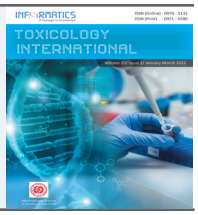
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Potential Protective Role of Curcumin on the Toxic Effect of Food Azo Dye Tartrazine on the Brain of Young Albino Rats

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Abstract

The use of coloring agents in food industry has dramatically increased. Children are the main consumers of colored food products and beverages and are more vulnerable to the effects of synthetic colorants. Curcumin, an active constituent of turmeric plant, has been the subject of extensive studies assessing its antioxidant properties. The present study was conducted to evaluate the protective potentials of curcumin against neurotoxicity induced by the synthetic food colorant; tartrazine. Thirty-six young male albino rats were divided into six groups; negative control (Group I), distilled water (Group II), corn oil (Group III), curcumin (Group IV), tartrazine (Group V) and tartrazine+curcumin (Group VI). At the end of the study, the rats were subjected to biochemical, histological and immunohistochemical staining for GFAP. Our results showed that tartrazine induced a significant decrease in GSH, serotonin and GABA levels and a significant increase in MDA levels in the brain as compared to control groups. Tartrazine induced apoptosis, vacuolations, congestion and cellular infiltration in the brain tissue. An immunohistochemistry study showed astrogliosis with increased expression of GFAP. Curcumin ameliorated all these effects. In conclusion, tartrazine is neurotoxic. Increased brain oxidative stress and cellular damage are contributing factors. Curcumin can prevent tartrazine-induced neurotoxicity.

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1. Introduction

Food additives are compounds that are added to food products to achieve a specific technological goal while not being consumed as food itself[1]. The Food and Drug Administration (FDA) has classified food additives into six main categories including coloring agents[2]. Food color is a feature that makes the food attractive and recognizable[3]. Over the last few years, there has been a progressive increase in the use of synthetic colorants that are added to food in Egypt without controlled use[4]. Although artificial food colorants are considered safe within the Acceptable Daily Intake (ADI), excess consumption may result in many hazards. Children, especially in developing countries, are at high risk because they most often exceed the prescribed ADIs[5].

Tartrazine (E102) is a monoazo synthetic dye that is commonly used as a colorant in food products, medicines and industrial products intended for human use[6,7]. The FDA has estimated the ADI of tartrazine for humans as 0 - 7.5 mg/kg body weight/day[8]. Tartrazine is reduced inside the body to aromatic amines mostly sulphanilic acid and aminopyrazolone that create Reactive Oxygen Species (ROS), which in turn accelerates oxidative stress[8,9]. This biotransformation is responsible for different disorders including anemia, liver, kidney and spleen pathological lesions, as well as allergic reactions, genotoxicity and cancer[10-16]. Even at the ADI level of tartrazine, oxidative damage and alteration of biochemical brain tissue markers can occur[17].

Because of its high content of Polyunsaturated Fatty Acids (PUFAs), rapid oxidative metabolic activity, relatively low antioxidant ability and insufficient neuronal cell repair function, the brain is highly vulnerable to the damage caused by free radicals from food additives[18]. When PUFAs get attacked by free radicals, lipid peroxides are generated[19]. Lipid peroxidation can alter cell membrane integrity. After a while, persistent oxidative stress will result in irreversible loss of the brain and spinal cord cells besides damage of the nerve endings leading to disturbances in neurotransmitter transport and subsequent alteration in CNS functions. So, antioxidant defense is very important for the protection of the nervous system[20].

Curcumin is the principal ingredient of the Curcuma longa plant; turmeric[21]. It has gained increasing attention for its multiple pharmacological activities, including antioxidant, anti-inflammatory, antibacterial and antiviral activities, as well as the therapeutic potential for cancer and Alzheimer's Disease (AD)[22]. Curcumin attenuates the neurotoxicity induced by different toxicants such as lead, ethanol and arsenic. It also offers neuroprotection against neurodegenerative diseases in cell and animal models. Furthermore, curcumin stimulates the proliferation and differentiation of neural stem cells, both in vitro and in vivo[23]. According to[24], curcumin is a powerful antioxidant that enhances the activities of endogenous antioxidant enzymes and prevents lipid and protein oxidation. Due to its high lipophilic nature, curcumin can cross the Blood-Brain Barrier (BBB) and the inner mitochondrial membrane easily and thus provides protection of mitochondria against oxidative stress[25,26] .

The aim of the present study was to evaluate the ameliorative role of curcumin on changes induced by oral administration of tartrazine on the brain of young male albino rats.

2. Material and Methods

2.1 Animals

Thirty-six young male albino rats (28 days old)[27] weighing about 60-80 gm each were included in this study. They were obtained from the Animal House of the Faculty of Medicine, Zagazig University. The study was conducted in the departments of Physiology and Forensic medicine and Clinical Toxicology, Faculty of Medicine, Zagazig University. Rats received care in compliance with the Animal Care Guidelines and Ethical Regulations in accordance with ''The Guide for the Care and Use of Laboratory Animals"[28]. Prior to the experiment, rats were apparently healthy. They were exposed to 7 days period of passive preliminaries so as to acclimatize themselves to their new environment. They were kept in separate plastic cages with an ambient temperature range of 22 ± 2 ºC, relative humidity of 50 ± 5 % and a 12 hr light/ 12 hr dark cycle and received a standard laboratory diet and water *ad libitum*. The design of the experiment was approved by The Institutional Animal Care and Use Committee (IACUC), Zagazig University on May, 2019 (ZU-IACUC/3/F/85/2018).

2.2 Chemicals

Tartrazine (orange powder with dye content > 85 %) and

curcumin (yellowish-orange powder) was obtained from Alfa Aesar, Germany, and Sisco Research Laboratories Pvt. Ltd (SRL), India respectively via The International Company for Scientific and Medical Supplies, Cairo, Egypt. Distilled water (as a solvent for tartrazine) was obtained from El- Nasr Co, Egypt while corn oil (as a solvent for curcumin) was obtained from the local market. Kits for estimation of reduced glutathione (GSH) and malondialdehyde (MDA) were purchased from Bio diagnostic Co., Egypt. Serotonin ELISA kit (Cat. No.RE59121) was obtained from IBL international (Immuno-Biological Laboratories), Germany and gamma-aminobutyric acid (GABA) ELISA kit (Cat. No.KA4912) was purchased from Abnova Corporation, Taiwan.

2.3 Experimental Procedure

After housing acclimatization, rats were randomly divided into six equal groups (6 rats per group) as follows:-

Group I (Negative control group): Each rat received only regular diet and tap water to measure the basic parameters.

Group II (Positive control group; distilled water): Each rat received 1 ml distilled water as a solvent of tartrazine[9].

Group III (Positive control group; corn oil): Each rat received 0.5 ml corn oil as a solvent of curcumin[29].

Group IV (Curcumin group): Each rat received 200 mg/kg B.W curcumin[30] dissolved in 0.5 ml corn oil.

Group V (Tartrazine group): Each rat received 320 mg/kg B.W tartrazine (represents 1/20 of LD_{50} of tartrazine in rat) dissolved in 1 ml distilled water. Oral LD_{50} of tartrazine for rats is 6375 mg /kg body weight[31].

Group VI (Tartrazine and curcumin-treated group): Each rat received 200 mg/kg B.W curcumin dissolved in 0.5 ml corn oil followed 1 hour later[32] by 320 mg/kg B.W tartrazine dissolved in 1 ml distilled water.

All treatments were given once daily by oral gavage for 28 days. At the end of the experiment (24 hours from the last dose of each substance), the rats of all groups were exposed to diethyl ether inhalation followed by decapitation[33]. Skulls of the sacrificed rats were opened, brains were immediately dissected out and grossly inspected to assess any gross-abnormalities and washed with cold normal saline to remove any excess blood. The left cerebral hemispheres were prepared for biochemical analysis while the right cerebral hemispheres were used for histopathological and immunohistochemical studies.

2.4 Biochemical Analysis

The left cerebral hemispheres from all groups were cut into small pieces for homogenate preparation. The homogenate was prepared using an electrical homogenizer by mixing 0.5 g of tissue with 5 ml phosphate-buffered saline (PBS) at 4 ºC. Then homogenates were centrifuged at 3000 rpm for 15 min. Then, the collected supernatant was conserved (at −20 ºC) until further use for the biochemical examination[34]. Reduced glutathione (GSH) concentration was measured by the method of Beutler *et al.,*[35] and malondialdehyde (MDA) concentration was assayed according to the method proposed by Ohkawa *et al.*,[36]. Serotonin level was assayed using serotonin ELISA kit obtained from IBL international, Germany[37]. Gamma amino-butyric acid (GABA) level was assayed using GABA ELISA kit obtained from Abnova Corporation, Taiwan[38].

2.5 Histopathological Studies

Brain specimens were fixed in 10 % neutral buffered formalin for 48 hours, washed in distilled water, dehydrated in ascending grades of ethyl alcohol (50, 70, 90 and 95 %) each for one hour then in absolute alcohol (100 %) for one hour, cleared in xylene for 2 hours then embedded in soft paraffin wax at 55 ºC for 2 hours and in hard paraffin at 60 ºC for 2 hours. Sections of 3-4 µm in thickness were obtained by rotatory microtome and stained with hematoxylin and eosin for histopathological examination[39].

2.6 Immunohistochemical Analysis

Immunohistochemical detection of GFAP was performed using streptavidin-biotin complex immunoperoxidase system: Serial sections of paraffin-embedded specimens were deparaffinized on charged slides, incubated in 0.1 % hydrogen peroxide (H_2O_2) for 30 min to block the endogenous peroxidase then incubated with the primary antibody; mouse anti-GFAP antibody (Cat. No. MS- 280-R7, Lab Vision Corporation, Fremont, USA) diluted in 1:100 in Lab Vision antibody diluent (Cat. TA- 125- UD) for 20 min at room temperature. After several washes with phosphate buffer solution (PBS), primary antibodies were detected by incubation with biotinylated goat anti-mouse IgG (Zymed Laboratories; South San Francisco, CA, USA) for 30 min at room temperature[40].

2.7 Histo-morphometric Analysis

The Leica Qwin 500 (Leica Ltd, Cambridge, UK) image analyzer computer system at the Image Analyzing Unit, Pathology Department, Faculty of Dentistry, Cairo University, Egypt, was used to evaluate the GFAP percentage area. Using the interactive measure menu, the Area % was measured. The measuring frame of a standard area equal to 118476.6 mm^2 was chosen so that the brown positive immunoreaction could be seen and masked by blue binary color to be measured. Ten readings from five non-overlapping sections from each rat of all groups were examined.

2.8 Statistical Analysis

Data were represented by mean ± standard deviation (SD). Data were analyzed using Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis[41]. Differences between multiple groups were detected by one-way analysis of variance (ANOVA) test followed by least significant difference (LSD) test. Probability (P-value) was set at ≥ 0.05 for non-significant results, <0.05 for significant results and <0.001 for high significant results.

3. Results

3.1 Oxidative Stress Biomarkers

There was no statistically significant difference (P≥0.05) between negative control, distilled water, corn oil and curcumin groups regarding brain tissue levels of GSH and MDA all over the period of the study (Table 1). So, the results of the negative (-ve) control group were used for comparison with other groups of the study.

Regarding GSH levels among (-ve) control (group I), tartrazine (group V) and tartrazine + curcumin (group VI), there was highly significant reduction in the mean value of GSH (ng/mg) in tartrazine group when compared with other groups of the study (P<0.001). There was highly significant elevation in the mean value of GSH (p< 0.001) in tartrazine + curcumin group when compared with tartrazine group and a significant reduction in the mean value of GSH (p<0.05) in tartrazine + curcumin group when compared with (-ve) control group (Table 2).

As regard MDA levels among (-ve) control group, tartrazine (group V) and tartrazine + curcumin (group VI), there was highly significant elevation in the mean value of MDA (nmol/mg) in tartrazine group when compared with other groups of the study (P<0.001).

Table 1. Statistical comparison between the mean values of GSH, MDA, serotonin and GABA of young male albino rats of the negative (-ve) control group, distilled water group, corn oil group and curcumin group using ANOVA test

Group / Variable	Group I (-ve) control	Group II (+ve) control; distilled water	Group III (+ve) control; corn oil	Group IV Curcumin	F	P
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
GSH (ng/mg):	5.09±0.49	5.04±0.54	4.9± 0.6	5.13±0.39	2.102	0.21 NS
MDA (nmol/mg):	52.18±2.33	52.2±1.7	52.105±1.32	52.2±1.6	1.22	0.39 NS
Serotonin (ng/mg):	16.91±0.52	17.16±0.27	17.02±0.507	17.15±0.47	0.984	0.598NS
GABA (ng/mg):	4.24±0.16	4.11±0.18	4.14±0.25	4.27±0.12	1.259	0.321 NS

Number of rats in each group=6; SD: Standard Deviation; NS: Non significant (P ≥ 0.05).

GSH: Glutathione; MDA: Malondialdehyde.

GABA: Gamma amino butyric acid.

There was highly significant reduction in the mean value of MDA ($p<0.001$) in tartrazine + curcumin group when compared with tartrazine group and highly significant elevation in the mean value of MDA ($p<0.001$) in tartrazine + curcumin group when compared with (-ve) control group (Table 2).

3.2 Neurotransmitters

There was no statistically significant difference ($P\geq0.05$) between negative control, distilled water, corn oil and curcumin groups regarding brain tissue levels of serotonin and GABA all over the period of the study

Table 2. Statistical comparison between the mean values GSH and MDA of young male albino rats among (-ve) control (group I), tartrazine (group V) and tartrazine + curcumin (group VI) using ANOVA test

Group / Variable	Group I (-ve) control	Group V Tartrazine	Group VI Tartrazine + curcumin	F	P
	Mean ± SD	Mean ±SD	Mean ± SD		
GSH (ng/mg):	5.09±0.49	2.12±0.11[a]	4.36±0.02[b]	18.321	<0.001**
MDA (nmol/mg):	52.18±2.33	102.6±0.43[a]	61.53±18.7[a,b]	43.1	<0.001**

Number of rats in each group=6; SD: Standard Deviation.

GSH: Glutathione; MDA: Malondialdehyde.

**: highly significant ($P<0.001$).

[a]: significantly different from -ve control group (LSD test; $p<0.001$)

[b]: significantly different from tartrazine group (LSD test; $p<0.001$)

Table 3. Statistical comparison between the mean values of serotonin and GABA of young male albino rats among (-ve) control (group I), tartrazine (group V) and tartrazine + curcumin (group VI) using ANOVA test

Group / Variable	Group I (-ve) control	Group V Tartrazine	Group VI Tartrazine + curcumin	F	P
	Mean ± SD	Mean ± SD	Mean ± SD		
Serotonin (ng/mg):	16.91±0.52	8.75±0.43[a]	15.91±0.07[b]	98.845	<0.001**
GABA (ng/mg):	4.24±0.16	1.52±0.19[a]	3.82±0.29[b]	49.521	<0.001**

Number of rats in each group=6; SD: Standard Deviation.

GABA: Gamma amino butyric acid

**: highly significant ($P<0.001$).

[a]: significantly different from -vecontrol group (LSD test; $p<0.001$)

[b]: significantly different from tartrazine group (LSD test; $p<0.001$)

(Table 1). So, the results of negative (-ve) control group were used for comparison with other groups of the study.

Regarding serotonin and GABA levels among (-ve) control (group I), tartrazine (group V) and tartrazine + curcumin (group VI), there was highly significant reduction in the mean value of serotonin (ng/mg) and GABA (ng/mg) in tartrazine group when compared with other groups of the study (P<0.001). There was highly significant elevation in the mean value of serotonin and GABA (p<0.001) in tartrazine + curcumin group when compared with tartrazine group and significant reduction in the mean value of serotonin and GABA (p<0.05) in tartrazine + curcumin group when compared with (-ve) control group (Table 3).

3.3 Haematoxylin and Eosin (H&E) Staining Results

Examination of H&E stained sections of the rat cerebrum of the negative control group, positive control groups (distilled water and corn oil) and curcumin group showed nearly the same histological picture. H&E stained sections of the negative control group showed neuronal cells of variable sizes and shapes. The most prominent cells were

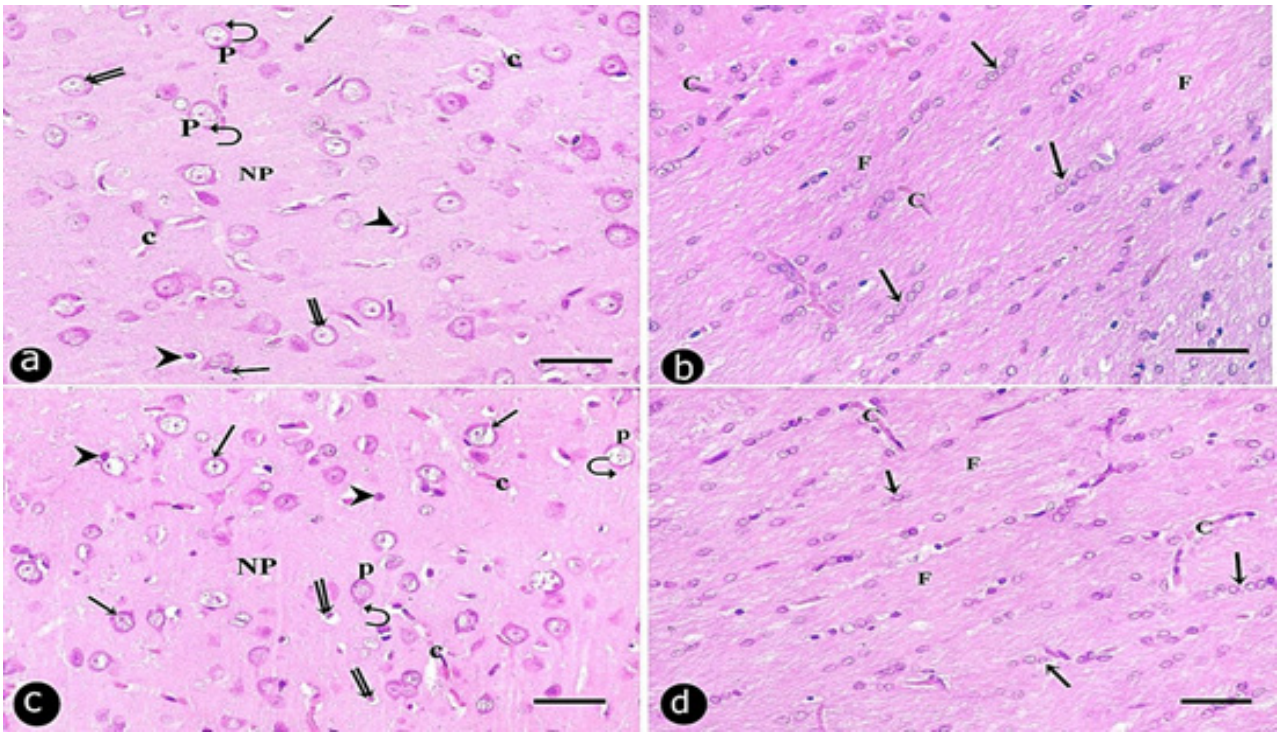


Figure 1. **a.** A photomicrograph of a section from the cerebral cortex of rat from negative control group showing acidophilic neuropil (NP). Pyramidal cells (P) have pale open-face nuclei and neuronal processes (curved arrow). Granular cells (double arrow) have large open face nuclei and prominent nucleoli. Oligodendrocytes (arrow) have dark-stained nuclei surrounded by a perinuclear halo. Astrocytes (arrow head) appear small with pale nuclei. Blood vessels (C) have narrow perivascular spaces. **b.** A photomicrograph of a section from the cerebral cortex white matter of negative control group showing neuropil with regularly packed nerve fibers (F). Rows of neuroglial cells (arrow) and normal capillaries (C) are also noticed. **c.** A photomicrograph of a section from the cerebral cortex of curcumin-treated group showing neuropil (NP), Pyramidal cells (P) have open face nuclei and neuronal processes (curved arrow), granular cells (double arrow), oligodendrocytes (arrow), astrocytes (arrow head) and blood vessels (C) are also detected. **d.** A photomicrograph of a section from the cerebral cortex white matter of curcumin-treated group showing neuropil with regularly packed nerve fibers (F). Rows of neuroglial cells (arrow) and normal capillaries (C) are also noticed (H & E×400, scale bar 20 μm).

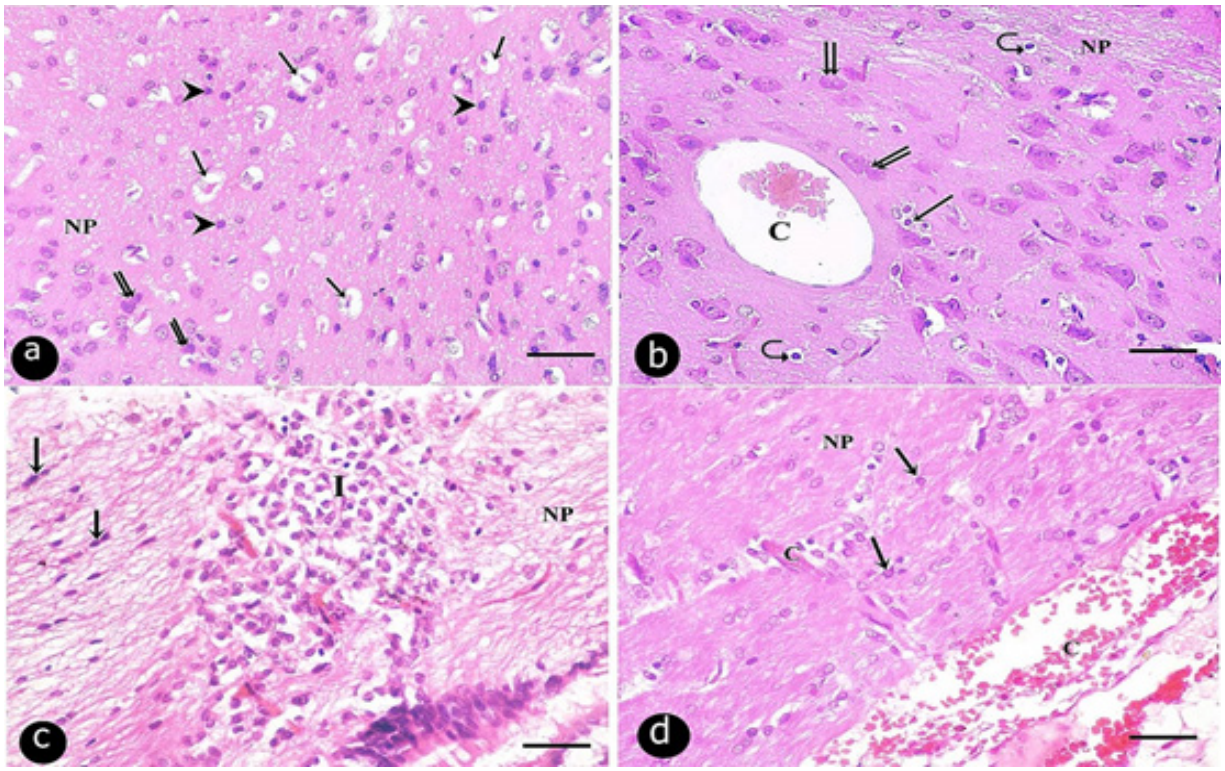


Figure 2. **a.** A photomicrograph of a section from the cerebral cortex of rat from tartrazine-treated group showing vacuolated neuropil (NP). Marked increase in the number of neuroglia cells (arrow head). Some neurons have dark nuclei (double arrow) and others have cytoplasmic remnants (arrow). **b.** A photomicrograph of a section from the cerebral cortex of tartrazine-treated group showing vacuolated neuropil (NP) with dilated congested capillaries (C). Neurons have dark nuclei (double arrow). Oligodendrocytes (curved arrow) and astrocytes (arrow) are also noticed. **c.** A photomicrograph of a section from the cerebral cortex white matter of tartrazine-treated group showing vacuolated neuropil (NP) with dark stained nuclei (arrow). Cellular infiltration (I) is also noticed. **d.** A photomicrograph of a section from the cerebral cortex white matter of tartrazine-treated group showing marked congested dilated capillaries (C). Neuropil (NP) has increased number of neuroglia cells (arrow) (H & E×400, scale bar 20 µm).

pyramidal cells. They had pale open-face nuclei and long processes. Granular cells had large open face nuclei and prominent nucleoli. Astrocytes, oligodendrocytes and peri-neural glial cells were noticed in the gray matter. Neuropil appeared acidophilic and contained blood vessels with narrow peri-vascular spaces among the neurons (Figure 1 a). The white matter contained aggregations of regularly packed nerve fibers and neuroglia cells that were noticed in rows in-between the fibers (Figure 1 b). H&E stained sections from the cerebral cortex of the curcumin group showed the same normal histological picture of gray and white matter (Figures 1 c and d).

Examination of H&E stained sections of the cerebral cortex of tartrazine treated group showed neuropil with marked vacuolations and apoptotic neuronal and neuroglia cells (Figure 2a). Also, sections revealed a marked increase in the number of neuroglia cells. Vacuolated neuropil showed apoptotic cells, cells with shrunken irregular dark nuclei and cells with cytoplasmic remnants (Figure 2b). Haematoxylin and Eosin (H & E) stained sections in the white matter of the same group showed marked cellular infiltration. Vacuolated neuropil showed dark-stained nuclei of glia cells and dilated congested capillaries (Figures 2c and d).

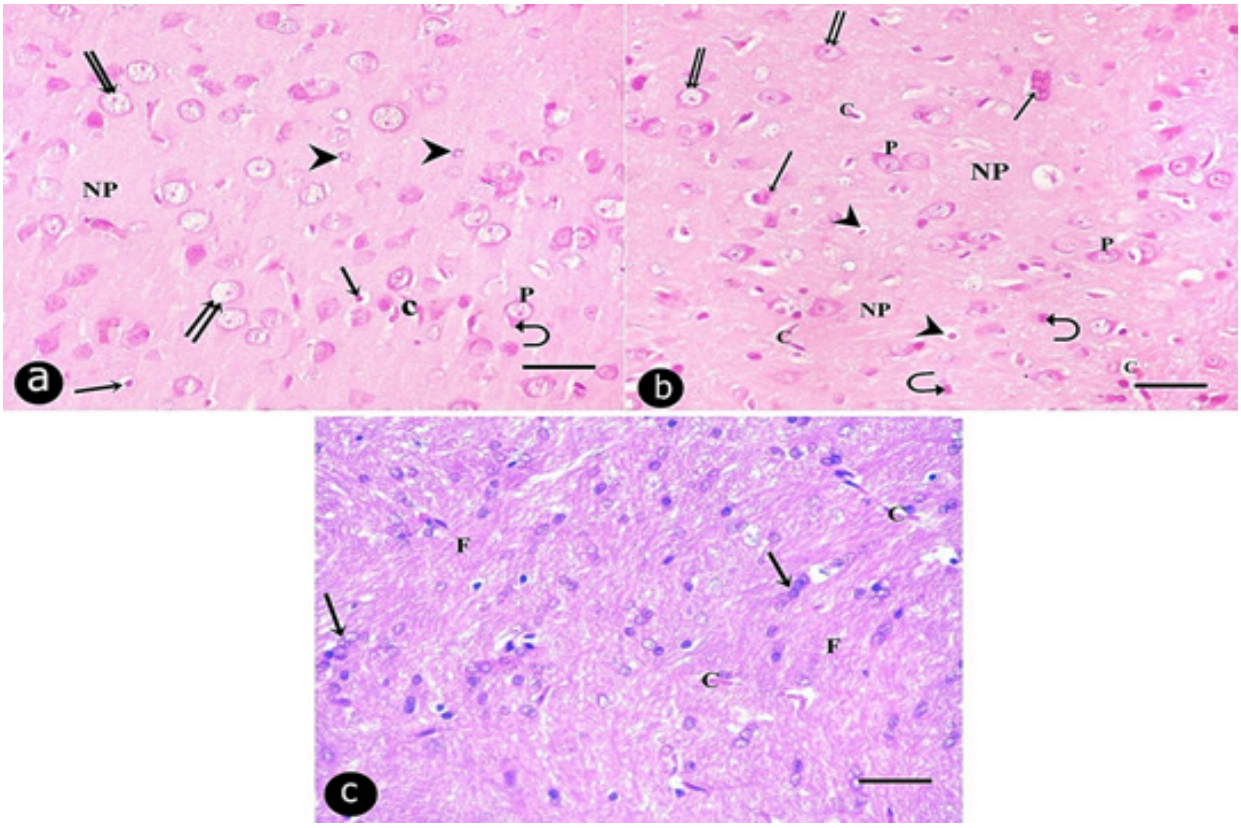


Figure 3. **a.** A photomicrograph of a section from the cerebral cortex ofrat from tartrazine + curcumin group showing normal histological picture. Neuropil (NP) with capillaries (C) having narrow perivascular spaces. Pyramidal cells (P) have open face nuclei. Granular cells (double arrow), oligodendrocytes (arrow) and astrocytes (arrow head) are also detected. **b.** A photomicrograph of a section from the cerebral cortex of tartrazine + curcumin group showing some neuronal cells with dark stained nuclei (arrow). Other cells are histologically normal. Pyramidal cells (P) with their processes, granular cells (double arrow) with their open face nuclei. Capillaries (C) with narrow perivascular space, astrocytes (curved arrow), oligodendrocytes (arrow head) and acidophilic neuropil (NP) are also detected. **c.** A photomicrograph of a section from the cerebral cortex white matter oftartrazine + curcumin group showing neuropil with regularly packed nerve fibers (F). Rows of neuroglial cells (arrow) and normal capillaries (C) are also noticed (H & E×400, scale bar 20 μm).

Examination of H & E stained sections of the rat cerebral cortex of tartrazine + curcumin group revealed sections with nearly the same histological picture as the control cortex; pyramidal cells had pale open face nuclei and long processes. Granular cells had large open face nuclei and prominent nucleoli. Astrocytes and oligodendrocytes were also noticed in the gray matter. Neuropil appeared acidophilic and had blood vessels with narrow peri-vascular spaces among the neurons (Figure 3 a). On the other hand, some sections of the same group showed some neurons with dark apoptotic nuclei (Figure 3b). The white matter contained aggregations of regularly packed nerve fibers and neuroglia cells arranged in rows in-between the fibers (Figure 3c).

3.4 Immunohistochemical Staining Results

Regarding the immune stained sections for identification of GFAP in the cerebrum of control and curcumin, groups revealed positive reactions in the cytoplasm and processes of astrocytes (Figures 4a and b). Group V (tartrazine group) cerebral cortex revealed a strong positive reaction

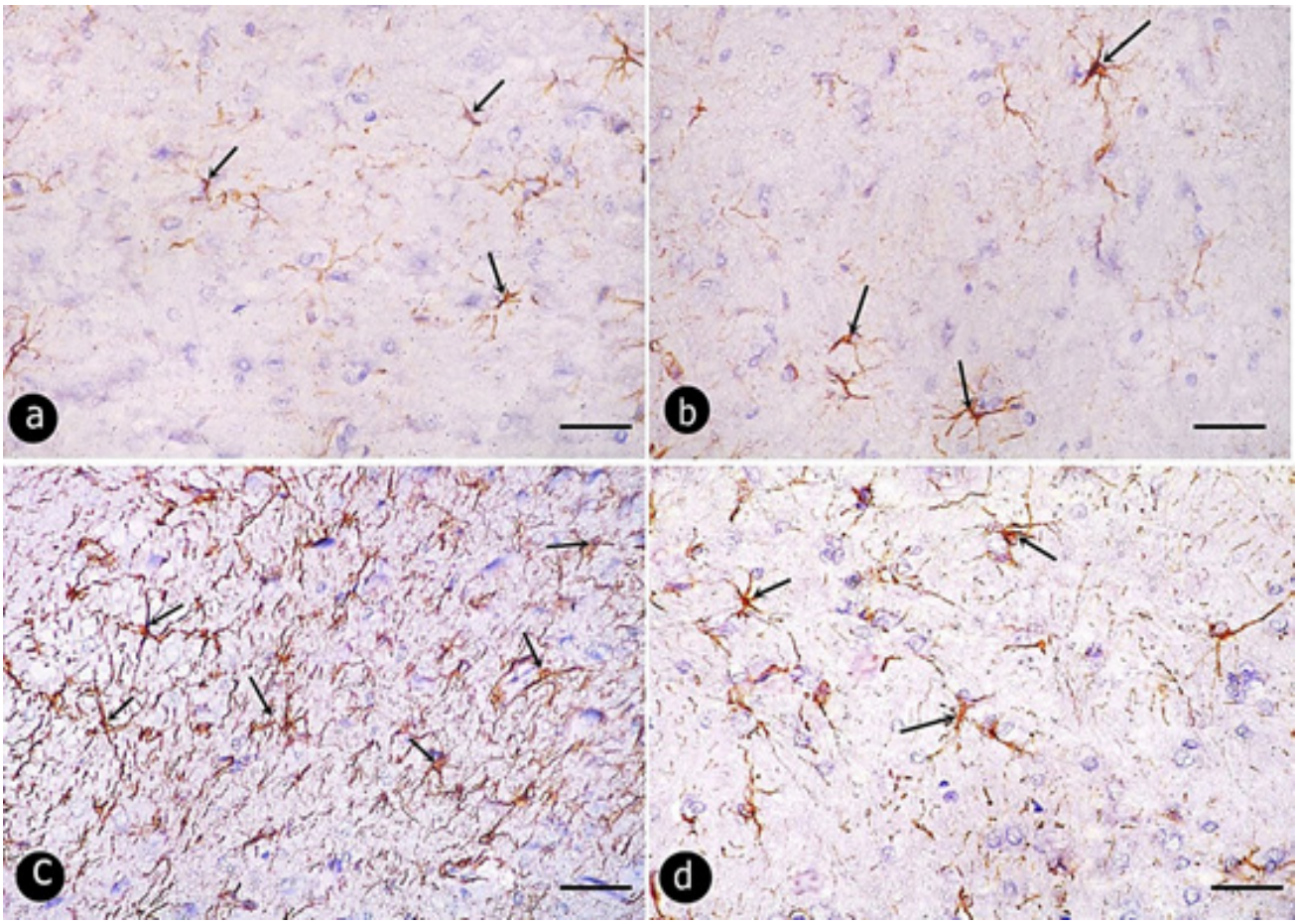


Figure 4. **a.** A photomicrograph of a section from the cerebral cortex of rat from the negative control group showing positive reaction for GFAP in the cytoplasm and processes of astrocytes (arrow). **b.** A photomicrograph of a section from the cerebral cortex of curcumin-treated group showing positive reaction for GFAP in the cytoplasm and processes of astrocytes (arrow). **c.** A photomicrograph of a section from the cerebral cortex of tartrazine-treated group showing strong positive reaction for GFAP in the cytoplasm and processes of astrocytes (arrow). **d.** A photomicrograph of a section from the cerebral cortex of tartrazine + curcumin group showing positive reaction for GFAP in the cytoplasm and processes of astrocytes (arrow) (Immunoperoxidase technique ×400, scale bar 20 μm).

in the cytoplasm and the processes of astrocytes (brown star-shaped cells with increased branching of their cytoplasmic processes) as compared to the control groups (Figure 4 c). Immunoperoxidase reaction for GFAP in group VI (tartrazine + curcumin group) revealed a positive immune reaction in the cytoplasm and the processes of astrocytes (Figure 4d).

3.5 Histomorphometric Results

There was a statistically significant increase in the mean area % of GFAP immunoperoxidase reaction in Group V (tartrazine-treated group) as compared to Group I (-ve control group), Group II (distilled water group), Group III (corn oil group), Group IV (curcumin group) and Group VI (tartrazine + curcumin group). No statistically significant difference was detected between Group I as compared to Groups II, III, IV and VI (Table 4).

Table 4. The mean area % of GFAP immune-reaction in sections of the cerebrum in the different studied groups

Groups / Parameters	Group I (-ve) control	Group II (+ve) control; distilled water	Group III (+ve) control; corn oil	Group IV Curcumin	Group V Tartrazine	Group VI Tartrazine + curcumin	F	P
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD		
Area % of GFAP immune-reactions	11.2±3. 2	12. 1±1.4	11.6±3.5	11.3±2.6	20.04±7.4*	13.1±8.1	12.9	< 0.05

Number of rats in each group=6, SD: standard deviation, GFAP: Glial fibrillary acidic protein, *statistically significant as compared with groups I, II, III, IV & VI at P <0.05.

4. Discussion

Food dyes are added to foods and food products in order to make them look more appetizing and attractive[42]. Tartrazine provides yellow color to pharmaceutical, cosmetic and several food products[43]. Its consumption in food has been reported to induce numerous side effects[44]. Curcumin is a bright yellow-colored spice that is widely used as coloring and flavoring agent[45]. It has the ability to improve the outcome of many diseases owing to its chemo-protective, anti-inflammatory and antioxidant properties[46].

The present study showed that tartrazine caused a significant decline in brain tissue reduced GSH level with a significant increase in the mean value of brain tissue MDA level when compared to control groups. This is consistent with[34] who found that 500 mg/kg of oral tartrazine given to rat pups on a daily basis for 30 days led to the significant decrease of reduced GSH level and significant increase of MDA level in the cerebral cortex as compared to the control group. [17]Added that oral intake of tartrazine at the ADI (7.5 mg/kg/day) for 40 consecutive days can produce oxidative damage in the brain of young rats as evidenced by significantly increased MDA levels in all studied brain sub-regions. Also,[47] revealed that perinatal exposure to oral tartrazine within the ADI (2.5 and 5 mg/kg daily) provoked a significant increase of MDA and a significant decrease of reduced GSH in different brain regions (cerebrum, cerebellum and medulla) in mice offspring.

The brain with its extensive capacity to consume large amounts of oxygen and production of free radicals is considered especially sensitive to oxidative damage[48]. According to Bawazir[49], the metabolism of tartrazine results in the generation of free radicals which in turn inhibit the endogenous antioxidant defense system. Reduced glutathione (GSH) is the major antioxidant in the brain. It has multiple roles in maintaining the redox balance as well as protecting cells against oxidative stress[50]. It acts by scavenging harmful ROS generated in the brain during different molecular processes. GSH level in the brain tissue provides indirect information on oxidative stress of the brain[51]. In the brain, reduced GSH acts as a substrate for several peroxidases including the glutathione peroxidase (GPx) enzyme. During scavenging of free radicals, glutathione disulfide (GSSG) is produced while reduced GSH level declines. So, in case of overproduction of free radicals, reduced GSH is exhausted and its level falls[52]. This may explain the reduced levels of the GSH after tartrazine intoxication[47]. Glutathione is converted into its oxidized form in the reaction catalyzed by GPx, and then it can be reduced back to GSH by the Glutathione Reductase (GR) enzyme. Consequently, the decrease of GSH may be due to a deficiency of the antioxidant enzyme system GPx and GR. This decrease in the antioxidant system indicates a free radical-mediated cellular degeneration[53]. As reported by Chauhan *et al.*,[54], the depletion of GSH promotes the further generation of ROS and oxidative stress with a cascade of effects thereby affecting functional as well as

the structural integrity of cell or organelle membranes. The decreased concentration of GSH in brain tissue was due to excess availability of H_2O_2 in the biological systems which, in turn, generate hydroxyl and peroxyl radicals, resulting in the initiation and propagation of lipid peroxidation[3]. Malondialdehyde (MDA) is the principal end product of the lipid peroxidation process in which ROS react with polyunsaturated fatty acids. It is frequently used for the assessment of oxidative stress in tissues[55]. On the other hand, Cemek *et al.,*[56] stated that there was no significant difference in the levels of brain tissue reduced GSH and MDA when compared to the control group on the administration of oral tartrazine at 3 and 15 mg/kg /day for 15 days in albino rats. Also, Alsalman *et al.,*[57] reported no significant change in the levels of reduced GSH and MDA in the brain tissue after oral administration of 700 mg/kg tartrazine for 2 weeks. The discrepancy between the results of these studies and the present study can be attributed to differences in doses and durations of the experiments. According to Scholpp *et al.,*[58], lipid peroxidation occurs in the late stages of oxidative stress.

In the current work, co-administration of curcumin resulted in a significant increase in the mean values of brain tissue reduced GSH with a significant decrease in the mean values of brain tissue MDA in comparison with tartrazine group. However, these values were still statistically different from those of control groups which indicate partial improvement. According to Abbaoui and Gamrani[59], the protective effect of curcumin could be attributed to its free radical scavenging ability and stimulation of GSH synthesis. Curcumin increases GSH levels by stimulating the activity of enzymes related to GSH biosynthesis like γ-glutamyl-cysteinyl synthetase and glutamate-cysteineligase[60]. The mechanism of neuro-protection involves the prevention of the formation of ROS by curcumin in the rat's brain and increased levels of antioxidant enzymes together with decreasing inflammatory markers such as interleukin-6, tumor necrosis factor alpha, and cyclo-oxygenase-2[61].

The results of the present study demonstrated a statistically significant decline in the mean values of serotonin and GABA in the brain tissue when compared to control groups. These results are in agreement with[34] who revealed that tartrazine administration led to a significant decline in the concentration of the brain neurotransmitters serotonin and GABA in studied rats in comparison to the control group. Marked decline in serotonin level was observed in different brain regions after 8 weeks of 1 % and 3 % oral tartrazine treatment in young albino rats[62]. According to Eman *et al.,*[63], 30 days of oral tartrazine at a dose of 50 mg/kg daily resulted in a significant decline of serotonin level in the cerebellar tissue of experimental albino rats. Oral administration of 7.5 mg/kg/day tartrazine to adult male rats for 30 days induced a remarkable decrease in levels of serotonin and GABA of the cerebellum compared to the control groups[3].

Serotonin is an important neurotransmitter that is involved in the regulation of numerous physiological functions such as food intake, stress and sleep-wakefulness. It is involved in various cognitive functions including learning, memory, and decision making, and is closely linked to social behavior[64]. Gamma amino-butyric acid (GABA) is the main inhibitory neurotransmitter within the central nervous system. It plays an important role in regulation of the balance state between excitation and inhibition of the brain[65]. According to[18], changes in neurotransmitters levels and their metabolites have been used as an indicator of neurotoxic effects of different environmental chemicals. Decline in the level of neurotransmitters was referred to the generation of free radicals when tartrazine is metabolized by intestinal bacteria. These free radicals inhibited the formation of adenosine triphosphate (ATP) and subsequently reduced neurotransmitter synthesis or re-uptake in the presynaptic cell[66]. According to Lee *et al.,*[67], reactive free radicals promote the production of proinflammatory cytokines such as interleukin (IL)-1b, 2, interferon-gamma (INF-γ), or TNF-α. These cytokines enhance the activity of indoleamine 2, 3-dioxegenase (IDO) enzyme which is responsible for tryptophan degradation. Tryptophan is the precursor of serotonin so, its degradation results in a decrease in serotonin synthesis. Oxygen-free radicals cause inhibition of glutamate decarboxylase enzyme and subsequent decrease in the brain cortex content of neurotransmitter GABA[68].

In the current work, upon supplementation with curcumin to tartrazine-treated rats, there was a significant increase in the mean values of serotonin and GABA when compared to tartrazine group. However, these values were still statistically lower than that of control groups which indicates partial improvement. Serotonin levels

in different brain regions significantly improved after 8 weeks of oral 200 mg/kg/day curcumin in tartrazine-treated weanling albino rats[62]. According to Murad *et al.,*[69], curcumin treatment has increased serotonin levels through inhibition of monoamine oxidase (MAO) enzymes and/or interaction of curcumin with serotonin receptors. Monoamine oxidase enzyme is a catabolic enzyme that catalyzes the degradation of serotonin[70]. According to Moghaddam *et al.,*[71] curcumin's ability to enhance GABA level is mediated by activation of glutamic acid decarboxylase enzyme that catalyzes the conversion of glutamic acid to GABA. They also proposed that curcumin could inhibit the GABA transaminase or succinic semialdehyde dehydrogenase enzymes (both of which are the main enzymes in catabolism of GABA).

In the current study, microscopic examination of the brain of rats treated with tartrazine showed areas of separation and marked vacuolations in the neuropil in the cerebral cortex. There were also excessive apoptotic neuronal and neuroglia cells with a marked increase in the number of neuroglia cells. Blood vessels showed dilatation, congestion with wide perivascular spaces. The white matter of the same group showed marked irregularly arranged nerve fibers and glia cells, vacuolations and thickened fibers in the neuropil, marked cellular infiltration, darkly stained nuclei (pyknosis) of glia cells and dilated congested capillaries. As reported by Gao *et al.,*[72], daily oral administration of tartrazine at a dose of 500 mg/kg for thirty days caused edema, vacuolations, karyopyknosis, and disappearance of the nucleoli as well as features of apoptosis in the brain of experimental rats**.** It was found that oral tartrazine at a dose of 500 mg/kg daily given to rats for 30 days resulted in extensive myelin vacuolation in the white matter with very few vacuolations of the grey matter of both the cerebrum and the cerebellum[73]. According to Mohamed *et al.,*[34], tartrazine administration led to pyknosis in the cerebral cortex of male rat pups which denotes irreversible chromatin condensation in the nucleus of cells undergoing necrosis. Daily oral 50 mg/kg of tartrazine given to adult male albino rats over 30 days induced pyknosis and necrosis of purkinje cells in the cerebellum[63]. Occurrence of hemorrhage and increase in the perivascular space in brains of rats treated with tartrazine was recorded by Alsalman *et al.*,[57]. According to[33], oral tartrazine at the ADI caused cerebellar pathological changes in adult rats in the form of subcortical edema, cytoplasmic vacuolations of the gray matter neurons and an increase in the astrocytes population. The intensity of damage is more with increasing dose of tartrazine. Cerebral neuronal degeneration in mice offspring exposed perinatally to oral doses of tartrazine within the ADI range was recorded by Albasher *et al.*,[47].

The brain is very sensitive to oxidative stress since it has a very poor anti-oxidant capacity[74]. According to Bhatt *et al.,*[17], the vulnerability of the brain to oxidative stress is region-specific. This may be the result of variation in the metabolic rate and distribution of the antioxidant enzymes among different brain regions. In this context, the frontal cortex is more susceptible to oxidative damage than other brain regions. The observed vacuolations can be the result of altered lipid metabolism and proteasome dysfunction[75,76].

In the present study, microscopic examination of the brain specimens of curcumin and tartrazine-treated rats revealed improvement in histopathological changes; almost the same histological picture as the control cortex; pyramidal cells had pale open face nuclei and long processes, granular cells had large open face nuclei and prominent nucleoli, astrocytes and oligodendrocytes were noticed in the grey matter, neuropil appeared acidophilic and had blood vessels with narrow peri-vascular spaces among the neurons. On the other hand, some sections of the same group showed some neurons with dark apoptotic nuclei. The white matter contained aggregations of regularly packed nerve fibers and neuroglia cells arranged in rows in-between the fibers.

Brain tissue contains large amounts of polyunsaturated fatty acids, which are predominantly vulnerable to free radical damage and subsequent vacuolations in the brain cortex[77]. Oxidative stress can promote cell death through various signaling pathways. The production of ROS may cause DNA damage and cellular apoptosis[78]. The neuroprotective effect of curcumin is due to inhibition of brain cytokines, oxidative stress, and reduction in cholesterol. Curcumin prevents protein oxidation and protects mitochondrial complex-I by restoring depleted antioxidant glutathione (GSH)[79]. According to Amer *et al.,*[61], curcumin has a direct neurogenic effect through induction of proliferation and migration of neural progenitor cells and enhances neural plasticity and repair. Curcumin can reduce brain edema through maintenance

of endothelial cells integrity and prevention of BBB disruption[80]. The neuroprotective potential of curcumin is mediated by its effect on the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. This pathway plays a remarkable role in antioxidant metabolism, regulation of inflammation, biotransformative reactions and protein degradation. Therefore, regulation of the Nrf2 pathway is important in maintaining homeostasis. Curcumin triggers the activation of the Nrf2 signaling pathway leading to a reduction in oxidative stress, neuronal apoptosis, neutrophil infiltration and microglial activation[81].

In the present study, the cerebral cortex of tartrazine-treated rats revealed a strong positive reaction for GFAP in the cytoplasm and the processes of astrocytes (astrogliosis) as compared to the control groups. According to El-Sakhawy *et al.*,[33], control group rats showed a weak positive reaction of astrocytes to GFAP while it was intense in the cerebellum of tartrazine-administered groups. This denotes activation of astrocytes in response to oxidative injury in order to inhibit the inflammatory response after injury and minimize the damage[82]. Glial Fibrillary Acidic Protein (GFAP) is an intermediary monofilament protein that is located in the cytoskeleton of astrocytes forming a network that provides structural support to the cell[83]. It is essential for the regulation of neuron-astrocyte communication, white matter myelination and maintenance of BBB integrity[84]. Astrocytes, the most abundant cell type in the human CNS, are supporting cells that react to insults or damage to the brain in an attempt to protect it. They respond to CNS injury and other disturbing conditions by a process called reactive astrogliosis[85]. According to Otoabasi *et al.*,[86], activation of astrocytes represents an exceptional mechanism of defense and plays an essential role in the protection of neurons. Reactive astrogliosis is a process whereby astroglial cells undergo cellular hypertrophy [increase of size and protein (GFAP) expression] and proliferation (increased number of glial cells)[87]. According to[88,89] increased GFAP expression is the hallmark of reactive astrocytes that denotes oxidative stress in the brain tissue.

In the present study, immuno-peroxidase reaction for GFAP in tartrazine and curcumin-treated group revealed positive immune reaction in the cytoplasm and the processes of astrocytes nearly similar to the control groups. Curcumin has a potent anti-inflammatory effect as it blocks the activated microglial cell-mediated neuronal cell damage and blocks the production of pro-inflammatory and cytotoxic mediators[90]. As reported by Yuan *et al.*,[91], NF-κβ signaling pathway is very important for the initial activation of astrocytes. They believe that curcumin prevents astrocytes activation, and associated upregulation of GFAP, through inhibition of NF-κβ signaling pathway.

5. Conclusion

In conclusion, the results of the present study confirmed each other as the neurotoxic effect induced by tartrazine on the brain of young rats which was explained by histopathological and immunohistochemical changes was associated with a significant decrease in the neurotransmitters. These changes could be due to oxidative stress as confirmed by the significant decrease in reduced GSH level and the significant increase in MDA level. Moreover, these changes were ameliorated by the natural antioxidant curcumin.

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7. Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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