Journal of Recent Advances in Medicine

Print ISSN: 2636-252X
Online ISSN: 2636-2538

Original Article

Sub-chronic toxic effects of tartrazine on the heart and brain of adult male albino rats and the protective effect of vitamin E

Forensic medicine & Clinical toxicology

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ABSTRACT

Background: Tartrazine is the most widely employed dye in food stuffs, medicines as well as cosmetics**.**

Objective: This study aimed to assess oral sub-chronic toxicity of tartrazine on the body weight, heart and brain of adult male albino rats using biochemical and histological studies and to evaluate the potential protection function of vitamin E.

Methodology: This controlled clinical trial study included 24 rats. They were randomly processed into four groups: control, vitamin E (100mg/kg/day), tartrazine (300 mg/kg/day), tartrazine + vitamin E groups. The treatment was given to all rats orally for 30 days. Weights of body, heart and brain, serum levels of Cardiac Troponin I (cTnI), Lactate Dehydrogenase (LDH) and Creatinine Kinase muscle-brain (Ck-MB), Superoxide Dismutase (SOD),Catalase (CAT) and histological changes of heart and brain were assisted.

Results: There was a significant increase in weights of body, heart and brain, cTnI, LDH and CK-MB levels, also a significant decrease in SOD and CAT activities and alternation in the normal histological structure of the heart and brain in the tartrazine group in comparison to the control group. Vitamin E co-administration with tartrazine produced an improvement in all previous changes caused by tartrazine.

Conclusion: The present study concluded that tartrazine has a toxic effect on the heart and brain. The use of vitamin E via its antioxidant properties leads to improvement of such toxicity.

JRAM 2022;3(1):19-30

Keywords: Tartrazine, toxic effect, vitamin E, heart, brain, cardiac biomarkers markers, oxidative stress markers, histology.

Submission Date: 19 June 2021 **Acceptance Date:** 11August 2021

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Please cite this article: Megahed RM, Barghash SS and Hasan RA. Sub-chronic toxic effects of tartrazine on the heart and brain of adult male albino rats and the protective effect of vitamin E. JRAM 2022; 3(1):19-30. DOI: 10.21608/jram.2021.69371.1116

INTRODUCTION

Tartrazine, which is a synthetic food added substance, is a shining yellow color azo pigment. It is more durable and less expensive option in contrast to natural food coloring agents. Different names of tartrazine incorporate C Yellow No. 5, FD and E 102 Europe [1]. Tartrazine is utilized in a variety of medications, cosmetics and food items including candy, cake batters, energy drinks, flavored corn chips, potato chips, cereals (corn flakes), jam, yogurt noodles, ice cream, ice pops, marzipan, jelly, biscuits, chewing gum, soft drinks and some honey products [2].

The tartrazine has been related to unfavorably allergic reactions [3] and has been appeared to impair animal learning and memory functions [4]. Some researchers in rat toxicology studies, have discovered that it has many adverse consequences on the kidney, liver and blood cells [5]. Irritability, restlessness, and sleep disturbances were observed in children who consumed tartrazine through the consumption of colored foods [2]. Children can develop hyperactivity syndrome [6] and thyroid tumors when tartrazine is combined with benzoate [7]. This dye causes oxidative stress in rats [8] and has been linked to the production of free radicals, lowering serum biochemical parameters and blood antioxidants significantly [9]. It can also cause agitation, infertility, confusion, rhinitis, and migraine by altering perception and behavior [10]. In humans, tartrazine doses of 0.14 to 750 mg, can cause peripheral nerve sequels, paresthesia as well as tooth changes [11].

Vitamin E (tocopherol) is a strong antioxidant which protects DNA from oxidative damage caused by free radicals. It prevents lipid peroxidation in cell membranes and thus protects them from the damage caused by free radicals by limiting their activity [12].

This study aimed to assess oral sub-chronic toxicity of tartrazine on the body weight, heart and brain of adult male albino rats using biochemical and histological studies and to evaluate the potential protection function of vitamin E.

MATERIALS AND METHODS

- **The experimental animals**

In this controlled clinical trial study, twenty-four adult male albino rats weighting between 160 and 200 grams (g) for each rat, were used. The animals were got from animal breeding farm in Helwan, Cairo, Egypt. They were maintained in stainless steel cages in a well-ventilated animal house at room temperature (22°C ±5°C) with a 12:12 light–dark cycle. They were provided regular food and water as well. Prior to the start of the experiment, the rats were held in suitable conditions for one week to allow for adaptation. Animals were handled according to experimental research ethics guidelines approved by the Research Ethics Committee at Al-Azhar University's Faculty of Medicine for Girls., Egypt. The guide for care and use of laboratory animals is followed [13].

- **Tested Substances**

1. **Tartrazine:** in the form of yellow powder was dissolved in distilled water. The powder was bought from Sigma-Aldrich Company, Egypt.
2. **Vitamin (vit.) E**: in oil form, was obtained from Egypt's Cairo Company for Pharmaceutical and Chemical Industries, Shubra Cairo.

- **Experimental design**

The rats were separated randomly into four groups (6 rats each) as follows:

- **Group (1),** control group (rats were fed normal food and drank distilled water).
- **Group (2),** vit. E group (vit. E was given to the rats by intragastric tube (100 mg/kg/day) [14].
- **Group (3),** tartrazine treated group (rats were administered tartrazine (300 mg/kg/day) [1].
- **Group (4),** rats received [tartrazine (300 mg/kg/ day) [1] + vit. E (100 mg/kg/day) [14].

All rats were received the treatment orally by intra-gastric tube for 30 days.

- **Serum and tissue collection**

At the end of experiment, the weight of rats was assessed and recorded, then rats were anesthetized by diethyl ether. Glass capillaries were used to collect blood samples from the medial canthus of rat's eyes. These samples were collected in clean, dry test tubes and allowed to clot for 20 minutes at room temperature. The sera were then separated by centrifugation at 4,000 rpm for 15 minutes, then stored at -20 °C for biochemical analysis that included cardiac biomarkers [Cardiac Troponin I (cTnI), Lactate Dehydrogenase (LDH), Creatinine kinase muscle-brain (Ck-MB)]; antioxidant markers [Superoxide Dismutase activity (SOD) and Catalase (CAT) **[15]**. Then all animals were sacrificed while they were under anesthesia by diethyl ether inhalation. The hearts and brains were dissected out carefully, their weights were assessed and recorded then they were washed by sterile saline then were preserved to be utilized for histological examination by light microscope, immunohistochemical study and morphometric study.

- **Biochemical evaluation**

a) Cardiac biomarkers:

1. **cTnI level:** serum levels of Cardiac Troponin I were tested with a specific rat enzyme-linked immunosorbent assay kits purchased from (KAMIYA BIOMEDICAL COMPANY Seattle, WA 98168, USA, E-mail: info@k-assay.com) in accordance with the manufacturer's recommendations. Standard curves were constructed.
2. **LDH:** Enzyme Colorimetric method was used to determine its serum level [16].
3. **Ck-MB:** serum level was assessed using Enzyme Colorimetric approach [17].

b) Antioxidant markers

1. **SOD activity:** The colorimetric approach was used to determine its serum level [18].
2. **CAT:** serum level was analyzed using colorimetric method [19].

c) Histological study

Following proper fixation of heart and brain tissues in 10% formaldehyde, the specimens were dehydrated in an alcohol sequence of 100%, 90%, 70%, 50%, cleared in xylene, infiltrated and embedded in paraffin, and then sectioned (5 µm thick) using a rotary microtome. (LEICA RM 2125 UK). They were further deparaffinised, stained with hematoxylin and eosin (H&E) [20]. Collagen fibers were stained by using Mallory's trichrome stain in heart specimens only [21]. A light microscope (Primo star, ZEISS, China) was used to examine the slides. The images were taken at Histology Department, faculty of Medicine for Girls, Al- Azhar University, using (Axiocam ERc 5s, ZEISS, China) camera.

- **Immunohistochemicsl study**

Heart and brain sections were processed and immunostained using peroxidase labeled streptavidin-biotin for nuclear factor Kappa ß using nuclear factor Kappa ß antibodies (Abcam, Anti-NF-KB antibody, Rabbit polyclonal "ab 16502, diluted 1:100)**.** Positive slide was delivered by the manufacture. The primary antibody was left out of the negative control areas [22].

- **Morphometric study**

A- Using Image J software, the following parameters were calculated (National Institute of Health, Bethesda, MD, USA). For setting scale and converting values from pixels to micrometers, an image of a known distance in micrometer was used [23].

1. Cardiomyocyte cross-sectional area to measure cardiac muscle fibre hypertrophy. It was determined by manually drawing a line around the circumference of cardiomyocytes with visible central nuclei in ten randomly selected transverse sections at ×400 magnification using H&E-stain. As a measure of cell size, the average cross-sectional areas obtained for each category were used. The findings were expressed in μm^2 [24].

2. In mallory trichrome stained sections, the area % of the stained collagen fibers in between the cardiomyocytes was measured in ten randomly selected fields per group at x400 magnification in ten randomly selected fields per group. The results were expressed as mean area % of collagen/µm² [25].

B. Immunohistochemical quantification for nuclear factor Kappa ß

d) The immunoreaction to the nuclear factor Kappa ß appeared as a brown nuclear and cytoplasmic reaction. In each group, the optical density/high power field was calculated in ten randomly chosen fields. The protein expressions and optical density of NF-B immunoexpression were determined using a color image analysis device (a Leica Qwin 500 image analyzer connected to a Leica microscope) [22].

Statistical analysis

The data was analysed statistically using means ± SD and analysis of variance using one-way [ANOVA] tests followed by Tukey's post-hoc test. The level of P-value> 0.05 is significant; non-significant P-value (≤ 0.05). According to the computer program SPSS V20 for Windows.

RESULTS

Effects of tartrazine on body weight, weight and relative weight of heart and brain of the rats:

Tartrazine group showed significant increase ($p<0.05$) in the body weight & weight and relative weight of both heart and brain when compared to control group, as a result of administration of vit. E with tartrazine there was significant decrease ($p<0.05$) in the body weight, weight and relative weight of both heart and the brain when compared with tartrazine group (table 1).

- **Biochemical evaluation**

1. **Regarding cardiac enzymes:** Tartrazine caused significant increase ($p<0.05$) in cTnI, LDH and CK-MB**,** in comparison with the control group. Administration of vit. E with tartrazine produced significant decrease ($p<0.05$) in cTnI, LDH and CK-MB by comparing with tartrazine group (table 2).
2. **Regarding antioxidant markers**: Comparing tartrazine and the control groups, tartrazine resulted in significant decrease ($p<0.05$) in SOD and CAT activities. Also, co-administration of vit. E with tartrazine caused significant increase in SOD and CAT activities in comparison to tartrazine group (table 2).
3. **Regarding morphometric histological results:** As regard the cross-sectional area of cardiomyocytes, Tartrazine group produced significant increase ($p<0.05$) by comparing with control group, administration of vit. E with tartrazine, revealed significant decrease ($p<0.05$) as compared with tartrazine group (table 3). Tartrazine caused significant increase ($p<0.05$) in collagen area % of heart when compared to control group. After administration of vit. E with tartrazine there was significant decrease ($p<0.05$) in comparison with tartrazine group (table 3). Tartrazine group expressed significant increase ($p<0.05$) in the optical density of nuclear factor Kappa B of both heart and the brain contrasted to control group, administration of vit. E with tartrazine, produced significant decrease ($p<0.05$) in comparison to tartrazine group (table 3).

- **Histological results**

A. H & E heart sections

Examination of H and E stained heart sections, the control group had the typical histological architecture of the myocardium, with longitudinally striated branching and anastomosing muscle fibers with centrally located oval vesicular nuclei and acidophilic sarcoplasm They are separated by narrow intercellular spaces. Fibroblast nuclei were detected in the interstitial tissue between myocytes. The cardiac muscle fibers appeared polygonal or polyhedral in transverse sections, with central rounded nuclei. The vitamin E-treated group's histological appearance was identical to that of the control group. However, there were several cardiac changes were observed in the tartrazine group, including hypertrophy of cardiac muscle fibers, destruction of some cardiomyocytes, and interruption and discontinuity of some cardiomyocytes. Also, vacuolization of the cardiomyocytes could be detected. Wide intercellular spaces separated these cardiomyocytes from each other. Between the cardiac muscle fibers, there was a large area of hemorrhage and mononuclear cellular infiltration. The combination of vitamin E and tartrazine alleviated all the adverse effects seen in the tetrazine group (figure 1).

B. Mallory trichrome- stained heart sections

In between the cardiomyocyte, a few collagen fibers (stained blue) were visible in control, vitamin E and vitamin E + tartrazine groups. While, in tartrazine-treated rats, there was a significant deposition of collagen fibers between cardiomyocytes and around blood vessels (figure 2).

- **H &E brain sections**

Examination of slides of rat cerebral cortex, from the control group and vitamin E group showed normal characteristic histological features of the cerebral cortex layers as groups of nerve cells arranged is well-organized six layers covered with pia mater, the molecular layer, the outer granular layer, the outer pyramidal layer, the inner granular layer, the inner pyramidal, and the polymorphic layers. The molecular layer was thick and contained dense plexus of nerve fibers with few cells. Whereas, the external granular and external pyramidal contained numerous granular cells and pyramidal cells. While the internal granular and internal pyramidal showed few granular cells and pyramidal cells. These layers were ill defined from each other. Higher magnification of the cortex showed many cells; the pyramidal cells had multipolar shape with open face nuclei, basophilic cytoplasm, and long apical dendrite while granular cells appeared rounded in shape with large rounded vesicular nuclei and

prominent nucleoli and little cytoplasm. In between neurons there were blood vessels and neuroglial cells. The ground substance between the nerve cells is normally occupied with homogenous eosinophilic background (neuropil). In tartrazine group, microscopic examination showed the six layers of the cerebral cortex, but the pia mater covered with dilated congested blood vessels. The pyramidal cells surrounded by haloes and appeared shrunken, loss their processes. There was also, extensive neuropil vacuolization. Dilated congested blood vessels and mononuclear cellular infiltration were seen within the neuropil. The histological structure of treated group with vitamin E was more or less similar to that in group control group (figure 3).

❖ **Immunohistochemical results**

- **Heart sections:** Nuclear factor Kappa ß expression appeared as dark brown nuclear and cytoplasmic reactions. Weak nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß in the cardiomyocytes were detected in control, vitamin E and vitamin E+ tartrazine groups, while strong nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß in the cardiomyocytes were seen in the tartrazine group (figure 4).
- **Brain sections** Weak nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß in the pyramidal cells were detected in control, vitamin E and vitamin E+ tartrazine groups, while strong nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß in the pyramidal cells were seen in the tartrazine treated rats (figure 5).

Table (1): Effect of tartrazine dye, vit E, tartrazine and vit E on body weight, weight and relative weight of heart and brain of adult male albino rats studied groups

Groups		Weight (wt.)				
		Body wt./g	Heart wt./g	Relative heart wt.	Brain wt./g	Relative brain wt.
		Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
Control		215.00±4.47	0.69±0.01	0.32±0.01	1.42±0.01	0.66±0.013
Vit. E		223.33±8.76 a	0.68±0.01 a	0.31±0.01 a	1.42±0.01 a	0.64±0.026 a
Tartrazine		263.00±2.53a*	0.95±0.05 a*	0.36±0.02 a*	1.99±0.01a*	0.76±0.006 a*
Tartrazine+ Vit. E		245.17±2.79 c*	0.79±0.02 c*	0.32±0.01 c*	1.49±0.03c*	0.61±0.016 c*
ANOVA	F	101.984	120.977	20.017	1597.697	86.757
	P-value	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*

Wt. = weight; Vit. E= vitamin E; a= (Vit E and Tartrazine groups) compared to the control group; c= (Tartrazine+ Vit E groups) compared to (Tartrazine group); *: significant at (P < 0.05).

Table (2): Effect of tartrazine dye, vit E, tartrazine and vit E on cardiac enzymes and antioxidant markers of adult male albino rats studied groups

Groups		Cardiac enzymes			Antioxidant markers	
		cTnI (ng/mL)	LDH (U/L)	CK-MB (U/L)	SOD (U/L)	CAT (U/L)
		Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
Control		0.018±0.001	244.83±4.79	31.83±2.79	189.67±5.68	501.17±15.58
Vit E		0.018±0.003a	241.50±7.50a	31.81±4.62a	196.83±5.85 a	528.33±34.07a
Tartrazine		0.745±0.088a*	615.50±23.71a*	71.67±2.81a*	105.00±6.07a*	225.50±48.81a*
Tartrazine+ VIT E		0.163±0.084c*	400.67±15.78c*	44.83±6.18c*	141.00±7.43c*	363.00±28.08c*
ANOVA	F	193.876	837.724	283.210	283.210	102.209
	P-value	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*

Vit. E= vitamin E; cTnI= Cardiac troponin I; LDH= lactate dehydrogenase; CK-MB =Creatinine Kinase muscle-brain; SOD= superoxide dismutase; CAT= catalase; a= (Vit E and Tartrazine groups) compared to the control group; c= (Tartrazine+ Vit E group) compared to (Tartrazine group); *: significant at (P < 0.05).

Table (3): Effect of tartrazine dye, vit E, tartrazine and vit E on morphometric measurements of both heart and brain of adult male albino rats studied groups

Groups		Cross sectional area of cardiomyocytes (µm²)	Collagen area % per (µm²) Heart	Optical density of nuclear factor kappa B Heart	Optical density of nuclear factor kappa B Brain
		Mean± SD	Mean± SD	Mean± SD	Mean± SD
Control		232.01±27.49	7.09±0.87	0.80±0.07	0.84±0.06
VIT E		241.13±58.83a	7.48±0.75a	0.80±0.08a	0.84±0.06a
Tartrazine		541.29±50.78a*	23.81±0.98a*	1.78±0.58a*	1.96±0.64a*
Tartrazine+ VIT E		327.96±48.84c*	9.27±0.33c*	0.91±0.08c*	0.91±0.08c*
ANOVA	F	90.078	1064.691	25.759	28.395
	P-value	<0.05*	<0.05*	<0.05*	<0.05*

Vit. E= vitamin E; a= (Vit E and Tartrazine groups) compared to the control group; c= (Tartrazine+ Vit E group) compared to (Tartrazine group); *: Significant p- value

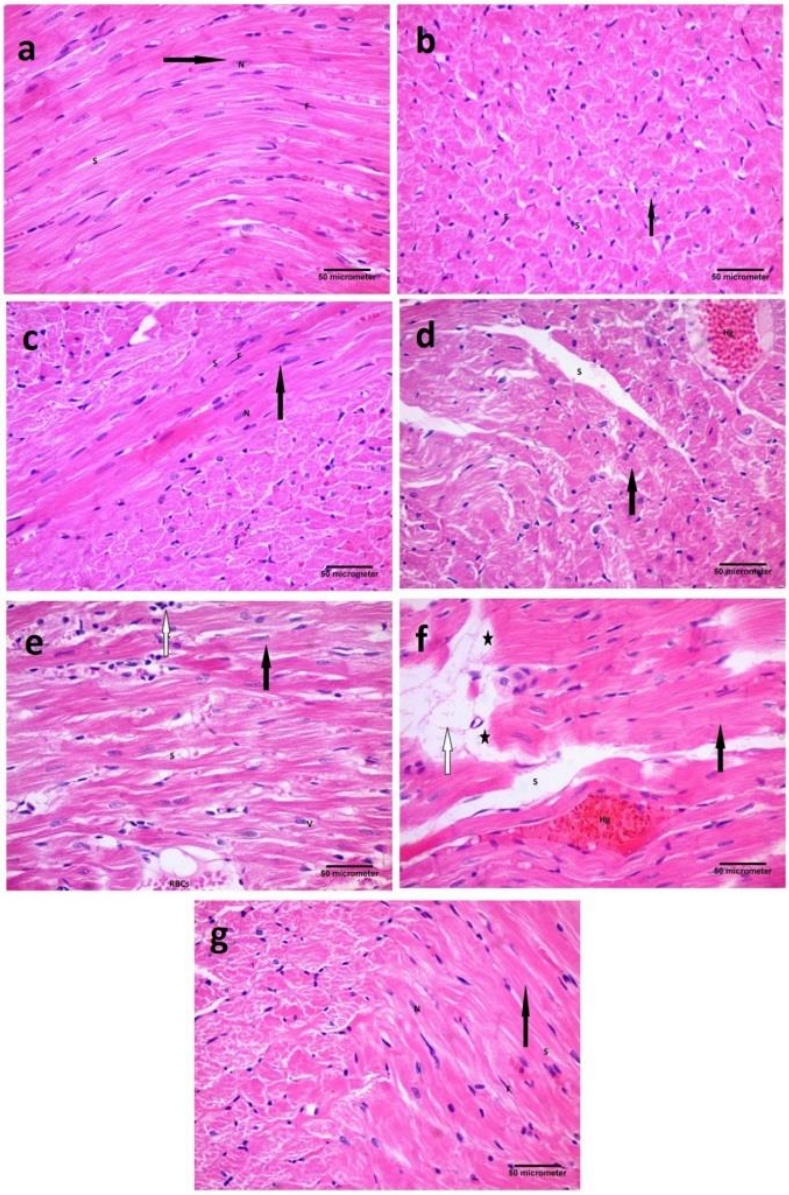

Figure (1): a) A photomicrograph of a longitudinal section in the cardiac muscle of a control group, showing the characteristic branching and anastomosing pattern of cardiac muscle fibers (arrow). Each cardiac muscle fiber has a single oval centrally located nucleus (N) and acidophilic sarcoplasm. They are separated by narrow intercellular spaces (S). Notice, the nuclei of fibroblast (F) in the interstitial tissue between the myocytes. **b)** A photomicrograph of a transverse section in the cardiac muscle of a control group, showing the polygonal or polyhedral appearance of cardiac muscle fibers (arrow) in cross section. The nuclei of cardiac muscle fibers appear central rounded. They are separated by narrow intercellular spaces (S). Notice, the nuclei of fibroblast (F) in between the myocytes. **c)** A photomicrograph of a transverse and longitudinal section in the cardiac muscle of a Vit. E group, showing the same histological structure as in control group. **d)** A photomicrograph of a section in the cardiac muscle fibers of a tartrazine group, showing that the cardiomyocytes are apparently hypertrophied (black arrow). The cardiomyocytes are widely separated (S). A large area of hemorrhage (Hg) can be seen. **e)** A photomicrograph of a section in the cardiac muscle fibers of a tartrazine group, showing that the cardiomyocytes are widely separated (S) and are apparently hypertrophied (black arrow). Also, some cardiac muscle fibers have vacuoles within their sarcoplasm (V). Mononuclear cellular infiltration (white arrow) and extravasation of blood (RBCs) can be seen. **f)** A photomicrograph of a section in the cardiac muscle fibers of a tartrazine group, showing that the cardiomyocytes are widely separated (S) and are apparently hypertrophied (black arrow). Also, some cardiac muscle fibers are interrupted and discontinuous (*). There is a large area of destructed cardiomyocytes (white arrow). Large area of hemorrhage (Hg) can be seen. **g)** A photomicrograph of a section in the cardiac muscle fibers of a tartrazine + vit. E group, showing that the histological structure is as the control group **(H&E X 400, scale bar=50µm).**

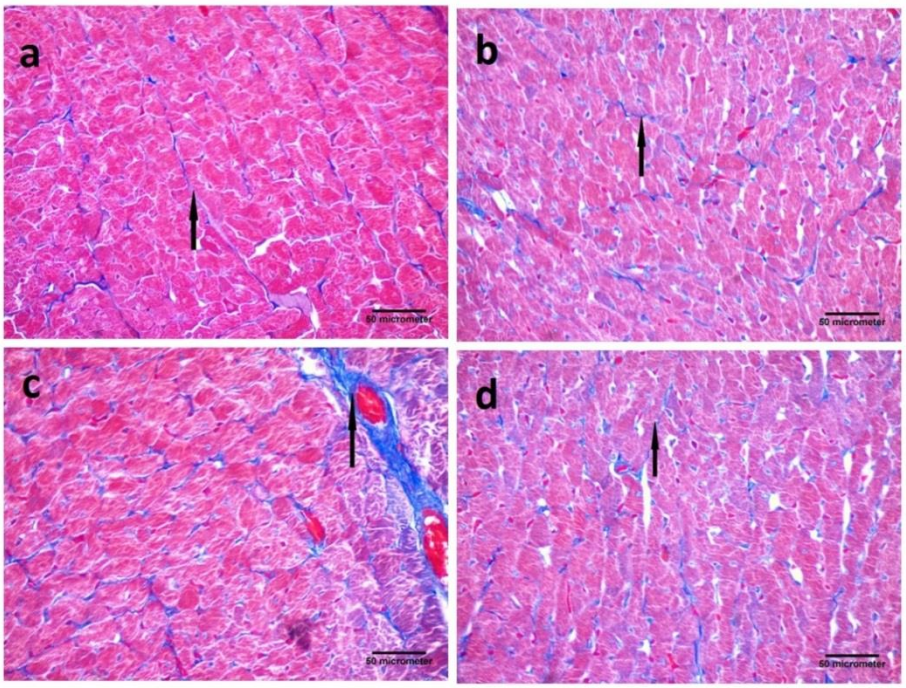

Figure (2) a) A photomicrograph of a section in the cardiac muscle of a control group, showing minimal collagen fibers deposition (arrow) in between the cardiomyocytes. **b)** A photomicrograph of a section in the cardiac muscle of a vit E group, showing minimal collagen fibers deposition (arrow) in between the cardiomyocytes. **c)** A photomicrograph of a section in the cardiac muscle of a tartrazine group, showing excessive collagen fibers deposition (arrow) in between the cardiomyocytes and around the blood vessels. **d)** A photomicrograph of a section in the cardiac muscle of a tartrazine +vit E group, showing minimal collagen fibers deposition (arrow) in between the cardiomyocytes **(Mallory trichrome X 400, scale bar=50µm).**

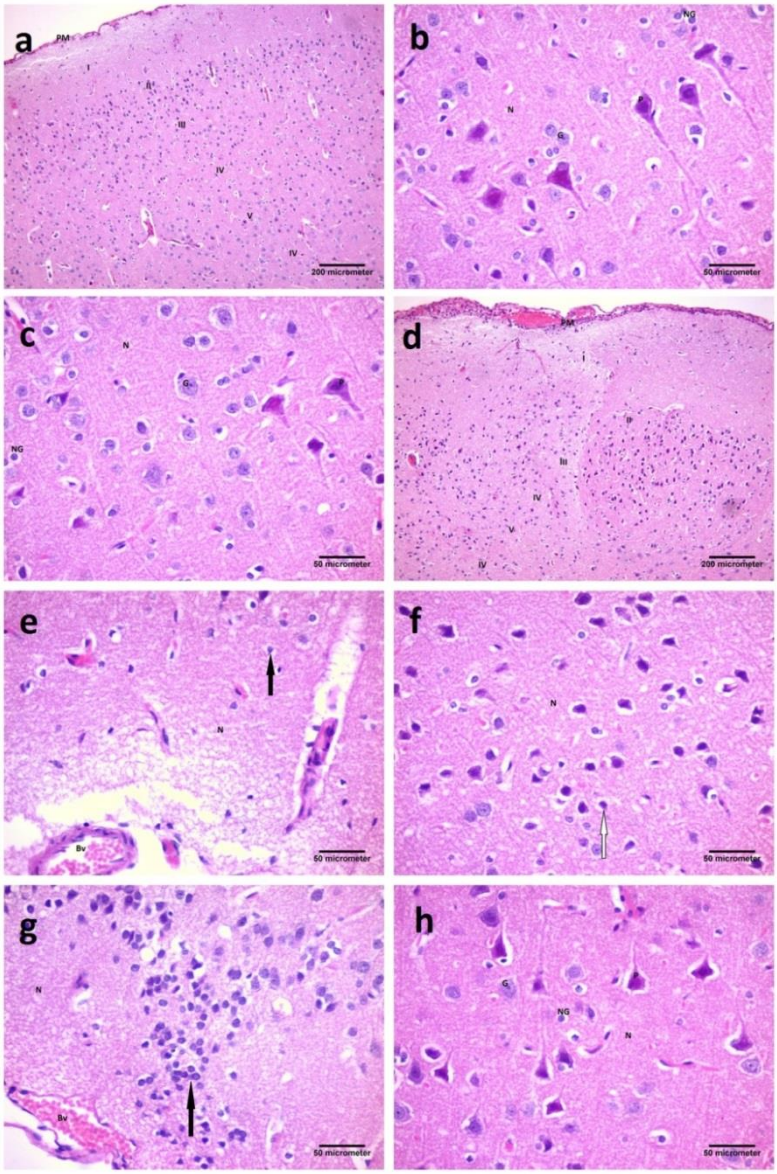

Figure (3) a) A photomicrograph of section in the cerebral cortex of adult male albino rat of control group showing the pia matter (PM) covers the outer molecular layer (I) followed by external granular layer (II), external pyramidal layer (III), inner granular layer (IV), inner pyramidal (V) and the polymorphic layer (VI). **(H&E X 100). b)** A photomicrograph of section in the cerebral cortex of adult male albino rat of control group showing normal pyramidal cell (P). This cell has multipolar shape with basophilic cytoplasm and large, rounded vesicular nucleus. Granular cells (G) can be seen with large open face nuclei, prominent nucleolus and little cytoplasm. Also, neuroglia cells can be seen (NG). The pink stained background is the neuropil (N) **(H&E X 400). c)** A photomicrograph of section in the cerebral cortex of adult male albino rat of vitamin E group showing that the histological structure of the cerebral cortex is similar to that of the control group **(H&E X 400). d)** A photomicrograph of section in the cerebral cortex of adult male albino rat of tartrazine group showing the pia matter (PM) which covered with dilated congested blood vessels. The outer molecular layer (I) followed by external granular layer (II), external pyramidal layer (III), inner granular layer (IV), inner pyramidal (V) and the polymorphic layer (VI) can be seen. **(H&E X 100). e)** A photomicrograph of section in the cerebral cortex of adult male albino rat of tartrazine group showing that the pyramidal cells are shrunken surrounded with empty spaces with loss of their processes (arrow). Dilated and congested blood vessel (Bv) can be seen. Notice, the neuropil (N) appears vacuolated **(H&E X 400). f)** A photomicrograph of section in the cerebral cortex of adult male albino rat of tartrazine group showing that the pyramidal cells are shrunken, surrounded with empty spaces with loss of their processes (arrow). Notice, the neuropil (N) appears vacuolated **(H&E X 400). g)** A photomicrograph of section in the cerebral cortex of adult male albino rat of tetrazine group showing that there is mononuclear cellular infiltration (arrow). Dilated congested blood vessel (Bv) can be seen. Notice, the neuropil (N) appears vacuolated **(H&E X 400). h)** A photomicrograph of section in the cerebral cortex of adult male albino rat of tartrazine +vit E group showing that there is marked improvement and the histological structure is as the control group **(H &E X 400)** [**X100, scale bar=200µm - X 400, scale bar=50µm].**

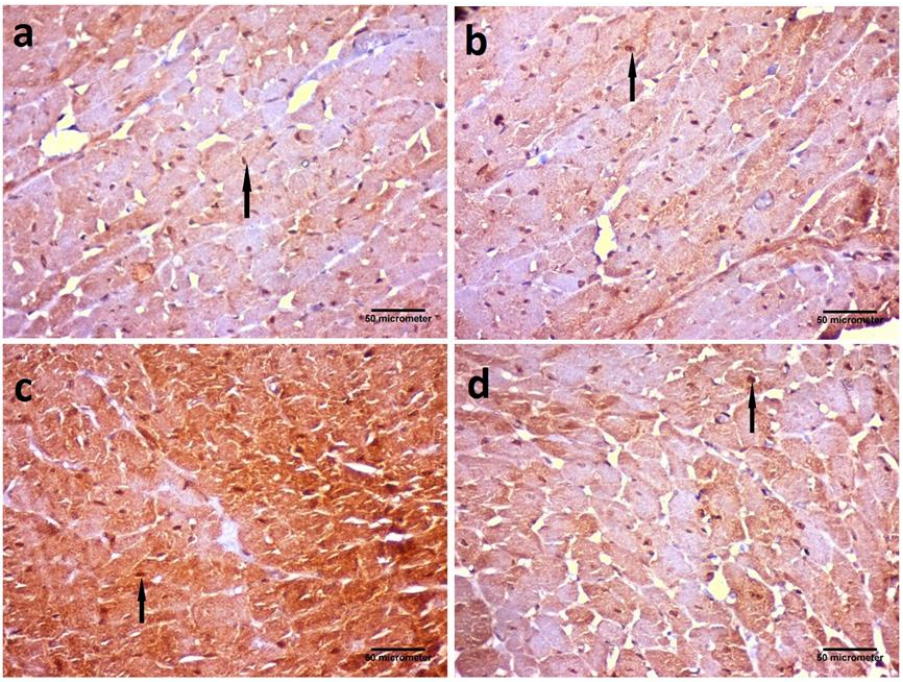

Figure (4) a) A photomicrograph of a section in the cardiac muscle of a control group, showing weak nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß (arrow) in the cardiomyocytes. **b)** A photomicrograph of a section in the cardiac muscle of a vit. E group, showing weak nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß (arrow) in the cardiomyocytes. **c)** A photomicrograph of a section in the cardiac muscle of a tartrazine group, showing strong nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß (arrow) in the cardiomyocytes. **d)** A photomicrograph of a section in the cardiac muscle of a tartrazine +vit E group, showing weak nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß (arrow) in the cardiomyocytes **(Avidine biotin peroxidase stain with Hx counter stain X 400, scale bar=50µm).**

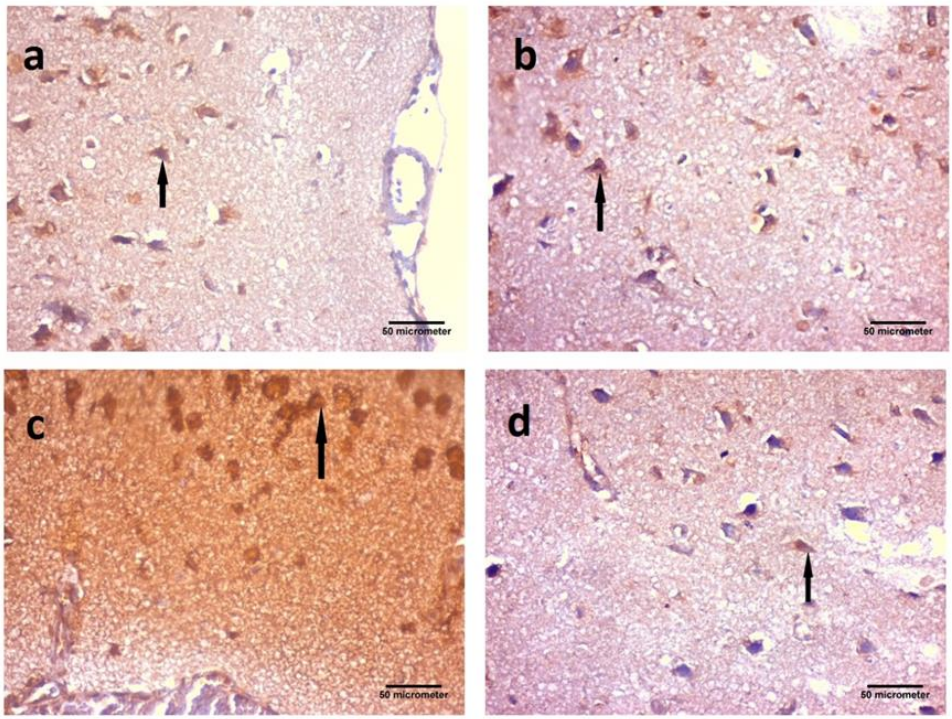

Figure (5) a) A photomicrograph of a section in the cerebral cortex of a control group, showing weak nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß (arrow) in the pyramidal cells. **b)** A photomicrograph of a section in the cerebral cortex of a vitamin E group, showing weak nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß (arrow) in the pyramidal cells. **c)** A photomicrograph of a section in the cerebral cortex of a tetrazine group, showing strong nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß (arrow) in the pyramidal cells. **d)** A photomicrograph of a section in the cerebral cortex of a vitamin E + tetrazine group, showing weak nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß (arrow) in the pyramidal cells **(Avidine biotin peroxidase stain with Hx counter stain X 400, scale bar=50µm)**.

DISCUSSION

One of the most used food dyes is tartrazine. It is a yellow-coloured [26]. Tartrazine has several adverse impacts on learning and memory functions in animals. induced behavioral changes, and has toxic effects of hepatocytes, renal function and lymphocytes. The usage of azo dyes for a long period could result in diseases such as eye defects, anemia, cancer, pathological lesions in spleen and brain [27]. Tartrazine toxicity was produced by formation of free radicals which disrupts antioxidant enzymes activities [28]. The gastrointestinal microflora metabolizes tartrazine to aromatic amine sulfanilic acid [3]. The reactive oxygen species (ROS) can be generated from the reaction between formed aromatic amines and sulfonic acid and nitrite and nitrate [29]. ROS, such as hydroxyl radical, superoxide anion, and hydrogen peroxide if not scavenged can produce oxidative stress and lipid peroxidation in the membranes [30].

This study aimed to assess oral sub-chronic toxicity of tartrazine on body weight, heart and brain of adult mail albino rats via biochemical and histological studies and to assess antioxidant properties of vit. E against these effects. According to the findings of that study, sub-chronic toxicity by tartrazine generates significant increase in body weight in comparison with control group. This result was agreed with Gautam et al. [31]; Mehedi et al. [25]. But dis-agreed with Himri et al. [32] who stated that tartrazine did not affect the body weight. Tartrazine induces significant increase in weight and relative weight of both heart and brain as opposed to control group, Similarly Arefin et al. [33] reported that tartrazine had significantly increased mice heart average weight and Balta et al. [34] observed that relative weight of brain was slightly increased in tartrazine treated rats.

In current work, tartrazine produced significant increase in cTnI, LDH and CK-MB, in comparison to control group. Same findings have also been described by Oyewole and Oladele [35] and they attributed this elevation to the stress condition and possible membrane damage which resulted in leaking of cardiac enzymes into the blood, these results also were confirmed by the histological results of the current work, Adesokan and Akanji [36] reported that The activity of enzymes in tissues is employed as a marker to detect early harmful effects of foreign chemicals given to experimental animals.

In this study, tartrazine provoked significant reduction in SOD and CAT activities as compared to control group. These outcomes were in accordance with that of El-Rabey et al. [37] and El-Desoky et al. [38] who also stated that the reduction in activities of SOD and CAT because of azo dye metabolism in the small intestine with production of genotoxic compounds. Hoseinpouran et al. [6] have concluded that, in rats, tartrazine can cause oxidative stress, which is linked to the formation of free radicals so reducing the antioxidants of blood and biochemical serum parameters, also Amin et al. [39] have come to the conclusion that tartrazine has adverse effects and alters the biochemical indicators s in vital tissues such as antioxidant enzymes activities.

As regards histological results tartrazine resulted in alternation in the normal histological structure of the heart in the form of hypertrophy of cardiac muscle fibers. Large area of mononuclear cellular infiltration and hemorrhage between the cardiac muscle fibers. Destruction and vacuolization of some cardiomyocytes, as some cardiomyocytes were discontinuous, interrupted and separated from each other by wide intercellular spaces. There were marked deposition of collagen fibers in between the cardiomyocytes and around the blood vessels. Strong nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß in the cardiomyocytes by immunohistochemical stain were noticed. These finding are correlated with biochemical analysis as elevation in the level of the cardiac biomarkers in the serum resulted from damage to cardiomyocytes. These finding are correlated with Oyewole and Oladele [35] who found that tartrazine caused deformation in shapes and sizes of the nuclei of the cardiomyocytes and disarranged pattern of cardiac myofibres.

In addition, tartrazine leads to the alternation in the normal histological structure of brain when contrasted to control group, in form of the six layers of cerebral cortex but the pia mater covered with dilated congested blood vessels. The pyramidal cells surrounded by haloes and appeared shrunken, loss their processes. There was also, extensive neuropil vacuolization. Dilated congested blood vessels and mononuclear cellular infiltration were noticed within neuropil. While strong nuclear and cytoplasmic immunoreaction for nuclear factor Kappa ß in the pyramidal cells by immunohistochemical stain were observed. Similarly, Balta et al. [34] and Rafati et al. [14] fount that tartrazine leads to alternation of microscopic examination of the brain tissue. Mohamed et al. [40] reported that, by utilizing an immunohistochemical staining with anti-ssDNA antibody as an apoptotic cell marker, there were numerous apoptotic cells in brain cortex as compared to rest of groups. The pathological changes that occur in the heart and the brain of the tartrazine treated rats in that study are attributed to the increase in the weight which observed in that organs.

Suzuki et al. [41] attributed the serious pathologies to the effect of the ROS on the membranes, as peroxidation of unsaturated fatty acids in biological membranes promotes disruption of membrane integrity and function and decrease of fluidity. Gao et al. [4] stated that the lipid peroxidation metabolites and ROS prevent the protective effect of endogenous antioxidant enzymes and lead to brain tissue injury. They also indicated that the diminution in the activities of SOD, CAT in brain of tartrazine treated rats were linked with oxidative brain damage.

In current study co-administration of vit. E with tartrazine led to significant decrease in body weight &

weight and relative weight of both heart and brain when compared with tartrazine group, also induced significant decrease in cTnI, LDH and CK-MB as compared tartrazine group and significant increase in SOD and CAT activities when compared with tartrazine group. Administration of vit. E with tartrazine produced correction in all histological changes in both heart and brain as compared with tartrazine group. Rafati et al. [14] reported that vitamin E with tartrazine prevented the changes that occur in the brain tissues. These results can be described by Elshama et al. [42] who declared that the vitamins are used as complementary or as protective antioxidant agent to prevent and treat the toxicity that is caused by numerous chemicals and drugs. Vitamin E has antioxidant effect as it decreases the lipid peroxidation, suppresses the oxidative stress and increases the activity of antioxidant enzymes. Vitamin E decreases the activity of free radicals, so it inhibits lipid peroxidation in cell membranes and protects the cell membranes from the damage [43].

CONCLUSION AND RECOMMENDATION

Tartrazine can generate biochemical and histological damage changes of both heart and the brain of albino rats that administered this dye. We elucidated the protective properties of vitamin E on biochemical and histological parameters of heart and brain. Vitamin E could have a protective effect against toxic changes of tartrazine due to its antioxidant properties. Tartrazine must be used with caution in humans. Vitamin E should be administered to humans to protect them from the toxic effect of tartrazine. Tartrazine as a dye has an impact on body weight and toxic effects on the heart and brain so the food which contain this dye should be avoided as much as possible. More study should be done to detect the hazardous effect of tartrazine on other body organs.

Financial support: none
Conflict of interest declaration: none

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الملخص العربي

الآثار السامة الشبه مزمنة للتارترازين على قلب ومخ الفئران البيضاء الذكور البالغين والتأثير الوقائي لفيتامين هـ

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ملخص البحث

الخلفية: يعتبر التارترازين الصبغة الأكثر استخدامًا في المواد الغذائية والأدوية ومستحضرات التجميل.

الهدف: هو تقييم السمية شبه المزمنة للتارترازين على وزن الجسم وقلب ومخ الفئران البيضاء الذكور البالغين باستخدام الدراسات البيوكيميائية الهستولوجية ولتقييم التأثير الوقائي المحتمل لفيتامين هـ.

الطرق: وقد اشتملت هذه الدراسة السريرية علي 24 فأرا. وتم تقسيمها بشكل عشوائي إلى أربع مجموعات: المجموعة الضابطة، ومجموعة فيتامين هـ (100 مجم / كجم / يوم) ، ومجموعة التارترازين (300 مجم / كجم / يوم) ، ومجموعة التارترازين (300 مجم / كجم / يوم)+ فيتامين هـ (100 مجم / كجم / يوم). وتم إعطاء العلاج لجميع الفئران عن طريق الفم لمدة 30 يومًا. وبعد ذلك تم تقييم أوزان الجسم والقلب والدماغ كما تم تقييم مستوى تروبونين القلب، واللاكتات ديهيدروجينيز، الكرياتين كيناز عضلات-المخ، وديسموتاز الأكسيد الفائق، والكاتالاز في الدم، وكذلك التغيرات الهستولوجية للقلب والدماغ.

النتائج: وقد أوضحت نتائج هذه الدراسة أن التارترازين قد أدى الي زيادة ذات دلالة إحصائية في أوزان الجسم والقلب والدماغ، وكذلك في مستويات تروبونين القلب، واللاكتات ديهيدروجينيز، الكرياتين كيناز عضلات-المخفف في الدم، كما أدي إلي انخفاض ذات دلالة إحصائية في نشاط كل من ديسموتاز الأكسيد الفائق، والكاتالاز. وكذلك اختلاف في التركيب الهستولوجي الطبيعي للقلب والدماغ في مجموعة التارترازين مقارنة مع المجموعة الضابطة. ونتج عن اعطاء فيتامين هـ مع التارترازين للفئران إلى تحسن في جميع التغييرات السابقة التي يسببها التارترازين.

الاستنتاجات: وقد خلصت هذه الدراسة الحالية إلى أن التارترازين له أثار سامة علي القلب والدماغ. ويؤدي استخدام فيتامين هـ الذي له خصائص مضادة للأكسدة إلى تحسن هذه السمية.

الكلمات المفتاحية: التارترازين، التأثير السام، فيتامين هـ، القلب، المخ، المؤشرات الحيوية للقلب، علامات الإجهاد التأكسدي، الهستولوجي.

الباحث الرئيسي

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