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# Effect of Synthetic Red Dye Orange Red and Natural Red Dye Alizarin on Biochemical and Hematological Parameters in Male Wistar Rats

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**ABSTRACT**

Nowadays synthetic food dyes are mostly preferred than natural plant derived dyes due to low cost and intense coloration. In this study hematological and biochemical parameters were determined in male wistar rats after 30 days treatment with synthetic red dye orange red and natural plant derived red dye alizarin. 25 male wistar rats were divided into 5 groups with 5 animals per group. Group I rats were taken as control treated with normal rat diet and distilled water. Group II and III rats (experimental) were oral gavaged with 50 mg and 150 mg/kg body weight of alizarin dye. Group IV and V rats (experimental) were gavaged with 50 mg and 150 mg/kg body weight of orange red dye. Treatment of group V rats with 150 mg/kg body weight of orange red dye produce significant changes in RBC, Hb, Hct, MCH, serum aminotransferase enzymes and serum protein fraction. In comparison to this in group IV rats a significant change was observed only in Hb, serum aminotransferase enzymes and serum protein fraction when compared with control (group I) rats. However in group II and III alizarin treated rats no significant change was observed in different biochemical and hematological parameters relative to their respective control. In conclusion synthetic orange red dye proved to be more toxic than natural plant derived red dye alizarin.

**Keywords:** Orange red dye, alizarin, hematological and biochemical parameters, wistar rats.

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## INTRODUCTION

From the last few decades there have been observed a tremendous increase in the usage of food additives mainly azo dyes due to increase in industrialization, population and new trends in food technology. Nowadays use of food dyes is an integral part of all food preparations and is mainly used to increase consumer attraction of food. [1] Food dyes are mainly used to change the physical condition of food stuff. Food dyes may be synthetic or natural plant derived but synthetic dyes are mostly preferred than natural dyes due to low cost and deep coloration. [2] Natural

dyes derived from plants are considered to be safe and also show potent antimicrobial activities. [3] During older times natural plant derived dyes were preferred food colorants but nowadays people use synthetic dyes due to their intense coloration and consumer attraction. Synthetic food colour additives are mostly azo dyes which when applied to food and drugs give new colour and make them more attractive and palatable. [4] There are more than 3000 azo dyes which are nearly used in every sector like cosmetics, textile firms, leather, pharmaceutical industries, food and paper production industries. [5] Many food dyes have the capacity to induce chromosomal abnormalities in mammalian cells with increase in dosage and exposure time. [6] The food items containing azo dyes have the potential to cause cancer. [7]

In our research work we select synthetic red coloured dye called orange red, which is a mixture of two azo colorants carmoisine and sunset yellow and natural red dye alizarin derived from roots of madder plant (*Rubia tinctorum*) and evaluate their effect on haematological and biochemical parameters in male albino wistar rats.

Orange red dye is an amalgamation of two azo colorants namely carmoisine and sunset yellow. Both these colorants are reported to be toxic. Carmoisine is Di-sodium salt of 2-(4 sulpho -1-naphthylazo)-1-naphthol-4-sulphonic acid with chemical formulla $C_{20}H_{12}N_2Na_2O_7S_2$ and molecular weight 458.459 g/mol. Although use of carmoisine is allowed in UK and India but it is not permitted in US. Regarding the toxicological status of carmoisine it was concluded that, high doses of carmoisine cause liver damage and also lowers the expression of some key enzymes. [8] However in one research finding it was found that carmoisine causes chromosomal aberrations in bone marrow cells. [9] Later on in another research work carmoisine was found to be safe in both *in vitro* and *in vivo* experiments. [10]

Sunset yellow (E 110) is an orange red coloured azo dye with chemical formula of $C_{16}H_{10}N_2Na_2O_7S_2$ and molecular weight 452.37 g/mol. In regard of sunset yellow an admissible daily intake (ADI) of 2.5 mg/kg was approved by the joint expert committee on food additives (JECFA) according to a three month experimental report in rats. It was reported that significant testicular toxicity was produced in rats when treated with sunset yellow at dose concentration of 250 and 1500 mg/kg for 90 days. [11] The toxicological status of sunset yellow receives both positive and negative mutagenic reviews. Lack of any toxicity report was observed in albino mice when dye sunset yellow was administered orally twice at 24 hours interval. [12] In an *in vivo* micronucleus assay in mice, sunset yellow at single dose concentration of 2000 mg/kg was found to be antimutagenic in action. [13]

During older times people mostly use plant derived alizarin as red coloring dye for food and textiles. Alizarin is an anthraquinone red dye pigment derived from roots of *Rubia tinctorum* commonly known as madder plant. [14] Chemically alizarin is 1,2-dihydroxyanthraquinone; 1,2- dihydroxy- 9,10-anthracenedione with molecular formulla $C_{14}H_{18}O_4$. It was reported that alizarin contain antimicrobial and antifungal activities. [15] However with advancement in food technology people start to replace use of natural red dye alizarin with synthetic red dyes. In this research work we made efforts to evaluate and compare the haematological and biochemical parameters in male albino wistar rats treated with natural red dye alizarin derived from roots of madder plant and synthetic orange red dye.

## MATERIALS AND METHODS

### Experimental Animals

In our study 25 mature male albino wistar strain rats (*Rattus norvegicus*) weighing about 150- 200 grams were used. These rats were procured from Indian Institute of Integrative Medicine (IIM), Canal Road Jammu. The animals were housed in stainless steel cages in the animal house of Zoology department, University of Kashmir under laboratory conditions at temperature 24 ± 2°C and 12/12 hours of light and dark cycle. The animals were first acclimatized for about one week and were starved for 50 minutes before the start of experiment. The approval for the experimental study was sanctioned by Institutional Animal Ethical Committee, and the whole experiment setup was performed as per the ethical laws of CPCSEA rules.

### Chemicals

Synthetic orange red dye (mixture of carmoisine and sunset yellow) was procured from local market under fssai license. Alizarin dye and other chemicals used in this study were of high analytical grade. A stock solution was prepared by dissolving the orange red dye and alizarin separately in 1000 ml of double distilled water. The dose concentration was maintained in such a way that the amount of orange red dye and alizarin administered per animal was according to their respective weight.

### Dosage and Design of Experimental Animals

The rats were randomly divided into 5 groups, each group comprise of 5 animals. The animals from each group were weighed on the first day of experiment and after the completion of experiment. The dose was given mostly in morning time between 9.30- 10.30 a.m. The animals were treated with the test chemical orally by gavage intubation once a day for 30 days. The animals were starved for about 12 hours before start of the treatment protocol.

Group I: (control group) treated with normal rat feed and distilled water.

Group II: Rats in this group were treated with low dose of alizarin 50 mg/kg b. wt/day orally for 30 days.

Group III: Rats in this group were treated with high dose of alizarin 150 mg/kg b. wt/day orally for 30 days.

Group IV: Rats in this group were treated with low dose of orange red dye 50 mg/kg b. wt/day orally for 30 days.

Group V: Rats in this group were treated with high dose of orange red dye 150 mg/kg b. wt/day orally for 30 days.

### Biochemical Analysis

Blood samples were collected from all group of rats and allowed to clot at room temperature in dry glass centrifuge tubes and centrifuged at 3500 rpm for 13 minutes. The supernatant serum obtained was used to estimate biochemical parameters. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by the method of [16], Serum alkaline phosphatase (ALP) was determined according to protocol of [17], Serum total protein and serum albumin concentrations were determined according to method of [18], Serum globulin was calculated by [19].

### Haematological Analysis

A part of blood samples were collected in heparinized tubes for hematocrit value, which was determined by the method. [20] Another part of blood was collected in EDTA for the haematological experimentation. The red blood cells (RBCs) and white blood cells (WBCs) counts were estimated by Neubauer haemocytometer method. The hemoglobin (Hb) concentration was determined according to [21], using the cyanomethaemoglobin method. Mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV), were determined according to [21]

### Statistical Analysis

All values were calculated by taking mean values with ± standard deviation. The results were evaluated by using one- way ANOVA followed by t- test. The value of $p<0.05$ was considered as significant value.

## RESULTS

The effect of orange red dye and alizarin on hematological and biochemical parameters after treatment of rats with 50 mg and 150 mg/kg bw of both above mentioned dyes for 30 days is shown in Tables 1-3.

Table 1 shows the effect of orange red dye and alizarin on some hematological parameters. Treatment of rats with orange red dye (150 mg/kg bw) produce a significant ($p<0.05$) decrease in RBC, Hb, Hct, MCH values when compared with their respective control. Furthermore a highly significant ($p<0.01$) increase in total WBC was observed relative to their respective control. However treatment with 50 mg and 150 mg/kg bw of natural dye alizarin did not produce any significant change ($p>0.05$) in different blood indices when compared with control group.

Table 2 shows the effect of orange red dye and alizarin on some important biochemical enzymes representatives of liver function. Dosage of 50 mg and 150 mg/kg bw of orange red dye in male rats produce a highly significant ($p<0.01$) increase in the value of ALT, AST and ALP when compared with control. However in group II and III rats treated with 50 mg and 150 mg/kg bw of alizarin a statistically insignificant ($p>0.05$) change in the value of serum aminotransferase enzymes except AST was observed relative to their respective control.

**Table 1: Effect of 30 days treatment with 50 mg and 150 mg/kg bw of orange red dye and alizarin on haematological parameters in male wistar rats**

| Groups | RBC (x10⁶/mm³) | Hb (%) | Hct (%) | MCV (fl) | MCH (pg) | WBC (×10³/mm³) |
|---|---|---|---|---|---|---|
| I (Control) | 8.09 ± 1.01 | 14.47 ± 1.20 | 44.47 ± 4.69 | 54.01 ± 5.32 | 18.34 ± 2.37 | 6.64 ± 1.95 |
| II (Alizarin) | 7.70 ± 0.82 | 13.60 ± 1.19 | 43.58 ± 4.76 | 53.22 ± 5.50 | 17.50 ± 2.35 | 6.48 ± 1.73 |
| III (Alizarin) | 7.10 ± 1.29 | 12.80 ± 1.50 | 42.88 ± 4.50 | 52.62 ± 5.64 | 16.90 ± 2.66 | 7.34 ± 1.59 |
| IV (Orange red) | 6.67 ± 1.56 | 12.26 ± 1.17* | 42.20 ± 4.41 | 51.96 ± 5.56 | 16.42 ± 2.59 | 7.54 ± 1.44 |
| V (Orange red) | 5.27 ± 1.86* | 10.66 ± 0.88** | 40.25 ± 4.41* | 50.02 ± 5.42 | 14.7 ± 3.00* | 10.44 ± 0.49** |

Values are expressed as mean ± SD of five animals per group. RBC: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, WBC: White blood cell; Level of significance: * significant, ** highly significant

**Table 2: Effect of 30 days treatment with 50 mg and 150 mg/kg bw of orange red dye and alizarin on some liver specific serum enzymes in male albino Wistar rats.**

| Groups | ALT (U/L) | AST (U/L) | ALP (U/L) |
|---|---|---|---|
| I (Control) | 28.14 ± 3.52 | 48.82 ± 2.77 | 64.4 ± 15.70 |
| II (Alizarin) | 28.99 ± 3.61 | 55.10 ± 3.14* | 66.76 ± 1.51 |
| III (Alizarin) | 30.98 ± 3.78 | 57.96 ± 3.69* | 72.56 ± 3.18 |
| IV (Orange red) | 60.40 ± 3.64** | 62.66 ± 4.50** | 130.0 ± 12.74** |
| V (Orange red) | 90.0 ± 15.81** | 70.84 ± 6.36** | 146.0 ± 20.73** |

All values are expressed as mean ± SD; ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, ALP= Alkaline phosphatase
Level of significance: * significant, ** highly significant

**Table 3: Effect of 30 days treatment with 50 mg and 150 mg/kg bw of orange red dye and alizarin on serum total protein, albumin and globulin in male albino Wistar rats.**

| Groups | Serum total protein (g/dl) | Serum albumin (g/dl) | Serum globulin (g/dl) |
|---|---|---|---|
| I (Control) | 7.44 ± 0.52 | 4.20 ± 0.49 | 2.48 ± 0.39 |
| II(Alizarin) | 8.80 ± 0.23 | 5.82 ± 0.25 | 3.84 ± 0.60 |
| III(Alizarin) | 9.32 ± 0.23 | 6.28 ± 0.57 | 4.24 ± 0.65 |
| IV(Orange red) | 11.66 ± 1.89* | 7.97 ± 1.89* | 6.96 ± 2.26* |
| V(Orange red) | 14.81 ± 2.45** | 9.91 ± 1.39* | 8.27 ± 0.82* |

Values are expressed as mean ± SD. Level of significance: * significant, ** highly significant

Table 3 shows the effect of orange red dye and alizarin on serum total protein, albumin and globulin levels in male wistar rats. The two doses 50 mg and 150 mg/kg bw of orange red dye produce a significant increase ($p$<0.05) in level of total protein, albumin and globulin when compared with the control group. However in group II and III rats treated with low and high dose of alizarin a statistically insignificant change was detected when compared with (group I) rats.

## DISCUSSION

Food dyes are important component of food in this present fast life. They are added to food for increasing consumer attraction and making it more palatable. [22] Coloring dyes are important kind of food additives synthesized or extracted from plants or other sources. [23] Synthetic food dyes are nowadays considered one of the prime suspects for causing cancer. [24] According to IARC (1983) most of the food coloring agents are toxic and are causing many types of damages in mammalian cells. Therefore more and more attention has been made to focus on short and long term toxicity, carcinogenicity and metabolic studies in view of food dyes. [25] However the plant derived natural dyes are considered as safe and also possess antimicrobial activities.

In this research work, an attempt was made to find out the effect of synthetic orange red dye and natural red dye called alizarin derived from roots of madder plant on hematological and biochemical parameters in male albino wistar rats. The dye orange red that we use in food is actually a mixture of two primary colorants carmoisine and sunset yellow both of these primary colorants have been listed as permitted food dyes. There are both positive and negative reports regarding toxicity of carmoisine and sunset yellow individually, but no research work had been done yet in view of toxicity of orange red dye.

Hematological parameters are considered important tools for assessing damage caused by certain chemicals. It is used for calculation of blood indices like RBC, MCV, MCH, Hb, Hct, WBC etc. Decreased RBC value is usually considered an anemia condition.

The present investigation showed that rats treated with high dose of orange red dye produce a significant decrease in RBC count, Hb, Hct and MCH values in comparison to control rats. However group II and III rats which were treated with low and high dose of natural red dye alizarin didn't produce any significant change in different blood indices when compared with control group rats. The decline of different blood parameters may be due to inhibition of hematopoietic organ system. The hematopoietic organs are considered most sensitive system to assess the toxicity of chemicals. [26] Due to orange red dye chemicals the hematopoietic organs may get affected and became unable to release normal RBCs and thus can be responsible for decline in different blood parameter values. The present investigation was in accordance with the finding of [27], who observes a decrease in value of different blood indices when albino rats were treated with allura red dye.

Furthermore it was reported that a significant increase in the value of WBCs was observed in rats treated with high dose of orange red dye and no significant change in WBC value was observed in alizarin treated rats when compared with control group. The increase in WBC count in rats treated with orange red dye may be due to activation of immune system in response to cellular damage caused by any chemical. [28]

Normally serum aminotransferase enzymes are considered as markers of liver damage caused by chemicals. [29] Increase in concentration of these serum enzymes is recognized as early sign of liver hepatitis. The present investigation study revealed that rats treated with both low and high dose of orange red dye produce highly significant increase ($p$<0.01) in serum ALT, AST and ALP activities when compared with group I rats (control). The elevation in concentration of these enzymes has been considered as indicators of liver damage. The present reports are in accordance with [30], who found that low and high doses of some synthetic dyes carmoisine, sunset yellow, erythrosine, ponceau, tartrazine, indigotine, fast green, brilliant blue and brilliant black produce an increase in level of AST, ALT and ALP. Furthermore these results coincides with the results of [31], who found that rats whose diets were supplemented with chocolate colors A and B show an increase in the level of serum aminotransferase enzymes. In contrast to this alizarin dye treated rats didn't produce any significant change in ALT and ALP except AST value which was considered as significant change when compared with control rats. The increase in concentration of serum aminotransferase enzymes due to orange red dye treatment may be due to hepatic damage which subsequently caused the release of these liver specific enzymes in greater concentration.

Our work revealed that rats administered with 50 mg and 150 mg/kg bw of orange red dye produce a significant change in serum total protein, serum albumin and serum globulin levels when compared with their respective control rats. On the other side rats treated with different doses of alizarin produce insignificant change in albumin, globulin and total protein levels. These results are in accordance with [32], who observe an increase in serum total protein, albumin and globulin when male rats were consumed with tartrazine at low and high dose for 30 days. Furthermore our results are also in accordance with [31], who found an increase in concentration of serum total protein and albumin when diet of rats was supplemented with chocolate colors A and B.

The increase in concentration of serum proteins due to orange red dye may be due to stimulation of translation to synthesize the specific proteins required for all processes. Similarly the increase in serum globulin concentration is correlated with increased

immunoglobulin synthesis, the defense system which works to keep the body protective from the negative effects of the food dye.

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