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Research article

Involvement of striatal oxido-inflammatory, nitrosative and decreased cholinergic activity in neurobehavioral alteration in adult rat model with oral co-exposure to erythrosine and tartrazine

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ABSTRACT

Overuse or overconsumption of food additive or colorant cannot be ignored in our society and there are several reports of it harmful effect on the body system. This study investigated the toxicity effect of tartrazine and erythrosine (ET, 50:50) on neurobehavioral alteration, striatal oxido-nitrosative and pro-inflammatory stress and striatal acetylcholinesterase activity in experimental rat model. Rats were co-exposed to ET (2 mg/kg, 6 mg/kg and 10 mg/kg) and distilled water (control), p.o for 6 weeks. The change in neurobehavioral function (Open field test, Forced swimming test and Tail suspension test), Lipid peroxidation (Malonaldehyde, MDA), Antioxidants (Glutathione, GSH; Catalase, CAT) Nitrite, Pro-inflammatory cytokine (Tumor necrosis factor-alpha, TNF-α) and Acetylcholinesterase (AChE) activity were evaluated. Results showed significant decrease in neurobehavioral functions after co-exposure to ET. Moreover, there were significant increase in MDA and Nitrite level, significant decrease in the concentration of GSH and CAT and a significant increase TNF-α concentration and AChE activity after co-exposure to ET. Oral co-exposure to tartrazine and erythrosine induced decrease in locomotion and exploration, increase anxiety and depression-like behavior and altered the cholinergic system through upregulation of oxido-nitrosative stress, pro-inflammatory cytokine and acetylcholinesterase activity.

1. Introduction

The prevalence of excess consumption of food additives or color is increasingly becoming an important area that needs to be generally focused on. Food additives have been seen to institute various alterations to the body's defense mechanism which then leads to damage to the tissues and organs. Reports claimed that food additives or color caused deranged renal system (Wopara et al., 2020), institute liver hepatotoxicity (Amin et al., 2010), tampers with the hemopoeitic system (Dafallah et al., 2015) and also interferes with enzymes activities leading to reduction in functions when experimented in rat model (Himri et al., 2011; Ai-Mashhedy and Fijer, 2016). However, the Food and drug acts (FDA), Food and Agriculture Organization (FAO), World Health Organization (WHO) and other food safety authorities reviewed scientific literatures regularly and reported that synthetic food colors/dyes possess no destructive effect when taken within the daily acceptable doses. Meanwhile, these authorities have also imposed strict regulations and standards for the approval and use of various artificial food dyes and how the producers and manufacturers of food products are permitted to use those dyes (FAO/WHO, 2014; Barrow et al., 2014). Food additives or food color was previously suggested to adversely tamper with neurobehavior in children. Suspicion about the commonly believed effect of food additives or food color is to initiate hyperactive, impellent and lack of attention behavior observed in children associated with attention-disorder/hyperactivity disorder (Doguc et al., 2013).

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Neuronal disorders in developmental stages including attention-disorder/hyperactivity disorder (ADHD), dyslexia, hypokinesia, autism, and other related cognitive declines tampering with the health of millions of children worldwide are yet to be fully understood (Grandjean and Landrigan, 2014). Examining the link between co-exposure to dyes and their effect on brain tissue development, neurobehavioral and neurological alterations as well as neurodegeneration is challenging to human health (Grandjean and Landrigan, 2014). However, ADHD is commonly depicted by ongoing hyperactivity, lack of attention and impulsivity (NIMH, 2019). Although humans, most especially children are extremely regarded as the major consumers of food additives or food color in beverages, candies, chewing gums etc. and therefore are vulnerable to the adverse effects caused (Albasher et al., 2020). Furthermore, the consumption of synthetic food colors prenatally can also exacerbate neurobehavioral alterations and teratogenicity (Albasher et al., 2020). Since Azo dyes have been reported to cause injury to the brain tissue by altering the brain neuronal morphology (Rafati et al., 2017; Wopara et al., 2021), the striatum could be targeted by their activities. Functionally, the striatum direct series of cognition, including processing and planning of motor behavior and actions, making of decision, motivation, perception of reward and reinforcement (Nishi et al., 2011; Yager et al., 2015). The loss of striatal dopaminergic neurons and its activities can result into neurodegenerative disorder such as Parkinsonism, Huntington, chorea, dyskinesia, choreoathetosis etc (Nishi et al., 2011; Yager et al., 2015). Thus, protecting the striatum would reduce any possible development of neuropsychiatric symptoms associated with loss or atrophy of the striatal neurons.

These food colors, tartrazine (E102) and erythrosine (E127) are synthetic azo dye most commonly used. According to JECFA (Joint FAO/WHO Expert Committee on Food Additives 82nd Meeting Summary and Conclusion, 2016), the acceptable daily intake (ADI) in human for tartrazine and erythrosine is 0–10 mg/kg and 0–0.1 mg/kg bw). Despite the stringent rules placed on the use and consumption of food color in food products, guidelines introduced by some organizations on their safety limit being liberally consumed are still under debate continually (Bhatt et al., 2018). Concern with respect to food colors on human health effects, more importantly in children is a serious issue in our present society (Bhatt et al., 2018). Tartrazine and erythrosine are adopted in food produce like drinks, sauces, beverages, drugs and other several non-food consumables (Wopara et al., 2020). Azo dyes are constantly being significantly given consideration since they can cause health defect and exert threat to the kidney, gastrointestinal tract and nervous system (Amin et al., 2010; Alsalman et al., 2019; Wopara et al., 2020). Tartrazine and erythrosine was previously reported to cause neurodegeneration and induced neuropsychiatric symptoms such as visual and spatial memory deficit, depression, anxiety, motor deficit as well as changes in dendritic morphology in laboratory rodents (Tanaka et al., 2008; Dalal and Poddar, 2009, 2010; Rafati et al., 2017; Bhatt et al., 2018; Wopara et al., 2021). However, recent studies have highlighted the ability of colors to induce oxidative-inflammatory stress (El-Desoky et al., 2017; Leo et al., 2018; Wopara et al., 2021). This present study was designed to investigate the effect of co-exposure to tartrazine and erythrosine on neurobehavioral alteration, striatal degeneration through oxido-nitrosative stress, pro-inflammatory cytokines expression and cholinergic activity in adult rat.

2. Material and methods

2.1. Drugs and reagents

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

2.2. Experimental animals

Twenty-four male Wistar rat (10–12 weeks old; weighing 180–200g) procured from PAMO University of Medical Sciences Central Animal Housing facility and housed according to the laboratory conditions (12hours light:dark cycle, 28 ± 2 °C) and acclimated for 2 weeks with free access to standard rat chow and water *ad libithum* before the commencement of the study. The experimental protocols supported the University research ethics and guidelines (PAMO University of Medical Sciences Animal Research Ethics Committee (PUMS-AREC)) which obeys the "Guide to the care and use of laboratory animals in research and teaching" as indicated in the NIH publications, volume 25 No.28 revised in 1996 (National Institute of Health, 1996).

2.3. Experimental protocol

The rats were assigned into 4 groups (n = 6). Equal quantity of erythrosine and tartrazine (50:50) were weighed, mixed and prepared in distilled water before administration. The rats in group 1, served as non-treated control given distilled water (10 ml/kg, p.o), while group 2–4 were orally (p.o) co-exposed to daily administration with 2 mg/kg, 6 mg/kg and 10 mg/kg of combined erythrosine and tartrazine with the use of oral gavage for 6weeks as established by JECFA. The dose selection was in accordance with our findings from previous reports and preliminary studies (Dalal and Poddar, 2010; Bhatt et al., 2018; Wopara et al., 2021). The experimental rats were subjected to behavioral analysis for locomotion, exploration and depression within the last two days of treatments followed by anesthesia, cervical dislocation and brain excision (Figure 1).

2.4. Behavioral assessment

The effect of co-exposure to Erythrosine and Tartrazine on neurobehavioral deficits: locomotion, exploratory activity and depressive-like behaviors were examined in the rats in the last two days of treatment.

2.5. Locomotor and exploratory test

One hour after treatment, the animals were exposed to the open field test paradigm to assess locomotion, exploration and symptoms of anxiety on day 41 between 8:00 am- 11:00 am. The rats were acclimated to the test room prior the assessment. Following acclimation, the rats were individually placed within the center of the open field chamber (72 × 72 × 36 cm, divided into 3 concentric zones) and allow free exploring by moving within the chamber. Spontaneous ambulation (taken as the number of line crossing with the whole four paws), freezing duration (expressed as the immobility time) and exploratory activity/anxiety-like behavior (expressed as the time spent rearing with the hind limb and paw and number of grooming) were recorded for 5-min. The chamber was constantly cleaned with 70% ethanol to prevent olfactory cue alteration after each behavioral assessment (Walsh and Cummins, 1976; Izidio et al., 2005).

2.6. Forced swimming test

A cylinder glass jar (30cm height x 20cm diameter) was filled with tap water (15cm from bottom) and allowed to sit overnight to achieve room temperature (approximately 25 ± 2 °C) according to Roy et al. (2007). The rats' tail was prevented from touching the bottom of the cylinder to reduce behavior alteration. One hour following co-exposure to Erythrosine and Tartrazine on day 41 between 1:00 pm- 3:00 pm, the rats were individually picked and placed in the water with the head downward to prevent the rats' head from going underwater. Immobility for 6 min was recorded as the cessation of all movements except those for a float behavioral or light paddling. The water was constantly changed after each successive rat's assessment to prevent contamination and chemical transmission (Roy et al., 2007).

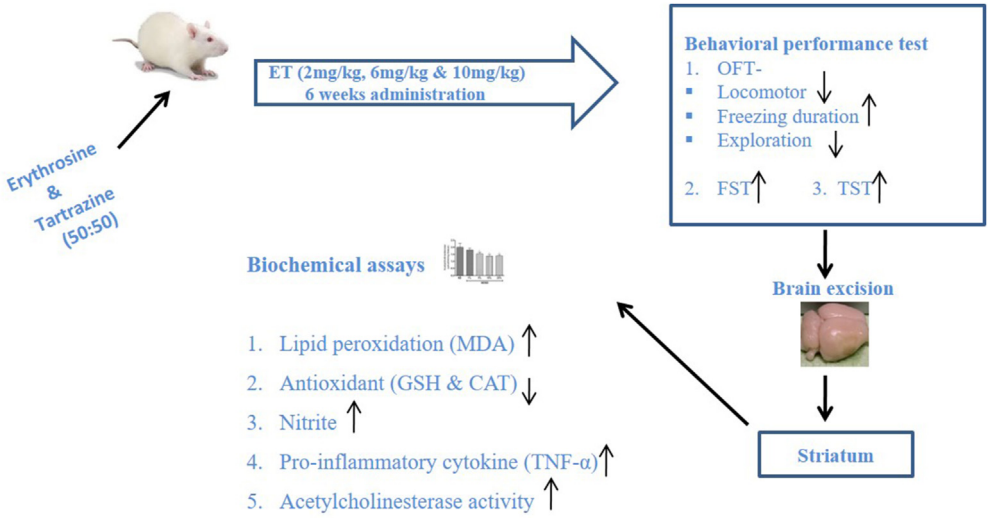

Figure 1. Schematic representation of the experimental protocol.

2.7. Tail suspension test

The tail suspension test (TST), usually adopted for screening procedure and evaluating novel compounds or drugs having anti-depressant activity in laboratory rodents (Adebayo et al., 2020). One hour following co-exposure to Erythrosine and Tartrazine on day 42 between 8:00 am-11:00 am, rat was individually hanged 50 cm above the floor held firm with a masking tape placed 1 cm approximately away from the tip of the rodent's tail. Immobility or freezing time(s) was taken within 6 min following the previous 4 min delay and the rats were accepted immobile when freeze or when remained stationary and hung passively (Adebayo et al., 2020; Dereli et al., 2018).

2.8. Brain tissue preparation for biochemical assays

Following the completion of the behavioral evaluation, all the rats were subjected to euthanasia using ketamine anesthetic agent (20 mg/kg, i.p.) and the brains were collected and then dissected for the isolation of the striatum. Thereafter, the isolated striatum was homogenized in 10% w/v phosphate buffer (0.1 M, pH 7.4), and then centrifuged at 10,000 rpm within 4 °C for 10 min. The supernatants were separated from the homogenate and then were instantly frozen and stored (−20 °C) for biochemical assessment of acetylcholinesterase and oxido-nitrosative activity, nitrite, and pro-inflammatory mediators. Reduced glutathione (GSH) was evaluated according to Jollow et al. (1974). Lipid peroxidation was assayed to quantify the concentration of malondialdehyde (MDA) according to Nagababu *at al* (Nagababu et al., 2010). Catalase (CAT) activity was evaluated as previously described by Goth (1991).

2.9. Estimation of protein content

The levels of protein in the rats' striatum were estimated as previously described by Gornall et al. (1949). Distill water (0.9 Ml), samples of the striatal supernatants (0.1 Ml) with biuret reagent (3 mL) were added thoroughly. The mixture was then further left undisturbed and incubated at 25 °C for 30 min. Thereafter, absorbance (540 nm) was obtained with the use of UV/Vis spectrophotometer (725N INESA, China). The standard (1 mg/mL bovine serum albumin) was later calculated taken within range of 0.01–0.1 mg/mL.

2.10. Estimation of striatal lipid peroxidation

The level of lipid peroxidation (MDA concentration) in the rats' striatum supernatants were measured according to previous description by Nagababu et al. (2010). One hundred microlitre of striatal supernatant was aliquoted, added to 900 μLs of Tris-KCl buffer and then 500 μL of 30% TCA was also mixed with it. Thereafter, 500 μL of thiobarbituric acid (TBA) (0.75%) was also added and was heated using a water bath for 45 min at 80 °C. Furthermore, the mixture was then cooled and centrifuged (3000 rpm g, 5 min) with the supernatant collected and the absorbance read at 532 nm with the UV/Vis spectrophotometer (725N INESA, China). The MDA formed was calculated taking the molar extinction coefficient of 1.56×10^5/M/cm and expressed as nanomole of MDA per mg protein (nmol MDA mg−1 protein).

2.11. Estimation of striatal reduced glutathione level

Determining the level of reduced glutathione (GSH) in the rats' striatum was done following Jollow et al. (1974). The striatal aliquot (100 μL) was mixed to 400 μL of TCA (20%) and further taken through centrifugation at 10,000 rpm g (10 min, 4 °C). After that, 250 μL of the centrifuged supernatants was then added to 2 mL of DTNB (0.6 M). The combine mixtures were incubated for another 10 min at room temperature. The level of absorbance was then obtained at 412 nm. The striatal glutathione content was calculated as nmol mg−1 protein.

2.12. Estimation of striatal catalase level

Catalase activity was assayed with the colorimetric method based on the formation of a yellow complex with ammonium molybdate and hydrogen peroxide as enzyme substrate prescribed by Goth (1991). The absorbance (405 nm) was obtained with a UV/Vis Spectrophotometer (INESA 750N, China) and thereafter, the striatal catalase enzyme reaction unit was calculated as kU/mg protein.

2.13. Estimation of striatal nitrite level

Striatal nitrite concentration was assessed using the Griess reagent, showing the total amount of nitric oxide production (Green et al., 1982). Greiss reagent (100 μL) (1:1 solution of 1% sulfanilamide mixed with 5% phosphoric acid and 0.1% of N-1- naphthyl ethylenediamine dihydrochloride) was thoroughly added to the striatal supernatant (100 μL). Thereafter, the absorbance was taken at 540 nm using the UV/Vis-spectrophotometer (752 N INESIA, China) (Sun et al., 2003). The striatal nitrite concentration was then calculated from the standard curve obtained from sodium nitrite (0–100 μM).

2.14. Estimation of striatal TNF-α level

The concentration and measurement of TNF-α in the rats' striatal supernatants was taken at room temperature (at a wavelength of 450 nm) and determined as prescribed by the manufacturer's procedure as described by Bio Legend protocol with a microplate reader. Shortly, the TNF-α enzyme immunoassay was taken by the addition of 100μL of the striatal supernatants, standards and the controls to each of the wells of an overnight (18 h, 4 °C) mouse TNF-α capture antibody incubated 96 well plate. After that, the plate was thoroughly sealed using an adhesive foil and incubated for 2 hours at a room temperature (25 °C) on a shaker (approx. 500 rpm). The, 100 μL of the biotinylated goat polyclonal anti-mouse TNF-α detection antibody and avidin-horseradish peroxidase (avidin-HRP) solutions were added to each well; the plate was later sealed and incubated for another 1–3 min at room temperature (25 °C) on a shaker (approx. 500 rpm). Afterwards, 100 μL of the chromogenic substrate [3, 3′, 5, 5′- tetramethylbenzidine (TMB)] was put into each of the well and further incubated in a dark environment for another period of 15 min at room temperature (25 °C) before mixing the stop solution (100 μL). Spectramax M-5 (Molecular Devices, Sunnyvale, CA) microplate reader installed with the Softmax Pro v 5.4 (SMP 5.4) was then used to obtain the mixture absorbance (450 nm) within 15 min. In addition, a log-log logistic 4-parameter curve-fitting was used to determine the regional striatal levels in pg/mL.

2.15. Estimation of striatal acetylcholinesterase activity

The activity of enzyme acetylcholinesterase was determined as described by Ellman. The aliquots of the striatal supernatant (0.4 mL) was mixed with a 2.6 mL of phosphate buffer (0.1 M, pH 7.4) followed by another addition of 0.1 mL of 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB). Additionally, 0.1 mL of acetylthiocholine iodide was then thoroughly mixed together with the reaction mixture. Spectrophotometer (412 nm) was then employed to take the absorbance level at 10 min interval. The absorbance changes at every 2min interval were collected and recorded. The activity of AChE was evaluated using an increase in the colour change from thiocholine after the reaction with DTNB. Conclusively, change in the absorbance per minute was determined and the AChE activity rate was recorded and expressed as the μmol/min/mg protein (Ellman et al., 1961).

2.16. Statistical analysis

Values are represented as the Mean ± SEM (Standard Error of Mean) with the use of a Graph pad Prism software, Inc., Lajolla, USA, version 5.0. All statistical difference level was accepted significant at $p < 0.05$. One-way and two-way ANOVA followed by the Bonferroni post hoc test for comparing experimental groups were adopted.

3. Results

3.1. Oral co-exposure to erythrosine and tartrazine induced hypolocomotion in rat

Continuous exposure to erythrosine and tartrazine significantly caused decreased locomotor ability (motor behavioral function) presented by reduction in the total number of distance covered and increased freezing duration within the open field when compared with the control group (Figure 2A, B). As represented in Figure 2A, ET (6 mg/kg and 10 mg/kg, p.o) caused hypolocomotion as indexed by a significant ($p < 0.05$) [$F(3, 11) = 9.792$, $p = 0.0019$, $n = 6$] decreased number of line crossing in the open field test (OFT) compared to the control rats. Also, as shown in Figure 2B, oral co-exposure to erythrosine and tartrazine (10 mg/kg, p.o) increased immobility by a significant ($p < 0.05$) increase in freezing duration [$F(3, 11) = 5.222$, $p = 0.0174$, $n = 6$] in the open field test (OFT) when compared to the control rats (Figure 2B).

3.2. Oral co-exposure to erythrosine and tartrazine altered exploratory profile in rat

The exploratory profile was assessed using the open field maze during a 5-minutes trial within a new environment. Identical to the locomotory profile, there was exploratory deficit observed in the rats treated with erythrosine and tartrazine as they did not explore the walls of the open field apparatus appropriately. As represented in Figure 2C, decreased exploratory activity as determined by two-way ANOVA reveals the statistical difference between the co-exposed rats: index of time spent rearing [Exploration: $F(3, 22) = 5.02$, $p = 0.0084$, Rearing: $F(1, 22) = 11.60$, $p = 0.0025$, $n = 6$] and index of time spent grooming [$F(3, 22) = 9.91$, $p = 0.0002$, $n = 6$]. Oral co-exposure to erythrosine and tartrazine (6 mg/kg and 10 mg/kg, p.o) reduced the rearing time and increased the time spent grooming (6 mg/kg ET) in the treated rat comparative to the control. However, the rat co-exposed to 10 mg/kg ET showed a significant ($p < 0.05$) reduced grooming time when compared to the control. The reduction in exploration indicated that co-exposure to erythrosine and tartrazine may affect locomotor behavior in the rats (Figure 2A–C).

3.3. Oral co-exposure to erythrosine and tartrazine induced depression in rat

Depressive-like effect of co-exposure to erythrosine and tartrazine was determined in the treated rats using the forced swimming and tail suspension test model. As revealed in Figure 3A, B by one-way ANOVA

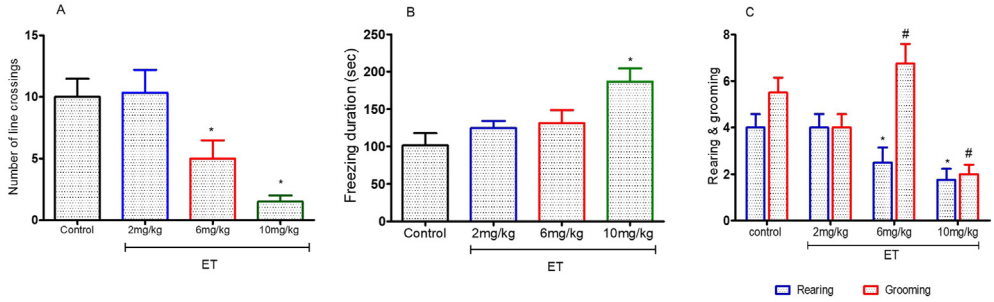

Figure 2. A-C: Oral co-exposure to Erythrosine and Tartrazine on locomotor and exploratory performance (Open Field Test) in rat ($n = 6$; *$p < 0.05$ versus control rat; #$p < 0.05$ versus control rat). A = Number of line crossing; B= Freezing duration; C= Rearing & Grooming; ET = Erythrosine and Tartrazine.

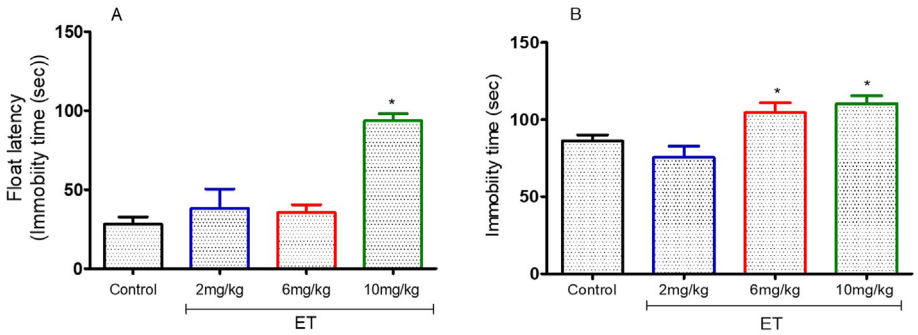

Figure 3. A-B: Oral co-exposure to Erythrosine and Tartrazine on depressive-like activity in rat (n = 6; *p < 0.05 versus control rat). A = Forced swimming test (immobility time); B= Tail suspension test (immobility time); ET = Erythrosine and Tartrazine.

revealed statistical difference after oral co-exposure to erythrosine and tartrazine in the treated rats. In Figure 3A, 10 mg/kg ET increased immobility (depressive-like behavior) as indexed by a significant ($p < 0.05$) [$F(3, 12) = 16.76$, $p = 0.0001$, $n = 6$] increase in duration of immobility in the forced swimming test (FST) when compared to the control rats. However, Figure 3B showed that oral co-exposure to ET (6 mg/kg and 10 mg/kg, p.o) also increased immobility (depressive-like behavior) by a significant ($p < 0.05$) [$F(3, 12) = 7.663$, $p = 0.0040$, $n = 6$] increase in freezing duration in the tail suspension test (TST) when compared to the control rats.

3.4. Oral co-exposure to erythrosine and tartrazine promote striatal oxidative stress in rat

Oxidative and antioxidant status evaluation from this study revealed obvious striatal oxidative imbalance in the animals when exposed to erythrosine and tartrazine as presented in Figure 4A, B. The oral co-exposure to erythrosine and tartrazine induced striatal oxidative stress by significant ($p < 0.05$) [$F(3, 12) = 9.562$, $p = 0.0017$, $n = 4$; ET (2 mg/kg, 6 mg/kg and 10 mg/kg)] increase in brain's striatal malonaldehyde (MDA) concentration (Figure 4A) followed by a significant ($p < 0.05$) [$F(3, 12) = 21.64$, $p < 0.0001$, $n = 4$; ET (2 mg/kg, 6 mg/kg and 10 mg/kg)] decrease in brain's striatal glutathione (GSH) concentration (Figure 4B) when compared to the control. Meanwhile, the brain's striatal catalase (CAT) concentration also decreased significantly ($p < 0.05$) [$F(3, 12) = 22.07$, $p < 0.0001$, $n = 4$; ET (2 mg/kg, 6 mg/kg and 10 mg/kg)] comparative to the control (Figure 4C).

3.5. Oral co-exposure to erythrosine and tartrazine elevated striatal nitrosative stress in rat

Similar to the oxidant and antioxidant status, the striatal nitrosative stress was also evaluated in the animals to further establish the effect of erythrosine and tartrazine co-exposure (Figure 5A). we recorded that oral co-exposure of erythrosine and tartrazine induced striatal nitrosative stress evident by significant ($p < 0.05$) [$F(3, 12) = 11.56$, $p = 0.0007$, $n = 4$] increase in the brain's striatal nitrite concentration after treatment with ET (6 mg/kg and 10 mg/kg) when compared to the control rat.

3.6. Oral co-exposure to erythrosine and tartrazine increased striatal concentration of pro-inflammatory cytokine in rat

Corroborating the oxidative and nitrosative stress recorded in the animals, we observed enhanced production of pro-inflammatory mediator (TNF-α) in the rats' striatum due to erythrosine and tartrazine co-exposure comparative to the control (Figure 5B). All the treatment groups co-exposed to erythrosine and tartrazine expressed significantly ($p < 0.05$) increased striatal concentrations of TNF-α [$F(3, 12) = 43.26$, $p < 0.0001$, $n = 4$; ET (2 mg/kg, 6 mg/kg and 10 mg/kg)] relative to non-treated controls (Figure 5B).

3.7. Oral co-exposure to erythrosine and tartrazine increased striatal acetylcholinesterase activity in rat

Furthermore, to establish the hypolocomotion, anxiety- and depressive-like effect recorded, we evaluated the acetylcholinesterase activity. We noticed that the erythrosine and tartrazine activity altered the striatal acetylcholinesterase activity in the treated rats (Figure 5C). Rat co-exposed to 10 mg/kg ET significantly ($p < 0.05$) [$F(3, 12) = 10.43$, $p = 0.0012$, $n = 4$] elevate striatal AChE activity when compared to the control.

4. Discussion

In this study, co-exposure to erythrosine and tartrazine exacerbate behavioral deficit and biochemical alteration in the rats' striatum through induced hypolocomotion, anxiety- and depressive-like activity, oxidative and nitrosative stress and upregulated acetylcholinesterase and pro-inflammatory activities. However, from our previous investigation following oral co-exposure to erythrosine and tartrazine, we reported decreased body and brain weight, loss of non-spatial working memory

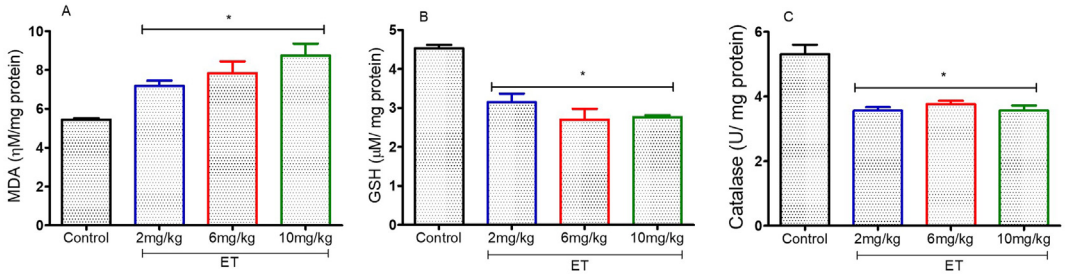

Figure 4. A-C: Oral co-exposure to Erythrosine and Tartrazine on lipid peroxidation and antioxidant concentrations in rat (n = 4; *p < 0.05 versus control rat). A = Malonaldehyde (MDA) concentration; B = Glutathione (GSH) concentration; Catalase concentration; ET = Erythrosine and Tartrazine.

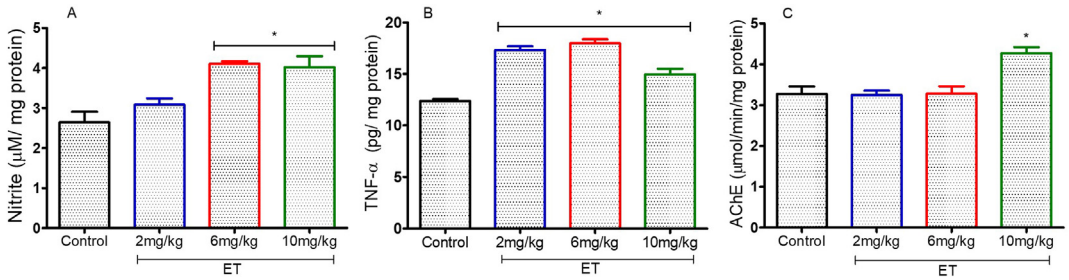

Figure 5. A-C: Oral co-exposure to Erythrosine and Tartrazine on: A = nitrosative stress, B = proinflammatory cytokine and C = acetylcholinesterase activity in rat (n = 4; *p < 0.05 versus control rat). ET = Erythrosine and Tartrazine.

with elevated level of cortico-hippocampal oxidative stress and neuronal inflammation (Wopara et al., 2021).

Impaired locomotion leading to hypolocomotion is a major feature experienced by rodents constantly predisposed to undetermined chronic stress in series of stress models (Umukoro et al., 2020). Clinically, the degree of motor activity is sometimes used to determine the intensity of chronic diseases or stresses. Reports claimed that motor function impairment affects animal motor behavior and thus could as well lead to decline in health of patients recuperating from post-traumatic injury or stress (Gaurav et al., 2013). The results from our study showed that the rats exposed to erythrosine and tartrazine indicated reduced locomotion as evident in the open field test. The numbers of line crossing decreased spontaneously with an increase in the freezing duration, thus, initiating a significant level of anxiety- and depressive-like behavior. Additionally, the exploratory activity observed in the rats during behavioral examination was recorded low. We noticed a reduction in time spent rearing with irregular grooming behavior; hence, developing hypolocomotion with symptoms of anxiety- and depressive-like behavior in the rats. Meanwhile increase or decrease in grooming behavior has also been used for assessing motor behavioral functions in laboratory rodents. Herein, we may suggest that continuous co-exposure to erythrosine and tartrazine tampered with the striatum, thus, reducing locomotion by inhibiting decision making and motivation in the rats. Additionally, the irregular grooming behavior observed in the medium and higher dose treated rats may be related to alteration of the striatal cholinergic activity in the animals. Although, previous findings reported hyperactivity of neurobehavior or no change in neurobehavior in rodents when treated with tartrazine or other food color (Kamel and El-lethey, 2011), but this result further confirmed other findings reported by Albasher et al. (2020), which revealed neurobehavioral alteration following the administration of food color. Our findings therefore suggested that the decreased ambulation might be accompanied with some level of ataxia, but no signs of significant or serious tremor was observed. Moreover, head twitching with increased aggressive behavior indicating anxiogenic and possible motor impairment effect of the food dye co-exposure especially in the high dose treated rats were noticed.

Some major symptoms noticed in patients with the progression of neurological disorder related to hypolocomotion are anxiety and depression. It is co-morbidity with several neuropsychiatric disorders (Adebayo et al., 2020) hence; forced swimming test (FST) and tail suspension test (TST) were used as paradigm for evaluating depressive-like behavior in this study. Our finding further showed that co-exposure to the food colors caused depressive-like behavior as evident in animals. The importance of FST and TST in assessing depressive-like behavior stands on the changes in immobility time, which is usually accepted as an index of 'behavioral despair' (Adebayo et al., 2020). Drugs or compounds increasing immobility duration are deemed to possess depressant-like activity in this paradigm (Adebayo et al., 2020). The results of this present study showed that oral co-exposure to erythrosine and tartrazine increased immobility time in rats suggesting a potential harmful effect by increased depressive-like behaviors. This effect may be attributed to oxidative and nitrosative stress observed in the rats' striatum.

Oral co-exposure to erythrosine and tartrazine for 6 weeks resulted in oxidative and nitrosative stress biomarkers in the striatum, evidenced by increased lipid peroxidation and nitregic pathway activity and decline in antioxidants. Cerebral apoptosis and histomorphologic alteration in rat model following food color exposure have been reported (Albasher et al., 2020). Accordingly, the brain tissue is prone to oxidative damage because of its high concentration of polyunsaturated fatty acids. Moreover, the increased lipid peroxidation and nitrite level with decreased endogenous antioxidant noticed in the striatum are elicited by the co-administration of erythrosine and tartrazine. These redox activities led to the increased oxidative and nitrosative stress in the rats. Therefore, oxidative and nitrosative stress represents major mechanisms underlying the toxicity of co-administrated erythrosine and tartrazine. Given the reported oxidative and nitrosative stress in the striatum of the erythrosine and tartrazine-treated rats, we therefore conclude that the redox imbalance implicated in the brain damage was induced by the colorant co-exposure. We confirmed the redox imbalance by the elevated MDA and nitrite level and reduced endogenous GSH and CAT concentration. These results tandem with previous reports and further strengthen the impact of reactive oxygen and nitrogen species in neuronal cell death in the rat's brain with negatively modulated effect of food color toxicity (Gao et al., 2011; Ceyhan et al., 2013; Bhatt et al., 2018). Our results also unfold the implication of biomarkers of oxidative and nitrosative stress in striatal neuronal toxicity induced by co-exposure to erythrosine and tartrazine.

Investigating the level of neuronal inflammation leading to neuronal degeneration in the brain regions as a result of erythrosine and tartrazine exposure is still ongoing. Meanwhile, neurobehavioral alterations are linked to neuronal inflammation characterized by microglial cell activation and inflammatory mediator expression in the brain tissue that direct and process the deterioration of the central cholinergic systems (Czerniawski et al., 2015; Ming et al., 2015). Neuronal degeneration and motor cognitive decline are being associated with increased oxidative/nitrosative stress which further exacerbate increased upregulation of cytokine storms. Neuroinflammation is initiated due to inflammatory challenges and is amplified by pro-inflammatory molecules released including cytokines in the brain parenchyma (Abd-Elhakim et al., 2019; Wopara et al., 2020). TNF-α has been established as a powerful pro-inflammatory cytokine from activated microphages and other cell types (Czerniawski et al., 2015; Ajayi et al., 2020). Previous clinical studies have reported elevated brain levels of TNF-α in laboratory rodents with neurological disorder, which perhaps produces a causative relationship with memory impairment (Frühauf et al., 2015; Wopara et al., 2020). Nevertheless, findings from this report revealed increased striatal TNF-α expression after oral co-exposure to erythrosine and tartrazine in the animals. The elevation of TNF-α expression coupled with increased oxidative and nitrosative activity as a result of the striatal inflammation may be the reason for the behavioral alteration recorded in the animals. Since we observed increased pro-inflammatory activity (TNF-α expression) in the rats' striatum associated with increased oxidative and nitrosative stress following continuous exposure to the food colors, this might further damage the striatal cells or neurons producing neurotransmitters responsible for initiating motor and exploratory behavior, thus cognitive and motor behavior is impaired.

Inhibiting the acetylcholinesterase activity induced increased acetylcholine (ACh) level in the brain. Acetylcholine is a powerful and important neurotransmitter substance that helps the development of memory retention and learning processes (Eduviere et al., 2015). Notably, employing the use of acetylcholinesterase inhibitors such as donepezil, galatamine etc. acts by significantly increasing ACh availability in the brain for treating of neurodegenerative disorders (Ajayi et al., 2020). In this study, we noted significant expression of acetylcholinesterase activity in the rats subjected to highest dose of oral co-exposure to erythrosine and tartrazine. This increase in acetylcholinesterase activity enhanced the hydrolysis of acetylcholine at the cholinergic synapse which we also suggested to be one of the causes of motor behavioral and exploratory deficit and depressive-like activity in the rats. The increased level of oxidative and nitrosative stress coupled with pro-inflammatory expression may tamper with the cholinergic system in the rat's striatum thereby limiting the degree of responsiveness to internal environmental stimuli. However, we established our facts on these findings that the neurobehavioral alteration recorded could be attributed to the oxido-nitrosative and pro-inflammatory stress and inhibition of the cholinergic synapse in the striatum of the rats orally co-exposed to erythrosine and tartrazine.

5. Conclusion

Our findings from this study revealed the deleterious effects associated with unregulated or continuous oral co-exposure to erythrosine and tartrazine consumption on the striatum. This co-exposure exacerbated neurobehavioral alterations by progressively increasing neuropsychiatric symptoms (anxiety and depression) through the mechanism related to oxido-nitrosative and pro-inflammatory cytokine release. However, our finding also revealed that unregulated or continuous oral co-exposure to erythrosine and tartrazine may provoke inflammatory mechanism as well as inhibit the striatal cholinergic transmission. Meanwhile, furthers molecular studies related to gene deregulation and induction of apoptosis in the striatum that may cause serious neurodegeneration should be focused on.

Declarations

Author contribution statement

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

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

Elizabeth B. Umoren: Analyzed and interpreted the data.

Wadioni Aduema; Adaku V. Iwueke; O.E. Etim: Contributed reagents, materials, analysis tools or data.

Egelege Aziemeola. Pius: Performed the experiments; contributed reagents, materials, analysis tools or data; Wrote the paper.

Woha Boobondah James: Analyzed and interpreted the data.

Joel Wodo: Performed the experiments.

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

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Additional information

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