Protective Effect of Riboflavin Against Tartrazine Induced Toxicity of Spleen in Albino Mice *(Mus Musculus)*

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Abstract

Introduction: Tartrazine, an azo colorant, is the most often used artificial food coloring in food items, including confectionery, soft drinks, canned juices, sauces, and many more. It cause several health diseases, including allergies, migraines, melancholy, and anxiety. In this study, the researchers aimed to investigate the effects of tartrazine withdrawal on the spleens of mice as well as the role that riboflavin plays in recovery. ***Methodology*:** 24 adult male albino mice used in this study, divided into three groups: the Control Group, the Dose Group I, and the Dose Group II. To evaluate lipid profiles, antioxidants, enzymes, and risk factors, blood samples were taken. After being removed, the spleen underwent metamorphic procedures. ***Results:*** Body and organ weights were higher in the tartrazine-treated group. Nevertheless, in comparison to the control and Dose Group II mice, the final body and organ weights were decreased. When compared to the control group, the tartrazine-treated group also showed notable abnormalities in the spleen surfaces. Nonetheless, there were not many notable differences between the Tartrazine and Riboflavin-treated group and the control group. ***Conclusion***: this study demonstrated that the recovery function of riboflavin, when combined with tartrazine withdrawal, is more successful in recovering spleen toxicity and other parameters. The findings of this study indicate that there should be close monitoring of the use of tartrazine in high-consumption meals, particularly for children.

Index terms: Toxicology, Tartrazine, Riboflavin, Spleen, Albino mice.

Introduction

Tartrazine, also referred to as E102, is a synthetic coloring agent that is a member of the mono-azo dyes group and has been linked to a number of health issues [1] In spite of this, it is frequently utilized in food preparations for desserts, candy fruits, soft drinks, pickles, ice cream, canned juice, and more. Animal studies have revealed that exposure to a hazardous dosage of Tartrazine may lead to inflammatory responses, bleeding Clogged blood vessels, necrosis, fibrosis, numerous granuloma lesions in internal organs, astrocyte proliferation, and gliosis in the brain [2] In addition to its application in food, Tartrazine is also employed in the pharmaceutical and cosmetic sectors for its yellow hue. However, several azo dyes, including Tartrazine, have been demonstrated to have carcinogenic and mutagenic activities and may induce allergies. The toxicity of components tends to grow with the number of benzene rings in their structure, and the carcinogenicity of azo dyes relies on the degradation process and the molecule's structure [3].

Consumption of Tartrazine in excessive doses may disrupt enzyme activity in vivo, which can have detrimental repercussions for digestion. Therefore, stringent management of tartrazine dosage in high-consumption meals, particularly among youngsters, is necessary. Tartrazine has been connected to many health conditions, such as hypoxia, sleeplessness, depression, anxiety, headaches, itching, weakness, and impaired vision [4].

Studies have also indicated that Tartrazine may produce diverse pathological abnormalities, including red pulp hemorrhages, vacuolation of specific splenic cells, localized hyperplasia of the white pulp, and capsular and parenchymal fibrosis. Additionally, there is evidence of a substantial rise in vascular endothelial protein (VEGF) and endothelial gas synthase (e-NOS) immunolabeling [5].

However, a single dose injection of Tartrazine at a dosage of up to 6250 mg /Kg B.W. did not result in any fatalities in animals and the drug was categorized as virtually harmless according to the Hodge & Sterner scale [6].

2. Research design and methods

2.1 Animal Housing:

To coordinate the evaluation, 30 Swiss Webster Mus musculus mice were obtained from the Veterinary Research Institute of Lahore and put in under-regulated circumstances, comprising a 12-hour day/night time cycle, temperatures of 27±2°C, and humidity of 40-50 percent. They were kept in iron cages of 24 inches in length and 12 inches in breadth and given with Chick diet No.14, a highly nutritious diet containing minerals, proteins, and vitamins. The mice were given water in glass bottles, which were replaced every day.

The research aims to evaluate the adverse effects of Tartrazine (Taz) on the spleen of mice. Four-week-old mice were utilized in the experiment, with the Control group labeled as (C) and the experimental group I designated as Tartrazine. Experimental group II was labeled as TARTRAZINE + RIBOFLAVIN and was given a dosage of 135 mg/kg B.W of mice. Tartrazine was dissolved in 10 ml of distilled water and

delivered to the mice intraperitoneally using an insulin syringe.

The Control group (C) was given clean water. In contrast, experimental group I was given Tartrazine, and experimental group II was given Tartrazine + Riboflavin orally at 135 mg/kg/B.W. for 21 consecutive days. The dosage was supplied daily using a syringe, with 0.1 ml injected into the body. Blood extraction, the spleen, ovaries, and kidneys were surgically removed from the mice for additional examination. These organs were moved to saline for subsequent treatments.

2.2 Morphological Assessment: The spleen was thoroughly examined under a binocular stereoscopic magnifying lens, and any morphological anomalies were reported. Once located, the spleen was captured using a sophisticated camera. ***2.3 Statistical Analysis:*** The data collected was examined using a computer-based system. Unpaired T-tests were run, and a probability level of $p<0.001$ was utilized for evaluation.

3. Results:

3.1 Morphological Analysis

Morphological analysis was performed on the spleens of treated mice, including the control group and dose groups TAZ I (Taz 135mg/kg B.W.) and TAZ II (Taz 135mg/kg B.W. and R.B. 2mg/kg B.W.). The investigation attempted to evaluate any morphological anomalies produced by TAZ.

Control Group: The spleens of the control group seemed normal, with a smooth and shiny appearance. The red and white pulp of the spleen were typical.

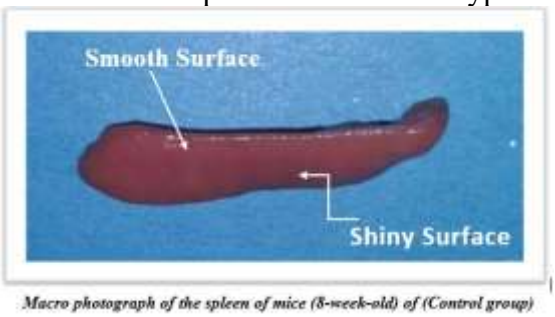

Figure 01: Micro photograph of spleen of mice (Control group)

Experimental Groups:

Dose Group TAZ I (Taz 135mg/kg B.W.): Morphological examination revealed different abnormalities in the spleens, such as abnormally shaped lobes and dullness in their shine. The organs revealed abnormalities, such as wrinkles and changes in surface color. Some of the treated animals showed a decrease in the right and median lobes and a distortion in the gall bladder's anatomy. Additionally, the spleen revealed several dark stains suggestive of the degradation of marginal zones positioned between the red and white pulp of the spleen in the treated TAZ I group (Taz 135mg/kg B.W.)

Figure 02: Micro photograph of spleen of mice (Dose group TAZ I)

Dose Group TAZ II (Taz 135mg/kg B.W. and 2mg/kg R.B.): The spleens in the TAZ II group seemed normal with a smooth and shiny surface, and the red and white pulp of the spleen was usual. These findings indicate that Riboflavin performed a significant function in minimizing the abnormalities reported.

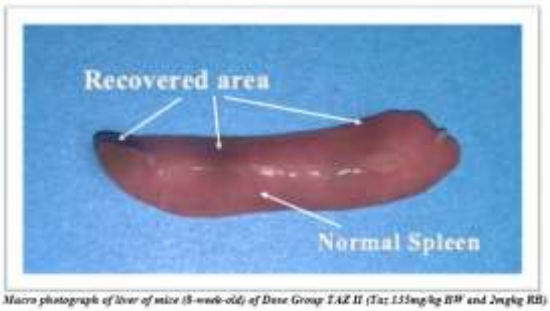

Figure 03: Micro photograph of spleen of mice (Dose group TAZ II)

Morphometric Analysis:

3.2.1Body weight:

Control group: The mice in the control group had an initial average body weight of 16.1 ± 4.6g, and their average body weight on the last day of oral treatment was 17.4 ± 4.8g, resulting in a calculated percentage body weight gain of 51.5%.

Experimental Groups

Dose Group TAZ I (135mg/kg B.W.):

Their initial and final body weights were recorded to measure the percentage body weight gain of the mice in dose group TAZ I (135mg/kg B.W.). The average initial body weight was 16.1 ± 4.6g, and the average final body weight was 17.4 ±

4.8g, resulting in a percentage body weight gain of 28.73g. This value was significantly lower ($P < 0.001$) than the control group.

Dose Group TAZ II (135mg/kg B.W. + R.B. 2mg/kg B.W.): The mice in dose group TAZ II (135mg/kg B.W. + R.B. 2mg/kg B.W.) had an average initial body weight of 18.1 ± 5.3g and an average end body weight of 26.4 ± 4.2g, resulting in a percentage body weight gain of 45.8%. This value was dramatically higher than dose group TAZ I (135mg/kg B.W.) with a lower proportion of weight gain. The spleen in this group had a normal appearance with smooth surfaces and no wrinkles, unlike dose group TAZ I (135mg/kg B.W.), and the black stains on the surface of the spleen were restored to normal after treatment with R.B.

Dose Group TAZ II (135mg/kg BW+ RB 2mg/kg BW):

The average initial body weight of mice of dosage group TAZ II (35mg/kg B.W. + R.B. 2mg/kg B.W.) was 18.1 ± 5.3g, whereas the average final weight of dose group TAZ II (135mg/kg BW+ RB 2mg/kg) was 26.4 ± 4.2g. It showed a %age body weight rise of 45.8 and was substantially more significant than the %age bodyweight of mice of dose group TAZ I (TAZ 135mg/kg) 28.73.

Weight	Control group (mean ± SEM)	TAZ I (135mg/kg B.W.) (mean ± SEM)	Dose Group TAZ II (mean ± SEM)
Initial body weight (g)	16.1 ± 4.6	17.4 ± 4.8	18.1 ± 5.3
Final body weight (g)	24.4 ± 4.8	22.4 ± 6.2***	26.4 ± 4.2***
Body weight (g) gain	8.3± 3.2	5± 2.2***	6.3±3.5***
% Age weight	51.5 %	28.73 %***	45.8%***

Table No: I Comparison of Body Weights of Mice of Control, Dose Group TAZ I (135mg/Kg BW) and Dose Group TAZ II (35mg/Kg BW + RB 2mg/Kg BW

Note. Results were represented as ***p < 0.001.

↕ Increase in value
Decrease in value

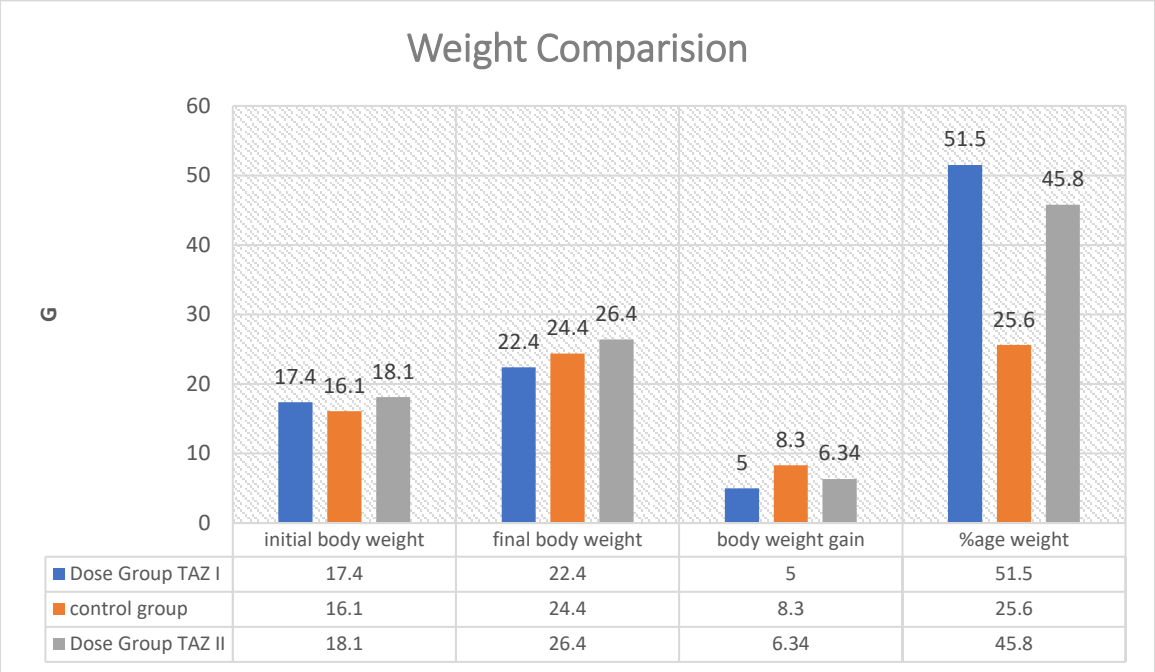

Figure 04: shows the comparison of body weights of the control group, dose group I, and dose group II.

Table 2: Comparison of the lipid profile of Control Group, Dose Group TAZ I (135mg/kg BW), and Dose Group TAZ II (135mg/kg BW + RB 2mgkg BW)

Groups	CHOLESTROL	TRIGLYCERIDES	LDL U/L	HDL U/L	vLDL U/L	TOTAL LIPIDS
	Comparison of the values of Control Group and Dose Group I					
Control	41.5 ± 0.56	33.0 ± 0.66	65.5 ± 0.52	16.5 ± 0.52	10.06 ± 0.11	435.10 ± 2.13
Tartrazine	70.2 ± 0.78*** ⬆	45.0 ± 2.10*** ⬆	44.65 ±0.08*** ⬇	12.7 ± 0.48*** ⬇	12.22 ±0.06*** ⬆	403.60 ± 1.42*** ⬇
	Comparison of the values of Dose Group TAZ I and Dose Group TAZ II					
Tartrazine	70.2 ± 0.78	45.0 ± 2.10	44.65 ±0.08	12.7 ± 0.48	12.22 ±0.06	403.60 ± 1.42
Taz and Riboflavin	42.6 ± 0.69*** ⬇	33.5 ± 0.52*** ⬇	67.35 ±0.17*** ⬆	16.8 ± 0.42*** ⬆	8.86 ± 0.52*** ⬇	429.60 ± 2.67*** ⬆

Note. ***p < 0.001 ⬆ **Increase in value** ⬇ **Decrease in value**

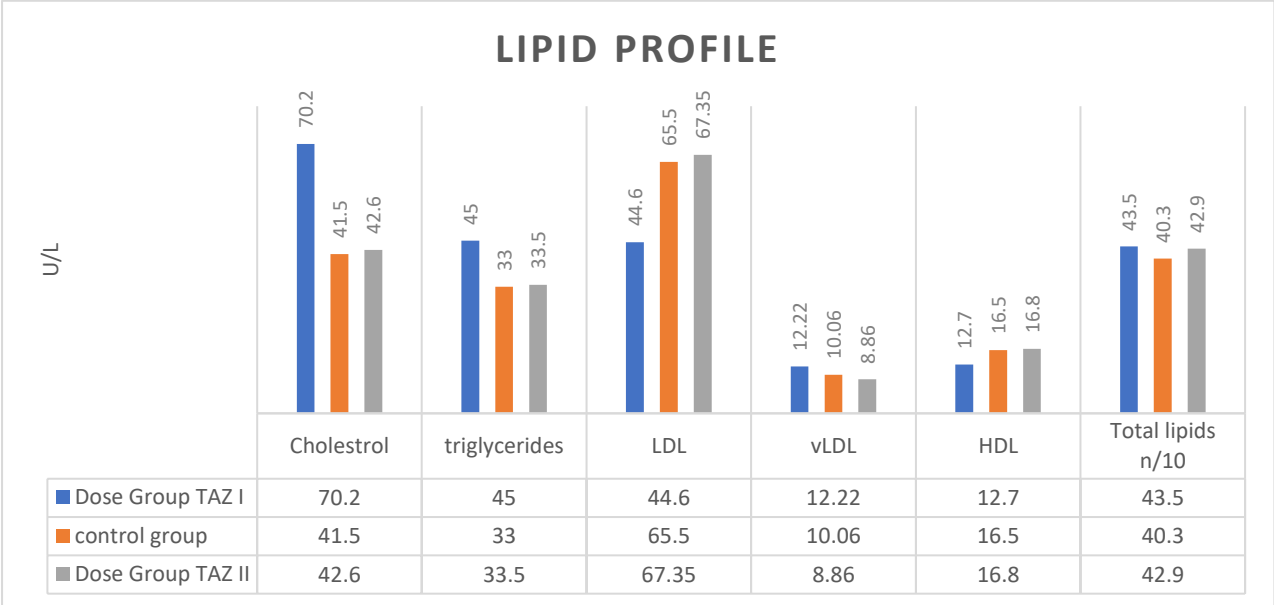

Figure 05: Comparison of the lipid profile of Control Group, Dose Group TAZ I and Dose Group TAZ II.

Table: 3. Comparison of the values of Protein Profile of Control Group, Dose Group TAZ I (TAZ 135mg/kg) and Dose Group TAZ II (TAZ 135mg/kg + RB 2mg/kg)

Groups	ALBUMIN (g/dl)	GLOBULIN (g/dl)	A/G RATIO (%)	BILIRUBIN (mg/dl)	TOTAL PROTEINS (g/dl)
	Comparison of the values of Control Group and Dose Group I				
Control	5.15 ± 0.10	4.24 ± 0.13	1.22 ± 0.04	6.04 ± .03	9.17 ± 0.11
Tartrazine	3.86 ± 0.02*** ⬇	3.21 ± 0.12*** ⬇	3.40 ± 0.16*** ⬆	8.24 ± 0.02*** ⬆	14.48 ± 0.11*** ⬆
	Comparison of the values of Dose Group TAZ I and Dose Group TAZ II				
Tartrazine	3.86 ± 0.02	3.21 ± 0.12	3.40 ± 0.16	8.24 ± 0.02	14.48 ± 0.11
Taz and Riboflavin	4.71 ± 0.19*** ⬆	4.23 ± 0.11*** ⬆	1.23 ± 0.01*** ⬇	6.34 ± 0.08*** ⬇	7.26 ± 0.12*** ⬆

Note. ***p < 0.001

⬆ **Increase in value**

⬇ **Decrease in value**

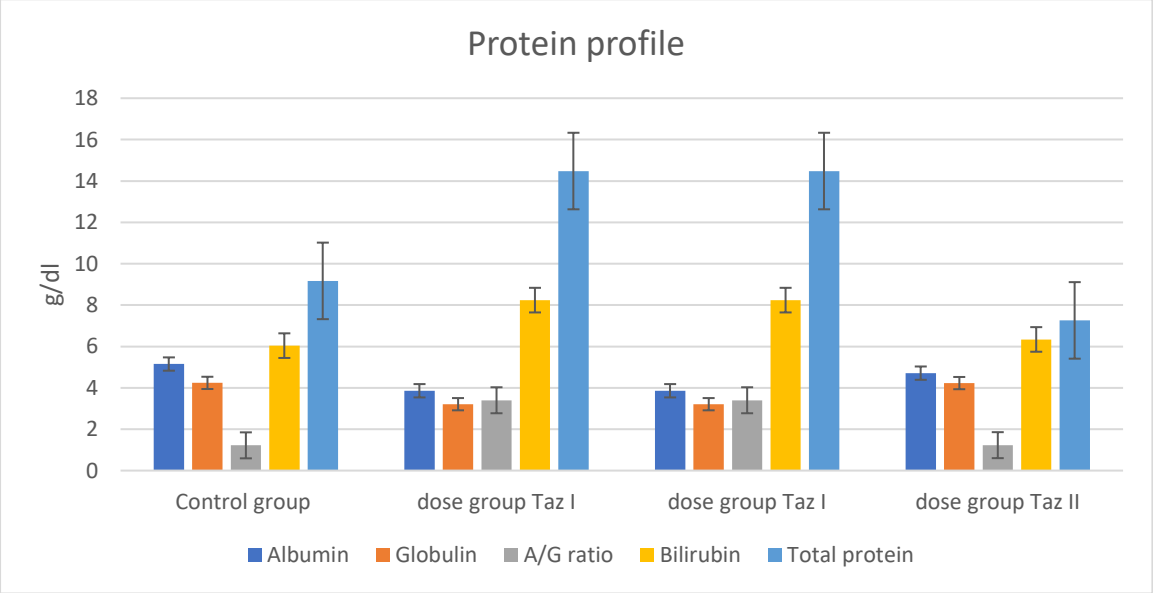

Figure: 06 Comparison of the values of Protein Profile of Control Group, Dose Group TAZ I (TAZ 135mg/kg) and Dose Group TAZ II (TAZ 135mg/kg + RB 2mg/kg)

Table: 4. Comparison of the values of Oxidant and anti-oxidant of Control Group, Dose Group TAZ I (TAZ 135mg/kg), and Dose Group TAZ II (TAZ 135mg/kg + RB 2mg/kg)

Oxidant/ anti oxi	CONTROL	TARTRAZINE	TARTRAZINE	TRZ + RIBOFLAVIN
MDA (µmol/ml)	0.87 ± 0.00	2.24 ± 0.69*** ⬆	2.24 ± 0.69	0.91 ± 0.1*** ⬇
GHS (mmol/L)	8.0 ± 0.11	7.23 ± 0.21*** ⬇	7.23 ± 0.21	7.98 ± 0.11*** ⬆

Note. Results were represented as ***p < 0.001.

⬆ **Increase in value**
⬇ **Decrease in value**

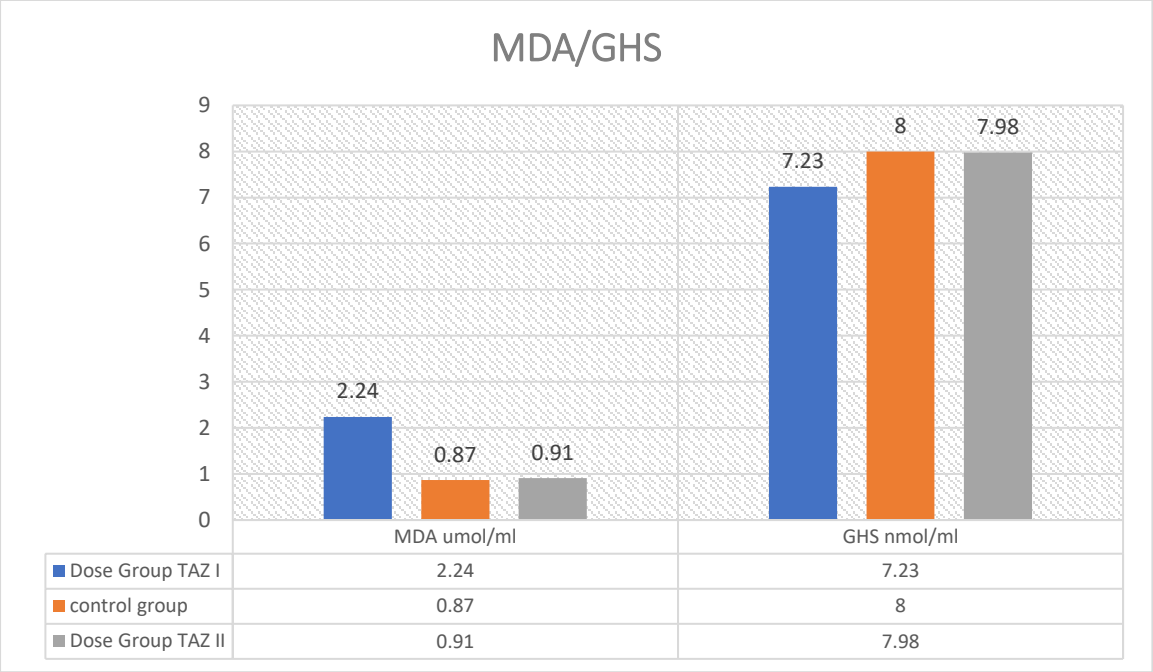

Figure: 07 Comparison of the oxidant and antioxidant of Control Group, Dose Group TAZ I and Dose Group TAZ II

3. Discussion

Tartrazine, a yellow azo color commonly used in edible food goods and the pharmaceutical industry, has been related to different health concerns, particularly among children. To determine the safe daily consumption of Tartrazine, we conducted toxicological tests to examine its effects on the spleen of albino mice [7]. Riboflavin, a water-soluble vitamin that plays a vital role in electron transfer and enzymatic activities in the body, has been discovered to alleviate oxidative stress.

Induced by free radicals and prevent tissue ischemia-reperfusion injury [8]. Recent studies have revealed that Riboflavin can help prevent kidney damage caused by harmful substances such as Tartrazine [9].

Our research focused on morphological and morphometric analysis, lipid and protein profiles, and levels of antioxidants

and stress indicators. Morphological investigations of control mice indicated no structural problems in the spleen, which appeared smooth and shiny. In contrast, mice in the experimental group (dose group TAZ I, Tartrazine 135mg/kg) displayed structural abnormalities in spleen architecture. This finding is confirmed by recent investigations showing that Tartrazine treatment can cause hepatic construction bias and degeneration in other organs of mice [10]. Fetuses treated with Tartrazine also exhibited dark brown pigmentation and destructed and necrotic renal tubules. However, the morphological deficiencies in the spleen were partially restored in mice treated with Tartrazine and Riboflavin (dosage group TAZ II, Tartrazine 135mg/kg and Riboflavin 2mg/kg), making the organ more similar to that of the control group [11]

In the present study Morphometric analysis In the dose group, TAZ I (TAZ 135mg/kg), the analysis exhibited a significant reduction in body weight as equated to the control group (TAZ I vs. Control Group 22.4 ± 6.2g vs. 24.4 ± 4.8g ± SEM) and on the contrary dose group TAZ II (TAZ 135mg/kg and RB 2mg/kg) exhibits significant ($P \geq 0.001$) increase in the bodyweight of mice when compared with the mice of the dose group I (TAZ 135mg/kg) [12]In contrast, dose group TAZ II displayed a significant increase in body weight compared to TAZ I but stayed near the control group's weight. In a prior study, tartrazine-treated mice likewise exhibited a considerable blockage in body weight increase compared to the control group, although riboflavin supplementation resulted in significant weight gain [13].

In the present study, serum enzymes (AST, ALT, ALP, and LDH) were used as a hepatic marker, the enzymatic activity in the mice of dose group TAZ I (TAZ 135mg/kg) was raised evidently as linked to the Control group. While the level of enzymes (AST, ALT, ALP, and LDH) in the dose group TAZII (TAZ 135mg/kg and RB 2mg/kg) represents a significant decrease as compared to dose group TAZ I (TAZ 135mg/kg) and also found to be was very close to the control group. Another study showed an increase in serum ALT, AST, and ALP activities using 500 mg of tartrazine/kg of BW/day in young mice during 30 days [14-15].

In the present study dose group TAZ II (TAZ 135mg/kg and RB 2mg/kg) showed a rise in complete protein profile, while albumin and globulin were significantly ($P \geq 0.001$) decreased, but the A/G ratio, bilirubin, and total protein significantly ($P \geq 0.001$) increased as compared to dose group TAZ I (TAZ 135mg/kg). In the previous studies Plasma enzyme activities of ALT and AST were greater in RD than in CPF, indicating the recovery role of Riboflavin (RF) in enzymatic activities [16].

In the present study, the lipid profile, Level of HDL, LDL, and total lipids content significantly ($P \geq 0.001$) decreased in the mice of the dose group TAZ I (TAZ 135mg/kg); however, the cholesterol, triglycerides, and vLDL were evidently ($P \geq 0.001$) increased as linked to the mice of the control group reported a decrease in blood serum cholesterol value but a rise in triglyceride value in mice after treatment with diverse Azo dyes. Previous studies described an increased value of triglyceride and cholesterol due to the impact of Azo-dyes [17].

Our studies showed a significant ($P \geq 0.001$) drop in the GSH value of the mice of the dose group TAZ I (TAZ 135mg/kg) as compared to the GHS value of the

control group. Regarding the antioxidant system, CAT, GSH-Px, and SOD enzymes served as the first line against oxidative injury and decomposed O2 and H2O2 before their interaction to form a more harmful hydroxyl radical. At the same time, it decreased the reduced glutathione (GSH) levels and total antioxidant capacity [18-19].Whereas the dose group TAZ II (TAZ 135mg/kg and RB 2mg/kg) showed a significant raise in the GSH value of the albino mice as compared to the TAZ I (TAZ 135mg/kg), thus indicating the RB had played a recovery role against toxicity [20]

4. Conclusion:

Vitamins like Riboflavin may play a significant function in dangerous

detoxification agents, which may cause severe disorders in the body of people. Long-term usage of Tartrazine in excess may affect the critical organs of albino mice, causing issues with splenic toxicity. This food dye must be used with care, particularly for goods under usage by children.

Acknowledgment

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