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Original Article

Curcumin's Neuroprotective Effects on the Neurons of the Rat Cerebrum's Motor Cortex against Tartrazine's Neurotoxic Effects

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Abstract

Objective: To assess the potential mitigating impact of curcumin on any potential neurotoxic effects induced by tartrazine.

Methods: This random control trial involved 45 adult male rats with weights ranging from 250-300g, randomly divided into III groups, each consisting of 15 rats (n=15). Group I was given a standard diet. Group II received tartrazine by dissolving in tap water, at a dose of 7.5 mg/kg of body weight. Group III was administered both 200 mg/kg of curcumin and tartrazine orally, daily for period of twenty-eight days. At the conclusion of the study, the animals were euthanized, and their brains were dissected. Following embedding, coronal sections of 5µm thickness were obtained and stained with haematoxylin eosin to visualize the degenerative changes in the neurons of rat motor cortex. Results were analysed using SPSS version 21.

Results: Histologically significant ameliorative effects of curcumin were observed in terms of improvement in pyramidal cell degeneration and granule cell necrosis in motor cortex of rat cerebrum. Encouraging the limitation of artificial dyes, particularly in children's diets, is advisable. Opting for a diet devoid of artificial food coloring or substituting tartrazine, with Curcumin, a natural alternative, is recommended.

Conclusion: Curcumin is a budding solution for alleviating tartrazine-induced neurotoxicity, thanks to its antioxidative, anti-neuroinflammatory, and anti-apoptotic attributes.

Keywords: Curcumin, Food dyes, Pyramidal neurons, Neuroprotection, Tartrazine

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Introduction

One of the most important qualities of food is its colour. Artificial colors may be incorporated to improve the visual appeal and vibrancy of food. Tartrazine (E 102, FD and C Yellow, is a lemon yellow-coloured powder widely used to colour food goods.[1] It's a synthetic azo dye made from coal tar. Frequently employed for coloring various human pharmaceuticals such as vitamin capsules, antacids, cosmetics, and hair products, it is also utilized in cake mixes, jams, jellies, flavored chips, chewing gums, biscuits, sauces, and ice cream to impart an appealing hue.[2] Tartrazine has a tolerable daily intake of 7.5 mg/kgbw. After intake, the gut microbiota and perhaps mammalian azo reductase in the liver or intestinal wall produce sulfanilic acid and aminopyrazolone as the main metabolites. The P450 enzymatic system oxidises these azo dyes to N-hydroxy derivatives once they have been reduced completely to aromatic amines.[3] This biotransformation mechanism in human, is responsible for a variety of disorders such as anaemia, pathological lesions in the brain, liver, kidney, and spleen, as well as allergic reactions, genotoxicity, neurotoxicity, and cancer. Due to its elevated polyunsaturated fatty acid content, limited antioxidant capacity, and inadequate ability for neuronal cell repair, the brain is highly susceptible to damage from free radicals.[4] Tartrazine-induced neurotoxicity has been found in the frontal cortex, hippocampus, and cerebellum, among other brain regions. Neuronal degeneration, vacuolation in grey and white matter, and other tartrazine-induced histological alterations in nervous tissue have been reported.[5,6] Oxidative stress is thought to be the cause of tartrazine-

induced neurotoxicity.[7] An increasing body of evidence indicates that supplementing the diet with antioxidants can inhibit this process, mitigate nervous tissue insult and promote the healthy neuronal functioning.[8]

Curcumin is a potent free-radical fighting superstar as well as a readily available food colour. Turmeric's major component is curcumin. It has anti-tumour, anti-inflammatory, antioxidant, and other pharmacological activities. Possessing neuromodulatory properties, it can traverse the blood-brain barrier. It protects the brain from heavy metal and chemical-induced neurotoxicity.[9] The present study explored the neuroprotective potential of curcumin in countering degenerative alterations induced by tartrazine in the motor nerve cells of the cortex of rats. This investigation was prompted by curcumin's therapeutic qualities.

Methods

The study's sample was acquired using a non-probability consecutive technique. After receiving approval from the Ethics Review Committee of Islamic International Medical College, this study was conducted in the department of Anatomy at Islamic International Medical College Rawalpindi in collaboration with the National Institute of Health (NIH) from September 2019 to December 2020.

The research included 45 adult male rats of two months old with a weight of 300 grams, while rats weighing less than 300 grams and female rats were excluded. Criteria for animal handling and care were established by the Islamic International Medical College's Ethical Review committee in Rawalpindi. Initially, an electronic weighing scale was used to weigh powdered tartrazine and curcumin at doses of 7.5 mg/kg and 200 mg/kg/day, respectively. After which it was diluted in tap water to create a 20 ml solution for each rat. This solution was then orally administered to 30 rats each day from experimental groups B and C.[5]

The rats were kept in cages at the Animal House of the National Institute of Health, Islamabad. Forty-five rats, weighing between 250 and 300 grammes, were placed in sterile cages, at a temperature of 22 ± 0.5˚C in an air-conditioned environment. For seven days, they had unlimited access to food and water to help them acclimatise. During the whole experiment, diet consisted of rat pellets and water. Fifteen male rats per group were included. Throughout the experiment, Group I (control group) was maintained on an oral conventional diet. Over the course of four weeks, experimental groups II received oral doses of tartrazine (0.031 g/day) and group III received tartrazine & curcumin (0.9 g/day) dissolved in tap water. The rat was euthanized by giving it a chloroform-soaked cotton ball anaesthesia 24 hours after the last dosage was administered. Following their removal, the brains were cleaned in cold saline. The brain tissues were preserved for histological examination after being fix in formalin and processed to create 5 µm-thick coronal slices.

The tissue was stained using Haematoxylin and Eosin. To evaluate the degree and existence of neuronal degeneration and necrosis, the sections were examined under 40X by using Image J software. The pyramidal cell degeneration was observed in motor cortex in four zones. The degenerated pyramidal cell was identified as bizarre shaped, shrunken and retracted neurons, from the surrounding neuropil. In some zones the fragmentation of pyramidal cells were also observed[10] Figure 1(A-C).

The necrosis was observed in granule cells in four zones. The necrotic neurons were characteristically 'red dead" neuron with hyper eosinophilic cytoplasm and absent nucleus[10] Figure 2(A-C). The criteria provided by the International Harmonisation of Toxicological Research were used for scoring11 (Figure 1). Four pictures of each slide were taken with the eye piece camera and sent to the image J programme. SPSS version 22 was used for data entry and analysis. The frequency and percentage of qualitative variables were used to express them. The Chi-square test was employed to compare qualitative data. Statistical significance was defined as a significance level of 0.05 or less.

Results

Microscopically examining sections of brain tissue stained with H&E showed that co-administration of curcumin effectively reduced the neuronal degeneration. (Figure 1, Table I) and necrotic changes of granule cells (figure 2, table II).

No pyramidal cell degeneration was seen in control group. However, in group II 60.0% rats showed severe degeneration and 26.7% showed moderate. In group III 66.7% rats showed negligible degeneration and 20% showed minimal (Figure 1, Table I).

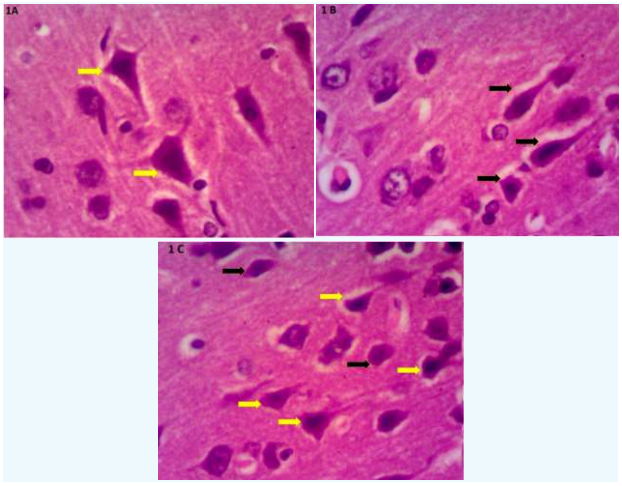

Figure-1: *(A-C): Histopathological analysis of gray matter of motor cortex of rat brain in control group I*

and experimental groups II & III. Figure 1A from control group showing normal pyramidal neurons (yellow arrow) in the motor cortex. While figure 1B showing significant degenerative changes in pyramidal neurons (black arrow) Figure 1 C showing the curcumin mediated amelioration in the neurons, in the motor cortex. (Approx.1600X H & E stain)

Table 1: *Comparison of pyramidal neurons degeneration between control group I and experimental groups II & III.*

Parameter	Grading	Group I n=15	Group II n=15	Group III n=15	p-value
Pyramidal cell degeneration	Grade 0	15(100%)	0 (0%)	10(66.7%)	<0.001**
	Grade 1	0 (0%)	1 (6.7%)	3 (20.0%)	
	Grade 2	0 (0%)	1 (6.7%)	1 (6.7%)	
	Grade 3	0 (0%)	4(26.7%)	1 (6.7%)	
	Grade 4	0 (0%)	9(60.0%)	0 (0%)	

The control group I showed pyramidal neuron degeneration (negligible grade 0). While in group II 60.0% rats showed severe (grade 4) necrosis and 26.7 % showed moderate (grade 3) necrosis. However, group III showed 66.7 % rats showed negligible (grade 0) necrosis and 20.0 % showed minimal (grade 1) necrosis in cortical neurons (Figure 2, table II)

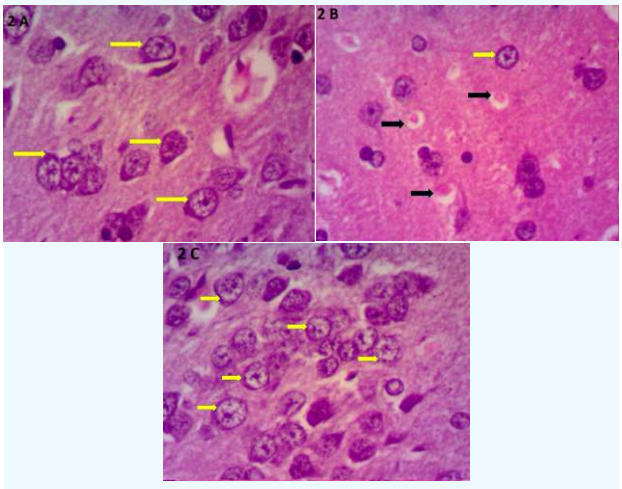

Figure-2: *(A-C): Histopathological analysis of grey matter of motor cortex of rat brain in control group I and experimental groups II & III. Figure 2 A from control group showing normal granule cells (yellow arrow) in the motor cortex. While figure 2 B showing significant degree of granule cell necrosis classically, red dead neurons, (black arrow) in the motor cortex of tartrazine treated group B. Figure 2 C showing the curcumin mediated amelioration in histological findings in the motor cortex. (Approx. 1600 X H & E stain)*

Table 2: *Comparison of granule cell necrosis between control group I and experimental groups II & III.*

Parameter	Grading	Group I n=15	Group II n=15	Group III n=15	p-value
granule cell necrosis	Grade 0	15(100%)	0 (0%)	9 (60.0%)	<0.001**
	Grade 1	0 (0%)	2 (13.3%)	4(26.7%)	
	Grade 2	0 (0%)	1 (6.7%)	1 (6.7%)	
	Grade 3	0 (0%)	5 (33.3%)	1 (6.7%)	
	Grade 4	0 (0%)	7 (46.7%)	0 (0%)	

The control group I showed no granule neuron necrosis (negligible grade 0). While in group II 46.7% rats showed severe (grade 4) necrosis and 33.3 % showed moderate (grade 3) necrosis. However, group III showed 60.0 % rats showed negligible (grade 0) necrosis and 26.7% showed minimal (grade 1) necrosis in cortical neurons (Figure 2, table II)

Discussion

Currently, consumers, nutritionists, and toxicologists are extremely concerned about safety limits and post-exposure health risks connected with the intake of food additives. Therefore, it is imperative to conduct a thorough and accurate assessment of the toxicity of food additives. Tartrazine is the most widely used azo dye.[12,13] Previous studies revealed that the long-term exposure of humans, to tartrazine even at ADI, can lead to damage to the vital organs including destructive changes at the cellular level in the brain.[7,14]

The findings of the present investigation shown that 28 days of curcumin treatment (200 mg/kg b.w.) significantly reversed the degenerative changes in the grey matter of the cerebral cortex. This antioxidant potency of curcumin was also observed in the study conducted by Wei Wei et al. In which they observed the neuroprotective role of curcumin against dichloroacetic acid induced dysfunction in hippocampus of rats.[15] In the present research, we provide evidence that tartrazine administration led to neuronal degeneration in the motor cortex, confirmed in the histological section in the form of bizarre, shrunken and reduced number of neurons but the curcumin treated group shown marked improvement in the number and morphology of neurons. This amelioration of neuronal destruction by curcumin is also observed in the cerebellar neurons of the Parkinson disease model of rat brain.[16] Curcumin exhibits anti-apoptotic, antioxidant, and anti-inflammatory activities, according to a study by Guo et al. that looked at the potential protective effect of curcumin in preserving the morphological aspects of the cerebral cortex following exposure to acrylamide.[17]

Among the different brain sub-regions the frontal motor cortex of the cerebrum was revealed to be more vulner-

able to tartrazine-induced oxidative stress as evident in the current histomorphological changes in the neurons of frontal motor cortex.[5,6] The current study has demonstrated the neuroprotective impact of curcumin by lowering neuroinflammation, due to the antioxidant property and restoring the histomorphological picture of the neurons in the rat frontal motor cortex. These findings are in consonance with the curcumin action on the cadmium induced damaged neurons of prefrontal cortex.[18] The effects of both natural and artificial food colours on motor cortex's grey matter neurons were first seen in our study. Due to its antioxidant effects, curcumin may lessen brain oedema and BBB permeability by significantly increased the activity of SOD and GPX enzymes, as well as reduced MDA concentration in the brain tissue.[19]

Conclusion

The tartrazine-induced oxidative stress and neuroinflammation were significantly reduced by the curcumin treatment, and this was reflected in the restoration of the number and morphology of grey matter motor cortex neurons. Hence, it was observed that the administration of curcumin mitigates histomorphological changes induced by tartrazine in the grey matter of rat motor cortex.

Conflict of Interest: *None*

Funding Source: *None*

References

1. Hashem MM, Abd-Elhakim YM, Abo-EL-Sooud K, Eleiwa MM. Embryotoxic and teratogenic effects of tartrazine in rats. Toxicol Res. 2019;35(1):75-81.
2. da Silva J, Fracacio R. Toxicological and ecotoxicological aspects of tartrazine yellow food dye: a literature review. Brazilian J Environ Sci. 2021;56(1):137-51.
3. Altinoz E, Erdemli ME, Gül M, Erdemli Z, Gül S, Turkoz Y. Prevention of toxic effects of orally administered tartrazine by crocin in Wistar rats. Toxicol Environ Chemistry. 2021;103(2):184-98.
4. Cemek, Mustafa, et al. "Effects of Food Color Additiveson Antioxidant Functions and Bioelement Contents of Liver, Kidney and Brain Tissues in Rats." Journal of Food and Nutrition Research 2.10 (2014): 686-691.
5. Bhatt D, Vyas K, Singh S, John PJ, Soni I. Tartrazine induced neurobiochemical alterations in rat brain subregions. Food Chem Toxicol. 2018;113(3):322-27.
6. El-Sakhawy MA, Mohamed DW, Ahmed YH. Histological and immunohistochemical evaluation of the effect of tartrazine on the cerebellum, submandibular glands, and kidneys of adult male albino rats. Environ Sci Pollut Res Int. 2019;26(10):9574-84.
7. Hosieny N, eldemerdash M, Ahmed S, Zayed M. Toxic Effects of Food azo dye Tartrazine on the Brain of Young Male Albino Rats: Role of Oxidative Stress. Zagazig J Foren Med., 2021; 19(1): 60-73.
8. Huang D. Dietary Antioxidants and Health Promotion. Antioxidants (Basel). 2018;7(1):9.
9. Farkhondeh T, Samarghandian S, Roshanravan B, Peivasteh-Roudsari L. Impact of curcumin on traumatic brain injury and involved molecular signaling pathways. Recent Patents Food Nutr Agri. 2020;11(2):137-44.
10. Bradley AE, Bolon B, Butt MT, Cramer SD, Czasch S, Garman RH, George C, Gröters S, Kaufmann W, Kovi RC, Krinke G. Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems: new and revised INHAND terms. Toxicol Pathol. 2020;48(7):827-44.
11. Mann PC, Vahle J, Keenan CM, Baker JF, Bradley AE, Goodman DG, et al. International Harmonization of Toxicologic Pathology Nomenclature: An Overview and Review of Basic Principles. Toxicol Pathol. 2012; 40(4):p7S-13S.
12. Kaya SI, Cetinkaya A, Ozkan SA. Latest advances on the nanomaterials-based electrochemical analysis of azo toxic dyes Sunset Yellow and Tartrazine in food samples. Food Chemical Toxicol. 2021; 156(1): 112524.
13. Silva MM, Reboredo FH, Lidon FC. Food colour additives: A synoptical overview on their chemical properties, applications in food products, and health side effects. Foods. 2022;11(3):379.
14. Zafar M, Ali S, Hussain N, Khursheed T, Beenish H, Yasser A. The neuroprotective effect of curcumin against tartrazine-induced neurobiochemical and histopathological changes in the gray matter of motor cortex of brain of sprague dawley rats. Pak Armed Forces Med J. 2021;71(4):1269-73.
15. Wei W, Dong Q, Jiang W, Wang Y, Chen Y, Han T, Sun C. Dichloroacetic acid-induced dysfunction in rat hippocampus and the protective effect of curcumin. Metab Brain Dis. 2021;36(4):545-56.
16. Fikry H, Saleh LA, Abdel Gawad S. Neuroprotective effects of curcumin on the cerebellum in a rotenone-induced Parkinson's disease Model. CNS Neurosci Therapeut. 2022;28(5):732-48.
17. Guo J, Cao X, Hu X, Li S. The anti-apoptotic, antioxidant and anti-inflammatory effects of curcumin on acrylamide-induced neurotoxicity in rats. BMC Pharmacol Toxicol 2020; 21(1): 1-10
18. Namgyal D, Ali S, Hussain MD, Kazi M, Ahmad A, Sarwat M. Curcumin Ameliorates the Cd-Induced Anxiety-like Behavior in Mice by Regulating Oxidative Stress and Neuro-Inflammatory Proteins in the Prefrontal Cortex Region of the Brain. Antioxidants. 2021; 10(11):1710.
19. Vastegani SM, Hajipour S, Sarkaki A, Basir Z, Navabi SP, Farbood Y, Khoshnam SE. Curcumin mitigates lipopolysaccharide-induced anxiety/depression-like behaviors, blood–brain barrier dysfunction and brain edema by decreasing cerebral oxidative stress in male rats. Neurosci Letters. 2022;782(1):136697.