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Histopathological Effects of Tartrazine on Rat Brain: Implications for Plant-Based Food Additives

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ABSTRACT

This study investigates the impact of tartrazine, a synthetic azo dye widely used as a food colorant and potential contaminant in plant-derived food products, on the histological structure of the brain in adult male rats. Understanding the neurotoxic effects of such additives is crucial, given their prevalence in processed foods and potential interactions with bioactive compounds from medicinal plants. Thirty adult male Sprague Dawley rats were divided into three groups: a control group (distilled water), a low-dose tartrazine group (7.5 mg/kg), and a high-dose tartrazine group (9 mg/kg). Treatments were administered orally for 60 days. Brain tissues were then collected for histological examination. Microscopic examination revealed significant changes in brain tissue in both tartrazine-treated groups. The low-dose group showed oligodendrocyte necrosis, astrocyte damage, and neuron degeneration. The high-dose group exhibited severe astrocyte atrophy and necrosis, macroglia necrosis, neuron degeneration, and hemorrhage. Long-term exposure to tartrazine at high concentrations induces substantial histological damage in rat brain tissue. Given the potential for tartrazine to be present in or interact with plant-derived food products, further research is warranted to understand the combined effects and potential mitigation strategies using plant-based protective agents.

Keywords: Tartrazine, Dye, Brain, Tissue, Toxic

INTRODUCTION

Human culture has traditionally incorporated color additives, with evidence of cosmetic colors dating back to 5000 B.C. and food colorings around 1500 B.C. Color is a crucial factor in food selection, and synthetic colorants are widely used in the food industry to enhance visual appeal due to their stability, affordability, and coloring capabilities. An estimated 80,000,000 tonnes of colorant are produced annually worldwide. However, concerns regarding the safety and potential health impacts of synthetic colorants have led to increased interest in natural alternatives, including those derived from medicinal plants and their by-products [1].

Synthetic colorants, including azo compounds (such as amaranth and tartrazine), quinoline yellow derivatives, triarylmethanes, xanthenes (like erythrosine), and indigo colorants, represent a significant portion of food colorings. However, growing concerns about their safety have spurred research into natural alternatives derived from plants, including by-products of medicinal plant processing [2]. Azo compounds, including tartrazine, represent a major class of synthetic colorants widely used in food coloring. Given their prevalence in processed foods, it is important to understand their potential interactions with plant-derived compounds and extracts that are increasingly incorporated for nutritional or medicinal purposes [3].

Artrazine (E102), a synthetic azo dye imparting a lemon yellow hue, is widely used as a food colorant in various products, including those enriched with plant-derived ingredients or extracts. Its presence in foods consumed alongside plant-based components raises questions about potential interactions and combined effects on human health, particularly in the context of traditional diets in developing nations. The dye consists primarily of trisodium 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[azo[(4-sulfophenyl)]-1H-pyrazole-3-carboxylate, with sodium chloride and/or sodium sulphate as the main uncolored components [4].

Artrazine, derived from petroleum and marketed as FD&C Yellow 5 in the US, is metabolized in the gut, potentially forming carcinogenic compounds. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommends a daily intake of around 7.5 mg/kg b.w. Given these concerns, research is exploring the potential of plant-derived compounds to mitigate the adverse effects of tartrazine and other synthetic food colorants [5, 6].

Beyond its use in cosmetics, tartrazine is employed in sanitizing solutions for food processing equipment, raising concerns about potential residues and interactions with plant-derived ingredients or extracts used in food formulations.

Tartrazine finds widespread use in the food industry, including in products that may also contain plant-derived ingredients or extracts, such as soft drinks, breakfast cereals, and flavored snacks. Notably, it is sometimes used as a cheaper substitute for saffron in cooking, particularly in developing nations, which raises questions about its impact on the overall nutritional and medicinal properties of traditional dishes. Furthermore, tartrazine is used in pharmaceutical coatings and, to a lesser extent, in leather dyeing within the agricultural sector [7].

Previous research suggests that tartrazine can contribute to behavioral issues in children and adverse health effects in humans, including genotoxicity and organ impairment. Given the widespread consumption of tartrazine in conjunction with plant-based diets, it is important to investigate potential interactions and explore the protective effects of plant-derived compounds against tartrazine's toxicity [8].

Sensitivity reactions to tartrazine can occur through ingestion or skin contact, with some individuals exhibiting symptoms even at low concentrations. Given the potential for tartrazine exposure in conjunction with plant-based diets, research is needed to explore whether certain plant-derived compounds can mitigate these sensitivity responses. Reported effects include rashes, asthma attacks, and potential links to thyroid issues and behavioral disorders in children [9]

Studies have shown that tartrazine can exacerbate atopic dermatitis by increasing sulphido-leukotriene production in susceptible individuals. Given the potential for plant-derived compounds to modulate immune responses and alleviate atopic dermatitis symptoms, further research is warranted to explore whether specific plant extracts can counteract tartrazine's effects in these individuals [10].

Given the widespread use of tartrazine, its potential to interact with plant-derived components in the diet, and the growing interest in plant-based solutions to mitigate toxicity, this study aims to evaluate the histological effects of tartrazine on brain tissue in rats. By characterizing the neurotoxic effects of tartrazine, this research seeks to lay the groundwork for future investigations into the potential of medicinal plants or their by-products to protect against or counteract the adverse effects of this synthetic food colorant.

MATERIAL AND METHODS

Animals for the Experience

1. **Experimental Animals:** Thirty adult male Sprague Dawley rats, weighing 190-200 grams and aged 8-12 weeks, were obtained from the College of Science, University of Kufa. Upon arrival at the animal facility in the Department of Life Sciences, College of Education for Girls, the rats were housed in aluminum cages (5 rats per cage) with metal mesh covers and allowed to acclimate for two weeks under controlled conditions (air, lighting, moderate temperature, water, and standard rodent chow). All procedures were conducted in accordance with ethical guidelines for animal research.
2. **Tartrazine Treatment:** Tartrazine coloring powder (Shanghai Color India) was obtained from Al-Tayf Office for Chemical Equipment (Baghdad). Two concentrations were prepared: a low dose of 7.5 mg/kg (equivalent to the human Acceptable Daily Intake) and a toxic dose of 9 mg/kg. The tartrazine was dissolved in 1 ml of distilled water and administered orally via a stomach tube daily for 60 days. The control group received only distilled water.
3. **Histological Preparation:** Following the 60-day treatment period, the rats were euthanized, and their brains were immediately extracted and fixed in 10% formalin solution. The tissue was then processed for histological sectioning following standard procedures [Suvarna et al., 2019]. Paraffin-embedded tissue was sectioned at 5 µm, stained with hematoxylin and eosin (H&E), and examined under a light microscope.
4. **Image Analysis:** Representative images of brain sections from each group were captured. Histopathological changes, including cell necrosis, degeneration, and hemorrhage, were assessed qualitatively based on established criteria.

Preparation of Tartrazine

Tartrazine coloring powder (Shanghai Color India) was obtained from Al-Tayf Office for Chemical Equipment/Baghdad. A low dose (7.5 mg/kg, equivalent to the human Acceptable Daily Intake) and a toxic dose (9 mg/kg) were prepared. The tartrazine was dissolved in 1 ml of distilled water and administered orally via a stomach tube, considering that the FEEDAP Panel committee confirmed the oral LD50 of tartrazine in rats is 6375 mg/kg b.w. This dosage approach allows for investigation into the effects of this synthetic colorant, often present in human diets, especially in conjunction with plant-based foods [11].

Histological Study

Following extraction, the brain tissue was immediately fixed in 10% formalin. After rinsing to remove the formalin, the samples were processed for histological sectioning according to standard methods described by Suvarna et al. (2019). This approach allows for the subsequent examination of potential tartrazine-induced changes, as well as any possible interactions with plant-derived compounds that may be present in the animals' diet or administered as part of future mitigation strategies [12].

RESULTS

Microscopic examination of brain tissue from rats treated with tartrazine (7.5 mg/kg for 60 days) revealed distinct histological changes, including neuron degeneration, astrocyte degeneration, oligodendrocyte necrosis, and astrocyte necrosis (Figure 1B, 1C, 1D). These findings suggest a disruption of normal brain cell structure and function following exposure to this synthetic colorant. Given the increasing prevalence of tartrazine in processed foods, further research is warranted to investigate whether plant-derived antioxidants or other bioactive compounds can mitigate these effects.

In rats exposed to the higher, toxic concentration of tartrazine (9 mg/kg), more pronounced and severe changes were observed. These included severe astrocyte necrosis and atrophy, macroglia necrosis, neuron necrosis and hemorrhage, severe oligodendrocyte necrosis, and macroglia atrophy (Figure 1E, 1F, 1G). These results highlight the potential neurotoxic effects of high-dose tartrazine exposure. Given the use of tartrazine as a cheap saffron substitute in some regions, there is a potential need to consider herbal remedies to counter these effects.

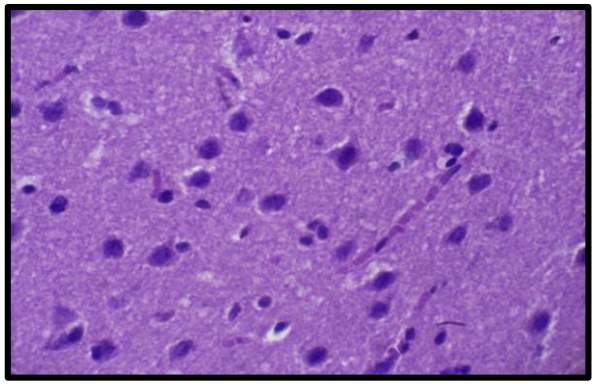

Fig. 1 A cross-section of the brain of rats in the control group. (H&E 400X).

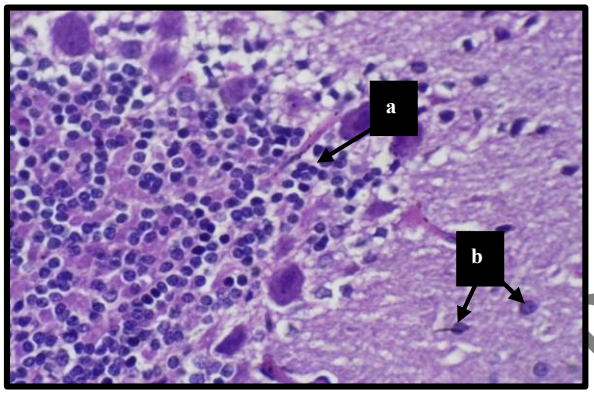

Fig. 2 A cross-section of the brain of male rats treated with Tartrazine concentration of 7.5 mg/kg, observed the presence of a: Neuron degenerate b: Astrocyte degenerate. (H&E 400X).

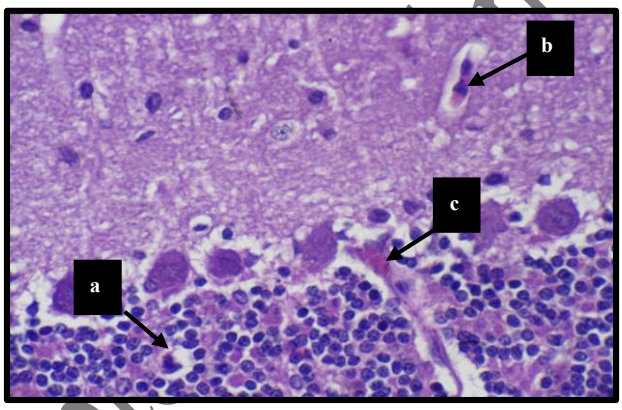

Fig. 3 A cross-section of the brain of male rats treated with Tartrazine concentration of 7.5 mg/kg, observed the presence of a: Astrocyte necrosis b: oligodendrocyte necrosis c: Hemorrhage. (H&E 400X).

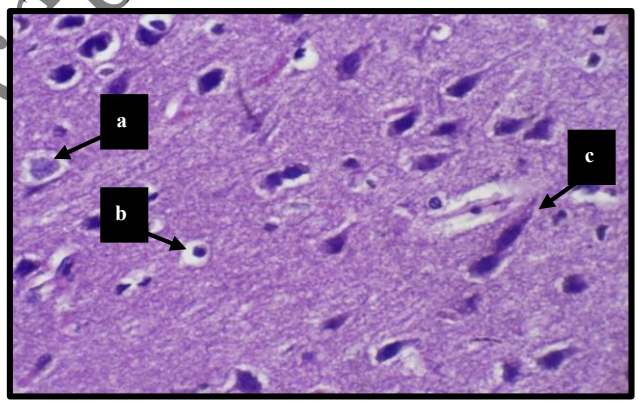

Fig. 4 A cross-section of the brain of male rats treated with Tartrazine concentration of 7.5 mg/kg, observed the presence of a: Astrocytes necrosis b: Neurocyte necrosis c: Oligodendrocyte necrosis. (H&E 400X)

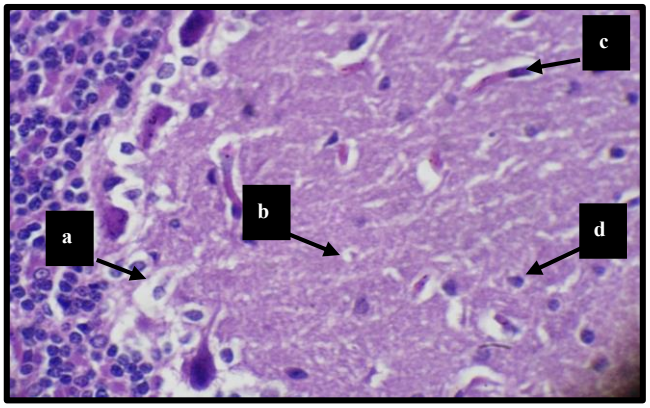

Fig. 5 A cross-section of the brain of male rats treated with Tartrazine concentration of 9 mg/kg, observed the presence of a: Severe necrosis of astrocyte b: Severe atrophy of astrocyte c: Macroglia necrosis d: Neuron necrosis. (H&E 400X).

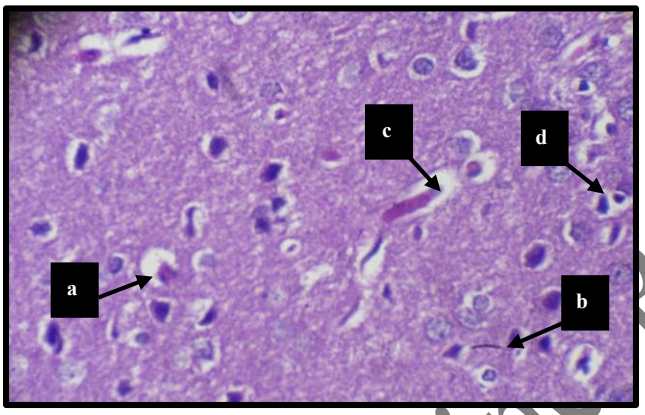

Fig. 6 A cross-section of the brain of male rats treated with Tartrazine concentration of 9 mg/kg, observed the presence of a: Necrosis and hemorrhage of astrocyte b: Severe Oligodendrocyte Necrosis c: Microglial necrosis d: Neuron degenerate. (H&E 400X).

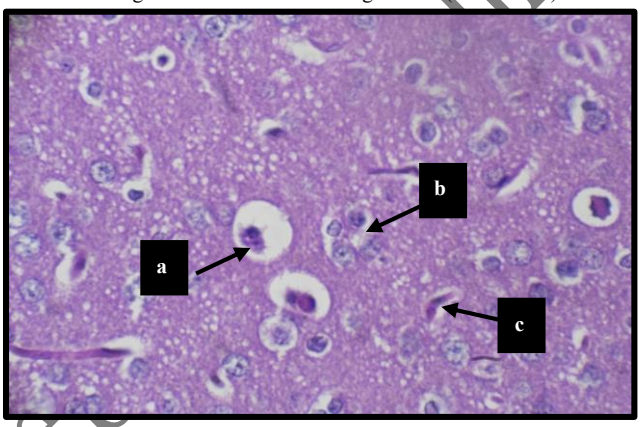

Fig. 7 A cross-section of the brain of male rats treated with Tartrazine concentration of 9 mg/kg, observed the presence of a: Severe astrocyte necrosis b: Oligodendrocyte necrosis c: Microglia atrophy. (H&E 400X).

DISCUSSION

Food colorings, including azo dyes, are added to food products to make them appear more attractive and appetizing [13]. Tetrazine imparts a yellow color to pharmaceutical, cosmetic, and food products, including soft drinks, ice cream, yogurt, and biscuits [14]. The harmful effect of tetrazine lies in its metabolized in the intestine and decrease the azo bond, producing free radicals in large quantities thus occurrence of oxidative stress that affects the unsaturated fatty acids present in brain tissue through attacking the free radicals of these fatty acids, generating lipid peroxides. These peroxides can alter the integrity of cell membranes [15]. Continued use of this process will increase the severity of oxidative stress, leading to irreversible loss of brain and spinal cord cells, as well as damage to nerve endings and disturbances in neurotransmitters, which in turn alters central nervous system function [16]. this is consistent with the results of our study as the result The results of the histological examination showed the occurrence of pathological tissue changes in the brain male rats' treated with tartrazine dye at concentration (7.5 mg/kg) of body weight, these changes included: oligodendrocyte necrosis, astrocyte necrosis, neuron degenerate, astrocyte degenerate, severe necrosis in oligodendrocyte and hemorrhage, while the brain tissue in the groups, which were treated with tartrazine dye at toxic concentration (9 mg/kg) of body weight were more affected, as these changes included the following: severe atrophy of astrocyte, severe necrosis of astrocyte, macroglia necrosis, neuron necrosis, neuron degenerate, necrosis and hemorrhage of astrocyte and oligodendrocyte necrosis .

Bawazir (2016) demonstrated similar findings when stating that artificial food colors such as tartrazine cause neurotransmitter disturbances and generate histopathological brain region change [17]. this study also agrees with Study Bhatt et al., (2018). which showed that ADI levels of tartrazine negatively affect and alter brain tissue biomarkers and cause oxidative damage [18].

Goldenring *et al.* demonstrated that sulfanilic acid a common metabolite of tartrazine dyes and increased behavioral activity in mice with direct oral administration for 80 days [19]. Other studies have also indicated that consuming artificial colors, including tetrazine, leads to increased lipid peroxides and free radicals, which inhibit antioxidant defense enzymes, causing damage to brain tissue and impaired learning and memory processes [20, 21].

CONCLUSION

In conclusion, our study demonstrates that long-term exposure to tartrazine at high concentrations induces significant histological damage in the brain tissue of rats, suggesting a potential for central nervous system dysfunction. Given the widespread use of tartrazine in processed foods and its potential to interact with plant-derived dietary components, further research is warranted to explore whether specific medicinal plants or their by-products possess protective effects against tartrazine-induced neurotoxicity. These studies may prove that certain herbs are beneficial to preventing this damage.

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