# Liver Toxicity Caused by Food Colors in Swiss Albino Arts

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**ABSTRACT**

A number of food coloring additives are added to improve the appearance of the food items and drinks. Colorant provides an aesthetic appearance to food stuff. In India 83.6% of the sample contained permitted colors and 16.4% of sample contained non-permitted colors.[1] Tartrazine and sunset yellow found to be the two most frequently used colors. These are used to color bakery goods, cereals, beverages, candies, gelatin and numerous other commodities. The drinks and juices contributed to the maximum exposure of tartrazine and metanil yellow. Metanil yellow is used extensively for coating of turmeric and pulses. Exposures to various food colors can cause hepatotoxicity.[2]

**INTRODUCTION**

To classify a color as a harmful agent to human depends on its ability to cause hepatocyte damage due to metabolic activation of the compounds to highly free radical products. These free radical products induce lipid peroxidation which is believed to be one cause of cell membrane damage leading to a number of pathological events. The principle effect of food colors may cause hepatic damage by increasing lipid peroxidation; decreasing activities of antioxidant enzyme such as superoxide dismutase (SOD), catalase (CAT), non-enzymatic reduced glutathione (GSH) and elevation of hepatic enzyme, alkaline phosphatase (ALP).[3] ALP is a reliable marker of hepatobiliary dysfunction due to damage. Malondialdehyde (MDA) is one of the end products of lipid peroxidation and a measure of free radical generation.

Thus, the aim of this study is to determine the toxicity of food colors in albino rat by determining some of the biochemical parameters including activities of ALP, total protein and albumin in serum as well as oxidative stress parameters such as SOD, CAT, GSH and MDA in liver tissue.

**MATERIALS AND METHODS**

**Experimental Animals**

Adult Swiss albino rats weighing 190–210 g were used in the present study. Animals were maintained under normal conditions and fed on a normal diet with free access to water *ad libitum*. Rats were randomly divided into four groups, six rats in each cage, as follows:

- **Group I**: The animals of this group were healthy normal rats and serves as untreated control group
- **Group II-IV**: On the basis of average consumption of food commodities and average level of detected colors, the animals of these groups were given blend of food colors at a dose of 25, 50 and 75 mg/kg body weight. The blend was prepared by mixing tartrazine, sunset yellow and metanil yellow in equal ratio and orally administered to experimental animals.

At the end of the experimental duration, the animals were weighed, anesthetized and sacrificed. The liver was removed and washed with cold normal saline and used to prepare liver homogenate.

**Blood Collection for the Estimation of Liver Functions**

Before sacrificing the animals, the blood was collected from retro-orbital plexus. The blood was incubated at 37°C for 10 min and centrifuged at 5000 rpm for 15 min. The resulting serum was used for analysis of total protein, albumin and ALP.

**Assay of Alkaline Phosphatase, Total Protein and Albumin**

The levels of ALP, total protein and albumin in the serum of all rats were determined by the commercially available kits (Beacon laboratory kit). All analysis was performed according to the instructions of the manufacturer.

**Liver Homogenate Preparation**

The liver of different groups was dissected and rinsed thoroughly with ice-cold normal saline. It was smashed in a homogenization buffer and solution was sonicated in an ice bath for 30 s followed by centrifugation at 13000 rpm for 4 min at 4°C. The supernatant was stored at − 80°C and used for determination of the level of lipid peroxidation, the concentration of reduced GSH and the activities of SOD and CAT.

**Assay of Antioxidant Markers of Liver Homogenate**

**Reduced Glutathione**

Reduced GSH was determined and the level of GSH is expressed as μg of GSH/mg protein.[4]

**Assay of Malondialdehyde**

Lipid peroxidation was evaluated on the basis of MDA level and MDA in liver was determined.[5]

**Assay of Superoxide Dismutase Activity**

SOD activity was determined by routine procedure and the activity of SOD was expressed as units/min/mg protein.[6]

**Assay of Catalase**

CAT activity was determined by the earlier method reported and the activity was expressed as the amount of $H_2O_2$ utilized/min/mg protein.[7]

**Histological Examination**

Liver tissues were collected after sacrificing the animals. Liver tissue was washed in normal saline and fixed in 10% neutral formalin and embedded in paraffin wax. Ten μm thick sections were prepared by rotary microtome and stained using H and E.[8]

Statistical analysis

All group values are expressed as a mean ± standard error of the mean. Statistical analysis was performed by one-way analysis of variance followed by Tukey's multiple comparison tests for comparison between different treatment groups. Statistical significance was set at $P < 0.05$.

**DISCUSSION**

The present study is concerned with the effect of blend of tartrazine, metanil yellow and sunset yellow on some biochemical parameters of Swiss albino rats.

The present study revealed a marked increase in serum protein, serum albumin and serum ALP at all dose levels of the blend of food colors. Tartrazine caused a highly significant increase in serum protein at a low dose and ALP at a low and high dose.[9] The high dose of tartrazine caused a significant increase in serum total protein and serum albumin.[10] The rats which consumed 7.5 and 10 mg/kg body weight of tartrazine showed a significant increase in serum total protein.[11] These results are in accordance with the present study. Our findings are also in agreement with the findings where rats treated with a higher dose of tartrazine showed an increase in total protein, albumin, and ALP.[2]

Food color may induce the oxidative stress in animal models leading to the generation of potent reactive oxygen species (ROS). Biological effect of ROS is controlled *in vivo* by wide spectrum of enzymatic and non-enzymatic defense mechanism, particularly by SOD which catalyzes dismutation of superoxide anions to hydrogen peroxide and CAT which then converts $H_2O_2$ into molecular oxygen and water.[3] SOD and CAT represents the front line of defense against oxidative damage. GSH is considered to be one of the most important component of the antioxidant defense of living cells. Lipid peroxidation is an autocatalytic free radical mediated destructive process, whereby polyunsaturated fatty acids in the cell membrane undergo degradation to form MDA.[4] MDA increase in rat liver is the indication of hepatotoxicity.

Hepatotoxicity not only initiates lipid peroxidation but also reduces the tissue GSH, CAT and SOD activities and this depletion may result from the oxidative stress of these proteins.[1] The current study is in agreement with these results which revealed that MDA was significantly higher in treated groups than the control group. In this study, significant decrease in SOD, GSH and CAT activities were observed in Group II, III and IV. MDA level was increased and GSH level was decreased with high tartrazine dose in liver homogenate which is in accordance with the present study.[9] When young male rats were given 500 mg/kg body weight dose of tartrazine, GSH level in tissue homogenate decreased significantly as compared to control.[10]

In the present study, food color caused hepatocellular necrosis and vacuolation and these results go in agreement with an earlier worker who found that the synthetic food dye brilliant blue revealed histopathological alteration in the liver of the rat.[2] This alteration includes necrosis of hepatocytes, infiltration and vacuolation. The food colors caused lymphocytic infiltration around central veins.[1] The hepatocellular necrosis in mice was observed when treated with commercial fruit drinks.[2] The present study is also in accordance with results where brown pigment deposition in the portal tract and Kupffer cells of the liver was observed.[3] Our results are also in accordance with the findings which described changes in the liver when guinea pigs received tartrazine in drinking water in a concentration of 1, 2 and 3% for 3 weeks.[4]

**CONCLUSION**

Our result indicates clearly that the daily intake of artificial food colorants that included the aromatic azo compounds impairs hepatic functions. The commodities consumed by children employ an excessive amount of colors as they attracted to colorful items so, excessive intake of food colorants may cause more risk to the health of children.

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