ORIGINAL ARTICLE

Curcumin's Neuroprotective Efficacy Against Tartrazine-Induced Nissl Rim Alterations in Adult Male Rats' Motor Cortex

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ABSTRACT

Objective: To evaluate the neuroprotective potential of curcumin against tartrazine made Nissl rim changes in neurons of the motor cortex in cerebrum of rats.

Study Design. Laboratory based experimental research.

Place and Duration of Study: Department of Anatomy, Islamic International Medical College, Rawalpindi from 30th Sep 2019 to 30th July 2020.

Materials and Methods: Forty-five adult male albino rats (250-300gm) were divided randomly into three groups. (n=15). Group A, rats were given standard rat diet. Group B, rats were given tartrazine orally by dissolving in tap water with dose of 7.5 mg/kg body weight. Group C, rats were given 200mg/kg of curcumin along with tartrazine orally by dissolving in tap water, daily for 28 consecutive days. At the end, animals were euthanized and dissected to remove the brain. Coronal section of 5mm thickness of rat motor (frontal) cortices in the precentral areas were taken for further processing. After embedding,5µm thick coronal sections were obtained and stained with Toluidine blue to visualize the Nissl substance. Results were analyzed using SPSS version 21.

Results: The curcumin administration significantly improved the Nissl rim thickness (p-value < 0.001 on intergroup comparison). Nissl's rim thickness was found to be 1.77 ±0.284µm, 1.01±0.974 µm and 1.92±0.193 µm in groups A, B and C respectively.

Conclusion: The curcumin significantly restores the Nissl rim thickness and alleviate the tartrazine induced neurotoxic changes in gray matter of motor cortex.

Key Words: *Curcumin, Food Azo Compounds, Nissl Bodies, Oxidative Stress, Tartrazine.*

Introduction

Food dyes are commonly used to enhance the food fascination among the consumers around the globe. With the rapid industrialization and color revolution a large variety of artificial food colors have been added into our diet. Tartrazine is one of most widely used food dye amongst all. It is extensively used in various daily edible food items as well as in non-food products. For human utilization, worthy every day (ADI) of this color is 0-7.5 mg/ kg.bw/day.[1] Orally ingested tartrazine undergoes azoreduction in gut and liver lead to production of aromatic amine sulfanilic acid which act as precursor of reactive oxygen species (ROS). This metabolite can cross the blood brain barrier (BBB) and results in oxidative stress mediated nervous tissue injury.[2,3] It is reported that its consumption even at ADI level can result in antagonistic health effects like genotoxicity, embryo toxicity and carcinogenicity.[4] Tartrazine induced neurotoxicity has been reported in various brain sub regions including frontal cortex, hippocampus and cerebellum[5].An immunohistochemical recoloring with the anti-ssDNA counteracting agent (apoptotic cell marker) detailed plenteous apoptotic cells in the brain cortex.[6] Tartrazine could be source of behavioral alterations in human children similar to rat progeny.[7,8]The brain is exceedingly helpless to the harm caused by free radicals since of its quick oxidative metabolic action, high polyunsaturated fatty acids content, quite low antioxidant levels. Various tartrazine induced histopathological changes in nervous tissue have been reported including neuronal degeneration, vacuolation in gray and in white matter.[9] Increasing research suggests that nutritional antioxidant supplementation can

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reduce oxidative stress, minimize brain damage, and improve behavioral functioning.[10]

Curcumin a compelling antioxidant and a naturally available food color. It is the main constituent of turmeric. It has anti-tumor, anti-inflammatory, and antioxidant properties, as well as other pharmacological characteristics.[11] It has the ability to pass the blood-brain barrier and has neuromodulatory effects.[12] It has a neuroprotective effect against heavy metals and chemical-induced neurotoxicity.[13,14]

Given curcumin's therapeutic properties, the current study looked into its neuroprotective potential against tartrazine-induced Nissl rim alterations in the gray matter of adult male rats' motor cortex neurons.

Materials and Methods

It was a laboratory-based experiment and was carried out in the department of Anatomy, Islamic International Medical College Rawalpindi in collaboration with the National Institute of Health (NIH), after seeking approval from the Ethics Review Committee [Riphah/IRC/19/0373], Islamic International Medical College, from 30th September 2019 to 30th July 2020.

The sample was drawn using a non-probability convenient sampling technique in a laboratory setting. The study was performed on 45 Albino Sprague Dawley adult male rats as a mammalian model. Two months old adult male rat weighing 300gm were included and rats weighing less than 300gm and female rats were excluded from the study. The ethical review committee of the Islamic International Medical College, Rawalpindi, defined the criteria for animal care and handling.

Tartrazine and curcumin in powdered form was weighed first on electronic weighing scale according to a dose of 7.5 mg/kg/day and 200mg/kg /day respectively. Then dissolved in tap water to make 20ml solution for each rat which was given daily to 30 rats of experimental group B & C via oral route[5].

The rats were housed in cages at the National Institute of Health's Animal House in Chak Shehzad, Islamabad. Forty-five rats weighing (250-300gm) were kept under standard temperature at 22 ± 0.5˚C in air-conditioned room and were shifted into clean stainless-steel cages under 12-hour light and dark cycle with 50% humidity. They were given food and water ad libitum for 7-days to acclimatize. Rat pellets and water were used as food during the whole experiment. Each group comprised of 15 male rats. Group A (control group) was kept on standard diet orally throughout the experiment. Experimental group B was given tartrazine at dose 0.031gm/day and those of group C same dose of tartrazine along with 0.9 gm/day curcumin by dissolving in tap water via oral route for four weeks. Following 24 hours of last dose administration, the rats were anaesthetized with chloroform-soaked cotton balls till they lost consciousness (euthanasia). The brains were removed and washed in with cold saline. The brain tissues were fixed in 10% formaldehyde solution which were processed to obtain paraffin blocks, from which 5-µm-thick coronal sections were prepared and conserved for histopathological analysis. Tissue was stained by using Toluidine blue staining. The Nissl's granules form a circular rim at the neuronal periphery. Its thickness was measured at 11, 12, 4, and 6 o'clock positions in the neurons of motor cortex, by the line tool of image J under 40x power in toluidine blue stained sections to indicate the Nissl's granules density in neurons. Four images of each slide were taken by using eye piece camera YW-100 2.0 MP and then all images taken, were transferred to image J software. SPSS version 21 was used to enter and analyze the data, and the findings were expressed as mean standard deviation. The mean comparison of quantitative variables between control and experimental groups was done using one way analysis of variance (ANOVA). The intergroup comparisons among groups was done using the post hoc tukey's test. Significant was defined as a *p*-value less than 0.05.

Results

The average standard deviation of Nissl's rim thickness was found to be 1.77 ±0.284µm, 1.01±0.974 µm and 1.92±0.193 µm in groups A, B and C respectively (Table I, Figure 1A-1C). Difference between the groups was analyzed by ANOVA, which yielded highly significant result (p-value < 0.001). On comparing the mean Nissl's rim thickness measurements of group B with the group C and A, the difference in mean between the groups was determined to be statistically significant (p-values of 0.001). While between A and C the mean difference was found to be insignificant (p-value of 0.126) (Table I).

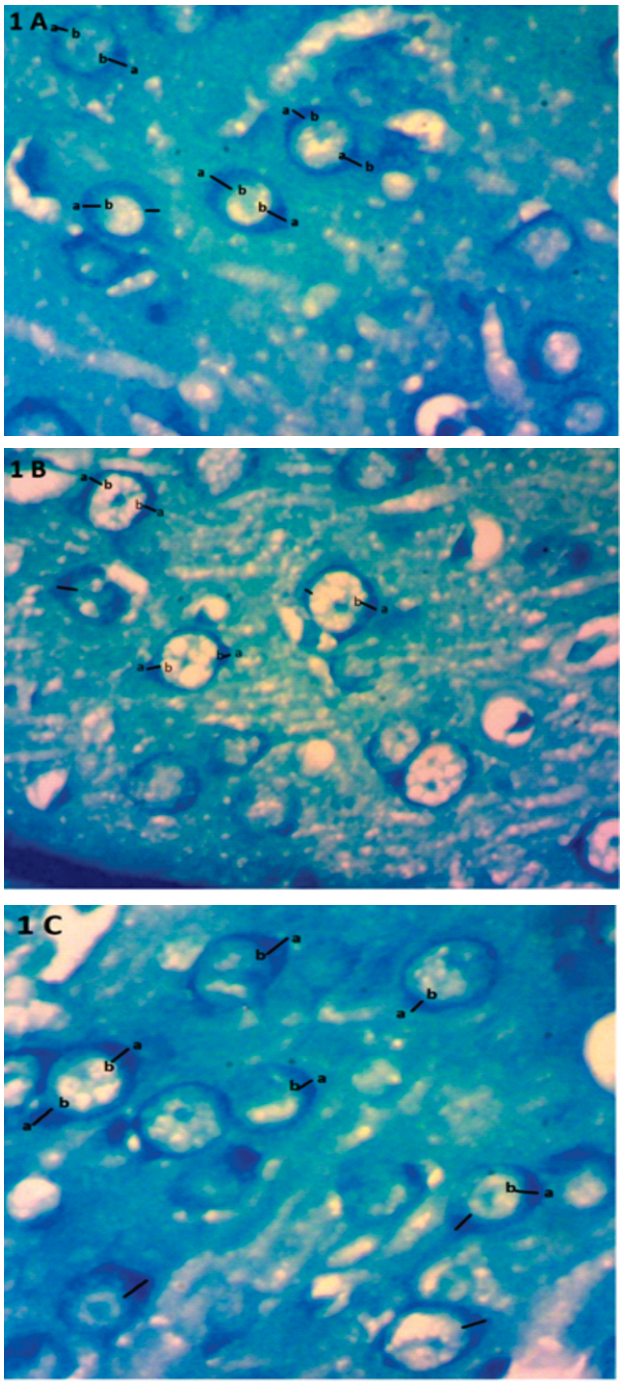

Fig 1: 1A-1C: Histopathological analysis of gray matter of motor cortex of rat brain in control group A and experimental groups B& C. Fig 1A from control group showing normal Nissl rim thickness represented as (a-b, a-outer margin to b-inner margin of rim) in the cortical neurons of motor cortex. While figure 1B showing significant decline in Nissl rim thickness represented as a-b. Fig 1 C showing the curcumin mediated amelioration in histological findings in the motor cortex. (Toluidine blue stains. Approximately 1600 X)

Table I: Mean Measurement of Nissl Rim Thickness in Micrometer among Control (A) Tartrazine Treated Group (B) Versus the Tartrazine + Curcumin Group (C).

Parameter	Group A n=15	Group B n=15	Group C n=15	p-value (ANOVA)
Nissl rim thickness µm	1.77 ±0.284	1.01±0.974	1.92±0.193	
Post-Hoc Analysis				
Parameter	B vs. A	B vs. C	C vs. A	
Nissl rim thickness µm	<0.001	<0.001	0.126	

Discussion

The inadvertent use of food colors in daily edible food items and other non-food products has augmented the need of determining the adverse effects on vital organs of the body including brain. Tartrazine has gained wide utilization in many foods such as canned juices, bakery items, jams and jellies, ice-cream and candies. The deleterious effects of tartrazine on the brain should be dealt with considerable attention because these can further lead to various behavioral and cognitive disorders during both childhood and adulthood [7].

In the present study, addition of the natural antioxidant, curcumin to diets containing tartrazine has improved cellular metabolism by increasing capacities of cellular antioxidants[15]. These ameliorating effects of curcumin on nervous tissue histology were in consonance with the previous reports[14,16].

Coadministration of curcumin in group C markedly improved the Nissl rim thickness highlighting its neuroprotective potential. Following the neuronal cell injury there is dispersion of rough endoplasmic reticulum in order to repair the cell. Central chromatolysis is the typical phenomenon involved, which is marked by a decrease in the number of Nissl bodies and eosinophilic cytoplasm. This is a reactionary alteration in injured neurons' perikarya[17]. Although the histological parameter of Nissl rim thickness on rat brain has not been studied with tartrazine but a study conducted by Heba R. Hashem in 2018 studied this parameter in rat cerebral cortex with complications of diabetes and it showed significant reduction in Nissl rim density[18].

Another study found that nutmeg's toxic effects on the primary visual occipital cortex in rats resulted in changes in Nissl rim density in rats[19]. Tartrazine causes neurotoxicity by triggering oxidative damage and peroxidation of lipids in cell membranes, affecting cellular activities by altering the physicochemical characteristics, fluidity, and integrity of cell membranes, making cells more vulnerable to lipid peroxidation and cell death[20]. In current study, a significant ameliorative effect of curcumin on Nissl rim thickness was detected in cortical neurons. This showed that antioxidants like curcumin improves the viability of neuronal cells by exerting its strong endogenous role of ROS scavenger[21]. Because of its antioxidant properties, it has been demonstrated that curcumin has proven to be useful in lowering neuronal death in the substantia nigra thus improving the functional outcomes in a variety of brain illnesses[22].

Thus, curcumin administration to tartrazine treated rats was found to significantly re-equilibrate antioxidant parameters back to normal values and restored the alteration in neuronal architecture[23].The limitation of study was lack of availability of special axonal staining procedures and settings at low cost. The current work highlights the need of future exploration of neurotoxicity induced by azo food dyes. It is recommended that the advanced investigation tools should be chosen to access toxic brain injury like immunohistochemistry, genetic polymorphism, gene expression analysis, portable EEG and diffusion MRI.

Conclusion

Curcumin administration markedly prevented the tartrazine induced oxidative stress and neuroinflammation which was appreciated in the form of restoration of Nissl rim thickness in the neurons of the gray matter of motor cortex. Therefore, it is concluded that curcumin administration improves the tartrazine induced Nissl rim changes in the gray matter of motor cortex of rat cerebrum.

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