EFFECT OF KESARI POWDER ON HAEMATOLOGICAL AND SEROLOGICAL PARAMETERS IN FEMALE SWISS ALBINO MICE

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Summary

Food colourants are used all over the world in large quantity and Kesari powder is one of the most commonly used permitted food colourant which impart orange-yellow colour to foods. It is a blend of tartrazine (FD&C Yellow 5) and sunset yellow (FD&C Yellow 6). Therefore, the current study is planned to evaluate the toxicity of Kesari powder because there is almost no literature available on the toxicity of this dye blend. The present study is aimed to evaluate toxic impacts of Kesari powder on some haematological and serological parameters of female *Swiss albino* mice. The body weights of mice were recorded daily. Experimental doses were calculated on the bases of LD_{50}. Kesari powder dose of 0.1 gm/kg b.wt (as low dose) and 0.2 gm/kg b.wt (as high dose) were given for 35 days to the experimental animals mixed with standard mice feed. The obtained data revealed a highly significant increase in the body weights of the experimental animals. Haematologically, a significant decrease was recorded in the Hb content, TEC count, haematocrit percentage, MCHC and TLC count. However, a significant increase was recorded in MCV and MCH values at both the dose levels when compared with their respective controls. The serological studies revealed a significant increase in serum glucose, alkaline phosphatase, triglycerides, total cholesterol and LDL-cholesterol levels. Whereas, a significant decrease was recorded in the levels of serum protein and HDL-cholesterol at both the dose levels.

Key words: food dye blend; kesari powder; *Mus musculus*; haemotoxicity; serotoxicity

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Introduction

Colour is one of the most important cues used by consumers to assess the quality of a food product. It may be defined as the individual's response to the visual signals generated by the light on a product. This important collection reviews how colour is perceived and measured, and ways in which it can be better understood and controlled in food.[1]

Food colours are generally classified as natural and synthetic (harries 1986)[2]. In India, according to the Prevention of Food Adulteration Act (1954) [3], eight synthetic colours viz. - tartrazine, brilliant blue, sunset yellow, ponceau-4R, carmoisine, erythrocine and indigo carmine are permitted to be used in the eatables and that too in a limited quantity. These food colours are frequently available in the market in the form of blends of two or more dyes and are widely encountered in a variety of eatables from both urban and rural market. (Khanna *et al.*, 1973)[4].

A blend of two or more dyes may produce an altogether different response than that observed with individual components (Singh *et al.,* 1988) [5.] Kesari powder is one of the most commonly used permitted food colourant which impart orange-yellow colour to foods. It is a blend of tartrazine (FD&C Yellow 5) and sunset yellow (FD&C Yellow 6).

Tartrazine (also known as E 102 and C.I 19140) is widely used in artificial foods, drugs and cosmetic dyes. It is a nitrous derivative and is known to cause allergic reactions such as asthma and urticaria [6]. Some authors have studied the carcinogenetic and mutagenetic effects of tartrazine with variable results [Maekawa *et al.* (1987) [7];

Borzelleca and Hallagan, (1988) [8]; Collins *et al.* (1990) [9], (1992) [10]; Reyes *et al.* (1996) [11]; Koutsogeorgopoulou *et al.* (1998) [12]; Walton *et al.* (1999)[13] and Sasaki *et al.* (2002) [14]]. Sunset Yellow FCF (also known as E110 and C.I. 15985) is a synthetic coal tar and azo yellow dye. It is used in variety of eatables such as orange squash, orange jelly, marzipan, Swiss roll, apricot jam etc. It may be responsible for causing an allergic reaction in people with an aspirin intolerance[15,] resulting in various symptoms including gastric upset, diarrhoea, vomiting, nettle rash (urticaria) and swelling of the skin (angioedema)[16.] The colouring has also been linked to hyperactivity in young children [17.] However, there is paucity of literature concerning to the toxicity of the blend of these dyes.

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 kesari powder 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 "orange yellow, IH 9140". The other chemicals used in the experimentation were of analytical grade.

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 0.1 gm and 0.2 gm of kesari powder per kg/b.wt/day as **low dose** (LD) and **high dose** (HD) respectively for 35 days. The animals of group I and III were served as control for experimental group 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 kesari powder 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	0.1	all food consumed
Group III Control (HD)	8	5	nil	all food consumed
Group IV (HD)	8	5	0.2	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 kesari powder.

The percentage of body weight gain was calculated as follow:

$$\frac{\text{Mean final weight - Mean initial weight}}{\text{Mean initial body weight}} \times 100$$

Clinical Toxicity

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) [18]. MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated according to Natelson (1951) [19].

Serum Analysis

The serum glucose was determined following the method described by Asatoor and King (1954) [20] ; Alkaline phosphates (ALP) activity was measured according to the method given by Kind and King (1954) [21]; Triglycerides were determined by the method given by Gottfried & Rosenberg (1973) [22] ; Serum total protein was determined according to the method given by Lowry *et al.* (1951) [23]; Total Cholesterol was estimated using the method described by Wybenga and Pileggi's (1970) [24] ; LDL (Low Density Lipoprotein) cholesterol was estimated by method of Schriewer *et al.* (1984) [25] and HDL (High Density Lipoprotein) cholesterol was estimated according to the method given by Assman *et al.* (1983) [26].

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)[27] guidelines were followed for maintenance and use of experimental animals.

Statistical analysis

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 increase in the body weights 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)	23±0.46	24.62± 0.37	7.04 ↓
Group II (LD)	23.62± 0.32 (P<0.28)	28.12 ± 0.55*** (P<0.0001)	19.05 ↑
Group III Control (HD)	24.00 ± 0.46	24.75 ± 0.41	3.12 ↓
Group IV (HD)	24.37± 0.42 (P<0.55)	29.5 ± 0.53*** (P<0)	21.05 ↑

*** 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 highly significant decrease was observed in the haemoglobin (Hb) content, TEC count and haematocrit percentage were observed at both the dose levels when compared with the respective controls. The decrease in MCHC was found to be non-significant at low dose but highly significant at high dose when compare to the respective controls. On the other hand, an increase in MCH was found to be non-significant at low dose but highly significant at high dose when compare to the respective controls. On the contrary, a highly significant increase was recorded in MCV values at both the dose levels when compared with their respective controls. (Table 3).

Table 3. Showing changes in haematological parameters in mice fed with kesari powder

Groups	Haemoglobin (gms/dl)	TEC (mil/cu-mm)	Haematocrit (%)	MCV (µ3)	MCH (µg)	MCHC (%)
Group I Control (LD)	12.7 ± 0.36	6.14 ± 0.26	37 ± 0.78	60.26± 0.97	20.68 ± 0.59	34.32 ± 0.68
Group II (LD)	9.5 ± 0.44*** (P<0.0001)	4.25± 0.37*** (P<0.001)	28 ±0.65*** (P<0)	65.88± 0.76*** (P<0.0005)	22.35 ± 0.62^{ns} (P<0.75)	33.92 ± 0.56 ^{ns} (P<0.65)
Group III Control (HD)	11.2± 0.41	6.21 ± 0.46	34± 0.59	54.75± 0.75	18.03 ± 0.65	32.94 ± 0.71
Group IV (HD)	8.2 ± 0.51*** (P<0.0004)	3.27 ± 0.36*** (P<0.0002)	31± 0.46*** (P<0.001)	94.80 ± 0.46*** (P<0)	25.07 ± 0.41*** (P<0.0001)	26.45 ± 0.66 *** (P<0.0000)

***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 caused a highly significant decrease in TLC and polymorphs count and a highly significant increase in lymphocytes count at both the dose levels. However, the non-significant changes were observed in monocytes count and an almost significant decrease was observed in eosinophils count at both the dose levels. (Table 4)

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

Groups	TLC Th/mm^3	DLC			
		Polymorphs (%)	Eosinophils (%)	Lymphocytes (%)	Monocytes (%)
Group I Control (LD)	9.2 ± 0.51	29 ± 0.81	2 ± 0.46	68 ± 0.56	1 ± 0.21
Group II (LD)	4.8 ± 0.39*** (P<0)	26 ± 0.51*** (P<0.006)	1 ± 0.21* (P<0.03)	72 ± 0.75*** (P<0.0008)	1 ± 0.37^{ns} (P<1)
Group III Control (HD)	8.8 ± 0.51	47 ± 0.84	2 ± 0.46	50 ± 0.73	1 ± 0.21
Group IV (HD)	4.3 ± 0.46*** (P<0.0000)	28 ± 0.53*** (P<0)	1 ± 0.21* (P<0.03)	70 ± 0.46*** (P<0)	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 increase in serum glucose, alkaline phosphates, triglycerides, total cholesterol and LDL-cholesterol at both the dose levels. Whereas, a decrease was recorded in the level of serum protein which was found to be highly significant at low dose but an almost significant at high dose when compare to the respective controls. Similarly, a decrease was recorded in HDL-cholesterol which was found to be an almost significant at low dose but highly significant at high dose when compared with the respective controls. (Table 5).

Table 5. Showing serological changes in mice fed with kesari powder

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)	246± 1.15	270 ± 1.46	145 ± 0.73	5.6 ± 0.28	166 ± 1.57	70 ± 0.56	40 ± 0.78
Group II (LD)	281 ± 2.32*** (P<0)	350 ± 1.94*** (P<0)	181 ± 1.58*** (P<0)	4.2 ± 0.17*** (P<0.0009)	175 ± 1.24*** (P<0.0005)	81 ± 0.46*** (P<0.003)	38 ± 0.42* (P<0.02)
GroupIII Control (HD)	235 ± 1.31	265 ± 1.07	113 ± 1.16	6.4± 0.38	158 ± 1.24	95 ± 0.68	39 ± 0.81
Group IV (HD)	290± 1.99*** (P<0)	390 ± 1.91*** (P<0)	146 ± 0.86*** (P<0)	5.2 ± 0.25* (P<0.02)	180 ± 2.04*** (P<0)	110 ± 1.93** (P<0)	26 ± 0.98*** (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 present work revealed a marked increase in the body weights at both the dose levels. It is in accordance with the findings of Mathur *et al.* (2005)[28] in rats fed with Metanil yellow; Sharma *et al.* (2005a)[29] in mice fed with Chocolate brown (a blend of tartrazine, carmoisine and brilliant blue); Sharma *et al.* (2005b)[30] in mice fed with Orange red (a blend of carmoisine and sunset yellow); Sharma *et al.* (2006c)[31] in mice fed with Apple green (a blend of tartrazine and brilliant blue); Sharma *et al.* (2006d)[32] in mice fed with Tomato red (a blend of carmoisine and Ponceau 4R) and Chakravarti *et al.* (2005)[33] in mice fed with malachite green. The present study also revealed a significant increase in the level of triglyceride and total cholesterol which may be the probable caused of increase in the body weights as triglyceride and cholesterol are the forms of fat present in

the body. Dongsheng *et al.* (2000)[34] reported that lower HDL is associated with obesity. Hence, this may be the another probable cause of increase in the body weights due to dye toxicity.

The present study revealed a marked decrease in the TEC count, haemoglobin content and haematocrit percentage at both the dose levels. Similar result have also been reported in albino mice fed with chocolate brown (a blend of tartrazine, carmoisine and brilliant blue) by Sharma *et al.* (2005a) [29]; in albino mice fed with orange red (a blend of carmoisine and sunset yellow) by Sharma *et al.* (2005b) [30]; in mice fed with malachite green by Chakravarti *et al.* (2005) [33]; in albino mice fed with apple green (a blend of tartrazine and brilliant blue) by Sharma *et al.* (2006c) [31]; in albino mice fed with tomato red (a blend of carmoisine and Ponceau 4R) by Sharma *et al.* (2006d) [32]; in rats fed with FO and C green no.3 (fast green) by Knezevich and Hogan (1981) [35] and in rats fed with fast green by Ashour and Abdelaziz (2009) [36]. It seems that kesari powder might have suppressed the haemopoietic system and caused a decreased in TEC, Hb concentration and haematocrit percentage. The decrease in the haemoglobin content might be due to the decreased rate of haemoglobin synthesis during erythropoeisis. 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) [37]. 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 blend 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. Further, the decrease in haematocrit percentage may be the effect of stress on animal health caused by dye toxicity (Larsson *et al.,*1985) [38] .

Red cell indicators like MCV, MCH and MCHC values depend on the TEC, haemoglobin concentration and haematocrit percentage. The present study revealed a marked increase in MCV and MCH values but a significant decrease in MCHC value. Similar results have also been reported in male albino rats fed with amaranth by Shinnawy (2009) [39.] A significant increase in MCV might be due to the anaemia as the increase in MCV tends to be roughly proportional to the decrease in haemoglobin concentration. (Lee *et al.,*1998) [40]. Increased in MCH is compatible with macrocytic anaemia. Macrocytosis may be abscured or masked by coexisting iron deficiency, inflammatory diseases, or thalasemia minor[41, 42.] MCHC is an expression of the average concentration of haemoglobin in red blood cells and give the ratio of the weight of haemoglobin to the volume of red blood cells. As decrease MCHC signifies that a unit-volume of packed red blood carpuscles contains less haemoglobin than normal or that haemoglobin has been replaced by erythrocytic stromal material as in iron deficiency. (Fischbach, 1984) [43]

The present study further revealed a decrease in TLC count at both the dose levels of the kesari powder. The decrease in TLC indicates that the dye toxicity might have caused blood poisoning (septicaemia) in which blood literary run out of WBC's. Similar results have also been reported in rats fed with sunset yellow by Mannel *et al*. (1958) [44]; in albino mice fed with chocolate brown(a blend of tartrazine, carmoisine and brilliant

blue) by Sharma *et al.* (2005a) [29] and in albino mice fed with orange red (a blend of carmoisine and sunset yellow)by Sharma *et al.* (2005b) [30] .

The present study revealed a decrease in the neutrophils and a decrease in neutrophils is known as neutropenia. (Lewis, 1970)[45]. Similarly, a decrease was recorded in eosinophils count. Such abnormalities are common in the megaloblastic anemia's (Kaplan and Basford, 1976) [46]. Eosinophils are associated with antigen-antibody reactions. A decrease in eosinophils might be due to the reduction of bone marrow eosinophils production or cessation of bone marrow release of eosinophils (Altman *et al.,* 1981) [47] due to dye toxicity. However, the exact cause of neutropenia and eosinopenia remain obscure.

Lymphocytes are the primary components of the body's immune system. In the present study an increase was observed in lymphocytes count and an increase in the lymphocytes count is termed as lymphocytosis (Lewis, 1970)[45]. It may be possible that the blend kesari powder might have stimulated the immune mechanism of the animal in order to eliminate the dyes components. This is in agreement with the findings of Samprath *et al.* (1993)[48].

The present study revealed an increase of serum glucose at both the dose levels of the dye blend. Similar finding has also been reported by Sharma *et al.* (2006c) [31] in mice fed with apple green (a blend of tartrazine and brilliant blue) and Shinnawy (2009)[39] in male albino rats fed with amaranth. The elevation of serum glucose level can be attributed to glycogenolysis and gluconeogenesis by liver with a temporarily loss of endocrine functions of pancreas leading to hyperglycemia due to dye toxicity.

Similarly, a highly significant increase was reported in serum alkaline phosphatase at both the dose levels of kesari powder. Similar results have been reported in mice fed with apple green (a blend of tartrazine and brilliant blue) by Sharma *et al.* (2006c) [31]; in mice fed with malachite green by Chakravarty *et al.* (2005) [33]; in mice fed with tomato red (a blend of carmoisine and Ponceau 4R) by Sharma *et al.* (2006d) [37] and in rats fed with amaranth by Shinnawy (2009) [39]. Alkaline phospahtase belongs to a group of enzymes that catalyze the hydrolysis of phosphomonoesters at alkaline pH and it is present in the cell surface in most human tissue (Gitnick *et al.,* 1992 [49]; Moss and Handderson, 1999 [50] and Ashour and Abdelaziz, 2009[36]). Further, the serum alkaline phosphatase is increased in response to a varity of drugs (Webner, 2003) [51]. Similarly, Chakravarty *et al.* (2005) [33] has been reported that the damaged or disease tissue release enzymes into the blood, so serum alkaline phosphatase measurements can be abnormal when liver cells are damaged or a billiary obstruction occurs.

Results also revealed an increase in the serum triglycerides at both the dose levels. Similar results have been seen in mice fed with orange red (a blend of sunset yellow and carmoisine) by Sharma *et al.* (2005b) [30] and in mice fed with apple green (a blend of tartrazine and brilliant blue) by Sharma *et al.* (2006c) [31]

A significant decrease was observed in total serum protein at both the dose level of kesari powder. Similar result has been reported in rats fed with fast green by Ashour and Abdelaziz (2009) [36] and in mice fed with malachite green by Chakravarty *et al.* (2005) [33]. The depletion in the serum protein might be attributed to the impared protein synthesis due to dye toxicity. The disturbance in liver functions also depresses serum protein and

thus results in hypoproteinemia in animals. Moreover, increase in amino acid deamination as a result of some toxic compound also caused hypoproteinemia (Varley, 1987) [52].

The total body content of cholesterol depends on the balance between amount of cholesterol formed in the body, plus that absorbed from the diet (Cook, 1958) [53]. In the present study, a highly significant increase was observed in the serum cholesterol at both the dose levels. Elevated cholesterol values in the serum can be considered as symptoms of liver diseases (Singh *et al.*, 1988) [5]. Similar results have been reported by in rats fed with tartrazine- containing dyes by Aboel-Zahab *et al.* (1997) [54] ; in rats fed with Paprika food colour by Kanki *et al.* (2003) [55] ; in mice fed with apple green (a blend of tartrazine and brilliant blue) by Sharma *et al.* (2006c) [31] ; in mice fed with malachite green by Chakravarti *et al.* (2005) [33] and in mice fed with chocolate brown (a blend of tartrazine, carmoisine and brilliant blue) by Sharma *et al.* (2005a) [20.]

The present study revealed a significant increase in LDL cholesterol while, a significant decrease in HDL cholesterol at both the dose levels. Carbohydrates break down into the triglycerides and can lead to formation of LDL cholesterol[56]. In the present study, the dye blend caused a significant increase in triglycerides. Thus, it seems that the high triglycerides might have caused high serum LDL cholesterol.

Conclusion

Present study revealed that intake of kesari powder (a blend of tartrazine and sunset yellow) has deleterious effect on body weight, haematological and serological

parameters. Hence, consumption of this dye would cause adverse effect on human health. Therefore, it is necessary to create consumer awareness regarding the ill effect of this dye blend.

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