

Further, the dye tartrazine caused a decrease in TEC and Hb content but an increase in MCV and MCH. However the MCHC remained unaltered at both the dose levels. These findings suggested the occurrence of normochromic macrocytic anemia. It is in accordance with the finding of Prasad and Rastogi (1983) [28] in mice fed with metanil yellow.

A decrease level of serum glucose was observed at both the dose levels of the dye. This hypoglycemia observed in the present investigation might be due to disturbance in the enzymatic function of the liver caused by tartrazine. Similar findings have been also reported by Sharma *et al.* (2005b) [23] in mice fed with orange red and Sharma *et al.* (2005a) [22] in mice fed with chocolate brown.

Webner (2003) [29] reported that the damaged or disease tissue release enzymes into the blood, so serum alkaline phosphatase measurements can be abnormal in many condition including bone and liver diseases. Moreover, serum alkaline phosphatase is increased in response to a varity of drugs. Hence, the increase in the alkaline phosphatase level in the present investigation can be attributed to the liver damage caused by the dye tartrazine.

Results also revealed an increase in the serum triglycerides. Most of the body's fat is in the form of triglycerides stored in fat tissue. Moreover, they are also present in blood plasma in association with cholesterol forming the plasma lipids (Heit, 2001) [30]. Breckenridge *et al.* (1982) [31]; Carlson *et al.* (1986) [32] and Connelly *et al.* (1990) [33] have reported that the defect of hepatic lipase increases serum triglycerides level in human beings.

Thus it seems possible that the damage hepatocytes could not produce the requisite amount of hepatic lipase in the animals fed with tartrazine and resulted in hypertriglyceridaemia.

Serum proteins are produced by the liver, and any general stimulation of protein biosynthesis by the liver may be reflected by increasing serum protein content. An increase in the total serum protein could be attributed to increase incorporation of amino acids into protein due to a rise in the amino acids pool size brought about by a blocking in the conservation of amino acids to keto acids in the damaged liver cells. It is accordance with findings of Bhatia *et al.* (1973) [34] in rats treated with dieldrin.

A significant increase in total serum cholesterol level was observed at both the dose levels. The cholesterol is an important part of healthy body because it is used to form cell membranes and to produce certain hormones. Body content of cholesterol depends on the balance between the amount of cholesterol formed in the body plus that absorbed from diet (Cook, 1958) [35]. The deviation from normal values of cholesterol, in the blood serum is considered as symptoms of liver disease (Singh et al., 1988) [36] and can be attributed to the changes in the activities of specific enzymes responsible for lipid metabolism. Ashakumary and Vijayammal (1993) [37] reported that enzyme lecithin cholesterol transferase is involved in the transfer of cholesterol in to the liver for degradation and the decrease activity of this enzyme is in agreement with the increased concentration of cholesterol. Hence, it is possible that the dye might have be inhibited the activity of enzyme lecithin cholesterol transferase which in turn resulted in increased serum cholesterol.

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