



**PROGRESSIVE EFFECT OF KESARI POWDER ON DIFFERENT HAEMATOLOGICAL AND SEROLOGICAL
PARAMETERS IN FEMALE WISTAR RATS****Ms.Divya.C.V^{*1}, Ms.Devika.V²**

¹Research Scholar, Department of Biochemistry, Dr.N.G.P Arts and Science College, Coimbatore-641048, TamilNadu.

²Assistant Professor, Department of Biochemistry, Dr.N.G.P Arts and Science College, Coimbatore-641048, TamilNadu.

ABSTRACT

Food additives are used for various purposes, including preservation, colouring, flavouring and sweetening. The present study aimed to investigate the possible influence impacts of kesari powder on some haematological and serological parameters in female wistar rats. Kesari powder dosage of 0.5 gm/kg b.wt (as low dose) and 1 gm/kg b.wt (as high dose) were given for 30 days to the experimental animals mixed with standard rat feed. The body weights of rats were recorded daily. The study revealed that the administration of kesari powder increases the body weight of experimental animals. Haematologically, a significant decrease was recorded in the haemoglobin content, total erythrocyte count, haematocrit, MCHC and a significant increase was recorded in MCV, MCH by the intake of kesari powder. The serological studies revealed a significant increase in serum glucose, urea, creatinine and enzymes such as ACP, ALP whereas; a significant decrease in serum protein was recorded. The present study showed that the permitted doses of food additives may be harmful.

Keywords: Food additives, kesari powder, haematological and serological parameters, experimental animals, harmful.

Correspondence to Author**Ms.Divya.C.V.**

Research Scholar, Department of
Biochemistry, Dr.N.G.P Arts and
Science College, Coimbatore-641048,
TamilNadu.

Email: divyachandran.nta@gmail.com**INTRODUCTION**

Food additives are products added to the basic foodstuffs with an aim of improving its aspect, flavour, taste, colour, texture, food value and conservation. The great bulk of artificial colourings used in food are synthetic dyes. For decades synthetic food dyes have been suspected

of being toxic or carcinogenic and many have been banned whenever possible, choice of foods without dyes. Synthetic food Colours, also known as artificial food Colours, are manufactured chemically and are the most commonly used dyes in the food, pharmaceutical and cosmetic industries. The permitted synthetic food colours

are available in the form of blends of one or more dyes that are combined in numerous ways to produce a huge array of shades. Blends of two or more dyes can produce altogether different effects than observed with individual components. The effects may be additive, synergistic or even antagonistic¹. It has been reported that the toxicity of tomato red, a popular food dye blend on male wistar rats². Several synthetic food colours used in food industry proved numerous side effects such as urticaria, genotoxic effects³, endocrinal disturbances⁴, behavioural disorders and neurological effects⁵. Most food and confectionery products available today are made using artificial food colour.

Kesari powder is a widely used permitted food colourant. Almost all the food contains kesari powder as a colourant. It gives a colourful appearance to food. Kesari powder is actually the leftover bits after harvesting pure saffron. It is also mixed with turmeric so it does impart colour & has some fragrance. It is a substitute for those who cannot afford the high price of pure saffron. A blend of two or more dyes may produce an altogether different response than that observed with individual components⁶. Kesari powder is one of the most commonly used permitted food colourant which impart orange-yellow colour to foods. For the present study orange red colour kesari powder is used. It is the blend of sunset yellow, carmoisine and sodium chloride.

The present study aimed to investigate the oral subchronic toxicity of kesari powder on some haematological and serological parameters in female wistar rats.

MATERIALS AND METHODS

Animals Used

The studies were conducted on female wistar rats, weighing 100-150g. The animals were

obtained from Govt. Veterinary College, Mannuthy, Trissur, Kerala, India. The animals were maintained at standard laboratory condition in large spacious cages and they were given food and water *ad libitum* during the course of the experiment. They were kept under conditions of ambient room temperature and relative humidity. The animal room was well ventilated and the animals had a 10±1 hour's night schedule, throughout the experimental period. The animals were cared for in compliance with the principles and guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Institutional Animal Ethics Committee (IAEC) (KMCRET/M.Phil/04/2011).

Diet Used

The commercial pelleted animal feed marketed by M/s Hindustan Lever Limited, Bangalore, India, under the trade name of "Gold Mohour rat feed" was used.

Chemicals Used

The dye kesari powder used in present study was manufactured and packed by Mallaya Fine-Chem Pvt Ltd, Bangalore, India and sold in the Indian markets with the trade name "Orange red".

Treatment Protocol

Animals were divided into three groups having seven animals in each group. The animals of group II and III were fed with standard rat feed mixed 0.5g and 1g of kesari powder per Kg/b.wt/day as low dose (LD) and high dose (HD) for 30 days. The animals of group I served as control for experimental groups and they were fed with only standard rat feed. The experimental doses of kesari powder were decided after calculating the LD₅₀ value.

Table 1: CONSUMPTION OF FOOD IN BOTH CONTROL AND EXPERIMENTAL GROUPS

Groups	No. of rat in a group (kept Individually)	Amount of food/rat/day (gm)	Dye added/ rat/ day (gm/kg/b.wt)	Food intake/rat/day
Group I (Control)	7	8	Nil	all food consumed
Group II (Kesari LD)	7	8	0.5	all food consumed
Group III (Kesari HD)	7	8	1	all food consumed

Clinical Toxicity**Haematological Examination**

Blood samples were collected and the values of haemoglobin content, total erythrocyte count (TEC) were estimated using the method described by Schalm *et al.* (1975)⁷. MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated according to Schalm *et al.* (1975)⁷. Haematocrit was estimated using the method described by Ramnik and Sood *et al.* (1987)⁸.

Serological Examination

The serum glucose was determined following the method described by Bergmeyer *et al.* (1974)⁹; Alkaline phosphates (ALP) activity was measured according to King and Armstrong *et al.* (1934)¹⁰; Acid phosphatase (ACP) was determined by the method given by King *et al.* (1965a)¹¹; Serum total protein was evaluated according to the method given by Lowry *et al.* (1957)¹²; Urea was estimated using the method described by Natelson

et al. (1951)¹³ and Creatinine was estimated according to the method given by Owen *et al.* (1954)¹⁴.

Statistical analysis

Statistical significance between the control and experimental data were subjected to one way analysis of variance.

RESULTS**Analysis of bodyweight**

The toxic effect of a dye could be analyzed by monitoring alterations in the body weight of the animals. During the whole tenure of the experiment, no apparent sign of toxicity was observed in any experimental animal. All animals were weighed at the beginning of the experiment. The data represented in table no: (2) displays the effect of treatment with kesari powder on the percentage of body weight of female wistar rats. In the present study administration of kesari powder caused a highly significant increase in the body weights which was observed at both the dosage levels when compared with the respective control.

Table 2: CHANGES IN THE BODY WEIGHT OF FEMALE WISTAR RATS FED WITH KESARI POWDER

GROUPS	INITIAL BODY WEIGHT(gm)	FINAL BODY WEIGHT(gm)
Group I (Control)	106.43 ± 5.71	156.14 ± 6.39
Group II (Kesari LD)	128.14 ± 11.82 ^a	198.86 ± 15.38 ^a
Group II (Kesari HD)	133.71 ± 17.41 ^a	216.71 ± 22.22 ^{ab}

Values are mean ± SD of seven samples in each group

Group comparison: **a** – Group I vs Group II and Group III, are significant at 5% level

b – Group II vs Group III are significant at 5% level

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Haematological Examination

Haematologically, results after the treatment period there was no significant changes in group control and group treated with kesari powder. But the haematological studies revealed a significantly lower haemoglobin (Hb) content, TEC count,

haematocrit percentage and MCHC at both the dose levels when compared with the respective controls. On the contrary, highly significant increases were recorded in MCV, MCH values at both the dose levels when compared with their respective controls.

Table 3: CHANGES IN HAEMATOLOGICAL PARAMETERS IN FEMALE WISTAR RATS FED WITH KESARI POWDER

Parameters	Group I (Control)	Group II (Kesari LD)	Group III (Kesari HD)
Haemoglobin (g/dl)	14.17±1.42	10.40±0.65 ^a	7.89± 1.43 ^{ab}
RBC (M/UL)	5.22±0.47	4.01±0.20	2.92±0.45 ^{ab}
Haematocrit (%)	41.26 ± 4.32	31.14±0.88 ^a	25.50±3.58 ^{ab}
MCV (FL)	56.57±2.41	70.97 ±1.56 ^a	93.17±1.23 ^{ab}
MCH (pg)	35.10±1.09	43.64 ±2.74 ^a	57.59±8.05 ^{ab}
MCHC (g/dl)	26.80±1.41	42.83±3.77 ^a	31.04±2.26 ^{ab}

Values are mean ± SD of seven samples in each group

Group comparison: **a** – Group I vs Group II and Group III are significant at 5% level

b – Group II vs Group III are significant at 5% level

Serological Examination

Serological investigation of effect of oral administration of kesari powder revealed a highly significant increase in serum glucose, urea, creatinine, acid phosphatase and alkaline

phosphatase, at both the dose levels. Whereas, a decrease was recorded in the level of serum protein which was found to be highly significant at both the dosage when compared to the respective controls.

Table 4: CHANGES IN SEROLOGICAL PARAMETERS IN FEMALE WISTAR RATS FED WITH KESARI POWDER

Parameters	Group I (Control)	Group II (Kesari LD)	Group III (Kesari HD)
Total protein (g/dl)	19.15±0.51	12.14±0.56 ^a	10.36±0.75 ^{ab}
Glucose (mg/dl)	107.29±11.93	168.14±3.98 ^a	177.43±2.37 ^{ab}
Urea (mg/dl)	15.53±7.17	42.07±5.33 ^a	76.29±3.45 ^{ab}
Creatinine (mg/dl)	0.40 ± 0.29	2.86 ± 0.90 ^a	4.71 ± 1.11 ^{ab}
ACP (U/L)	3.76 ± 0.60	9.50 ± 0.74 ^a	21.73 ± 0.58 ^{ab}
ALP (U/L)	28.93±1.25	54.54±1.22 ^a	78.94±1.10 ^{ab}

Values are mean ± SD of seven samples in each group

Group comparison: **a** – Group I vs Group II and Group III are significant at 5% level

b – Group II vs Group III are significant at 5% level

DISCUSSION

The present work revealed a marked increase in the body weights at both the dose levels. It is in accordance with the findings in rats fed with metanil yellow¹⁵.

The present study revealed a marked decrease in the TEC count, haemoglobin content and haematocrit percentage at both the dosage levels. Similar results have been reported in rats fed with FO and C green no.3 (fast green)¹⁶ and in rats fed with fast green¹⁷ and changes induced by

kesari powder may be due to the prevention of red blood cell synthesis via inhibition of erythropoiesis in the bone marrow. Studies were reported that, there is a decrease in RBC may be due to impairment of biosynthesis of heme in bonemarrow^{18, 19}. Vitamin B12 and folic acid deficiency causes maturation failure in the process of erythropoiesis. Lynch *et al.*, 1969²⁰ 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 decrease in the haemoglobin content might be due to the decreased rate of haemoglobin synthesis during erythropoiesis. 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 ferritin²¹. The synthesis of haemoglobin requires iron, which is obtained from the stored ferritin and from the dietary sources. 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.

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. It has been reported that a fall in the rate of haemoglobin synthesis during all the stages of maturation of erythrocytes when the supply of iron is not adequate²².

The study was correlated with M.S.Khan *et al.*, 2008²³ was stated in rat that decreases in PCV due to sign of anaemic condition. The present study also revealed a decrease in haematocrit percent 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 synthesis²⁴. The decrease in haematocrit percentage may be the effect of stress on animal health caused by dye toxicity²⁵.

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Red cell indicators like MCV, MCH and MCHC values depend on the TEC, haemoglobin concentration and haematocrit percentage. 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 concentration²⁶. Increased mean corpuscular volume (MCV) indicates the cells to be macrocytic²⁷. Increase in MCV is seen in pernicious anemia (normochromic) and megaloblastic anemia (hypochromic)²⁸. Increased in MCH is compatible with macrocytic anaemia. Macrocytosis may be obscured or masked by coexisting iron deficiency, inflammatory diseases, or thalasemia minor^{29, 30}. 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

The present study revealed an increase of glucose, urea, creatinine and a significant decrease of serum total protein at both the dosage levels of the dye blend. These results find good support of the studies carried out by Sharma (1989)³¹ and Mackenzie *et al* (1992)³² who illustrated a marked decrease of serum protein in rats after treatment with metanil yellow or caramel colour. The inhibition of protein synthesis is reversible and not specific. The present findings since serum total protein were diminished after treatment with kesari powder.

The present study revealed an increase of serum glucose at both the dose levels of the dye blend. Similar finding has also been reported in rat fed with apple green (a blend of tartrazine and brilliant blue)^{33,34}. 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. This is in agreement with Helal *et al.* (2000)³⁵ who found a significant elevation in serum Creatinine and Urea in rats which consumed a synthetic or natural food colourants after 30 days of treatment. Moreover, the present results are in accordance with data reported was observed a significant elevation in

serum Creatinine and urea level of rats dosed with organic azo dye (fast green) orally for 35 days¹⁷.

The effect of kesari powder recorded a significant increase of acid phosphatase and alkaline phosphatase. The elevation of ACP level in serum these has been reported to be associated with prostatic cancer^{36, 37, 38}. The findings were not confirmed by Goldfisher *et al* (1963)³⁹ who, using both the lead sulfide method and azo dye method, found abundant acid phosphatase activity in livers of patients with hepatolenticular degeneration.

These result are in accordance with 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 toxicity⁴⁰.

CONCLUSION

In conclusion, the present study indicates that the consumption of kesari powder not only causes changes in hepatic and renal parameters but also their effect become more risky at higher doses because it can induce a deleterious effects on the body weight, haematological and serological parameters and the prolonged consumption of the blend appears to be of greater severity. Hence, consumption of this dye would cause adverse effect on human health. Therefore, it is necessary that people should be aware about the hazardous effects of consuming food additives.

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