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Using vitamin E to prevent the impairment in behavioral test, cell loss and dendrite changes in medial prefrontal cortex induced by tartrazine in rats

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ABSTRACT

Tartrazine is a food color that may adversely affect the nervous system. Vitamin E is a neuro-protective agent. This study aimed to evaluate the effects of tartrazine and vitamin E on the performance of rats in memory and learning tests as well as the structure of medial Prefrontal Cortex (mPFC). The rats were first divided into seven groups which received the followings for a period of seven weeks: distilled water, corn oil, vitamin E (100 mg/kg/day), a low dose (50 mg/kg/day) and a high dose (50 mg/kg/day) of tartrazine with and without vitamin E. Behavioral tests were conducted and the brain was extracted for stereological methods The high dose of tartrazine decreased the exploration time of novel objects ($P < 0.01$). The low and high doses of tartrazine led into an increase in working and reference memory errors in acquisition and retention phases (eight-arm radial maze) compared to distilled water group ($P < 0.01$). Additionally, the high dose of tartrazine induced a reduction in the volume of mPFC (~13%) and its subdivision. Not only that, but the number of neurons and glial cells (~14%) as well as the mushroom and thin spines per dendrite length declined. The length of dendrites per neuron also reduced in comparison to the distilled water group ($P < 0.01$). Nonetheless, concomitant treatment of the rats with vitamin E plus tartrazine prevented the above-mentioned changes. An acceptable daily dose of tartrazine could induce impairment in spatial memory and dendrite structure. Moreover, a high dose of tartrazine may defect the visual memory, mPFC structure, the spatial memory and also cause dendrite changes. Vitamin E could prevent the behavioral and structural changes.

1. Introduction

For a long time, chemical additives have been an option for human beings intended to cover the improper quality of blemished food. However, a wide range of food colors used today are synthetic because they are produced easily and provide cheaper and better coloration (Mohamed et al., 2015). Tartrazine (E 102, FD, and C Yellow) is an orange-colored powder, which is widely applied to products to yield a lemon yellow color. It is a water soluble artificial azo color obtained from coal tar. Drinks and beverages contain a maximum amount of tartrazine (Gao et al., 2011; Saxena and Sharma, 2015). Furthermore, tartrazine has been illegally used as an alternative to saffron for cooking in some countries. Its application has not been confined to food industry; rather, it has been widely used to color some pharmaceutical products, such as capsules of vitamins, antacids, cosmetics, and hair products (Mohamed et al., 2015). As for its side effects, it has been found that different doses of tartrazine in the diet of mice caused adverse effects leading to hepatocellular damage, reproductive alterations, genotoxicity of lymphocytes and inflammation of the stomach lining (Mohamed et al., 2015; Tanaka, 2006; Moutinho et al., 2007). In addition, a number of undesirable effects on nervous system, including anxiety, migraines, clinical depression, blurred vision, and sleep disturbance have been observed following tartrazine ingestion (Rowe and Rowe, 1994). Gao et al. (2011) also indicated that tartrazine could cause learning and memory deficits in mice and rats.

Prefrontal cortex and hippocampus are the essential parts of the brain that play important roles in learning and memory. Park et al. (2009) showed that a variety of food colors can adversely affect on both hippocampal cells and prefrontal cortex.

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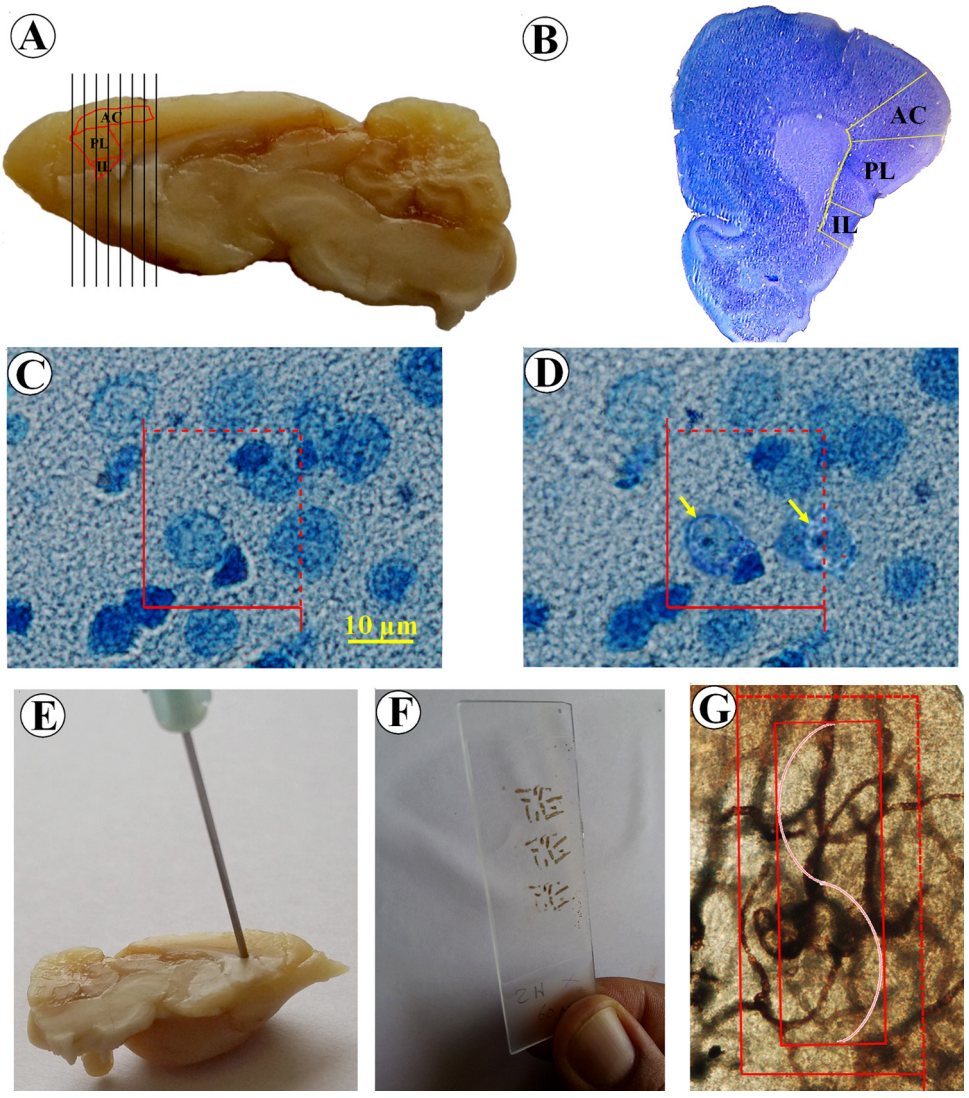

Fig. 1. Stereological estimation of the mPFC volume, cells number, and dendrites length. A. The design of sectioning used to estimate the volume of the mPFC using Cavalieri's method. Different parts of mPFC (AC: anterior cingulate, PL; prelimbic, IL: infralimbic) are indicated. B. The area of each part was determined using the software. C&D. An unbiased counting frame was superimposed on the images of the mPFC sections stained with Giemsa. The cells'nuclei appearing during scanning of the height of the "optical disector" were counted using the unbiased counting frame (arrow). E. The vertical cylinders were punched out from the mPFC perpendicular to its pial surface using a trocar. F. The cylinder was randomly rotated along its vertical axis, sectioned using a microtome, and mounted on a slide. G. Four cycloids were placed at a rectangle. The number of the cell bodies of the neurons was counted using the unbiased counting frame while the sections were scanned. The total number of the intersections between the dendrites axes and the cycloids were counted.

Therefore, the present study was conducted to fulfill two main purposes. The first aim of the present study was to evaluate the effects of tartrazine on the medial Prefrontal Cortex (mPFC), which plays a vital role in memory and learning. Further, it intended to find a protective agent to be consumed in the case of exposure to tartrazine. The main mechanism of the side effects of tartrazine has been proposed by Gao et al. (2011). They showed that after exposure to tartrazine the actions of antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase) would decrease as well as the level of malonaldehyde (as a marker of oxidative stress) increase in the brain of rats. Therefore, it seems to be rational to use an anti-oxidant compound to protect the side effects of tartrazine.

Vitamin E, a group of fat-soluble compounds with prominent antioxidant activities, is a well-known agent considered to fulfill the aforementioned purpose (Nuoya et al., 2015; Sakr et al., 2015). Vitamin E serves as a neuro-protective agent and is considered to be a therapeutic agent in neurodegenerative diseases (Sakr et al., 2015). It was considered to be evaluated in this survey because it can be found naturally in some foods and is also available as a nutritional supplement. In this study, a low and a high dose of tartrazine were defined as 5 and 50 mg/kg/day respectively. Five mg/kg body weight lies in the range of Acceptable Daily Intake (ADI) for tartrazine (Moutinho et al., 2007). The high dose was selected considering the fact that individuals' exact intakes during the day and in different dietary habits are hard to record. The dose of vitamin E was also selected according to its suggested neuro-protective effects in previous surveys (Nuoya et al., 2015; Sakr et al., 2015). Therefore, the present survey was performed using a rat model with the consumption of tartrazine to answer the following questions:

1. Does the exposure to tartrazine (low and high doses) influence the rats' memory and learning (visual and spatial)?
2. Does the tartrazine exposure have any effects on the volume of the mPFC (anterior cingulated, PL, and IL cortices)?
3. Does the exposure to tartrazine cause any changes in the number of neurons and glial cells in the mPFC?

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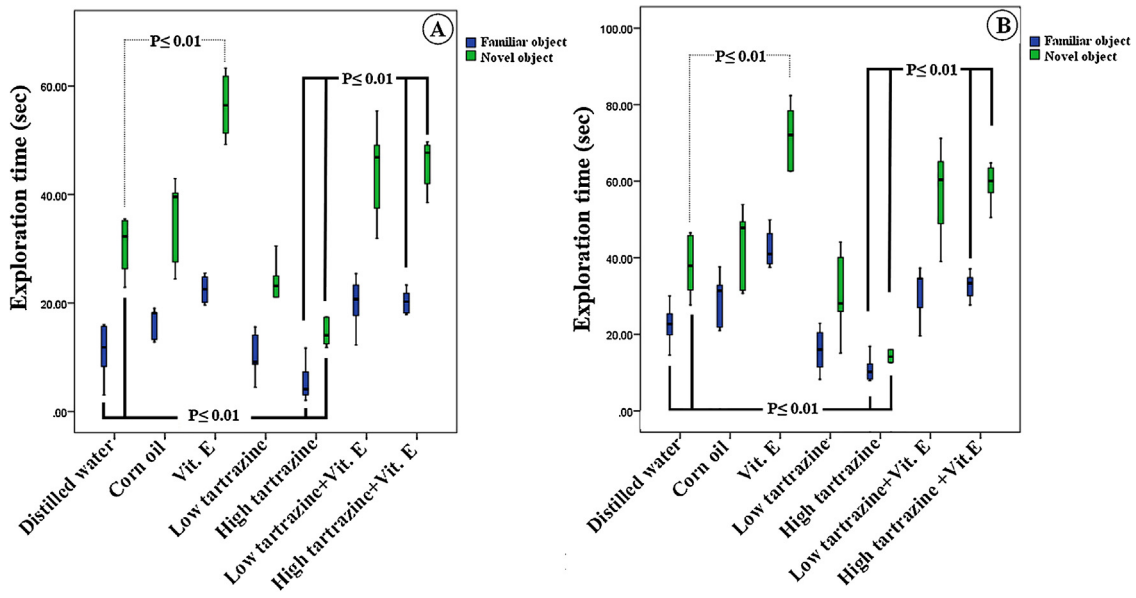

Fig. 2. Novel object recognition test. The box plot shows the exploration time for the familiar and novel objects during short-term (A) and long-term (B) memory tests. Different groups treated with distilled water, low and high doses of tartrazine, and vitamin E. The significant differences have been indicated on the plots. Number of animals per group = 10.

4. Does the dendrites length and its spines morphology (mushroom, thin, and stubby) undergo any changes after exposure to tartrazine?
5. Can vitamin E protect the alteration in behavioral tests and mPFC structure of the tartrazine-treated animals?

To examine the structural changes of mPFC, the tissue was evaluated using stereological techniques.

2. Materials and methods

2.1. Animals

In this study, we opted for a rat model consisting of seventy two-month old male Sprague-Dawley rats weighing 250–280 g which were obtained from The Center of Comparative and Experimental Medicine of the University. All of the animal procedures were carried out under the standard rules established by the Animal Care and Ethics Committee of the University (Agreement License No. 94–7521).

2.2. Experimental design

The animals were randomly divided into seven groups each containing ten rats. All of them underwent the behavioral tests but only 6 rats were included in stereological studies. Through daily gastric gavages, the rats in groups I to VII received the followings for 7 weeks: group I: distilled water; group II: corn oil; group III: vitamin E (100 mg/kg/day, CAS No. 10191-41-0 Sigma-Aldrich, Germany); group IV: the low dose of tartrazine (5 mg/kg/day, Colour Index Number 19140, Sigma-Aldrich, Germany); group V: the high dose of tartrazine (50 mg/kg/day); group VI: the low dose of tartrazine + vitamin E; and finally group VII: the high dose of tartrazine + vitamin E. Distilled water and corn oil were used as the solvents of tartrazine and vitamin E respectively (Tanaka, 2006; Moutinho et al., 2007; Nuoya et al., 2015).The behavioral tests started at the end of the 4th weeks and the animals were scarified at the end of the 7th week.

2.3. The novel object recognition test

In order to evaluate the recognition memory, the novel object recognition test was performed according to the previous study (Stranahan, 2011). The first phase (aka the habituation phase) was carried out in an empty box where each animal could explore the box (Stranahan, 2011). In the second (aka the acquisition phase) the animals were exposed to two similar objects. In the third phase (aka the short-term memory test) the animals were exposed to a familiar and a novel object. Finally, the fourth phase (aka the long-term memory test) was performed later by exposing the animals to the familiar and another novel object. After each trial, the objects and the box were cleaned with ethanol to decrease the olfactory cues. All the behaviors were recorded using a camera for offline evaluation (Stranahan, 2011).

2.4. Eight-arm radial maze test

The eight-arm radial maze test was performed to evaluate spatial learning and memory according to the details that have been given by Karkada et al., 2012. In the first (aka adaptation phase, the animals were allowed to explore the baited arms of the maze. In the second phase (aka learning career), the animals were given two trials per day until they attained the learning criterion which was defined as reaching 80% correct choices. This session lasted for eight to fifteen days. Entrances into the unrewarded arms were recorded as reference memory errors, while re-entrances into the rewarded arms were recorded as working memory errors. The rats' actions were scored by the number of the correct choices and errors. The third phase (aka the retention session) started ten days after the acquisition. The average of the two trials and the number of the correct choices and errors were used for data analysis (Karkada et al., 2012; Justin Thenmozhi et al., 2016).

2.5. Tissue preparation

At the end of the 6th week, the dissected right hemispheres were processed and embedded in paraffin, and serially sectioned (25 μm thickness). The tissue slides were stained using Giemsa (Lifshitz and

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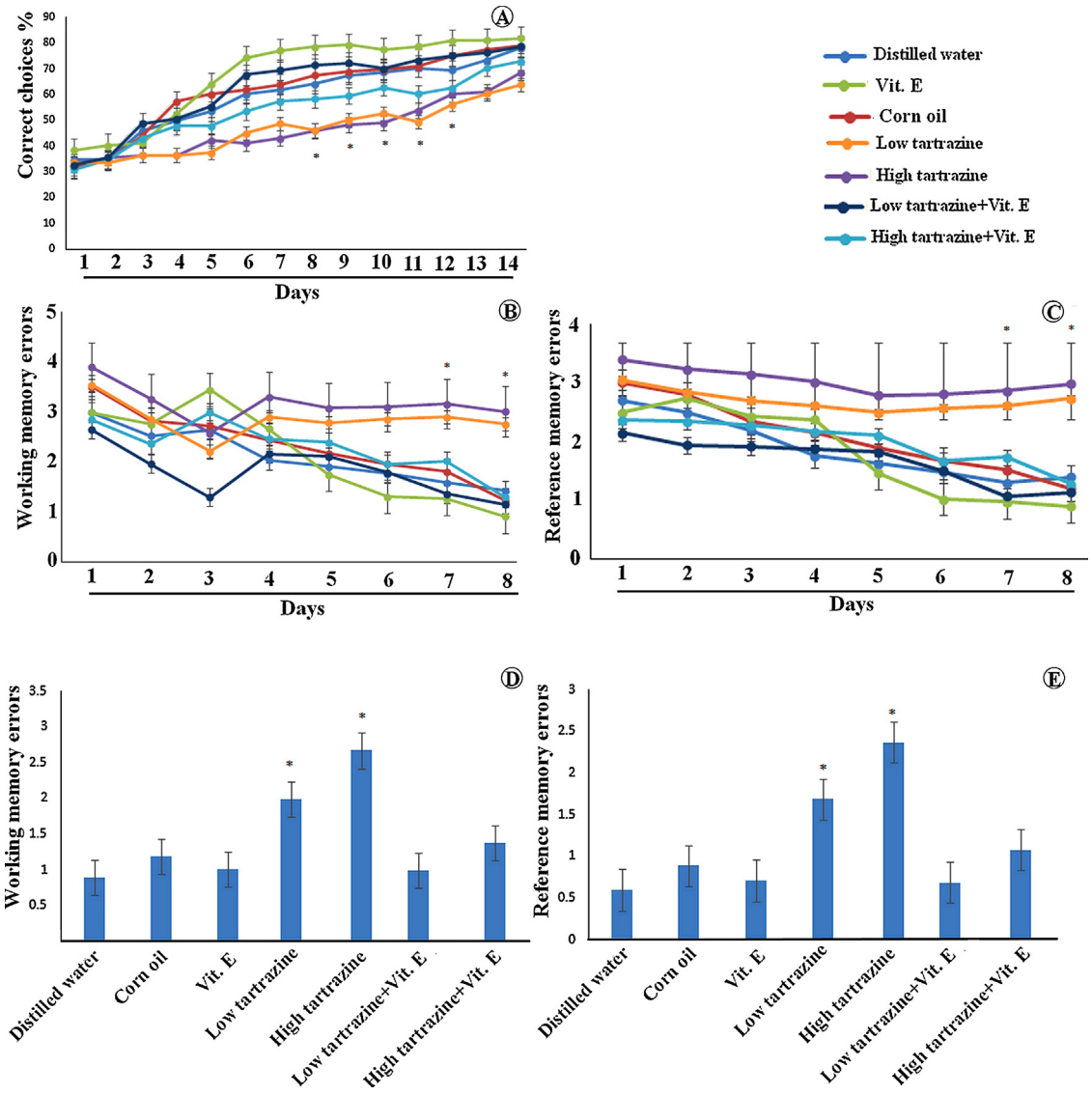

Fig. 3. Eight-arm radial maze test. Mean ± SEM of the days required for reaching the average criteria of 80% correct choices in different groups (A); the animals treated with low and high doses of tartrazine did not reach the criterion in 8–12 days in comparison to the distilled water group.The animals treated with vitamin E showed a batter performance in reaching the 80% criterionin comparison to the distilled water group. The mean ± SEM of the working memory errors (B) and reference memory errors (C) during the acquisition session, the animals treated with low and high doses of tartrazine vs. distilled water. The mean ± SEM of the working memory errors (D) and reference memory errors (E) during the retention session, the animals treated with low and high doses of tartrazine vs. distilled water group. The significant effects in working memory error can be seen. [$P < 0.01$), $F(7315) = 18.612$], "animal groups" [$P < 0.01$), $F(4, 45) = 33.75$], and the "training days" by "animal groups" interaction [$P < 0.01$), $F(28,315) = 5.60$]. The significant effect can also seen for reference memory errors during the acquisition [$P < 0.01$), $F(7315) = 19.12$], "animal groups" [$P < 0.01$), $F(4, 45) = 4.34$], and the "training days" by "animal groups" interaction [$P < 0.01$), $F(28,315) = 4.06$]. Number of animals per group = 10.

Lisembee, 2012). The slabs of the left hemispheres were also stained using Golgi impregnation procedure (Madeira et al., 1999).

2.6. Estimation of the mPFC volume

mPFC was evaluated according to the atlas of rat brain (Paxinos and Watson, 2006). Overall, 11–13 sections per mPFC were sampled and analyzed (Fig. 1). Generally, the mPFC can be subdivided into dorsal anterior cingulate, PL, and IL cortices (Fig. 1) (Dalton et al., 2016). The total volume of the mPFC was estimated using Cavalieri's method (Koss et al., 2012; Gundersen et al., 1988a,b; Kristiansen and Nyengaard, 2012). A video-microscopy system and using the software designed at the Histomorphometry and Stereology Research center of the University, stereological probes were overlaid on the image. In doing so, the areas of the sections ($\Sigma A\,(mPFC)$) were multiplied by the distance between the sections (d). Then, the area was estimated using the software and the volume was computed using the following formula:

$$V\,(mPFC) = \Sigma A\,(mPFC) \times d$$

2.7. Estimation of the number of neurons and glial cells

Using the video-microscopy system and the "optical disector" was applied to estimate the number of neurons and glial cells in the sections of mPFC Fig. 1 (Gundersen et al., 1988a,b; Kristiansen and Nyengaard, 2012; Koss et al., 2012). Post-shrinkage section thickness was measured during cell counting and was used to determine an average thickness "t" of 19 μm. The following formula was used

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in order to determine the numerical density of the cells (neurons or glia):

$$Nv\left(cells/mPFC\right) = \frac{\Sigma Q^{-}}{\Sigma P \times \left(\frac{a}{f}\right) \times h} \times \frac{t}{BA}$$

where "ΣQ^{-}" is the number of the sampled nuclei during scanning the "*h*", "ΣP" indicates the total counting of the unbiased counting frame in all fields, "*h*" represents the height of the disector, "*a/f*" is the frame area, "*t*" indicates the mean section thickness, and "*BA*" is the block advance of the microtome set at 25 μm. The total number of the cells was estimated by multiplying the numerical density by*V* (*mPFC*).

2.8. Estimation of the coefficient of error (CE)

The "CE" is defined as the standard error of the mean of repeated estimates divided by the mean. Meanwhile, the cross-sectional areas of the mPFC were estimated by the aforementioned software and CE (V) was calculated through the following formula (Gundersen et al., 1999):

$$\mathrm{CE(V)} = (\sum \mathrm{A})^{-1} \times [1/12 \times (3 \sum \mathrm{AiAi} + , \sum \mathrm{AiAi} + 2 - 4 \sum \mathrm{AiAi} + 1)]^{1/2}$$

The CE for the estimate of the total number of neurons, CE (N), was also calculated using CE (V) and CE (Nv) as follows:

$$CE\,(N) = \left[CE^2\,(Nv) + CE^2\,(V)\right]^{\frac{1}{2}}$$

$$CE\,(Nv) = \left[\left(\frac{n}{n-1}\right) \times \left[\left(\frac{\sum (Q^{-})^2}{\left(\sum Q^{-}\right)^2}\right) + \left(\frac{\sum (P)^2}{\left(\sum P\right)^2}\right) - \left(\frac{2\sum (Q^{-}P)}{\sum Q^{-} \sum P}\right)\right]\right]^{\frac{1}{2}}$$

2.9. Estimation of the dendrites length spine density and morphology

The video-microscopy system used for estimating the dendrites length on the "vertical uniform random sections" (Howard et al., 1992 Kristiansen and Nyengaard, 2012). Eight to ten cylinders were punched out perpendicular to the pial surface of the cortex (Fig. 1). The cylinders were fixed and stained with Golgi and after embedding 100 μm thickness sections were taken. Using a cycloid grid and a counting frame, the number (Q^{-}) of the neurons and the intersections (I) between the dendrites' axes and the cycloid were counted (Fig. 1). The following formula was used to estimate the dendritic length:

$$\bar{\mathrm{I}}_{\mathrm{N}} = 2.\frac{\mathrm{a}}{\mathrm{l}} \cdot \frac{1}{\mathrm{asf}}.\mathrm{M}^{-1}.\frac{\sum \mathrm{I}}{\sum \mathrm{Q}-}$$

where "$\frac{a}{l}$" is the area per cycloid test length, "asf" represents the area associated with cycloid grid divided by the area of the counting frame, and "M" indicates the final magnification (Howard et al., 1992). Density and morphology of the spine (thin, stubby and mushroom) were quantified and stated as the number of spines per neuron (Chapleau et al., 2008).

2.10. Statistical analysis

Two-way repeated measures ANOVA and Bonferroni's post hoc test were used to evaluate the behavioral tests. The quantitative stereological data were analyzed using non-parametric tests, including Kruskall-Wallis and Mann–Whitney *U* test. Besides, $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Novel-object recognition test

The short- and long-term memory functions were evaluated in different groups of rats Fig. 2. The results showed a decrease in the exploration time of the familiar and novel objects during the short- and long-memory tests in the group treated with the high dose of tartrazine compared to the distilled water group ($P < 0.01$). However, no significant differences were observed between the low-dose and distilled water groups. Moreover, treatment of the rats with the high dose of tartrazine plus vitamin E led to a performance similar to that of the distilled water animals ($P < 0.01$). Finally, consumption of vitamin E alone increased the exploration time for the novel object during the short- and long-memory tests.

3.2. Eight-arm radial maze test

In comparison to the distilled-water group, the performance of the tartrazine-treated rats (low and high doses) degraded in the acquisition phase of the task. In other words, it took significantly more days for this group to acquire the criterion of 80% correct choices ($P < 0.01$) Fig. 3. Compared to the tartrazine-treated rats, it took significantly fewer days for the rats that received tartrazine + vitamin E to reach the criterion of 80% correct choice ($P < 0.01$) Fig. 3. In addition, vitamin E consumption resulted in a better performance in reaching the criterion compared to the rats treated with distilled water group.

3.3. Working and reference memory errors during the acquisition phase

A two-way repeated measures ANOVA was considered with the "training days" as within-subjects factor and the "animal groups" as between-subjects factor.

A significant result was observed for working memory error [$P < 0.01$), $F(7315) = 18.612$], "animal groups" [$P < 0.01$), $F(4, 45) = 33.75$], and the "training days" by "animal groups" interaction [$P < 0.01$), $F(28,315) = 5.60$].

A significant effect was seen for reference memory errors during the acquisition [$P < 0.01$), $F(7315) = 19.12$], "animal groups" [$P < 0.01$), $F(4, 45) = 4.34$], and the "training days" by "animal groups" interaction [$P < 0.01$), $F(28,315) = 4.06$] Fig. 3. More specifically the results showed, the rats in the tartrazine-treated groups (low and high doses) exhibited more working and reference memory errors compared to the distilled water animals on the 7th and 8th days Fig. 3. On the other hand, fewer errors were observed in the tartrazine (low and high doses) + vitamin E groups in comparison to the tartrazine-treated (low and high doses) groups [Fig. 3]. Besides, the rats that consumed vitamin E alone made fewer errors in comparison to those treated with distilled water group ($P < 0.01$) [Fig. 3].

3.4. Working and reference memory errors during the retention phase

The results of ANOVA showed significant differences among the groups regarding the number of memory errors ($P < 0.01$). A significant difference was observed among the groups on the subject of the number of working and reference memory errors [$P < 0.01$), $F(4, 45) = 38.02$] and the number of working memory errors [$P < 0.01$), $F(4, 45) = 44.77$] [Fig. 3]. However, no significant differences were found between the tartrazine + vitamin E groups (high and low

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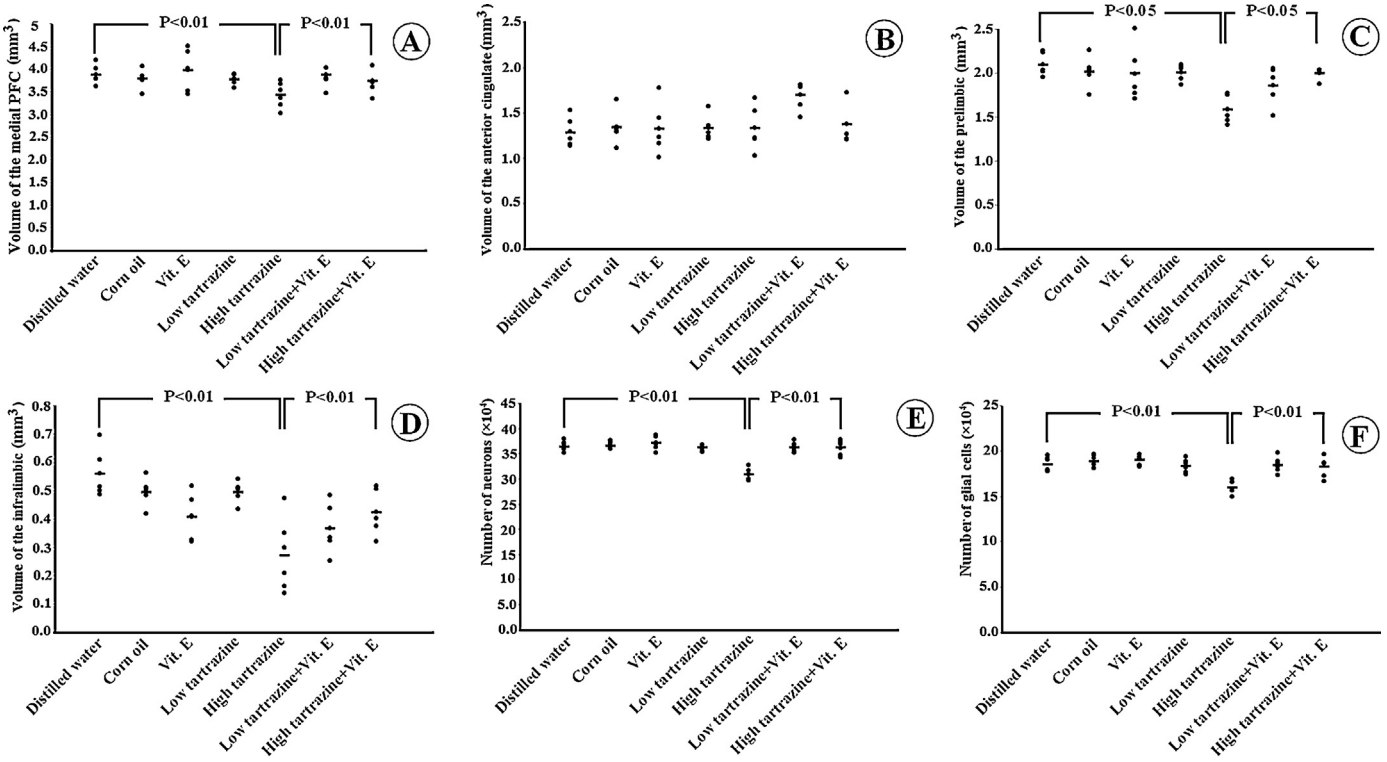

Fig. 4. The dot plot of the rats' mPFC parameters. The total volume of the mPFC (A), anterior cingulate (B), prelimbic (C), and infralimbic (D), sub-regions and number of neurons (E) and glial cells (F) in the mPFC of the rats receiving distilled water, corn oil, vitamin E, and tartrazine. Each dot represents the parameter in an animal. The horizontal bar indicates the means of the parameters in each group. Number of animals per group = 6.

doses +vitamin E) and the distilled water group in this regard. In addition,vitamin E consumption induced a better performance and fewer errors in comparison to distilled water rats ($P < 0.01$) [Fig. 3].

3.5. Stereological evaluation

The average CE was 0.04 for estimating the volume of the mPFC and 0.05 for number estimation in all the groups. The average coefficient of variation (CV) in all groups was 0.027 for the number of neurons and 0.032 for the glial cells. The average CV in all groups for the volume of anterior cingulate cortex was 0.137, for the prelimbic cortex was 0.082, for infralimbic cortex was 0.168 and for total volume of mMPC was 0.129.

The results indicated a significant reduction (~13%) in the total volume of mPFC in the rats treated with the high dose of tartrazine compared to the distilled water animals ($P < 0.01$, Fig. 4). The volume of the IL and PL regions of the animals that received the high dose of tartrazine was also decreased (~52%, 12%) compared to the distilled water rats ($P < 0.05$). However, no significant differences were observed with respect to the anterior cingulated cortex.

Feeding the animals with corn oil, low dose of tartrazine, tartrazine (low and high doses) + vitamin E and vitamin E alone did not affect the volume of mPFC and its subdivisions. Nevertheless, concomitant treatment with the high dose of tartrazine + vitamin E prevented the loss of mPFC volume induced by the high dose of tartrazine.

3.6. Neurons and glial cells counts

The study findings revealed a significant decrease (~14%) in the total number of neurons and glial cells in the animals exposed to the high dose of tartrazine compared to the distilled water group ($P < 0.01$, Fig. 4). However, feeding the animals with corn oil, low dose of tartrazine, tartrazine (low and high doses) + vitamin E, and vitamin E alone did not affect the number of neurons and glial cells of mPFC and its subdivisions. The findings further demonstrated that concomitant treatment with thehigh dose of tartrazine + vitamin E prevented the loss of the neurons and glia in mPFC induced by the high dose of tartrazine.

3.7. Qualitative evaluation of mPFC

As illustrated in Fig. 5, distilled water, vitamin E, and low dose of tartrazine maintained a normal shape. In fact, neurons and glial cells with normal nucleus and cytoplasm were observable. On the other hand, the mPFC of the rats treated with the high dose of tartrazine showed a lower population of cells. The remaining cells also seemed shrunk, pyknotic, and smaller in comparison to the distilled water group. The mPFC of the low dose of tartrazine + vitamin E group indicated a normal shape. The recovered tissue was observed in the cortex of the animals treated with the high dose of tartrazine + vitamin E.

3.8. Dendritic length measures

On average, 5% and 12% decreases were observed in the length of dendrites per neuron in the rats treated with low and high doses of tartrazine respectively in comparison to those that received distilled water ($P < 0.01$). In addition, concomitant treatment with vitamin E plus tartrazine (high and low doses) prevented the loss of dendritic length in the rats ($P < 0.01$, Fig. 6).

3.9. Density and morphology of the spines

The results showed that the number of mushroom spines per dendrite reduced by ~47% and 48% respectively in the groups treated with low and high doses of tartrazine in comparison to the distilled water group ($P < 0.01$, Fig. 6). The density of the thin spines

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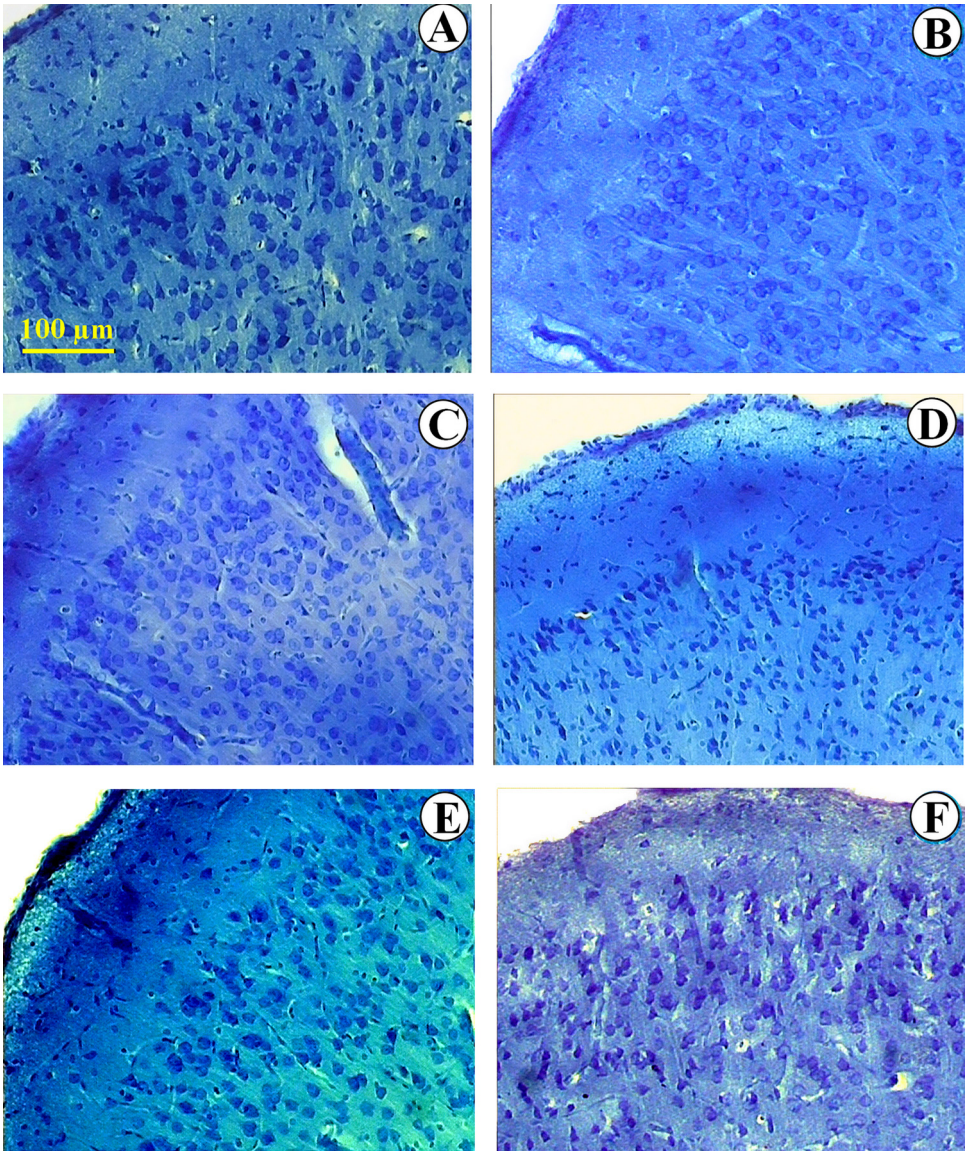

Fig. 5. A micrograph showing the mePFC of the rats in different groups. The mPFC of the rats receiving distilled water (A), vitamin E (B), and low dose of tartrazine (C) showed a nearly even appearance. The neurons and glial cells with normal nucleus and cytoplasm were also observable. The mPFC of the rats receiving the high dose of tartrazine (D) showed a lower population of cells. The cells also seemed shrunken, pyknotic, and smaller compared to the distilled water rats. The mPFC of the rats treated with the low dose of tartrazine + vitamin E showed a normal appearance. A recovered tissue could be observed in the cortex of the animals treated with the high dose of tartrazine + vitamin E.

also reduced by 10% and ~19% respectively in the rats treated with low and high doses of tartrazine compared to the distilled water group ($P < 0.01$, Fig. 6). Nonetheless, the number of stubby spines of the mPFC remained unchanged (Fig. 6). Concomitant treatment with vitamin E plus tartrazine (high and low doses) prevented the loss of dendritic spines in the mPFC ($P < 0.01$).

4. Discussion

The present study revealed the consequences of tartrazine consumption on some brain functions and its subsequent structural changes in the prefrontal cortex in an animal model. Based on the results, more extensive assessment of food additives in the current use is warranted.

The novel-object recognition and eight-arm radial maze allow parallel measurement of responses related to visual memory and spatial memory. The first part of the present study showed the effects of tartrazine (low and high doses) on these tasks. These changes might be due to the impaired neural function resulting from tartrazine toxicity in the brain. This finding is in line with the previous studies reporting the toxic effects of consumed tartrazine on the nervous system. Recently, Mohamed et al. (2015) showed the destructive effects of consumption of 500 mg/kg body weight tartrazine for 30 days on the brain of the rat pups. Likewise, Gao et al. (2011) reported that when administered for 30 days, tartrazine could induce remarkable learning and memory impairment in mice. The changes in neurobehavioral parameters after exposure to tartrazine were also shown by Tanaka (2006). Similarly, Ceyhan et al. (2013) showed that tartrazine reduced the receptor expressions related to learning and memory in rats.

The second part of the present experiment showed that treatment with tartrazine caused a reduction in the volume of mPFC, significant loss of neurons and glial cells, and a reduction in dendrites length as well as spines in mPFC. This finding supports the hypothesis that structural changes can occur after tartrazine treatment. Behavioral measurements also showed that tartrazine could induce impairment in learning and memory. In general, mPFC plays a key role in boosting the performance in tasks. Therefore, phys-

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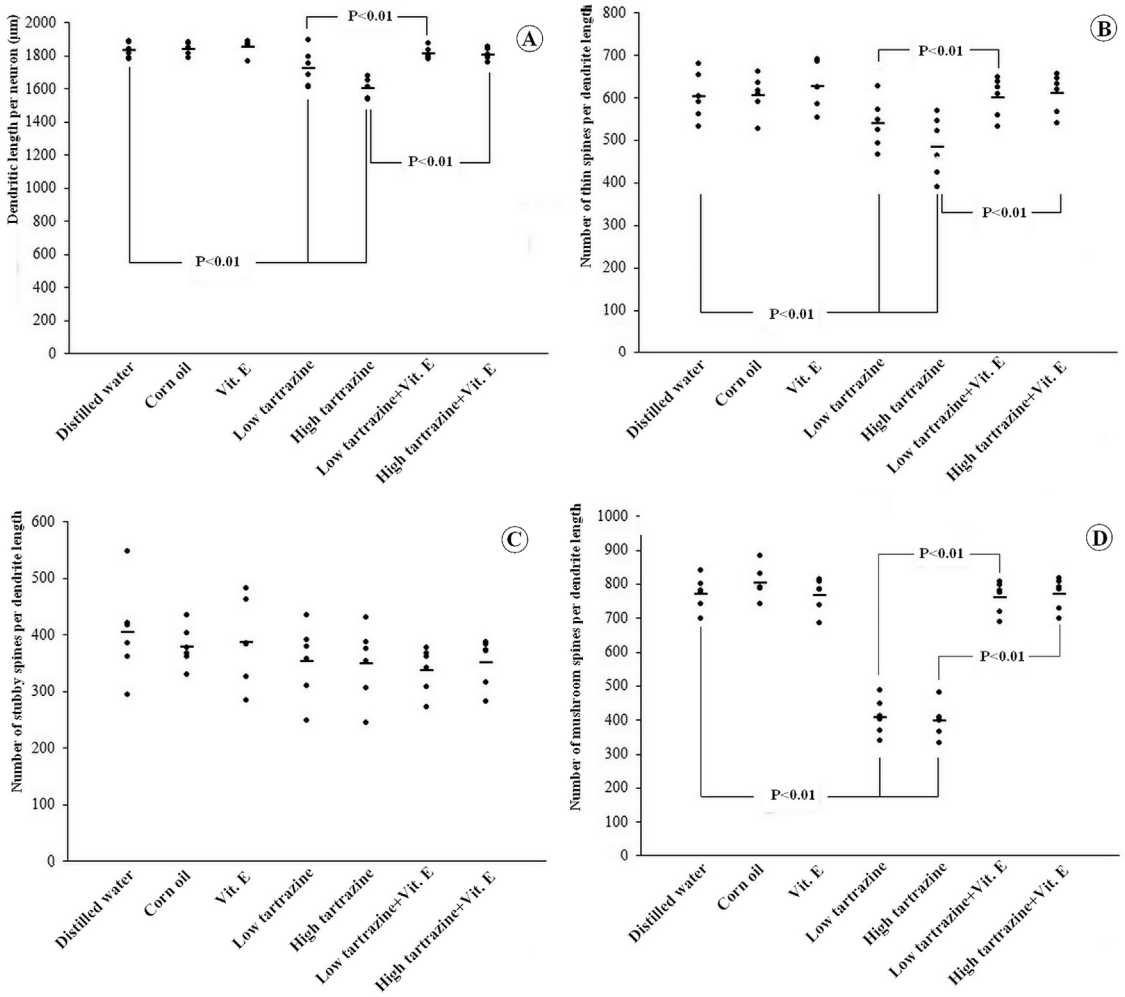

Fig. 6. Density of the dendritic spines. The density of thin (A), mushroom (B), and stubby (C) spines per neuron and total spine density (D) in the mPFC of different groups. The significant difference between tartrazine and other groups is indicated. *P < 0.01.

iological changes are accompanied by histological alterations. Of course, neurons loss and physiological alterations are not limited to mPFC. One study reported that tartrazine could cause brain weight loss in Swiss mice (Nabila et al., 2013).Toxic effects of tartrazine have also been reported in many studies. For instance, presence of apoptotic cells nuclei in the cerebral cortex was shown by Mohamed et al. (2015). They also reported the destructive effects and emergence of numerous apoptotic cells in the brains of rat pups when 500 mg/kg body weight tartrazine was consumed for 30 days (Mohamed et al. 0.2015). Moreover, investigation of the cerebellar sections obtained from Wistar rats treated with 500 mg/kg body weight tartrazine for 30 day showed that the myelin sheath in the white matter of both cerebrum and cerebellum, especially medulla, was more affected and represented the pattern of "myelin sheath splitting". Besides, they reported some pyknotic and necrotic neurons (Ghonimi and Elbaz, 2015). Furthermore, El-Nabarawy et al. (2015) indicated that tartrazine administration could reduce the monoamines formation that is likely to induce alterations in neurobiological substrates and brain tissue damage.

The mechanism of effect of tartrazine could be explained according to the findings of the previous researches. Tartrazine is a synthetic azo dye. Azo dyes are catalyzed by azo reeducates and peroxidases while semiquinone radicals and aromatic amines are the products of reactions. Semiquinone radicals in turn generate superoxide radicals, hydroxyl radicals, and H2O2 that possibly weaken the cellular defense, consequently opening the door for a variety of oxidative stress-related disorders. Brain tissue also contains large amounts of polyunsaturated fatty acids, which are predominantly vulnerable to free radical damage (Mohamed et al., 2015). Gao et al. (2011) also attributed the mechanisms of tartrazine to promotion of lipid peroxidation products and reactive oxygen species, which inhibits endogenous antioxidant defense enzymes and brain tissue damage.

Another finding of this study indicated that vitamin E prevented tartrazine-induced learning and memory impairment in the animals. This is the first study demonstrating that vitamin E had a protective role in learning and memory in the rats subjected to novel-object recognition and eight-arm radial maze after exposure to tartrazine. In addition, consuming vitamin E alone could improve the performance of the normal rats. The previous studies also reported the neuron-protective effects of vitamin E in different conditions. One of the earlier studies claimed that vitamin E supplementation prevented chronic sleep deprivation induced by impairment of hippocampal learning and memory via its antioxidative properties in the water maze (Alzoubi et al., 2012). It has been also reported that vitamin E could prevent silver-induced memory deficit and neurotoxicity in rats (Nuoya et al., 2015). In addition, it has been reported that vitamin E had anti-apoptotic effects on neurons (Heaton et al., 2004). Therefore, the protective effects of vitamin E against tartrazine in the present study might be explained by its antioxidant and anti-apoptotic effects reported in the previous studies.

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5. Conclusion

The low dose of tartrazine could induce impairments in spatial memory and dendrites structure. On the other hand, the high dose of tartrazine defected the visual memory and the structure of the mPFC as well as the spatial memory and caused dendritic changes. However, vitamin E could prevent the behavioral and structural changes.

Disclosure

Authors report no conflict of interest.

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