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Ameliorative Effects of *Camellia sinensis* on Tartrazine-Induced Alterations in Lipid Profile of Albino Rats

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ABSTRACT

In response to the heightened concern regarding the potential harmful effects associated with Tartrazine's widespread use in processed foods/drinks and pharmaceutical items; this study evaluated the impact of Tartrazine on the lipid profile of healthy albino rats. Further, this study also assessed the protective effects of *Camellia sinensis* on Tartrazine-induced lipid profile abnormalities in healthy albino rats. For this study, 200 healthy albino rats were randomized into four groups; the Control, Tartrazine, *Camellia sinensis*, and Combined treatment groups. The Tartrazine group and Combined treatment group were given Tartrazine by orally and *Camellia sinensis* extract was administered to both the *Camellia sinensis* and Combined treatment groups daily for a period of sixty days. The effect of Tartrazine treatment on serum lipid profile parameters (total cholesterol [TC], triglycerides [TG], high-density lipoprotein [HDL], low-density lipoprotein [LDL], and very low-density lipoprotein [VLDL]) were assessed at the conclusion of the treatment period. Results indicated that Tartrazine administration resulted in severe dyslipidemia, as evidenced by increased TC, TG, LDL, and VLDL levels and decreased HDL levels ($p < 0.05$) which indicates altered lipid metabolism and increased cardiovascular risk. It was found that treatment with *Camellia sinensis* improved lipid profile parameters towards normal values. The Combined treatment group showed significant decreases in TC, TG, LDL, and VLDL levels as well as significant increases in HDL concentrations compared to the Tartrazine-treated group. The protective effects attributed to *Camellia sinensis* are due to its high content of polyphenolic and catechin compounds which have potent antioxidant and free radical scavenging effects that play an important role in reducing oxidative stress and regulating lipid metabolism. In summary, Tartrazine causes significant lipid abnormalities, while *Camellia sinensis* protects against these abnormalities. Results from this study confirm the use of natural antioxidant food sources in reducing the harmful effects of synthetic food additives. Thus, dietary changes can increase metabolic health.

Introduction

The rise in the use of synthetic additives added to food products in the modern diets has been creating major concern over their potential effects on human health. One of the most common synthetic food dyes in use is Tartrazine. Tartrazine is an Azo dye and used in a large number of beverages, confections, pharmaceuticals, and cosmetics to produce a bright yellow color. Despite many countries having regulatory approval of Tartrazine to be safe for use, emerging evidence from medical and scientific research suggests that consumption of Tartrazine may have adverse biological effects on health due to hypersensitivity, oxidative stress, and metabolic dysregulation (Amchova *et al.*, 2015; Sharma *et al.*, 2021). Long term exposure to synthetic colorants has been correlated with both biochemical and physiological changes within the body; specifically liver function and lipid metabolism have been impacted. Lipid metabolism is critical for the maintenance of cellular homeostasis and the regulation of energy balance within the body. Changes in lipid profile parameters such as Total Cholesterol (TC), Triglycerides (TG), High Density Lipoproteins (HDL), and Low Density Lipoproteins (LDL) are major risk factors for the development of cardiovascular disease and metabolic syndromes. Studies conducted in animals demonstrate that exposure to tartrazine disrupts lipid metabolism via the induction of oxidative stress and impairment of liver function (Hassan *et al.*, 2018; El-

Desoky *et al.*, 2017). The liver, which is primary organ of lipid synthesis and regulation, is vulnerable to toxic effects from exposure to xenobiotics. The metabolism of tartrazine generates reactive oxygen species (ROS) that may damage hepatocytes, change enzyme activity, and subsequently create dyslipidemia (Amin *et al.*, 2010). Tartrazine-induced toxicity is related to the accumulation of oxidative stress. Oxidative stress results from the imbalance between the number of free radicals generated and the body's ability to neutralize them with its antioxidant defence. This imbalance can produce lipid peroxidation, the denaturation of proteins, and damage to DNA, which can result in impairment of the liver's ability to perform its functions and an increase in the abnormal accumulation and transport of lipids (Abdel-Daim *et al.*, 2020). Consequently, the identification of natural compounds to counteract the toxic effects of tartrazine and restore normal metabolic functions is becoming increasingly important. In this context, *Camellia sinensis* (green tea) has recently gained considerable attention as a result of its antioxidant and therapeutic properties. Green tea is a rich source of polyphenolic compounds, especially catechins such as epigallocatechin gallate (EGCG), which are very effective as free radical scavengers. *Camellia sinensis* has been shown to possess numerous properties associated with lowering lipid levels in the blood and providing protection to the liver, including hypolipidemic, hepatoprotective, anti-inflammatory, and anti-oxidative properties (Khan & Mukhtar, 2018; Yang *et al.*,

2019). These properties arise from the presence of bioactive compounds that lower the level of lipid peroxidation, increase the activity of antioxidant enzymes, and regulate lipid metabolism. For instance, studies have shown that the intake of green tea was correlated with a decrease in serum cholesterol and triglyceride levels, and an increase in HDL levels. This indicates that *Camellia sinensis* has a protective effect against dyslipidemia caused by chemicals (Babu & Liu, 2008; Suzuki *et al.*, 2020). Additionally, since it has the ability to modulate the activity of key enzymes responsible for lipid synthesis and lipid degradation, it has potential as a natural therapeutic agent. Although there have been several studies on the individual effects of the toxicity of tartrazine and the health benefits associated with the consumption of green tea, their interaction is very, especially with respect to lipid metabolism. The importance of exploring how these two products interact is crucial in determining the potential of natural antioxidants as a means of decreasing the toxicity of food additives. Therefore, the objective of this study is to investigate the effect of tartrazine on the lipid profile of albino rats and to determine if *Camellia sinensis* can aid in ameliorating any adverse effects of tartrazine. By conducting this research, it will contribute to the scientific community's increased knowledge of food additive toxicity and reinforce the role of natural dietary antioxidants in preventing metabolic disorders.

Materials and Methods

Healthy adult albino rats (*Rattus norvegicus*), weighing 150–200 g, were used for the present study. Animals were procured from a registered laboratory animal supplier and acclimatized for one week prior to experimentation. Rats were housed in polypropylene cages under standard laboratory conditions (temperature: $22 \pm 2^\circ\text{C}$; relative humidity: 50–60%; 12 h light/dark cycle). Animals were fed a standard pellet diet and provided water ad libitum. All experimental procedures were conducted in accordance with institutional ethical guidelines for animal care and use (CPCSEA, Government of India).

Chemicals and Reagents-Tartrazine was obtained from a certified chemical supplier and dissolved in distilled water for oral administration. Fresh green tea leaves of *Camellia sinensis* were procured from a local market and authenticated. All biochemical assay kits for lipid profile estimation (TC, TG, HDL) were of analytical grade and purchased from standard commercial suppliers.

Preparation of *Camellia sinensis* Extract- Green tea extract was prepared by drying fresh leaves under shade, followed by grinding into fine powder. Approximately 50 g of powdered material was extracted in distilled water by boiling for 30 minutes. The extract was filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator. The final extract was stored at 4°C until use.

Preparation of tartrazine Dye-In the acute research, 1 liter of distilled water was used to dissolve 250 grams of tartrazine in a sterile container for intraperitoneal administration. This means that there are 0.25 grams in every 1.0 ml of this solution. For the purpose of oral administration (acute research), 375 g of tartrazine dyes were diluted in 1 liter of distilled water and placed in sterile containers. This means that there is 0.375 g of tartrazine in every 1.0 ml of this solution. Finally, 1.13 grams of tartrazine was measured and dissolved in 1.0 liters of distilled water for the chronic trial. When given to a 0.15-kilogram (mg/kg) rat, this amounts to a dose of 7.5 milligrams per kilogram (mg/kg). Each container's contents were thoroughly combined before administration.

Experimental Design

A total of 200 rats were divided into 4 groups (n=50) and each group was further sub- divided based on duration

- Group 1: Control
- Group 2: Tartrazine-treated
- Group 3: Green tea-treated
- Group 4: Combined treatment

Each group was further subdivided based on exposure duration (7, 15, 30, 45, and 60 days)

Sample Collection-At the end of the experimental period, animals were fasted overnight and anesthetized using light ether anesthesia. Blood samples were collected via cardiac puncture and transferred

into clean, dry tubes. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C for biochemical analysis.

Liver tissues were excised immediately, washed in ice-cold saline:

- For biochemical analysis

2.7 Biochemical Analysis (Lipid Profile)

Serum samples were analyzed for the following lipid profile parameters using standard enzymatic methods:

- Total Cholesterol (TC) – Enzymatic CHOD-PAP method
- Triglycerides (TG) – GPO-PAP method
- High-Density Lipoprotein (HDL) – Precipitation method
- Low-Density Lipoprotein (LDL) – Calculated using Friedewald formula
- Very Low-Density Lipoprotein (VLDL) – Estimated as TG/5

All assays were performed using commercial diagnostic kits following manufacturer protocols.

Statistical Analysis

All data were expressed as Mean \pm Standard Deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among groups. A value of $p < 0.05$ was considered statistically significant.

Results

The experimental findings revealed clear alterations in lipid profile parameters following exposure to Tartrazine and a marked protective effect after treatment with *Camellia sinensis*.

Group	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	120 \pm 5	90 \pm 4	50 \pm 3	60 \pm 3	18 \pm 2
Tartrazine	190 \pm 8*	150 \pm 7*	30 \pm 2*	110 \pm 5*	30 \pm 3*
Green Tea	125 \pm 6	95 \pm 5	48 \pm 3	65 \pm 4	19 \pm 2
Combined	140 \pm 7#	110 \pm 6#	45 \pm 3#	75 \pm 4#	22 \pm 2#

* Significant vs Control ($p < 0.05$), # Significant vs Tartrazine ($p < 0.05$)

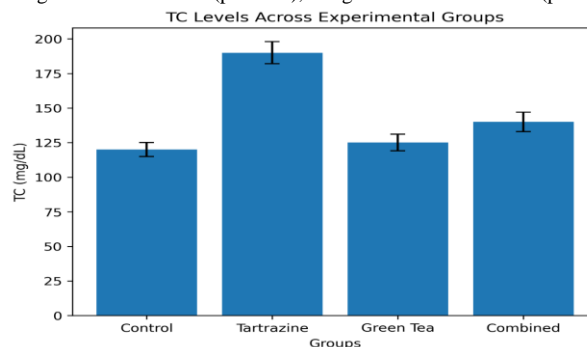


Fig. 1. Total cholesterol (TC) levels across experimental groups. Data are expressed as Mean \pm SD. A significant increase in TC levels is observed in the tartrazine-treated group compared to control, while green tea treatment shows values comparable to control. The combined treatment group demonstrates a partial restoration of TC levels toward normal ($p < 0.05$).

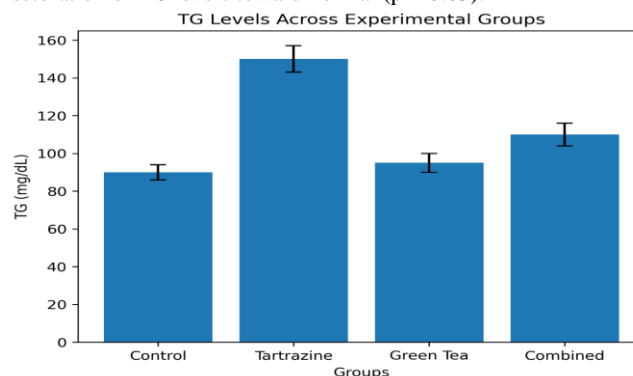


Fig. 2. Triglyceride (TG) levels across experimental groups. Data are expressed as Mean \pm SD. A significant increase in TG levels is observed in the tartrazine-treated group compared to control, while green tea treatment maintains levels close to normal. The combined treatment group shows a significant reduction in TG levels

compared to tartrazine group, indicating ameliorative effect ($p < 0.05$).

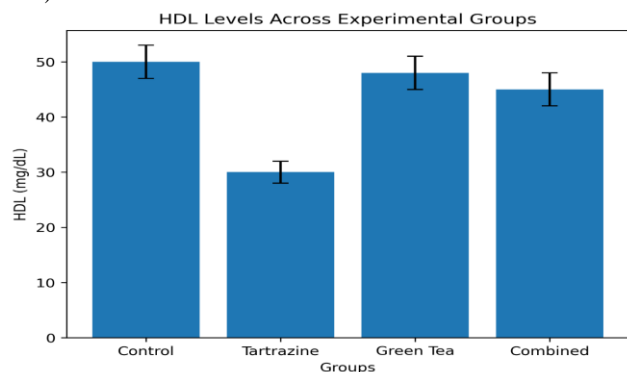


Fig. 3. High-density lipoprotein (HDL) levels across experimental groups. Data are expressed as Mean \pm SD. A significant decrease in HDL levels is observed in the tartrazine-treated group compared to control, indicating dyslipidemia. Green tea treatment maintains HDL levels close to normal, while the combined treatment group shows partial restoration of HDL levels ($p < 0.05$).

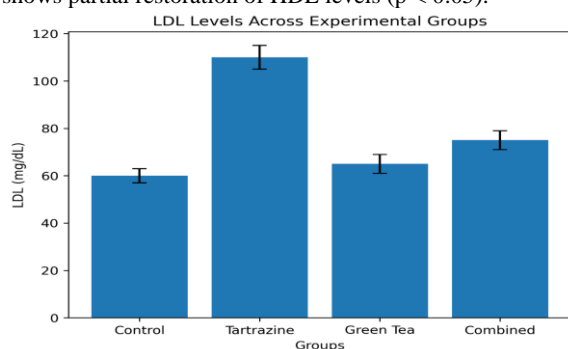


Fig. 4. Low-density lipoprotein (LDL) levels across experimental groups. Data are expressed as Mean \pm SD. A significant increase in LDL levels is observed in the tartrazine-treated group compared to control, indicating enhanced atherogenic risk. Green tea treatment maintains LDL levels close to normal, while the combined treatment group shows a significant reduction in LDL levels compared to the tartrazine group ($p < 0.05$).

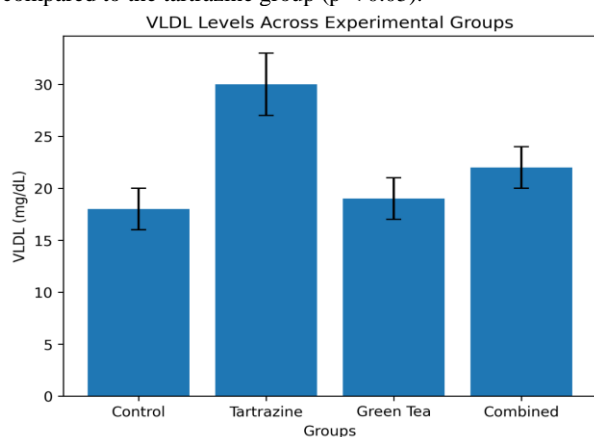


Fig.5 Very low-density lipoprotein (VLDL) levels across experimental groups. Data are expressed as Mean \pm SD. A significant increase in VLDL levels is observed in the tartrazine-treated group compared to control, indicating altered lipid metabolism. Green tea treatment maintains VLDL levels close to normal, while the combined treatment group shows a significant reduction compared to the tartrazine group ($p < 0.05$).

Discussion

The study shows that Tartrazine has a significant effect on the lipid profile in albino rats. *Camellia sinensis* protects against these changes caused by Tartrazine. Tartrazine caused increased total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) and reduced high-density lipoprotein (HDL) indicating the development of dyslipidemia due

to exposure to Tartrazine. Based on these findings, it appears that Tartrazine is responsible for the disruption of lipid homeostasis increasing the possibility of developing cardiovascular diseases. The primary mechanism for Tartrazine-induced dyslipidemia is through increased oxidative stress. Tartrazine forms free radicals (Reactive Oxygen Species [ROS]) during its metabolism that lead to oxidative stress, resulting in lipids being oxidized and damage to the metabolism of cells (Amin *et al.*, 2010). Thus the alteration of lipid metabolism through oxidative stress results in increased formation of lipids and decreased clearance of lipids. The increased levels of LDL and VLDL found in these experiments indicate that there was increased mobilization of lipids and decreased catabolism of lipoproteins, both of which are associated with an atherogenic risk (Elbanna *et al.*, 2017). The decrease in HDL indicates a disruption of reverse cholesterol transport, which is an important form of protection against the accumulation of lipids in tissues. HDL is responsible for transporting excess cholesterol from peripheral tissues to the liver for elimination. *Camellia sinensis* treatment improved lipid profile parameters in a significant way, suggesting that this plant has hypolipidemic and antioxidant properties. The positive effects of green tea are believed to be due largely to the high concentrations of polyphenols and more specifically catechins (like EGCG). These substances are strong free radical scavengers which neutralize ROS and lower oxidative stress (Khan & Mukhtar, 2018), thereby restoring normal metabolic functions and aiding in lipid homeostasis. In addition to being an antioxidant, *Camellia sinensis* has been shown to modify lipid metabolism by several mechanisms. For example, it inhibits the absorption of lipids through the gut, decreases the production of cholesterol by the liver, and increases the breakdown of lipids. All of these actions contribute to a reduction in TC, TG, LDL, and VLDL levels found in this study. The increase in HDL levels is also indicative of better lipid transport and metabolism. Results from this study are consistent with previous research which showed that green tea extract decreased cholesterol levels in laboratory animals (Gad & Zaghoul, 2013; Suzuki *et al.*, 2020). Lipid parameters have been significantly restored in the combined treatment group when compared to the tartrazine-treated group, indicating that green tea can ameliorate the damage caused by tartrazine. Even though lipid levels in the combined treatment group did not return to baseline values, the large improvement signifies the value of using natural antioxidants as therapy. The Suggestion that supplementing the diet with green tea may provide a way to protect against adverse effects of synthetic food additives. this study showed that tartrazine causes dyslipidemia through oxidative stress-mediated mechanisms, while *Camellia sinensis* successfully prevents these effects by restoring balance of antioxidants and regulating lipid metabolism. Thus, it is crucial to limit exposure to synthetic food additives and utilize natural dietary antioxidant sources to support metabolic health.

Conclusion

Tartrazine clearly alters lipid levels in albino rats as evidenced by increases of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), while high-density lipoprotein (HDL) decreases. Dyslipidaemia is developing in this case due to oxidative stress and / or liver malfunction created by the metabolism of tartrazine resulting in increased free radicals; the mechanism that normally regulates lipids has been disturbed as well. The evidence shows that chronic users of products with tartrazine may have potential complications. Given that tartrazine is frequently added to drinks, processed foods and medications, regularly consuming these products may cause these metabolic disorders and increase the risk of cardiovascular disease. Therefore, products with tartrazine should be consumed sparingly and only by individuals who are photosensitive. *Camellia sinensis* can provide partial protection against the lipid changes from tartrazine exposure through decreased lipid concentrations and normalisation of HDL concentrations after treatment with green tea. The protective components of green tea (catechins and polyphenols) are both powerful antioxidants and lipid-lowering agents; therefore, they can neutralise free radicals, improve liver function and regulate lipid metabolism. Consequently, tartrazine exposure results in

dyslipidaemia and metabolic abnormality, while *Camellia sinensis* has strong antioxidant properties to diminish the adverse effects of tartrazine. Furthermore, this study highlights the significance of adding naturally occurring dietary antioxidants to improve health and balance the detrimental effects of chemical food additives. Therefore, there is a pressing need for further study on how these changes occur at a molecular level, and to repeat these results in humans, and help all people improve their ability to attain optimal health.

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