# Toxicological Assessment of *Azo* Dye Brown HT *In Vivo*

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## Abstract

Background: Azo dyes, widely used in food, pharmaceuticals, textiles, and cosmetics, raise significant safety concerns due to their potential toxicological effects. Specifically, the biotransformation of azo dyes into aromatic amines, which may have carcinogenic potential, necessitates an investigation into their impact on health, especially for vulnerable populations like children. Methods: This study assessed the effects of Brown HT (E155) azo dye on juvenile rats. Male Long-Evans rats were divided into control and treatment groups, receiving varying doses of Brown HT over six weeks. Parameters measured included body weight, hematological and biochemical indices, and histopathological changes in liver and kidney tissues. Results: The results indicated a significant dose-dependent effect of Brown HT on body weight, blood parameters, and organ function. The high-dose group exhibited significant weight reduction, elevated liver enzymes, and abnormal lipid profiles. Histopathological examination revealed severe liver damage and renal impairment in high-dose groups, indicating potential long-term health risks associated with azo dye consumption. Conclusion: Brown HT azo dye demonstrates dose-dependent toxicity in juvenile rats, impacting physiological, biochemical, and histopathological parameters. These findings underscore the need for regulatory scrutiny regarding azo dye usage in food products, particularly for children, to mitigate associated health risks and promote safer food practices.

**Keywords:** Azo dyes, Brown HT, Juvenile Rats, Toxicology, Health Risks.

***Significance*** | This study demonstrated the adverse effects of azo dye Brown HT on juvenile health, underscoring the need for safer food practices to protect vulnerable populations.

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## Introduction

Azo dyes are synthetic amphoteric compounds widely used in industries such as food, pharmaceuticals, textiles, and cosmetics. However, their potential toxicological effects on humans and animals have become a significant concern (Watabe et al., 1980). Known for their vibrant colors, these dyes are commonly found in everyday products. Despite their widespread use, concerns have emerged regarding their safety, particularly when used as food additives. Azo dyes are metabolized in the body to form aromatic amines, compounds believed to have carcinogenic potential. This biotransformation is especially concerning because non-toxic parent compounds may become harmful after ingestion (Brown & De Vito, 1993; Chung, 2016).

The regular consumption of azo dyes through food products has raised alarms about their toxic effects, including physiological, biochemical, and histopathological changes (Al-Shinnawy & Elkattan, 2013; Amin et al., 2010). Physiologically, azo dyes have been shown to cause significant weight loss in animal studies, a common sign of systemic toxicity. This weight reduction may result from poor appetite, nutrient malabsorption, or an increased

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metabolic rate due to the body's attempts to eliminate toxic substances. Studies have revealed that azo dyes disrupt normal metabolic processes, leading to these adverse effects (Chung et al., 1992; Elbanna et al., 2017). On a biochemical level, azo dyes can severely affect metabolic processes, often reflected in abnormal biochemical values. For instance, elevated levels of liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicate liver damage caused by azo dye exposure (Reza et al., 2019). Since the liver is responsible for detoxification, it is particularly vulnerable to these compounds. Histopathological studies also revealed liver tissue changes, including degeneration, necrosis, and inflammation. Additionally, the kidneys, another key detoxifying organ, were affected by azo dyes, showing signs of tubular necrosis and glomerular damage, which imply impaired renal function (Elbanna et al., 2017; Elekima et al., 2019).

These biochemical and histopathological alterations suggest that azo dyes can reduce organ functionality, posing long-term health risks. The carcinogenic potential of azo dyes is especially alarming, given their use in food products. Studies have linked chronic exposure to these dyes with cancers of the gastrointestinal tract, liver, and bladder (Brown & De Vito, 1993; Golka et al., 2012). Carcinogenicity is believed to result from the formation of reactive intermediate metabolites, which can bind to DNA and initiate mutations that lead to cancer development. Animal studies, particularly in rodents, have shown an increased risk of liver and breast cancer with prolonged exposure to azo dyes (Arkan Majhool et al., 2023).

Furthermore, azo dyes have been found to impair the immune system. Their immunosuppressive properties make the body more susceptible to infections and diseases. This immunotoxicity is likely due to systemic inflammation and oxidative stress caused by the metabolic byproducts of azo dyes. Oxidative stress plays a critical role in the development of various diseases, including cancer, cardiovascular disease, and neurodegenerative disorders (Bladin, 2014; John et al., 2022). Reactive oxygen species (ROS) generated during azo dye metabolism contribute to lipid peroxidation, protein oxidation, and DNA damage, all of which are significant factors in disease development (Josephy & Allen-Vercoe, 2023).

The aim of this study is to assess the histopathological, hematological, and biochemical effects of azo dyes, specifically Brown HT, in juvenile rats. By evaluating the adverse impacts of this dye, the study seeks to establish a safe physiological dose and recommend optimal intake levels that minimize health risks, particularly in children. The ultimate goal is to promote safer food practices and protect public health by mitigating the potential hazards associated with azo dye consumption.

In recent years, the use of artificial food dyes has raised increasing concerns, particularly regarding their impact on consumer behavior and health. Brightly colored foods, such as chocolates, cakes, and biscuits, are often more appealing to children due to their visual attractiveness rather than their nutritional content (Schouteten et al., 2018). Among these, azo dyes, including Brown HT, are commonly used to enhance the appearance of food products. However, in countries like Bangladesh, many local bakeries use azo dyes in unsafe concentrations, which may pose serious health risks. These risks are especially concerning for children, as exposure to excessive amounts of azo dyes like Brown HT has been linked to potential adverse effects on growth, cognitive development, and overall health. This investigation focuses on the toxicity of Brown HT to better understand its impact on children's health and to promote safer food practices.

## 2. Materials and Methods

### *2.1 Study Area*

This study was conducted at the Department of Applied Nutrition and Food Technology, Islamic University, Bangladesh. The biochemical examinations were carried out at Doctor's Lab, Kushtia, and histological analyses were performed in the anatomical laboratory of National Medical College, Dhaka, Bangladesh.

### *2.2 Chemicals and Reagents*

The chemicals and reagents used in the study included Chocolate Brown HT (E155) sourced from ECHO Food Color and Aroma LTD, Uric Acid Liquicolor from Human GmbH, Germany, Creatinine Liquicolor from Diasys, Germany, SGOT (serum glutamic oxaloacetic transaminase) from Chronolab Ag, Switzerland, SGPT (serum glutamic pyruvate transaminase) from Tulip Group, India, Direct Bilirubin from Randox, UK, and Hematoxylin and Eosin (H&E) stain kits from Bio Lab Diagnostics I Private Limited.

### *2.3 Animal Grouping and Experimental Design*

Male Long-Evans rats, aged 4-5 weeks and in apparent good health, were obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The rats were acclimatized for one week under a controlled 12-hour light/dark cycle and provided with distilled water and standard laboratory feed, adhering to the guidelines of the National Research Council (2010). Following acclimatization, the rats were divided into four groups based on the study's objectives, designed to assess the effects of various concentrations of the azo dye Brown HT (E155) on physiological, biochemical, and histopathological parameters.

JC (Juvenile Control Group): No Brown HT exposure.

JLD (Juvenile Low Dose Group): Received 200 mg/kg body weight of Brown HT.

JMD (Juvenile Medium Dose Group): Received 400 mg/kg body weight of Brown HT.

JHD (Juvenile High Dose Group): Received 600 mg/kg body weight of Brown HT.

Each group consisted of four rats. The experimental period lasted six weeks, after which the rats were humanely sacrificed for further analysis of the study's parameters.

### *2.4 Blood Sampling*

At the end of the six-week period, the rats were sacrificed using ether anesthesia after being fasted for 12 hours. Blood samples were collected from the thoracic aorta, allowed to clot at room temperature for approximately 20 minutes, and centrifuged at 3000 rpm for 10 minutes at 4°C. The resulting serum was carefully aliquoted and stored at -80°C until needed for biochemical testing (Eraslan et al., 2007).

### *2.5 Hematological and Biochemical Analysis*

Hematological parameters were analyzed using the Sysmex XN-1000™ Hematology Analyzer, known for its reliability and precision. The hematological parameters measured included red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and differential counts for neutrophils, lymphocytes, monocytes, eosinophils, basophils, and reticulocytes.

Biochemical analyses were conducted using the Roche Diagnostics HITACHI cobas-c 311 analyzer, recognized for its accuracy and dependability. The following biochemical parameters were evaluated: serum triglycerides, serum cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), serum creatinine, SGOT (AST), SGPT (ALT), serum uric acid, and serum bilirubin. These tests provided comprehensive insights into the physiological changes induced by varying doses of Brown HT.

### *2.6 Histopathological Examination*

Histopathological analysis focused on the liver and kidney tissues of the rats. After dissection, small tissue samples were taken from each organ and immediately placed in 10% neutral buffered formalin to preserve cellular integrity. The tissues underwent a series of dehydration steps in graded ethanol solutions, followed by embedding in molten paraffin wax to form paraffin blocks. These blocks provided structural support for sectioning with a microtome. Tissue sections, approximately 5 µm thick, were carefully cut and placed on glass slides for staining. The sections were stained using Hematoxylin and Eosin (H&E), which highlights cellular structures and tissues, allowing for better visualization under a light microscope. The stained sections were then examined for any pathological changes, such as degeneration, necrosis, inflammation, or structural alterations in the liver and kidney tissues (Yousef et al., 2010).

### *2.7 Statistical Analysis*

The data from both the control group and the Brown HT-exposed groups were analyzed using one-way ANOVA, followed by Tukey's post-hoc test to compare the differences between groups. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS software (version 21 for Windows), ensuring robust interpretation of the study's findings.

This comprehensive methodology allowed for the accurate assessment of the physiological, biochemical, and histopathological effects of Brown HT in juvenile rats, providing crucial insights into the potential health risks associated with azo dye exposure.

## 3. Result

### *3.1 Physical and Biochemical Overview*

#### *Effects of Chocolate Brown HT on Body Weight Changes of Juvenile Rats*

The body weight changes of the juvenile rats over the course of the experiment were carefully monitored, as shown in Table 1. The control rats exhibited a consistent and gradual increase in body weight, which was expected given their healthy condition. However, the experimental groups exposed to Brown HT displayed a slower rate of weight gain compared to the control group. The low-dose group (JLD) did not show any significant deviation from the control group, indicating that the lower concentration of Brown HT had little to no effect on body weight. In contrast, the medium-dose (JMD) and high-dose (JHD) groups demonstrated significant differences in weight gain. Notably, the JHD group exhibited the slowest increase in body weight, with a rise of only 128.37% by the sixth week, compared to the control group. This suggests that higher doses of Brown HT may interfere with normal growth patterns in juvenile rats.

### *3.2 Effects of Chocolate Brown HT on Blood Counts*

The effects of Brown HT on the hematological parameters of juvenile rats were profound, as shown in Table 2. The administration of Brown HT significantly impacted red blood cell (RBC) counts, hemoglobin (Hb) levels, and indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The JHD group showed the lowest values for RBC, MCV, MCH, and MCHC, suggesting anemia-like symptoms in the rats exposed to higher doses of the dye. Conversely, the reticulocyte count increased proportionally with the dosage, indicating a compensatory response to the reduced RBC levels. The white blood cell (WBC) count, particularly neutrophils, monocytes, and basophils, also increased significantly in a dose-dependent manner. This rise in WBC suggests an inflammatory or immune response triggered by the dye. Interestingly, lymphocyte counts decreased significantly in all treated groups, indicating potential immunosuppression at higher dosages.

### *3.3 Effects of Chocolate Brown HT on Lipid Profile*

Table 1. Effects of chocolate brown HT on juvenile's body weight (gm)

| Groups | 1st week | 3rd week | 6th week |
|---|---|---|---|
| JC | 41.25±1.91[a] (100%) | 52.26±1.53[a] (126.69%) | 64.72±1.91[a] (156.90%) |
| JLD | 41.11±2.39[a] (100%) | 50.90±1.25[a] (123.81%) | 61.76±2.47[ab] (150.23%) |
| JMD | 41.34±2.28[a] (100%) | 48.96±1.93[ab] (118.43%) | 57.31±3.62[bc] (138.63%) |
| JHD | 41.07±2.71[a] (100%) | 45.84±1.64[bc] (111.61%) | 52.72±4.22[c] (128.37%) |

*Each value in the table is represented as mean ± SD (n = 4). Values in the same column followed by a different letter ([a-c]) are significantly different (P < 0.05).*

**Table 2.** Effects of chocolate brown HT on blood count of different groups of juvenile rats

| Hematological parameters | JC | JLD | JMD | JHD |
|---|---|---|---|---|
| RBC ($10^6$/μL) | 7.68±0.26[a] | 6.80±0.08[b] | 6.55±0.13[b] | 6.48±0.22[b] |
| WBC ($10^3$/μL) | 7.40±0.22[a] | 8.28±0.10[b] | 8.20±0.08[bc] | 8.55±0.21[b] |
| Hb (g/dL) | 8.93±0.26[a] | 8.13±0.17[b] | 8.10±0.14[b] | 8.13±0.24[b] |
| MCV (fL) | 66.08±1.97[a] | 60.18±1.40[b] | 59.58±0.58[b] | 58.08±1.34[b] |
| MCH (pg) | 10.68±0.22[a] | 9.73±0.10[b] | 9.58±0.28[b] | 9.40±0.22[b] |
| MCHC (g/dL) | 21.78±1.03[a] | 16.80±2.03[b] | 17.90±0.52[b] | 16.15±1.95[b] |
| Platelet Count ($10^3$/μL) | 632.50±18.93[a] | 700.00±8.16[b] | 747.50±12.58[c] | 767.50±15.0[c] |
| Neutrophils (% of WBC) | 20.00±1.41[a] | 24.25±1.50[b] | 26.75±1.89[bc] | 29.25±1.26[c] |
| Lymphocytes (% of WBC) | 70.00±1.41[a] | 60.75±2.06[b] | 60.50±1.73[b] | 56.50±1.91[c] |
| Monocytes (% of WBC) | 8.50±0.58[a] | 10.00±0.82[b] | 11.00±0.82[bc] | 11.75±0.50[c] |
| Eosinophils (% of WBC) | 1.25±0.50[a] | 1.00±0.82[a] | 1.25±0.96[a] | 0.75±0.50[a] |
| Basophils (% of WBC) | 0.25±0.50[a] | 1.00±0.00[ab] | 0.75±0.50[ab] | 1.50±0.58[b] |
| Reticulocyte Count (% of RBC) | 0.71±0.06[a] | 0.79±0.01[b] | 0.85±0.04[bc] | 0.90±0.02[c] |

*Each value in the table is represented as mean ± SD (n = 4). Values in the same row followed by a different letter ([a-c]) are significantly different (P < 0.05).*

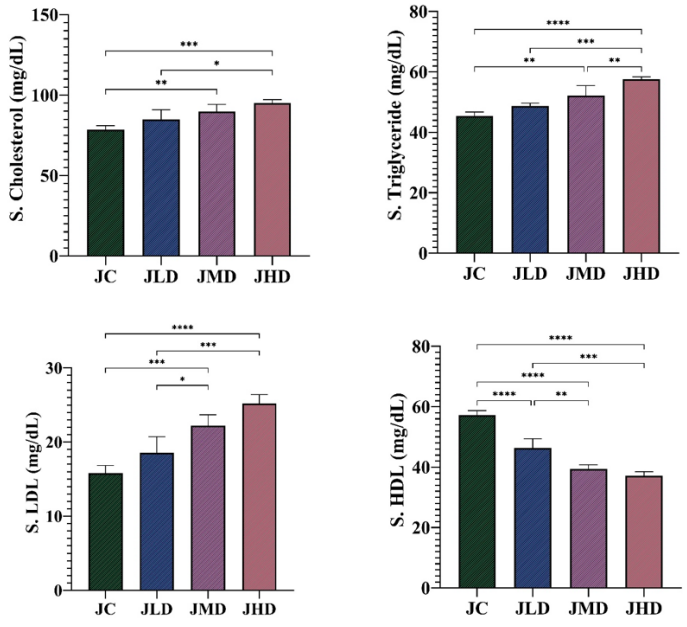


**Figure 1.** Effects of brown HT on lipid profile of juvenile rats from different groups. Asterisks (*) represent significant differences between groups (****p<0.0001, ***p<0.001, **0.01, *p<0.05)

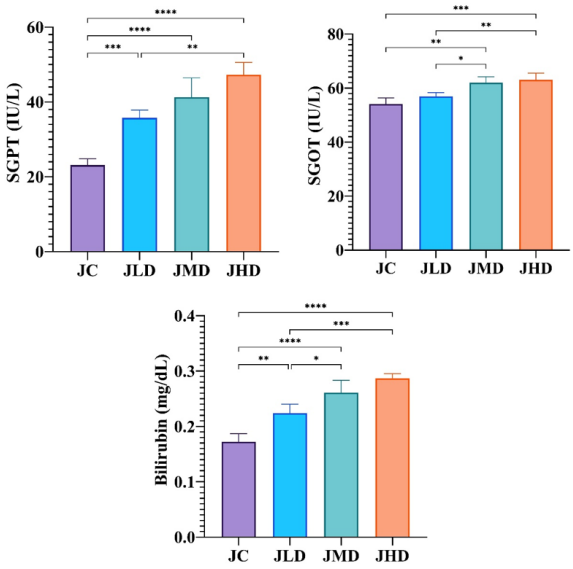


**Figure 2.** Effects of brown HT on liver function of juvenile rats. Asterisks (*) represent significant differences between groups (****p<0.0001, ***p<0.001, **0.01, *p<0.05).

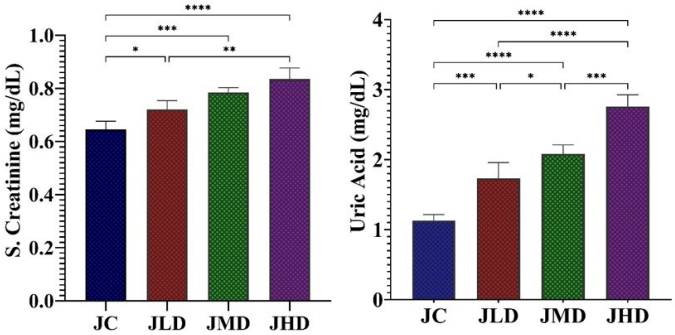


**Figure 3.** Figure 2: Effects of brown HT on renal function of juvenile rats. Asterisks (*) represent significant differences between groups (****p<0.0001, ***p<0.001, **0.01, *p<0.05)

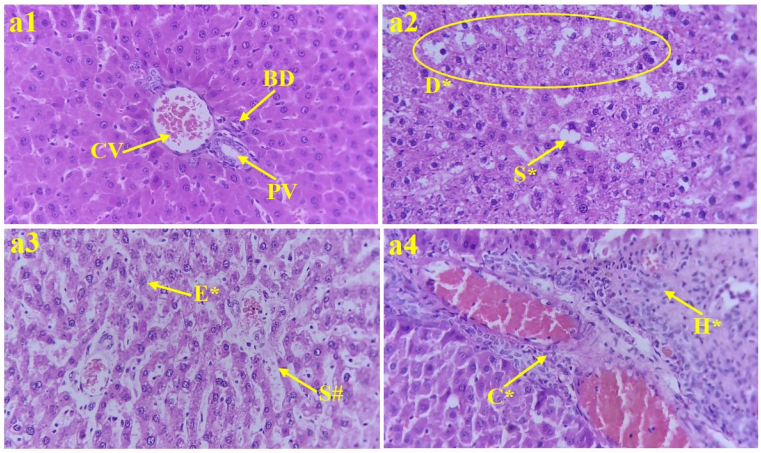


**Figure 4** An investigation into the hepatic histological changes induced by Brown HT (E-155) in juvenile rats. In the visualization of object x40 (Light Microscope Model: DC5V500mA) a section of the liver. (a1) Control Rats, (a2) Dose 200 mg/kg body weight, (a3) Dose 400 mg/kg body weight, (a4) Dose 600 mg/kg body weight. BD (Bile duct), CV (Central vain), C* (Centrilobular Necrosis), D* (Hepatocellular Damage), E* (Endothelial Cell Damage), H* (Hepatic Hemorrhage), PV (Portal vain), S* (Steatosis), S# (Sinusoidal Obstruction).

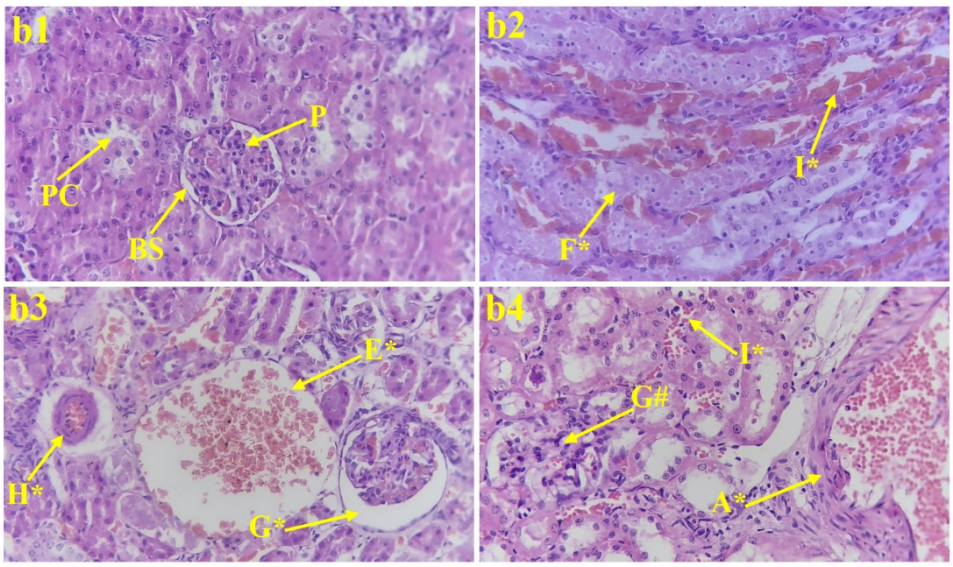


**Figure 5.** An investigation into the renal histological changes induced by Brown HT (E-155) in juvenile rats. In the visualization of object x40 (Light Microscope Model: DC5V500mA) a section of the liver. (b1) Control Rats, (b2) Dose 200 mg/kg body weight, (b3) Dose 400 mg/kg body weight, (b4) Dose 600 mg/kg body weight. A* (Arteriosclerosis), BS (Bowman's Space), E* (Interstitial Edema), F* (Interstitial Fibrosis), G* (Glomerular Atrophy), G# (Glomerulosclerosis) H* (Arteriolar Hyalinosis), I* (Interstitial Inflammation), P (Podocyte), PC (Proximal Convoluted Tubule).

The lipid profile of the experimental groups, presented in Figure 1, highlights the impact of Brown HT on cholesterol, triglycerides, and lipoprotein levels. The high-dose group (JHD) displayed the highest levels of serum cholesterol (94.98±2.24 mg/dL), triglycerides (57.6±0.77 mg/dL), and low-density lipoproteins (LDL) (25.2±1.22 mg/dL). These values were significantly higher than those of the control group ($p<0.001$), indicating that Brown HT may negatively affect lipid metabolism. High-density lipoproteins (HDL), which are considered beneficial, showed a marked decrease as the dosage of Brown HT increased. The alterations in lipid metabolism suggest a possible link between Brown HT exposure and an increased risk of cardiovascular diseases.

***3.4 Effects of Chocolate Brown HT on Liver Function***

The liver function of the juvenile rats, as indicated by the levels of liver enzymes and bilirubin, was significantly affected by the ingestion of Brown HT. The serum glutamic pyruvic transaminase (SGPT) levels peaked at 47.3±3.31 IU/L in the JHD group, with a dose-dependent increase observed across all treatment groups (Figure 2). Similarly, the serum glutamic oxaloacetic transaminase (SGOT) levels also showed a significant rise, with the JHD group reaching 63.03±2.48 IU/L, a highly significant difference compared to the control group ($p<0.001$). The elevated levels of these enzymes are indicative of liver stress or damage. Bilirubin concentrations also increased significantly in all treated groups, further confirming that Brown HT may have hepatotoxic effects in juvenile rats.

***3.5 Effects of Chocolate Brown HT on Renal Function of Juvenile Rats***

The renal function of the juvenile rats was evaluated through serum creatinine and uric acid levels. While the low-dose group (JLD) did not show significant changes in serum creatinine levels compared to the control, the medium-dose (JMD) and high-dose (JHD) groups exhibited highly significant differences ($p<0.001$). The creatinine levels in the JHD group reached 0.84±0.04 mg/dL, indicating impaired kidney function (Figure 3). Additionally, serum uric acid levels increased across all treated groups, with the JHD group exhibiting the highest level (2.76±0.17 mg/dL). These findings suggest that higher doses of Brown HT can induce renal stress, potentially leading to long-term kidney damage.

The physical, biochemical, and hematological data collected from the juvenile rats indicate that Brown HT exerts dose-dependent toxic effects, particularly on growth, blood parameters, liver, and kidney function. These findings highlight the potential health risks associated with the use of high doses of azo dyes like Brown HT in food products, particularly for vulnerable populations such as children.

**Histopathological Overview**

***3.6 Liver Specimen***

The histological analysis of liver tissues across treatment groups is depicted in Figure 4. In the control group (JC), normal liver architecture was evident, with well-defined bile ducts (BD), central veins (CV), and portal veins (PV), showing no signs of damage (Figure 4a1). However, early indications of toxicity were observed in the low-dose group (JLD, 200 mg/kg), as shown in Figure 4a2. The liver exhibited hepatocellular damage (D*) and moderate steatosis (S*), indicating fatty changes and early signs of liver injury. In the medium-dose group (JMD, 400 mg/kg), the lesions became more pronounced, with increased endothelial cell damage (E*) and sinusoidal congestion (S#), suggesting impaired blood flow and compromised liver vasculature (Figure 4a3). The high-dose group (JHD, 600 mg/kg) demonstrated the most severe pathological changes. This included extensive centrilobular necrosis (C*) and hepatic hemorrhage (H*), indicating widespread cell death and significant vascular disruption (Figure 4a4). These findings suggest that higher doses of Brown HT result in progressively worsening liver damage, with the most severe changes seen in the highest dose group.

***3.7 Kidney Specimen***

The kidney tissues from the control group (JC) exhibited intact architecture, with healthy podocytes (P), proximal convoluted tubules (PC), and Bowman's space (BS), all functioning normally (Figure 5b1). In contrast, the low-dose group (JLD, 200 mg/kg) displayed signs of interstitial fibrosis (F*), characterized by excessive extracellular matrix deposition, leading to tissue distortion and impaired kidney function. Additionally, interstitial inflammation (I*) was observed, indicating an immune response or ongoing injury to the kidney tissue (Figure 5b2).

The medium-dose group (JMD, 400 mg/kg) showed more severe damage, including interstitial edema (E*), which resulted in tissue swelling and potentially hindered renal function. Chronic changes such as glomerular atrophy (G*) were also noted, reflecting a reduction in the filtration surface area of the glomeruli. Arteriolar hyalinosis (H*) was present, marked by thickening of arterioles and the deposition of hyaline material, which restricted blood flow to the kidneys (Figure 5b3).

In the high-dose group (JHD, 600 mg/kg), slight arteriosclerosis (A*) was observed, indicating the thickening and hardening of arteries, which could further reduce blood flow and exacerbate renal dysfunction. This group also displayed severe interstitial fibrosis, similar to the low-dose group, but with greater severity. Additionally, glomerular sclerosis (G#) was evident, indicating irreversible kidney damage and significant loss of function (Figure 5b4). These findings suggest a dose-dependent worsening of kidney pathology, with the highest dose leading to substantial and potentially irreversible damage.

**4. Discussion**

Food manufacturers frequently add dyes to their products to enhance visual appeal, particularly in foods aimed at children. Azo dyes, like chocolate brown HT (E155), are popular for their vivid color and long-lasting appearance. However, these additives may carry health risks, especially in growing children. This study investigated the toxic effects of brown HT on juvenile rats, focusing on its impact on body growth, blood health, liver, and kidney functions.

The findings revealed a dose-dependent effect of brown HT on the body weight of juvenile rats. High dosages significantly hindered weight gain, aligning with previous studies, such as Hassan & Salman (2016), which found that prolonged brown HT exposure led to reduced body weight in treated animals. This reduction could be due to the dye's interference with metabolic processes or nutrient absorption. The rats in the low-dose group did not show significant weight differences compared to the control group, suggesting that the impact of brown HT becomes more pronounced at higher doses.

The blood analysis results provided further insight into the toxic effects of brown HT. The study found a marked reduction in hemoglobin levels and red blood cell counts, pointing to a risk of macrocytic anemia. This is likely due to the suppression of erythropoiesis, the process of red blood cell production, in the bone marrow. Similar findings have been observed in studies using other synthetic azo dyes, including the work of AL-Shinnawy (2009). The disruption in red blood cell production mirrors results from other azo dye variations, such as sunset yellow and tartrazine, as documented by Aboel-Zahab et al. (1997).

Conversely, white blood cell (WBC) counts, particularly lymphocytes, increased significantly in the treatment groups. The elevated WBC levels suggest an immune response triggered by the dye, which may be the body's defense against the harmful effects of the dye (Koller & Roan, 1980). An increase in platelet counts was also observed, which could indicate an immune response to tissue damage or inflammation caused by brown HT exposure (Reza et al., 2019).

Liver enzyme levels, particularly serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT), were significantly elevated in the medium and high-dose groups. Elevated enzyme levels are well-established markers of liver damage, with prior studies (Hassan & Salman, 2016; El-Wahab & Moram, 2013) confirming similar trends. The rise in these enzymes suggests that brown HT may cause liver cell damage, prompting the release of these enzymes into the bloodstream (Westlake et al., 1981).

Lipid profile abnormalities further indicated hepatic dysfunction. High levels of serum cholesterol and triglycerides in the treatment groups point to impaired lipid metabolism, a common outcome of liver damage. Sharma et al. (2009) found that high triglyceride levels could result from the liver's inability to produce adequate hepatic lipase, leading to fat accumulation. The study's findings also aligned with Singh et al. (1988), who reported that elevated cholesterol levels signal disrupted enzyme activity in lipid processing.

Additionally, elevated serum creatinine and uric acid levels, particularly in the high-dose group, suggest impaired renal function. These results are consistent with those of Crawley et al. (2022) and Elbanna et al. (2017), who also reported kidney damage following azo dye administration in rats. The dye's impact on kidney function, even at low doses, raises concerns about its safety, especially in vulnerable populations such as children.

The histopathological analysis of liver and kidney specimens from juvenile rats treated with various doses of chocolate brown HT (E155) provides significant insights into the toxic effects of azo dyes on these vital organs. The control group (JC) demonstrated a healthy liver architecture, with intact bile ducts (BD), central vein (CV), and portal vein (PV) systems, indicative of normal liver function (Figure 4; a1). This finding aligns with established histopathological standards, where the absence of pathological changes suggests proper liver function (Palipoch & Punsawad, 2013; Tsomaia et al., 2020).

In contrast, the low-dose group (JLD), receiving 200 mg/kg body weight, exhibited initial signs of hepatic lesions, including hepatocellular damage (D*) and steatosis (S*). Hepatocellular degeneration is a concerning indicator of potential hepatitis, which is consistent with the findings of Soltan & Shehata (2012) regarding toxic insults to liver cells. Steatosis, characterized by excessive lipid accumulation in hepatocytes, is often the first indication of non-alcoholic fatty liver disease (NAFLD) and results from metabolic disruptions caused by toxic substances (Elekima et al., 2019). These findings underscore that even at low concentrations, azo dyes can initiate pathological changes that may lead to impaired liver function.

The effects of E155 were more pronounced in the medium-dose group (JMD), which received 400 mg/kg body weight. This group displayed significant endothelial cell damage (E*) and sinusoidal obstruction (S#) (Figure 4; a3). Endothelial cell damage is critical as it highlights vascular injury within the liver, resulting in diminished blood flow and compromised liver function (Himri et al., 2011). Sinusoidal obstruction, characterized by blockage of small liver capillaries, exacerbates these issues by causing congestion and increased stress on liver tissues. These observations corroborate findings from Şensoy et al. (2024), which reported similar vascular impairments associated with toxic agents.

## Author contributions

T.I. and N.C.M. conceptualized the study and designed the research methodology. I.A.S. and A.K.T. were responsible for data collection and contributed to the analysis. S.A.R. and M.A.S. performed data

interpretation and provided critical revisions to the manuscript. I.S. supervised the overall research, provided intellectual input, and contributed to manuscript writing. All authors reviewed and approved the final version of the manuscript.

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## Competing financial interests

The authors have no conflict of interest.

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