Indian J. Applied & Pure Bio. Vol. 41(3), 2006-2014 (2026). **ISSN: 0970-2091**

Biochemical and Histopathological assessment of tartrazine-induced hepatic alterations in male albino rats (*Rattus norvegicus*)

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Abstract

Tartrazine is a synthetic coloring agent extensively utilized in the food, pharmaceutical and cosmetic industries. The current investigation examined the hepatotoxic effect of tartrazine in rats by analyzing body weight, hepatosomatic index, liver function enzymes, biochemical parameters and histopathological changes. Tartrazine treated rats showed significant reduction in body weight and increased hepatosomatic index compared to the control group. A considerable rise in serum ALT, AST, ALP and bilirubin concentration indicated impaired liver function. Biochemical analysis revealed decreased protein and carbohydrate contents, while DNA, RNA, and cholesterol level were significantly increased in liver tissue of treated rats. Histological observation also revealed degeneration, necrosis and inflammatory changes in liver tissues. These finding suggest that prolonged intake of tartrazine may adversely affect liver health.

Key words : Tartrazine, albino rat, hepatotoxicity, oxidative stress, liver enzymes, histopathology.

The use of food additives is considered crucial in the contemporary food industry by improving the quality, preservation, flavour, texture and appearance of food products to meet consumer preferences. The use of food additives is an ancient practice that has existed for many centuries. In earlier times, humans used natural method such as smoking, salting and fermentation to preserve food and improve in taste. With the development of food processing technologies and industrialization, the use of additives gradually increased. In the twentieth century, especially with the growth of processed food industries, both natural and synthetic additives became widely incorporated into food production. At present, food additives have become an essential part of daily life, helping in transportation, storage and maintaining

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food quality. However, their increasing use has raised public awareness and concern regarding their safety, health effects and regulatory control[25].

A large number of individuals regularly consume different food additives, which offer both beneficial and harmful effects. Many people consume various food additives every day, which have both advantages and disadvantages. In recent years the consumption of food additives has increased significantly due to their widespread use in processed and packaged food. These substances contribute to improving the stability, flavour, colour and shelf life of food products, making them more acceptable to consumer. Despite these advantages, some additives may pose health risk when consumed in excess or over a long period. Certain chemical compound, including monosodium glutamate and nitrite derivatives, have been linked with toxic and carcinogenic effects. The biological impact of these additives depends on how they influence normal metabolic activities such as absorption, digestion and elimination. Furthermore, establishing safe consumption limits remain challenging because multiple additives may interact with each other and produced combined toxic effects[5].

Artificial colourants are commonly added to confectionery, beverages, and pharmaceutical products to improve visual appeal and consumer acceptance[3]. Although food additives provide several advantages, they also have certain limitations and the regular consumption is widespread. The beneficial role of food additives differs from one additive to another. On the other hand, some compound like monosodium glutamate and nitrous substance have been linked to cancer causing effects and may be found in food additives. The level of toxicity of a food product depends on how strongly it affects normal metabolic pathways, absorption, mechanism and waste elimination process[5]. Many people are sensitive to food additives, which can lead to symptoms such as diarrhoea, skin irritation, stomach disorders, vomiting or an increase in body heat[18].

The visual appeal of food is greatly influenced by colour and food contributing to its attractiveness, while colouring agents are commonly used to enhance the appearance of various food items and drinks[23]. Several studies have reported that both natural and artificial food colourants may produce toxic effects when evaluated under experimental condition. There has been a substantial rise in the utilization of synthetic dyes in food products during recent years. Azo dyes make up the major share of this artificial colourant and may contribute to environmental pollution during processing.

Tartrazine, an azo-based synthetic colouring agent, is extensively utilized in food industries because of its bright yellow appearance and cost-effectiveness. It is commonly present in beverages, confectioneries, snacks, dairy items and chewing gums because of its bright colour and low cost. In some developing countries, it is also used as a cheaper substitute for saffron in food preparation. Among food additives, synthetic colourants are considered more harmful because of their toxic effects on body tissues[2].

When tartrazine enters the digestive

system through food, it is mainly absorbed by the intestinal lining and metabolized by azo reductase enzyme in the liver and intestinal wall into sulfanilic acid, a compound associated with harmful effects. The World Health organization has set its acceptable daily intake at 7.5 mg/kg body weight. Previous studies have linked tartrazine exposure with various health disorder, including immune toxic effects and damage to organ such as the liver, kidney and stomach in experimental animals. Earlier studies on tartrazine revealed its effects on DNA damage, particularly in the liver and kidney[17].

The study was conducted on albino rat (*Rattus norvegicus*) weighing between 220- 280 grams were brought from Central Animal Preclinical Research Facility DMIHER (DU) Sawangi, Wardha (Reg No.571/02/a/ CPCSEA), in PGTD Of Zoology RTM Nagpur University Nagpur. the experimental rats were maintained under standard laboratory condition with unrestricted access to food and water during the acclimatization period. A total of eighteen albino rats were selected and equally distributed into three experimental groups, with six rats in each group. Group I was maintained as the untreated control, whereas Groups II and III were treated with oral administration of tartrazine at concentration level of 50 mg/ kg BW and 100 mg/kg BW, respectively, for 30 days. Body weights were measured before treatment and at the end of the experiment. After sacrifice, the liver was removed, washed and weighed, while blood samples were collected in non-EDTA tubes for enzyme concentration. Liver tissues were also collected, washed and preserved for biochemical analysis to determine protein, DNA, RNA, carbohydrate and cholesterol levels using standard protocols. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities were determined using the ERBA biochemical assay kit by following the standard procedure provided by the manufacturer. SGOT and SGPT activities were determined using the ERBA diagnostic kit by following the standard procedure provided by the manufacturer. The absorbance was measured using a semi-automatic biochemical auto-analyser at Saraswati Pathology Laboratory and Research Centre, Nagpur. Protein content in liver tissues was estimated using Lowry's method[21], carbohydrate concentration in liver tissue was estimated using the method described by Dubois[11], DNA concentration in liver tissue was estimated using the diphenylamine method as described by Burton[24] and cholesterol levels were determined using Zake's method[26]. All observed parameters were analysed and represented as individual data value and mean ± standard error (SE), while significance among group was determined at $p<0.05$. Statistical analysis was done using the statistical (GraphPad software, 2024).

Body weight and Liver Weight :

Table-1. Body weight and liver weight of control and tartrazine treated albino rats for 30 days duration

Groups	Initial BW (g) Before treatment	Final BW (g) After treatment	Liver Weight (g) After treatment
Group I (Control)	270±5.77	288.67±11.77	3.18 ± 0.18
Group II (Treated)50mg/kg BW (Tz)	240±2.88	216±6.28***	3.24 ± 0.16**
Group III (Treated)100mg/kg BW (Tz)	235±7.96	209±4.55***	4.28 ± 0.06***

Values are presented as Mean ± SE, N=06, for each group, $p<0.05$*, $p<0.001$**, $p<0.0001$***.

The table-1 presents data on body weight, liver weight of control group and tartrazine treated albino rat for 30 days duration. Group II and III (50 mg/ kg BW, and 100 mg/kg BW). The initial body weights were comparable among all groups before the experiment. After the experiment, the control group showed slight increase in body weight. In contrast, all treated groups demonstrated a dose-dependent decline in body weight. The group treated with 50 mg/ kg BW and 100 mg /kg BW of tartrazine showed a highly significant reduction in final body weight compared to the control group.

Additionally, liver weight increased in the treated groups compared to the control group after the experiment. The increase was slight in the 50 mg/kg BW treated group and more pronounced in the 100 mg/kg BW treated group, indicating dose-dependent liver enlargement.

The result showed that continuous exposure of tartrazine induces significant physiological changes in albino rats. Rats given higher doses of tartrazine resulted in marked reduction in body weight and increased liver weight. These finding suggest that tartrazine may affect normal metabolism and liver activity.

Table-2. Concentration of liver function enzymes (SGOT, SGPT, Alkaline phosphate and Total bilirubin) in the serum of control and tartrazine treated albino rats after 30 days of treatment

Group	SGOT (IU/L)	SGPT (IU/L)	Alkaline phosphate	Total bilirubin
Group I (Control)	135.3 ± 3.28	50.2 ± 5.72	203.85 ± 11.8	0.22 ± 0.02
Group II (Treated) 50mg/kg BW (Tz)	155.8 ± 2.89**	68.8 ± 4.88**	221.0 ± 4.84**	0.32 ± 0.01**
Group III (Treated) 100mg/kg BW (Tz)	239.1 ± 7.07***	114.0 ± 2.06***	293 ± 3.77***	0.63 ± 0.1**

Values are presented as Mean ± SE, N=06, for each group, $p<0.05$*, $p<0.001$**, $p<0.0001$***.

The table-2 presents data on the levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate and total bilirubin in the control group and tartrazine treated groups (50 mg/kg BW and 100 mg/kg BW). In the rat treated with 50 mg/kg BW of tartrazine, showed a significant increase in all biochemical parameters compared to the control group. A further increase was observed with the higher dose (100 mg/kg BW) of tartrazine showed a highly significant rise in SGOT, SGPT, alkaline phosphate and total bilirubin levels. These changes suggest that tartrazine affects liver function in a dose dependant manner and may cause liver damage at higher concentration.

Table-3. Concentration DNA, RNA, Protein, Carbohydrate and Cholesterol in the hepatic tissue of control and tartrazine treated albino rats after 30 days of treatment

Group	DNA (mg/100mg)	RNA (mg/100mg)	Protein (mg/100mg)	Carbohydrate (mg/100mg)	Cholesterol (mg/100mg)
Control	0.478 ± 0.05	0.838 ± 0.051	22.25 ± 0.85	2.836 ± 0.100	236.5 ± 11.54
50mg/kg BW (Tz)	1.553±0.115***	0.801±0.050***	18.98±0.30**	2.040±0.075***	312.8 ±5.322***
100mg/kg BW (Tz)	2.393±0.091***	2.62 ± 0.105***	15.97±0.24***	1.296±0.104***	366.9 ±3.935***

Values are presented as Mean ± SE, N=06, for each group, $p<0.05$*, $p<0.001$**, $p<0.0001$***.

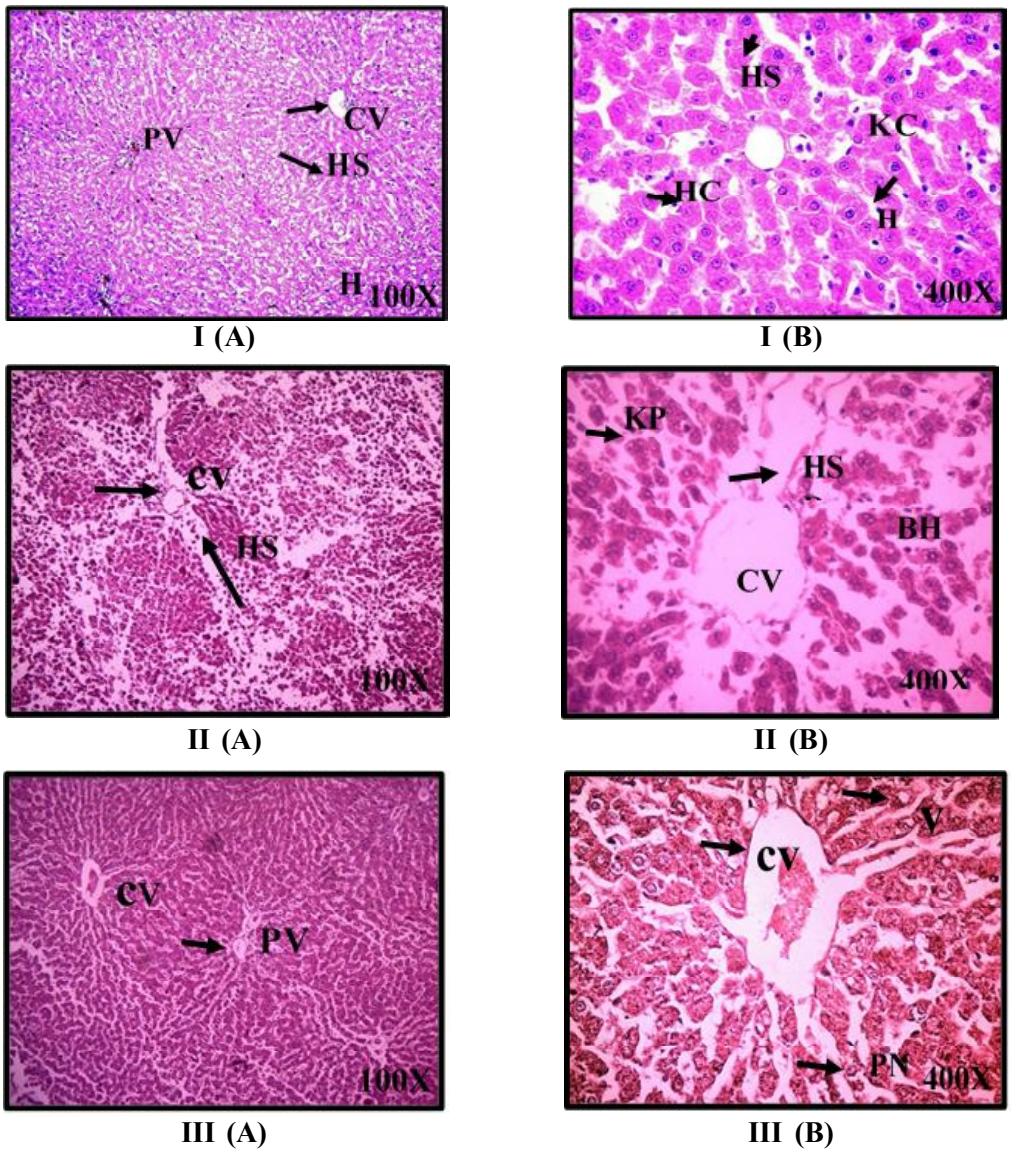

Fig. 1. Photomicrograph showing transverse section of liver in photo plate I (control), photo plate II (50 mg/kg BW) and photo plate III (100 mg/kg BW) male albino rat, *Rattus norvegicus* for 30 days duration (HE staining, magnification 100X and 400X).

Abbreviations used :

CV: Degeneration of central vein
HS: Hepatic sinusoidal dilation
PV: Portal vein congested
H: Irregular hepatocytes
V: Vacuolization
PN: pyknotic nucleus
BH: Binucleated hepatocytes
KP: Kuffer cell proliferation

The present study evaluated the effect of tartrazine on biochemical parameters in albino rats. The result clearly revealed dose-dependent alterations in DNA, RNA, protein, carbohydrate, and cholesterol level.

DNA content was found increase significantly in treated groups, with a greater increase at higher dose (100 mg/kg BW), which may indicate changes in nucleic acid levels and cellular activity. RNA levels also showed an increased significantly at the higher dose, suggesting increased transcriptional and metabolic activity, while only slight changes were observed at the lower dose.

In contrast, protein levels showed a significant decrease with increasing dose of tartrazine, which may indicate reduced protein synthesis or enhanced protein breakdown. Similarly, carbohydrate level also declined gradually in the treated group, suggesting disturbances in energy metabolism and increased utilization of carbohydrate to meet the body metabolic need.

On the other hand, cholesterol levels increased significantly with increasing doses of tartrazine, suggesting disturbance in lipid metabolism and possible accumulation of cholesterol in the liver tissue.

Overall, the result indicate that tartrazine exposure induces significant biochemical and metabolic activities. it was observed that nucleic acid and cholesterol level increased, while protein and carbohydrate contents decreased with increasing doses.

Liver tissue sections from the control group showed normal histological organization, with hepatocytes properly arranged in hepatic cords surrounding the central vein. The hepatic sinusoids appeared normal and uniformly distributed between the hepatic cords, while Kupffer cells were clearly visible along the sinusoidal lining. The portal vein and central vein showed normal morphology without congestion or structural abnormalities (Photoplate I-A, B). These observations indicate healthy liver tissue and normal physiological function.

In contrast, Liver tissue of rat treated with 50 mg/kg body weight of tartrazine showed moderate structural changes compared to the control group. These included congestion of the portal vein, degeneration of the central vein, dilated sinusoids, and irregular hepatocytes with vacuolation and pycnotic nuclei. Binucleated hepatocytes and increased Kupffer cells were also observed (Photoplate II-A, B), indicating early liver damage and inflammation caused by tartrazine exposure. Liver tissue of rat treated with 100 mg/kg body weight of tartrazine showed more severe histopathological changes, indicating greater liver damage at the higher dose. The liver sections revealed marked congestion and dilation of the portal and central veins, extensive degeneration of hepatocytes and widened sinusoids. The normal arrangement of hepatic cords was disturbed, leading the loss of normal liver structure. Cytoplasmic vacuolation, enucleated hepatocytes, and necrotic areas were also clearly observed (Photoplate III-A, B). These findings suggest severe hepatotoxic effects of tartrazine at higher concentration.

Tartrazine is a widespread synthetic dye, derived from coal tar, orange coloured, water soluble powder used as a food additive

to colour the food product, especially fruit juices, soft drinks, flavoured chips, soups, candies, ice cream and gum. The use of tartrazine extends beyond the food sector and has been widely employed in several medicinal and cosmetic products, including vitamin supplements, antacids and hair products[2,4,8,16].

Liver is the main important organ which serves as the centre for metabolism. It is also most vulnerable organ to toxicity as it involves in the detoxification of the drug and xenobiotics[8]. In the present study, significant increase in hepatosomatic index of rat treated with tartrazine as compared to the control rats were reported. Similar findings have been reported by [9,15,19]. In contrast, some workers such as[14] have documented an increase in body weight. The decrease in body weight may be due to generalized toxicity, reduced appetite, and decreased food intake, as reported by[5]. On the other hand[12] stated that an increase in body weight exceeding 20% above the mean is indicative of obesity which is one of the indicators of metabolic disruption.

Liver function test is an important parameter use to analyse the function of the liver which leads to the metabolic disorders[12]. Biochemical analysis showed elevated liver enzymes (ALT, AST, ALP, and bilirubin), indicating hepatocellular damage and impaired liver function, consistent with earlier findings[1,15]. Significant alterations were also observed in proteins, DNA, RNA, cholesterol, and carbohydrates, reflecting disrupted metabolic processes[5,13,14].

The Present study reported that tartrazine causes significant alteration in key biochemical parameters, including protein, DNA, RNA, cholesterol and carbohydrate in liver as compared to control. Similar observations also reported by [2,6,7,19,20,22].

In the present study, an increase in DNA and RNA concentration in liver tissue was observed in experimental animals treated with Tartrazine. significant biochemical alterations in rats exposed to synthetic food dyes, including changes in nucleic acid metabolism reported by[5]. Thus, the tartrazine induced increase in the level of the DNA and RNA may associated with cellular response to the toxic stress caused by the tartrazine.

Cholesterol in the liver is crucial for maintaining systemic lipid homeostasis, structural integrity of cell membranes, and metabolic function. Significant increase in liver cholesterol concentration was observed in experimental animals treated with Tartrazine indicates disturbance in lipid metabolism and hepatic dysfunction, suggesting that exposure to the food dye alters normal biochemical processes in the liver. Similar finding[6,19] reported that synthetic food dyes, including tartrazine, caused significant increases in cholesterol levels in treated rats, indicating disruption of lipid metabolism.

The liver being a primary metabolic organ, regulates carbohydrate levels through processes like glycogenesis and glycogenolysis, which are control by the hormonal level. A decrease in carbohydrate concentration in the liver was observed after exposure to tartrazine may be attributed to oxidative stress and altered liver metabolism caused by tartrazine leading to reduced carbohydrate content. decreased glycogen levels in liver associated with hepatic stress caused by food dyes[6].

In the present study, exposure to food dye resulted in marked histopathological alterations in liver sections, including hepatocellular degeneration, necrosis, sinusoidal dilatation, and infiltration of inflammatory cells. These changes indicate clear structural damage to hepatic tissue and suggest impairment of normal liver function due to toxic insult. Similar findings have been reported in earlier studies. Also reported by[5] hepatic damage characterized by dilatation of central veins, haemorrhage, and necrotic changes in the liver of rats exposed to tartrazine, indicating vascular and cellular injury.

Overall, the study clearly demonstrates that tartrazine induces significant hepatotoxic effects through oxidative stress, biochemical alterations, and structural damage to liver tissue. The observed changes in physiological, biochemical, and histological parameters indicate impaired liver function, which may worsen with prolonged or high-dose exposure.

In conclusion, tartrazine exposure leads to pronounced metabolic and structural disturbances in the liver, highlighting its potential health risks. These findings emphasize the need for cautious and regulated use of synthetic food colorants and support the consideration of safer natural alternatives to minimize adverse health effects.

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