



Research article

Anxiogenic and memory impairment effect of food color exposure: upregulation of oxido-neuroinflammatory markers and acetyl-cholinesterase activity in the prefrontal cortex and hippocampus



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ARTICLE INFO

Keywords:

Neurobehavior

Pro-oxidants

Anti-oxidants

Tumor necrosis factor-alpha acetyl-cholinesterase

Erythrosine and tartrazine

ABSTRACT

Erythrosine and tartrazine are one of the synthetic azo dye mostly consumed in food, drugs and other industrial compounds. This study was designed to investigate the adverse effect of combine erythrosine and tartrazine on cognitive and neurobehavioral functions, pro-oxidants, endogenous antioxidants, cholinergic system and pro-inflammatory cytokines in rats.

Erythrosine and tartrazine (2 mg/kg, 6 mg/kg, and 10 mg/kg, b.w., p.o, 50:50) was administered to rats (n = 6) for 6 weeks. Memory and neurobehavioral assessment using Novel object recognition test (NORT) and Elevated plus maze (EPM) and biochemical (pro-oxidants and anti-oxidant enzymes) and pro-inflammatory cytokine measurement from the brain sub regions namely, hippocampus and prefrontal cortex were done at the end of treatment. The results showed (p < 0.05) significant decreased memory and neurobehavioral function, increased acetyl-cholinesterase and pro-oxidants activity (Malonaldehyde level and Nitrite), decreased endogenous anti-oxidants (Glutathione and Catalase) and increased pro-inflammatory cytokines (Tumor necrosis factor-alpha, TNF- α). We suggested that the mechanism by which this oxidative and neuro-inflammatory damage and cholinergic system alteration occur might be related to the release of metabolite in fission of the azo dyes of the combined erythrosine and tartrazine administration in the animals. However, we concluded on these findings that erythrosine and tartrazine dyes significantly provoke the release of oxido-nitregic and neuroinflammatory stress markers and also may incite acetyl-cholinesterase activities in different brain regions leading to memory and neurobehavioral impairment.

1. Introduction

Tartrazine and erythrosine are two synthetic azo dye primarily adopted as food colorant to improve the quality of food products. These dyes are certified as colorants by FDA for its food, drug and cosmetic use but it is also generally used in other chemical industries. Toxicological studies have shown that high dose consumption of these colorants is toxic in rats and mice (Abd-Elhakim et al., 2018; Marwa et al., 2019). Synthetic food dyes or colorants include pigments occurring naturally. Given their properties and availability such as it low cost, stability and it colorful attraction, large quantities are produced yearly. However, high stringent

guidelines are been placed on the use and consumption of dye whereby the safety limit used in food and other industrial products is still under continuous debate till today.

There are significant concerns regarding the effect of food colorants on human health, most especially in children in which hyperactivity have been reported (Neeta, 2018). Other reports have shown behavioral impairment, psychological deficit and biochemical alterations in organs of laboratory rodents when exposed to artificial food additives or colorants in experimental based study (Albasher et al., 2020). Additionally, continuous exposure to food colorants in experimental rodents reveals reproductive alterations and teratogenicity in newborns, testicular

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<https://doi.org/10.1016/j.heliyon.2021.e06378>

Received 9 November 2020; Received in revised form 25 January 2021; Accepted 23 February 2021

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damage, cardiomegaly, kidney and liver damage, immune alteration, gastrointestinal toxicity and also toxic to the hematopoietic system (Boussada et al., 2017; Abd-Elhakim et al., 2018; Neeta, 2018; Marwa et al., 2019).

Emerging evidence shows that severe exposure to colorants may become neuro-invasive capable of inciting neurodegeneration and or disorders related to neuropsychiatric disease produced through increased biomarkers of oxidative and neuroinflammatory stress (Albasher et al., 2020). These evidences have led to experimental models using animals exposed to colorants to mimic several features of different neurodegenerative disease (Albasher et al., 2020).

Moreover, neuronal inflammation and oxidative stress has individual features associated with neurobehavioral deficit observed in neurodegenerative disease conditions. Activating the body's immune system in response to neurotoxicity, the food colorants led to a profound change in behavior and physiology of the nervous system (Doguc et al., 2019). Therefore, the over-administration of food colorants or dyes could promote the generation and release of inflammatory biomarkers (Doguc et al., 2019). Although, there seem to be paucity of investigation on the adverse effect of food colorants or dyes on the cholinergic neurotransmission system and pro-inflammatory cytokine infiltration in both clinical and pre-clinical study. Therefore, the aim of this study was to investigate the adverse effect of combine erythrosine and tartrazine on cognitive and neurobehavioral functions, endogenous antioxidants, cholinergic system and pro-inflammatory cytokines in rats.

$$DI = \frac{\text{Difference in time exploring the novel object and familiar object (Z - X)}}{\text{Total time spent exploring both objects (X + Z)}} \quad (1)$$

2. Material and methods

2.1. Drugs and reagents

Tartrazine (T0388-100G) and erythrosine (1159360025), acetylthiocholine, Ellman Reagent [5', 5'-dithiobis-(2- nitrobenzoate) DTNB] and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich, St. Louis, USA. Trichloroacetic acid (TCA) was obtained from Burgoyne Burbidges & Co., Mumbai, India. Tumor necrosis factor-alpha (TNF- α) ELISA kit was procured from BioLegend (USA).

2.2. Experimental animals

Twenty-four male Wistar rat 210–230g purchased from PAMO University of Medical Sciences Central Animal Housing facility and kept within laboratory standard and conditions and acclimated for 2 weeks with free access to standard rat chow before commencing the study. The experimental procedures follow strictly with the University research guideline (PAMO University of Medical Sciences Animal Research Ethics Committee (PUMS-AREC)) according to the "Guide to the care and use of laboratory animals in research and teaching" as prescribed in NIH publications volume 25 No.28 revised in 1996 (NIH, 1996).

2.3. Experimental protocol

The rats were partitioned into 4 groups (n = 6): group 1, taken as control and given distilled water (10 ml/kg), while group 2–4 were treated with 2 mg/kg, 6 mg/kg and 10 mg/kg of Erythrosine and Tartrazine (50:50, p.o) daily for 6 weeks. The rats were subjected to behavioral performance test: learning and memory assessment and anxiety-like behavior. At the end of the performance test, the rats were

anesthetized, euthanized by cervical dislocation and then followed by brain excision.

2.4. Behavioral assessment

The impact of Erythrosine and Tartrazine consumption on neuro-behavioral performance: learning and memory assessment and anxiety-like behavior were examined in the rats during the last five days of treatment.

2.5. Learning and memory assessment

Non-spatial short-term memory was examined in the rats after treatment with Erythrosine and Tartrazine using ORT. We adopted an open-field box (60 cm \times 50 cm \times 40cm) as earlier described by Steckler et al. (1998) and Ennaceur (2010) consisting of two phases: the training and test phase. The training phase lasted a period of 2 days in which the rat was positioned in the box and allowed to explore the two identical objects (X and Y) placed oppositely 8cm from the walls and 34cm apart for 5 min. The object exploration time (seconds) was recorded. The exploration behaviors include object climbing, touching and sniffing. The rats were placed back into their cages for 24 h period. After 24 h retention time interval, the rats were positioned back into the open-field box for the test phase for 3 min in which object Y was replaced with object Z (novel object). Two parameters were recorded: discrimination index (DI) (Equation 1) and percentage exploration preference (PEP)

(Equation 2) which was used as index of memory retention.

$$PEP = \frac{\text{Time spent exploring the novel object (Z)}}{\text{Total time spent exploring both objects (X + Z)}} \times 100\% \quad (2)$$

2.6. Anxiety assessment

The anxiolytic or anxiogenic effect of Erythrosine and Tartrazine was assessed at the end of treatment using the elevated plus maze model (EPM) described by Handley and Mithani (1984) and Pellow et al. (1985). The use of EPM in examining the anxiolytic effect in rodents was validated by Lister (1987). Rodents were tested for the possible aversion of the open arm space and height. The EPM made of two open arms (30 \times 5 \times 15 cm) and two closed arms (30 \times 5 \times 15 cm) extend from the central platform (5 \times 5 cm) and mounted above the floor (38.5 cm) was adopted. The open arms, the central platform, and the floor of the closed arms were painted black. One hour after treatment with Erythrosine and Tartrazine, each rat was positioned at the centre platform facing one of the closed arms and examined for 5 min. The number of open and closed arm entries and the time spent in open and closed arm were scored during the experiment. We repeated the procedure for all the control and treated groups. At the end of each rat's assessment, the maze was cleaned with 70% ethyl alcohol to remove any lingering olfactory cues.

2.7. Brain tissue preparation for biochemical assays

Following the behavioral procedure, the rats were euthanized under ketamine anaesthesia and the brains were excised and dissected for hippocampus and prefrontal cortex isolation, homogenized with 10% w/v phosphate buffer (0.1 M, pH 7.4), centrifuged at 10,000 rpm at 4 $^{\circ}$ C for

10 min and then the supernatants were immediately frozen and stored (-20°C) for biochemical assays of acetyl-cholinesterase and antioxidant activity, nitrite, lipid peroxidation, and pro-inflammatory cytokines.

2.8. Estimation of protein content

The concentration of protein in the hippocampus and prefrontal cortex samples were measured according to [Gornall et al. \(1949\)](#). 0.9 mL of distilled water, 0.1 mL of the brain samples and 3 mL of biuret reagent were added together and allowed to incubate (25°C , 30 min). Thereafter, the absorbance was read at 540 nm with UV/Vis spectrophotometer (725N INESA, China). The standard (1 mg/mL bovine serum albumin) was calculated within the range of 0.01–0.1 mg/mL.

2.9. Estimation of acetylcholinesterase activity

Acetyl-cholinesterase enzyme activity was measured according to [Ellman \(1961\)](#). Aliquots of supernatant (0.4 mL) of the hippocampus and prefrontal cortex homogenate was added to 2.6 mL of phosphate buffer (0.1 M, pH 7.4) followed by 0.1 mL of 5, 5-dithio-bis(2-nitrobenzoic acid) (DTNB). Then, 0.1 mL of acetylthiocholine iodide was added to the reaction mixture. Spectrophotometer (412 nm) was later employed to read the absorbance (10 min). Absorbance changes at 2min interval were recorded. The activity of AChE was measured following increase in colour produced from thiocholine after reacting with DTNB. The change in absorbance per minute was determined and the rate of AChE activity was calculated and expressed as $\mu\text{mol}/\text{min}/\text{mg}$ protein [Ellman et al. \(1961\)](#).

2.10. Estimation of Malondialdehyde level

The MDA level in the hippocampus and prefrontal cortex was measured as previously described [Nagababu et al. \(2010\)](#). 100 μL of tissue supernatant aliquots were mixed with 900 μL s of Tris-KCl buffer and then 500 μL of 30% TCA was added. Thereafter, 500 μL of thio-barbituric acid (TBA) (0.75%) was added and allowed to be heated in a water bath (45 min, 80°C). The mixture was centrifuged (3000 rpm g, 5 min) and the supernatant's absorbances were read at 532 nm. The MDA formed was calculated considering the molar extinction coefficient of $1.56 \times 10^5/\text{M}/\text{cm}$ and expressed as nanomole of MDA per mg protein (nmol MDA mg^{-1} protein).

2.11. Estimation of reduced glutathione level

Estimating the reduced glutathione (GSH) level in the hippocampus and prefrontal cortex were assayed according to [Jollow et al. \(1974\)](#).

Aliquot (100 μL) of the hippocampus and prefrontal cortex was added to 400 μL of TCA (20%) and then centrifuged at 10,000 rpm g (10 min, 4°C). Thereafter, 250 μL of the supernatants was added to 2 mL of DTNB (0.6 M). The mixtures were allowed to be incubated for 10 min at room temperature and absorbance was taken at 412 nm. The brain (hippocampus and prefrontal cortex) content of glutathione was expressed as nmol mg^{-1} protein.

2.12. Estimation of catalase level

Catalase activity was determined using the colorimetric assay based on yellow complex formation with ammonium molybdate with hydrogen peroxide as enzyme substrate according to [Goth \(1991\)](#). The absorbance at 405 nm was taken in a UV/Vis Spectrophotometer (INESA 750N, China). The catalase enzyme activity unit was expressed kU/mg protein for the brain tissue sections.

2.13. Estimation of nitrite level

Hippocampal and prefrontal cortex nitrite level were determined with Griess reagent, indicating the amount of nitric oxide generation [Green et al. \(1982\)](#) 100 μL of Griess reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% of N-1- naphthyl ethylenediamine dihydrochloride) was mixed to supernatant (100 μL) and absorbance was recorded at 540 nm ([Sun et al., 2003](#)). The hippocampal and prefrontal cortex nitrite concentration was determined from the standard curve taken from sodium nitrite (0–100 μM).

2.14. Estimation of TNF- α level

The level of TNF- α in the supernatants of the hippocampus and prefrontal cortex was estimated as described by the manufacturer's protocol. TNF- α was estimated by specific mouse TNF- α (BioLegend ELISA MAX™ Deluxe kit, USA) with sensitivity limit of 4 pg/mL . Measurement was done at room temperature as described by BioLegend protocol using microplate reader at a wavelength of 450 nm filter. The level of TNF- α in the striatum was taken from the standard curve of TNF- α kits. The levels of TNF- α in the striatum was then expressed as pg/mg protein.

2.15. Data analysis

Data were presented as Mean \pm SEM (Standard Error of Mean) using Graph pad Prism software, version 5.0 and all the statistical difference at level $p < 0.05$, were considered significant. Analysis of the data was done using one-way (ANOVA) and two-way analysis of variance followed by

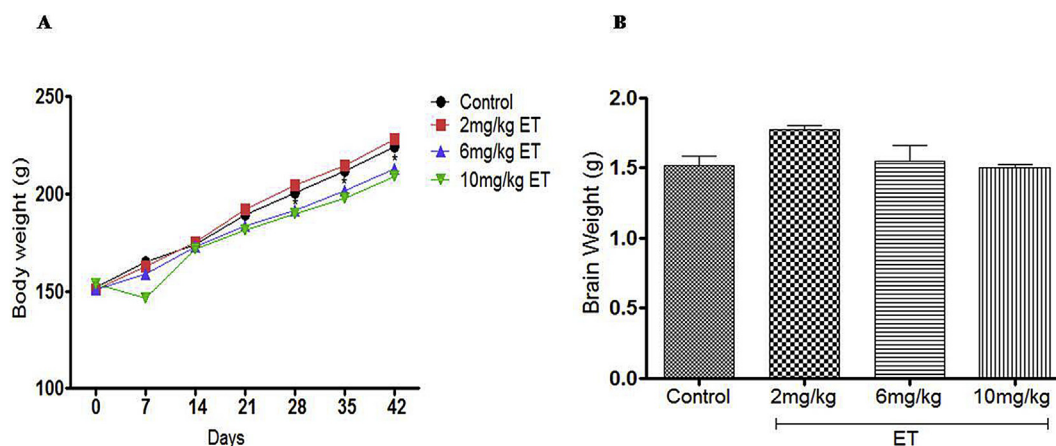


Figure 1. A–B: The effect of erythrosine and tartrazine on body weight and brain weight in rats ($n = 6$; $*p < 0.05$ versus control rat). A = Body weight; B= Brain weight.

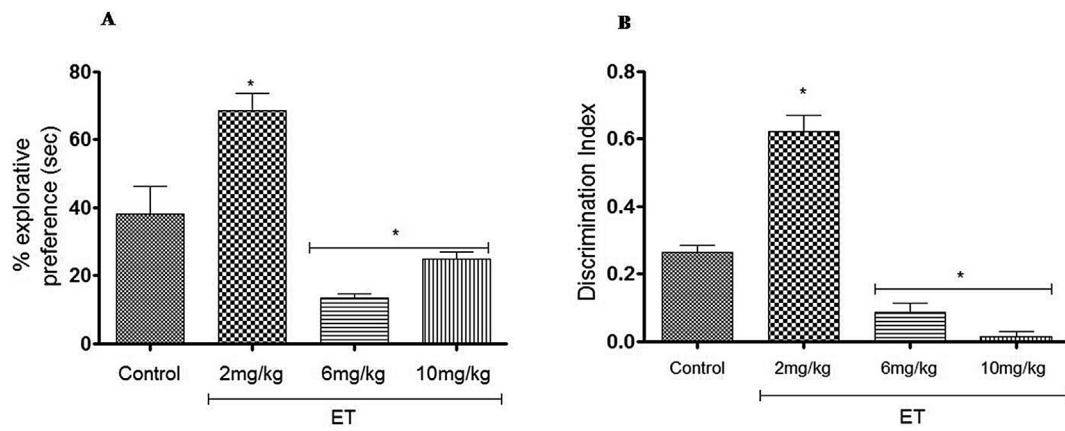


Figure 2. A–B: The effect of erythrosine and tartrazine on non-spatial working memory in rats (n = 6; *p < 0.05 versus control rat). A = Percentage exploratory preference (PEP); B = Discrimination index (DI).

Newman-Keuls and Bonferroni post hoc test for the comparison of experimental groups.

3. Results

3.1. Erythrosine and tartrazine effect on body weight and brain weight

The effect of combine consumption of erythrosine and tartrazine was shown in Figure 1(A, B). The rats subjected to continuous consumption of Erythrosine and Tartrazine exhibited significantly (p < 0.05) decreased body weight from the 4th week of treatment to the last week (28–42 days) of treatment in the rats administered 6 mg/kg ET and 10 mg/kg ET comparative to the control (Figure 1A). However, the oral treatment with the combined color did not show any significant difference (p > 0.05) in the brain weight of the treated rats when compared to the control (Figure 1B).

3.2. Erythrosine and tartrazine decreases non-spatial working memory in rats

As shown in Figure 2A, B, the lowest dose (2 mg/kg) of erythrosine and tartrazine consumption for 6 weeks (p < 0.05) significantly enhanced non-spatial memory retention as observed in increased percentage exploration preference (PEP) and increased discrimination index (DI) (Figure 2A). Meanwhile, treatment with (6 mg/kg and 10 mg/kg) ET

impaired non-spatial memory retention with decreased percentage exploration preference (PEP) and discrimination index (DI) (Figure 2B).

3.3. Erythrosine and tartrazine increases anxiety-like behavior in rats

The effect of erythrosine and tartrazine consumption on anxiety-like behavior was shown in Figure 3A, B. Erythrosine and tartrazine (6 mg/kg and 10 mg/kg) caused significant increase (p < 0.05) in anxiety-like behavior as evidenced in decreased time spent in the open arm and increased time spent in the closed arm comparative to the control (Figure 3A). However, the number of entries into the open arm decreased significantly (p < 0.05, 6 mg/kg and 10 mg/kg) (Figure 3B). Moreover, there was (p < 0.05) significant increase in the number of entries into the closed arm in the rats treated with 2 mg/kg ET when compared with the control (Figure 3B).

3.4. Erythrosine and tartrazine elevate acetylcholinesterase enzyme activity in rat's brain

The results showed a dose dependent significant increase in acetylcholinesterase activity in hippocampal tissues in the rats treated with (p < 0.05, 6 mg/kg and 10 mg/kg) respectively. Also, the activity acetylcholinesterase in the prefrontal cortex was more pronounced (p < 0.05) in the rats treated with 10 mg/kg ET alone after 6 weeks (Figure 4).

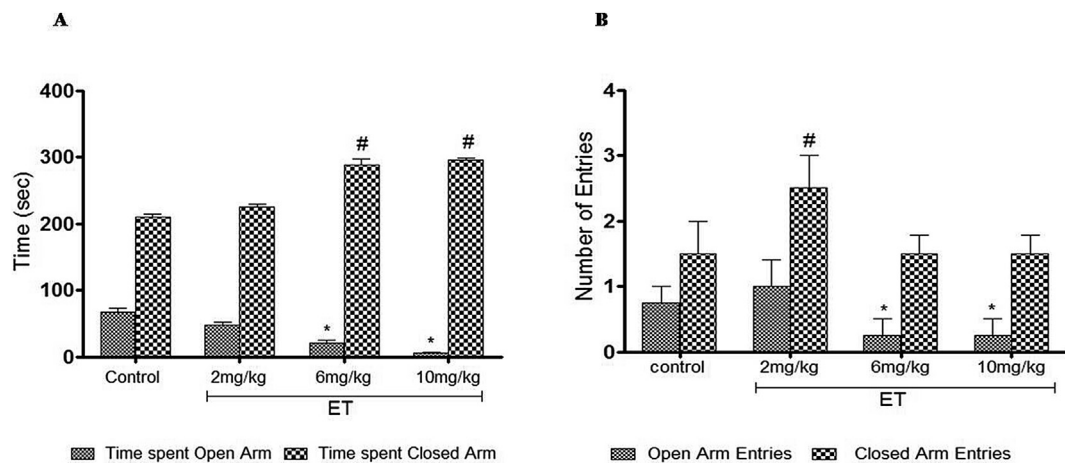


Figure 3. A–B: The effect of erythrosine and tartrazine on anxiety-like behavior in rats (n = 6; *p < 0.05 versus control rat; #p < 0.05 versus control rat). A = Time spent in open and close arm; B= Number of entries in the open and closed arm.

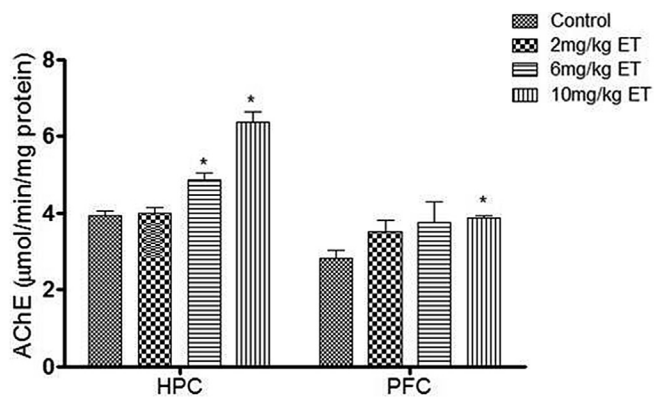


Figure 4. The effect of erythrosine and tartrazine on acetylcholinesterase enzyme activity in rat's brain (n = 4; *p < 0.05 versus control rat). ET = Erythrosine and Tartrazine.

3.5. Erythrosine and tartrazine provoke up-regulation of oxidative stress markers in rat's brain

Brain tissue (Prefrontal cortex and hippocampal tissues) MDA concentration increased significantly after 6 weeks exposure to erythrosine and tartrazine (p < 0.05, 6 mg/kg and 10 mg/kg) when compared to the control (Figure 5A). Erythrosine and tartrazine (p < 0.05, 2 mg/kg, 6 mg/kg and 10 mg/kg) reduces glutathione concentration significantly in the hippocampal tissue whereas significant decreased concentration of glutathione in the prefrontal cortex was only recorded after treatment with (p < 0.05, 2 mg/kg, and 10 mg/kg) (Figure 5B). Treatment with 2 mg/kg ET only, caused significant depletion of hippocampal catalase concentration. Meanwhile, there was no significant difference in the level of catalase concentration in the prefrontal cortex after exposure to erythrosine and tartrazine (Figure 5C).

3.6. Erythrosine and tartrazine increased nitregeric stress in rat's brain

Treatment with erythrosine and tartrazine (p < 0.05, 2 mg/kg, 6 mg/kg and 10 mg/kg) significantly elevated the brain nitrite level in the hippocampus. However, treatment with 6 mg/kg ET alone produced (p < 0.05) significant increase in brain nitrite level in the prefrontal cortex (Figure 5D).

3.7. Erythrosine and tartrazine provoked tumor necrosis factor-alpha (TNF-α) in rat's brain

Figure 6 showed the effect of erythrosine and tartrazine on the pro-inflammatory cytokine (TNF-α) in the rat's brain tissues. There was pronounced elevation of TNF-α in the two brain regions (hippocampus and prefrontal cortex) after treatment with ET (p < 0.05, 2 mg/kg, 6 mg/kg and 10 mg/kg) comparative to the control.

4. Discussion

This study investigated the neurotoxicity effect of erythrosine and tartrazine on memory and neurobehavior, endogenous antioxidant system and neurotransmitter alteration in rat's brain regions. Erythrosine and tartrazine impaired neurocognitive functions which lead to decreased percentage exploratory preference (PEP) and discrimination index (DI) in the novel object recognition paradigm. Increased time spent and number of entries in the closed arm indicated anxiety-like behavior in rat. Erythrosine and tartrazine altered cholinergic neurotransmission by provoking the elevation of acetylcholinesterase enzyme activity. There was up-regulated oxido-nitregeric stress as evidenced by increased concentration of prooxidants- MDA and nitrite, and reduced concentrations of anti-oxidant- GSH and CAT in the different regions of rat's brains.

Food colors are widely consumed on a regular basis due to their availability in food and industrial products. The continuous over-application of the food color in industrial food production has created

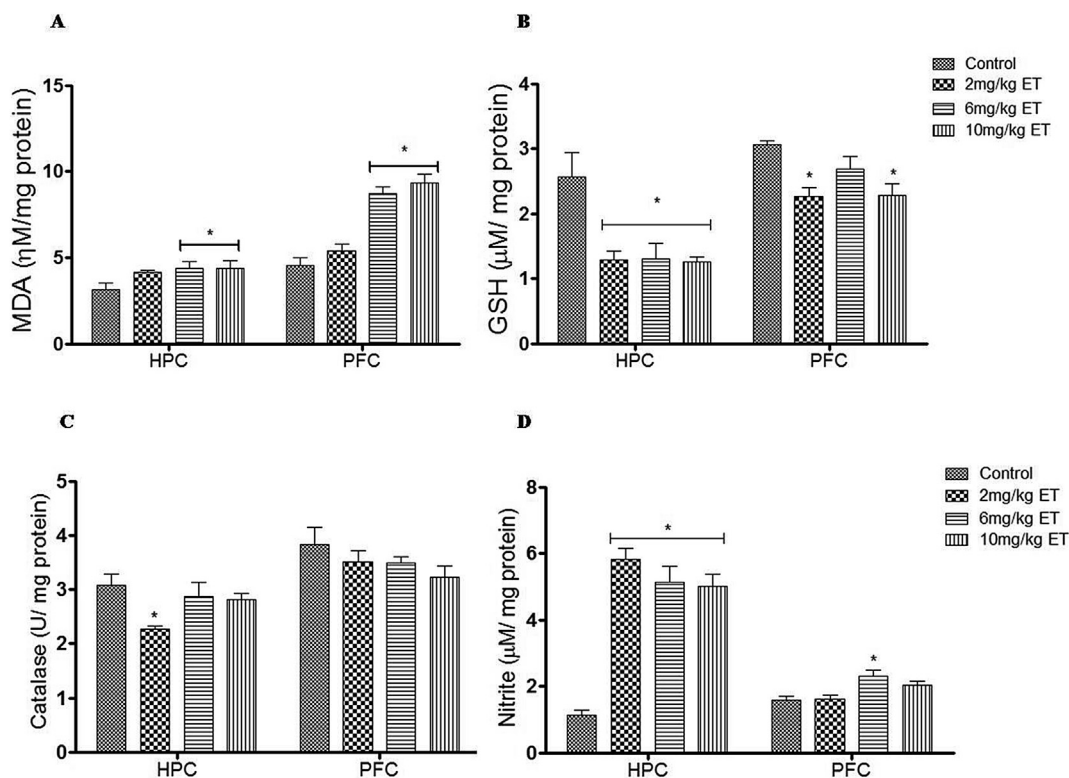


Figure 5. A–D: The effect of erythrosine and tartrazine on pro-oxidants and endogenous anti-oxidant concentrations in rat (n = 4; *p < 0.05 versus control rat). A = MDA concentration; B = Glutathione concentration; C= Catalase concentration; D = Nitrite concentration; ET = Erythrosine and Tartrazine.

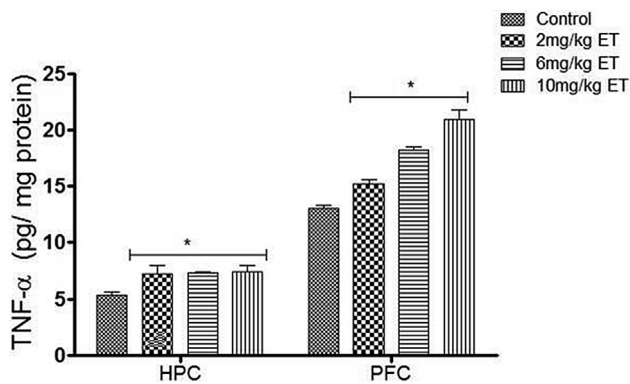


Figure 6. The effect of erythrosine and tartrazine on proinflammatory cytokine in rat ($n = 4$; * $p < 0.05$ versus control rat). ET = Erythrosine and Tartrazine.

serious interest towards its use in food products. Meanwhile, there are literature reviews available on the toxic effect of food colors at high and low doses but not on its moderate (ADI).

Post erythrosine and tartrazine treatment decreased the body weight gain from the 4-6th weeks which may be accountable to the food color adverse effect, as decrease or loss in body weight gain is accepted as an indicator of sensitive toxicity (Bhatt et al., 2018). However, the brain weight did not produce any significant changes after completing the treatment with the dyes for six weeks. This agrees with the findings of Bhatt et al. (2018) who reported decreased body weight gain in rats after oral consumption of food dyes.

Results obtained from the novel object recognition test (NORT) and elevated plus maze (EPM) paradigm in this present study revealed a decrease in cognitive and behavioral function in rats exposed to 6 weeks erythrosine and tartrazine consumption. The NORT paradigm is used to assess non-spatial working memory which is hippocampal and prefrontal cortex dependent (Steckler et al., 1998; Ennaceur, 2010), while the EPM was used to assess anxiogenic or anxiolytic effect (Lister, 1987). Erythrosine and tartrazine treatment resulted in reduction of the amount of time spent exploring the novel objects comparative to the familiar object in the NORT and increased amount of time spent in the closed arm in the EPM. Erythrosine and tartrazine treatment increased cognitive impairment and anxiety associated with the 6 weeks administration to the rats, implying its potential to mediate memory deficit conditions which is accordant with previous reports of erythrosine or tartrazine on rodents (Ceyhan et al., 2013; Albasher et al., 2020), confirming that both erythrosine and tartrazine may have the potentials to develop amnesic and anxiodepressive characteristics (Kamel and El-Iethy, 2011; Ceyhan et al., 2013; Doguc et al., 2019; Albasher et al., 2020).

Memory impairment resulting from neurodegeneration is related to biochemical alterations such as the increase expression of acetylcholinesterase activity, elevated lipid peroxidation, and anti-oxidant- GSH and CAT depletion in the brain (Ajayi et al., 2020; Elizabeth et al., 2020) Evaluating the activity of acetylcholinesterase enzyme in the different brain regions of the rats indicated an indirect means of measuring the neurotransmitter acetylcholine which plays an important role in memory retention and learning processes (Umukoro et al., 2020). Drugs that improve memory have been well studied to mediate their effect by partly inhibiting the activities of acetylcholinesterase in the brain. However, our study demonstrated an elevated activity of the acetylcholinesterase enzyme in the hippocampus and the prefrontal cortex of rat's brain treated with increased dose of erythrosine or tartrazine. Food colors or dyes that enhanced acetylcholinesterase or inhibited the biosynthesis of neurotransmitters like dopamine, acetylcholine, and serotonin facilitating the mechanisms of learning and memory processes have been reported to induced cognitive impairment and could produce neurodegenerative conditions such as Parkinson's and Alzheimer's

diseases (Dalala and Poddar, 2009; Mohamed et al., 2015; Doguc et al., 2019).

Another maker of pro-oxidants: nitrite and malonaldehyde were measured and we recorded elevated levels in the hippocampal and cortical tissues. Malonaldehyde level is measured to evaluate the end product of lipid peroxidation, a consequence resulting from the elevated reactive oxygen species (ROS) or reactive nitrogen species (RNS) generation in the rat's brain (Bhatt et al., 2018; Albasher et al., 2020). The increased nitrite and malonaldehyde concentrations were accompanied by the endogenous antioxidant depletion, glutathione (GSH) and catalase (CAT). The erythrosine and tartrazine -induced oxido-nitrgeric stress may be related to the memory deficit recorded in this study. Furthermore, the erythrosine and tartrazine treatment caused oxidative and nitrgeric damage to the brain macromolecules-lipids and proteins, inhibits cognition and neurobehavioral performance, and increases the accumulation and deposition of amyloid. In brain regions, activated inflammatory cytokines release (TNF- α) is associated with oxido-nitrgeric stress. Report suggests that stress induced TNF- α overproduction is implicated in the development of cognitive and neurobehavioral deficit (Weber et al., 2017; Ajayi et al., 2020). Activated pro-oxidant and anti-oxidant makers in turn, causes continuous generation of pro-inflammatory cytokine and except the sustained exposure to stress is inhibited or blocked, pro-inflammatory cytokine release increases further, stimulating an inflammatory response (Ajayi et al., 2020). Evidences indicated that turning on the pro-inflammatory signaling pathway in the brain led to negative impact on cognition, emotion and evokes anxiodepressive-like behaviors (Weber et al., 2017). However, reduction of brain inflammatory cytokine level produces a significant improved behavior and neuronal stability (Elizabeth et al., 2020). Based these findings, our result indicated activated pro-inflammatory pathway in the rat's hippocampal and cortical region subjected to erythrosine and tartrazine treatment across all doses. Furthermore, this could also be attributed to the contributory factors noticed in the memory impairment in erythrosine and tartrazine -rats.

5. Conclusion

Our study confirmed that food colors or dyes such as erythrosine and tartrazine induced biochemical alterations and exacerbates cognitive and neurobehavioral decline through increased oxidative and nitrgeric stress, acetylcholinesterase activity and release of pro-inflammatory cytokine in the rat's brain.

Declarations

Author contribution statement

Wopara Iheanyichukwu: Conceived and designed the experiments; Performed the experiments.

Modo Emmanuel U.: Analyzed and interpreted the data.

Olusegun G. Adebayo: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mobisson Samuel K.; Nwigwe Jovita O.; Prince I. Ogbu: Contributed reagents, materials, analysis tools or data.

Nwankwo Vincent U.: Analyzed and interpreted the data.

Ejeawa Constance U.: Performed the experiments.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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