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

Maleeha Zafar, Shabana Ali, Nabiha Hussain, Tooba Khursheed, Huma Beenish, Ayesha Yasser

Islamic International Medical College, Rawalpindi Pakistan

ABSTRACT

Objective: To study the neuroprotective effect of curcumin against tartrazine-induced neurobiochemical and histopathological changes in the gray matter of motor cortex of rat brain.

Study Design: Lab-based experimental study.

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

Methodology: Forty five adult male albino rats (250-300 gm) 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 in the dose of 7.5 mg/kg body weight. Group C, rats were given 200 mg/kg of curcumin along with tartrazine orally by dissolving in tap water, daily for 28 consecutive days. At the end, all animals were dissected and their brain were removed. The neurobiochemical parameters including lipid peroxidation marker level along with microscopic changes widening of Virchow's robin space and neuropil vacuolation in neurovascular unit were observed. Results were analyzed using SPSS version 21.

Results: The curcumin administration significantly improved the MDA levels (p-value <0.001 on intergroup comparison) and widening of Virchow's robin space (group A=3.57 ± 0.78 µm, group B=19.39 ± 0.68 µm, group C12.1 ± 0.83 µm; p-value <0.001). Neuropil vacuolation was reduced to negligible in 60% rats of curcumin treated group.

Conclusion: Curcumin displayed a neuroprotective role in improving the tartrazine induced neurobiochemical and histopathological changes in the motor cortex of rat brain.

Keywords: Antioxidant, Curcumin, Oxidative stress, Synthetic food colors, Tartrazine.

INTRODUCTION

Tartrazine, a synthetic azo dye that is certified by FDA as a colorant (E102, FD&C Yellow 5) is among the most commonly used food color to impart lemon yellow color in various food products[1]. The World Health Organization (WHO) has approved the acceptable daily intake for tartrazine to be 7.5mg/kg/day or less[2]. Azo-fission of orally ingested tartrazine in intestine and liver lead to formation of aromatic amines, sulfanilic acid, which can cross the blood brain barrier and generate reactive oxygen species (ROS) in brain[3]. Various neurotoxin may lead to damage to neurovascular unit (NVU) that includes cellular and extracellular components (neurons, perivascular astrocytes, microglia, pericytes, endo-thelial cells, and basement membrane) that are involved in regulating cerebral blood flow and blood-brain barrier function[4]. Tartrazine induces marked neuroinflammatory changes in the neurovascular unit of brain by promoting the marked rise in malondialdehyde (MDA), lipid peroxidation metabolites and ROS in the brain cortex even at prescribed accepted daily intake (ADI) levels. Histopathological changes in the brain of tartrazine-treated rats included significant widening of Virchow robins space (perivascular space) and vacuolation in neuropil[5]. The former is associated with tartrazine induced oxidative stress and inflammation which stimulate the release of pro-inflammatory cytokines. These cytokines in turn augment neutrophil aggregation and IL-6 synthesis by vascular endothelium, leading to increased blood flow in blood vessels[6]. Tartrazine by enhancing lipid peroxidation alters the vascular membrane permeability leading to accumulation of cellular infiltrates in the perivascular space surrounding the blood capillaries in neuropil[7]. Vacuolization is an initial histological indicator of interference in fluid balance between the neuronal cell membrane and surrounding interstitium. This may be attributed to the exhaustion of the antioxidants in combating ROS that were generated by tartrazine administration. Vacuolation in astrocytes (glial cells) results in neuropil vacuolation[8]. As these cells constitute the blood brain barrier, this may alter the permeability across their foot processes (aquaporin 4 gated channels) and lead to abnormal fluid diffusion and aggravate the neuroinflammation[9].

Curcumin, a natural food color, the main bioactive component in the rhizome of the turmeric plant is

Correspondence: Dr Maleeha Zafar, Department of Anatomy, Islamic International Medical College, Rawalpindi Pakistan

Received: 12 Feb 2021; revised received: 15 Apr 2021; accepted: 21 Apr 2021

a safe and highly pleiotropic molecule with multiple biological targets. Being a potent antioxidant it exerts its effects primarily by up regulating the brain antioxidants enzymes and neuroprotective markers. It enters brain tissue in biologically relevant concentrations, reduces acute and transient microglia activation, pro-inflammatory mediator production, and the behavioral symptoms of sickness. By hindering the activation of the transcription factor, nuclear factor kappa B (NF-KB) the curcumin play a vital role in the cell survival, cytokine production, and other cellular functions[10]. Considering the beneficial effects of curcumin a current study was undertaken to investigate the neuroprotective potential of curcumin against tartrazine induced neurobiochemical and histological alteration in the (neurovascular unit) gray matter of motor cortex of adult male rats.

METHODOLOGY

It was a laboratory based experiment and non-probability consecutive sampling technique was used to draw the sample. This study 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 September 2019 to September 2020.

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 <300gm and female rats were excluded from the study. The animal care and handling was done according to the guidelines set by the ethics review committee, Islamic International Medical College, Rawalpindi.

Tartrazine and curcumin in powdered form was weighed first on electronic weighing scale according to a dose of 7.5mg/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[2]. For neurobiochemical assays, MDA calorimetric assay kits and ELISA test kits were purchased from Multi LinkX Enterprises. Phosphate buffer saline PBS was purchased from local chemical shop.

Rats were kept in cages under the supervision of Animal house of NIH, Chak Shehzad Islamabad. Forty five rats weighing approximately 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 0.031 gm/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 rat was anaesthetized with chloroform soaked cotton balls till they lost consciousness (euthanasia). Brains were taken out, rinsed in cold saline.

A 2mm section of each rat brain in region of motor (frontal) cortex was removed and shifted to phosphate buffer saline (PH 7.5) containers for biochemical assays. While the rest of brain tissues were fixed in 10% formaldehyde solution which were processed to obtain paraffin blocks, from which 5µm thick coronal sections were prepared and conserved for histopathological analysis.

Tissue was stained by using H&E staining. For the assessment of qualitative parameter of neuropil vacuolation all the slides were observed under 10X magnification by using image J software to assess the presence and degree of neuropil vacuolation. Vacuolation was identified in neuropil as single or clustered, oval to round, colorless spaces (fig-1(a-c)). Scoring was done according to criteria given by International Harmonization of Toxicological Research (fig-1)[11]. For the assessment of quantitative parameter of Virchow

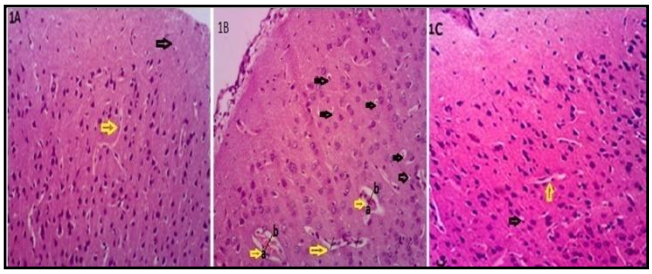

Figure-1: Histopathological analysis of gray matter of motor cortex of rat brain in control group A and experimental groups B & C. A): from control group showing normal caliber brain capillary (yellow arrow) and negligible vacuolation in neuropil (black arrow) in the motor cortex. B): showing significant widening of Virchow robin's space represented as a-b (yellow arrow) & Vacuolation in neuropil (black arrow) in the motor cortex of tartrazine treated group B. C): showing the curcumin mediated amelioration in histological findings in the motor cortex. (Approx. 400 X H & E stain).

Robin space measurement, the widening of maximally dilated vascular zone (a-b) was measured in the transverse plane of blood vessel in the four zones under 10X magnification and then mean of four reading was taken (table-I). Four images of each slides were taken by using eye piece camera YW-100 2.0 MP and then all images were taken transferred to image J software.

Lipid peroxidation metabolite MDA level was estimated by calorimetric assay kits through TBA method. A 10% tissue homogenate of motor cortex was prepared in PBS (pH 7.4) and was used for the determination of MDA. The brain tissue (motor cortex) was minced into small pieces, then weighed and homogenized in normal saline on ice, the volume of normal saline: weight of tissue (gm)=9:1. The tissue homogenate is centrifuged for 10min at 10000g. The supernatant was collected was used to estimate the level of MDA with the help of spectrophotometer.

Data was entered and analyzed in SPSS-21 and results were expressed as mean ± SD. One way analysis of variance (ANOVA) was applied for the mean comparison of quantitative variables between control and experimental groups. Post hoc tukey's test was applied for the intergroup comparisons among groups. Qualitative variable (vacuolation) was expressed by frequency and percentage. Chi square test was used for intergroup comparison among this variable. A *p*-value of ≤0.05 was considered significant.

RESULTS

An examination of hematoxylin/eosin-stained brain tissue sections by microscopy revealed that co-administration of curcumin significantly lessened the widening of the Virchow robin's space (perivascular space) (fig-1, table-I) and vacuolation in neuropil (fig-2, table-II) as compared to tartrazine intoxicated rats. A significant improvement was also observed in neurobiochemical assay in curcumin administered group.

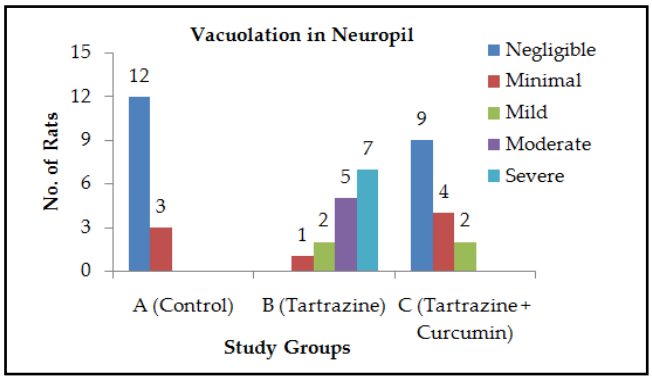

Figure-2: Frequency of vacuolation in neuropil in control group A and experimental groups B & C.

The mean measurements of Virchow's Robins Space was found to be 3.57 ± 0.78µm, 19.39 ± 0.68µm and 12.1 ± 0.83 µm respectively. On comparing the mean measurements of Virchow's Robins Space between all the three groups ANOVA showed significant results (*p*-value <0.001). On intergroup comparison

Table-I: Mean measurement of Virchow's Robins space µm among control (A) tartrazine treated group (B) versus the tartrazine ± curcumin group (C).

Parameter	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	*p*-value
Virchow's Robins space µm	3.57 ± 0.78	19.39 ± 0.68	12.1 ± 0.83	<0.001
Post-Hoc Analysis				
Parameter	B vs. A	B vs. C	C vs. A	
Virchow Robins space µm	<0.001	<0.001	<0.001	

Table-II: Comparison of neuropil vacuolization between control group A and experimental groups B & C.

Parameter	Grading	Group A n=15	Group B n=15	Group C n=15	*p*-value
Vacuolation in Neuropil	Grade 0	12 (80%)	-	9 (60.0%)	<0.001
	Grade 1	3 (20%)	1 (6.7%)	4(26.7%)	
	Grade 2	-	2 (13.3%)	2 (13.3%)	
	Grade 3	-	5 (33.3%)	-	
	Grade 4	-	7 (46.7%)	-	

Table-III: Mean measurement of neurobiochemical assay MDA among control (A) tartrazine treated group (B) versus the tartrazine ± curcumin group (C).

Parameters	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	*p*-value
Melandialdehyde nmol/ml	156.6 ± 0.757	172.8 ± 0.687	159.4 ± 1.24	<0.001
Intergroup Comparison	B vs A <0.001	B vs C <0.001	C vs A <0.001	

a significant widening of Virchow Robins space was observed in the experimental group B when compared with the group A (*p*-value <0.001) (fig-1b). While the comparison of group C with the groups B and A were also found to be statistically significant (*p*-values of <0.001) (fig-1, table-I).

Regarding neuropil vacuolization the control group A showed negligible (grade 0) neuropil vacuolization in 80% rats. 46.7% rats in experimental group B showed severe (grade 4) vacuolization and 33.3% rats showed moderate (grade 3) vacuolization in neuronal connective tissue. In experimental group C 60% rats showed negligible (grade 0) vacuolization and 26.7% showed minimal (grade 1) exhibiting the neuroprotective role of curcumin (fig-2, table-II).

Regarding the neurobiochemical assays the mean MDA level in group A was found to be 156.6 U/ml ± 0.757 while a significant elevation was observed in mean MDA levels of group B with the value of 172.8 U/ml ± 0.687 (*p*-value <0.001). The group C presented a significant decrease 159.4 U/ml ± 1.24 (*p*<0.001) in MDA concentration as compared to tartrazine exposed rat highlighting the neuroprotective role of curcumin. The *p*-value for intergroup comparison was <0.001, which was statistically significant (table-III).

DISCUSSION

Among the food colorants, tartrazine is considered as toxic to the human beings if consumed in excess amount and even within ADI limit[12,13]. In this study the ADI dose of tartrazine, 7.5 mg/kg body weight was used[2].

Addition of the natural antioxidant, curcumin to diet improved cellular metabolism by increasing capacities of cellular antioxidants. Several studies supported the anti-inflammatory, antioxidant, and neuroprotective effects of curcumin as an active constituent of turmeric. Because of its biological properties, curcumin is an appropriate candidate for improving injuries after any toxic insult to brain tissue[14-16]. Curcumin has a broad range of biological actions. Because of its lipophilic property, orally administered curcumin seem to cross the blood brain barrier (BBB) and inhibit lipid peroxidation (as a chain-breaking antioxidant) and to enhance endogenous antioxidant mechanisms[10,16]. Curcumin possesses antioxidant property by virtue of its phenolic hydroxyl group in its structure that donates electrons to quench free radicals[17].

Among the brain sub-regions the frontal motor cortex of cerebrum was found to be more prone to oxidative stress induced by tartrazine as evident in the current findings of neurobiochemical test in experimental rat frontal motor cortex[2]. The curcumin treated group showed significant decline of MDA level in the frontal motor cortex of brain tissue in our study as compared to the tartrazine intoxicated group.

Guo *et al* carried out a study to see the possible protective effect of curcumin in conservation of morphological and biochemical features of cerebral cortex after exposure to acrylamide and concluded that curcumin display anti-apoptotic, antioxidant and anti-inflammatory effects[18]. Dolatabadi *et al* conducted a study to ruled out that curcumin could improve memory and neurological deficits and restore irregular neuronal distribution following the cerebral ischemia[14]. Curcumin response toward the amendment of widening of perivascular space and neuropil vacuolation were not observed previously. Our study witnessed the neuroprotective potential of curcumin in these parameters.

Curcumin administration to tartrazine treated rats were found to significantly re-equilibrate antioxidant parameters back to normal values[19]. Curcumin administration improved the structural and serological alterations of the frontal motor cortex with significant reduction in tissue MDA levels[18]. Results of Bhat *et al* showed a significant protective effect of curcumin in neurodegenerative conditions, which may be due to amending lipid peroxidation and augmenting antioxidant defense system[20]. The limitation of study was the lack of immunohisto-chemical marker GFAP to rule out the astrocytes injury.

ACKNOWLEDGMENT

We are highly obliged to Riphah International University, Rawalpindi for providing us with immense help and opportunities for carrying out our research. We are also thankful to Dr. Hussain Ali, Scientific Officer, National Institute of Health, Islamabad for his immense help in animal handling and housing of animals. We thank Mr. Ijaz Lab technician in NIH, provision and establishing protocols of MDA assay kits.

CONCLUSION

The curcumin administration markedly halted the tartrazine induced oxidative stress and neuroinflammation which was replicated in the form of restoration of neurovascular unit of gray matter of motor cortex. Therefore, it was concluded that curcumin administration improves the tartrazine induced neurobio-chemical and histopathological changes in the gray matter of motor cortex of rat cerebrum.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Khaled E, Osama M S, Manal K, Mohamed E, Hussein HA, Rabie S, et al. Microbiological, histological, and biochemical evidence for the adverse effects of food azo dyes on rats. J Food Drug Anal 2017; 25(3): 667-70.
2. Bhatt D, Vyas K, Singh S, John PJ, Soni I. Tartrazine induced neurobiochemical alterations in rat brain sub regions. Food Chem Toxicol 2018; 113(1): 322-27.
3. Cemek M, Emin Buyukokuroglu M, Sertkaya F, Alpdagtaş S, Hazini A, Onul A, et al. Effects of food color additives on antioxidant functions and bioelement contents of liver, kidney and brain tissues in rats. J Food Nutr Res 2014; 2(10): 686–91.
4. Bell AH, Miller SL, Castillo-Melendez M, Malhotra A. The neurovascular unit: effects of brain insults during the perinatal period. Front Neurosci 2020; 13(1): 1–19.
5. Alsalman N, Aljafari A, Wani TA, Zargar S. High-dose aspirin reverses tartrazine-induced cell growth dysregulation independent of p53 signaling and antioxidant mechanisms in rat brain. Biomed Res Int 2019; 7(1): 1-8.
6. Cobley JN, Fiorello ML. Redox biology 13 reasons why the brain is susceptible to oxidative stress. Redox Biol 2018; 15(2): 490-503.
7. Nabila M, Nawel M, Omar A, Soraya AT, Chahinaize Z, Omar K, et al. A thirteen week ad libitum administration toxicity study of Tartrazine in Swiss mice. Afr J Biotechnol 2015; 12(28): 4519-29.
8. Ghonimi W. Histological changes of selected westar rat tissues following the ingestion of tartrazine with special emphasis on the protective effect of royal jelly. J Cytol Histol 2016; 6(4): 1-6.
9. Papadopoulos MC, Verkman AS. Aquaporin water channels in the nervous system. Nat Rev Neurosci 2013; 14(4): 265–77.
10. Ghasemi F, Bagheri H, Barreto GE. Effects of curcumin on microglial cells. Neurotox Res 2019; 36(1): 1-20.
11. Peter CM, John V, Charlotte MK, Julia FB, Alys EB, Dawn GG, et al. International harmonization of toxicologic pathology nomenclature: an overview and review of basic principle. Toxicol Pathol 2012; 40(4): 7-13.
12. Abd-Elhakim YM, Hashem MM, El-Metwally AE, Anwar A, Abo-EL-Sooud K, Moustafa GG, et al. Comparative haematoimmunotoxic impacts of long-term exposure to tartrazine and chlorophyll in rats. Int Immunopharmacol 2018; 63(7): 145–54.
13. 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.
14. Dolatabadi LK, Emamghoreishi M, Namavar MR, Sarkala HB. Curcumin effects on memory impairment and restoration of irregular neuronal distribution in the hippocampal CA1 region after global cerebral ischemia in Male rats. Basic Clin Neurosci 2019; 10(5): 527–39.
15. Koo BB, Calderazzo S, Bowley BGE, Kolli A, Moss MB, Rosene DL, et al. Long-term effects of curcumin in the non-human primate brain. Brain Res Bull 2018; 142(2): 88–95.
16. Sorrenti V, Contarini G, Sut S, Dall'Acqua S, Confortin F, Pagetta A, et al. Curcumin prevents acute neuroinflammation and long-term memory impairment induced by systemic lipopolysaccharide in mice. Front Pharmacol 2018; 5(9): 1–12.
17. Ahmad RS, Hussain MB, Sultan MT, Arshad MS, Waheed M, Shariati MA, et al. Biochemistry, safety, pharmacological activities, and clinical applications of turmeric: a mechanistic review. Evid Based Complement Alternat Med 2020; 10(1): 1-5.
18. 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.
19. Alsamydai A, Jaber N. Pharmacological aspects of curcumin. Int J Pharm 2018; 5(6): 313–26.
20. Bhat A, Mahalakshmi AM, Ray B, Tuladhar S, Hediyal TA, Manthiannem E, et al. Benefits of curcumin in brain disorders. Biofactors 2019; 45(5): 666-89.