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Comparative Study of Some Natural and Artificial Food Coloring Agents on Hyperactivity, Learning and Memory Performance in Weanling Rats

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Abstract

Color additives are used in a wide variety of foods, Food azo-colourstartrazine (Tar) is one of the most widely used artificial foods, drugs and cosmetic yellow dyes, its E number is E102 while Curcumin (Cur, an active ingredient of turmeric) is brightly yellow colored which routinely used as spice, food preservative and coloring material in different parts of the world; its E number is E100. The present study aimed to Compare between artificial yellow coloring additive Tar and natural one Cur (has the same color) on hyperactivity, learning and memory and the possibility of using Cur instead of Tar or at least in combined with Tar to protect against Tar behavioral disorder in weanling rats. To characterized biochemical and behavioral parameters the study was assessed the effect of Tar (1%, 3% of diet) alone or in combination with Cur (200 mg/kg/b.wt) for 8 weeks on Open field test to assess the potential hyperactivity and Morris water maze test to assess learning and memory. Furthermore, biomarkers of oxidative stress malondialdhyde (MDA, end product of lipid peroxides), nitric oxides (NO, as nitrite to nitrate ratio), GSH (reduced glutathione), and oxidized glutathione (GSSG) in addition to some neurotransmitter, monoamines [dopamine (DA), norepinephrine (NE) and serotonin (5-HT)] were also measured in three different brain areas (frontal cortex, Striatum and hippocampus).

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These brain regions are important because they are involved in important behavioral functions, such as emotion, motivation, learning and memory. The results indicated that Tar extract significantly enhanced active behavioral response. Tar-treated rats showed hyperactivity in open field test presented by increasing horizontal locomotionas well as depletion in learning and memory by increased the escape latency in Morris water maze test and decreased the retention latency in probe test. Tar alone disturb oxidative stress marker by causing significant increase in serum NO and serum and tissue MDA and GSSG while it caused significant decrease in serum and tissue GSH as well as it inhibited neurotransmitters releases especially in striatum and hippocampus area. While combined treatment of Cur significantly ameliorated all the behavioral and biochemical alterations in serum and different brain regions of Tar-treated weanling rats. This study provides scientific evidence that there is a relationship between Tar and inflection of hyperactivity and depletion in learning and memory in weanling rats while coadministration of Cur attenuates the potential hazards of Tar.

Keywords: color additives; Tartrazine; Curcumin; hyperactivity; learning and memory; oxidative stress; monoamines.

1. Introduction

Color additives are used in a wide variety of foods such as beverages, dairy products, cereals, bakery goods, snack foods and ice creams. Although there are strict guidelines for chemicals to be approved as food additives, the safety of food colorants has not been rigorously proven, and acceptable daily intake (ADI) has been used to minimize any possible unfavorable effect of the dyes. Tar (E 102, FD & C Yellow N°5) is an azo dye used as a foodstuff additive and in various human drugs. Since its first safety assessment, conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1964, more than 300 new studies on laboratory animals and clinical trials on human beings have been conducted [32]. A number of data has described Tar related to hyperactivity behavior in children [8, 6]. Moreover, noticeable adverse effect of Tar on the behavior of young mice has been reported [46, 47]. In addition, Tar evaluates toxic effects on the learning and memory functions in mice and rats [51].

On the other hand, Cur, a yellow pigment from *Curcuma longa*, is a major component of turmeric and is commonly used as a spice and food-coloring agent. It is also used as a cosmetic and in some medical preparations. The desirable preventive or putative therapeutic properties of Cur have also been considered to be associated with its antioxidant and anti-inflammatory properties [1, 50]. Cur has also been found to be effective in the treatment of Alzheimer's dementia, neuroleptic-induced tardive dyskinesia and chemical-induced neurotoxicity resulting from lead and cadmium [36, 41, 28].Therefore, the goal of this study was to Compare between artificial yellow coloring additive Tar and natural one Cur (has the same color) on hyperactivity as well as learning and memory performance in weanling rats.

2. Materials and Methods

2.1. Animals and housing

For performing the present work, ninety six weanling albino rats' *rattusrattus* weighting 40 – 50 g were used.

The animals were brought from laboratory animal breeding of national organization of drug control and research (NODCAR), Giza, Egypt. They were kept under strictly hygienic condition for acclimatization. They were fed with a standard basal diet formulation in accordance with composition authorized by association of official analytical chemist (AOAC) (1988), which consists of about 78.5%carbohydrate (including about 50% crude cellulose fibers) 15.2% protein, 3.2% lipids, 2.1% salt mixture and 1% multi vitamins.

2.2. Materials

Tar (FD and C Yellow No. 5) was obtained from Sigma chemical Company (Sigma, Aldrich, USA) and dissolved in tap drinking water at a different concentrations; namely 1% of diet (low dose) and 3 %of diet (high dose) [33]. Cur powder 95.02 % Curcumoids was obtained from Sigma chemical Company (Sigma, Aldrich, USA) and suspending in 0.5 % carboxy-methylcellulose (CMC) just before administration [18].

Ninety six weanling albino rats will randomly assign into 6 groups, each group of 16 rats, 8 per cage will be administrated our treatment daily for 8 weeks as follow:

G1, +ve control group (CMC): orally administrated 0.5 % CMC.

G2, Tar-treated group in low dose (low Tar): orally administrated low dose (1% of diet) of tar.

G3, Tar-treated group in high dose (Higher Tar): orally administrated high dose (3% of diet) of tar.

G4, Cur-treated group (cur): orally administrated cur (200mg/kg/B.w.).

G5, Cur + lower Tar treated group: orally administrated cur (200mg/kg/B.w.) plus 1% of tar.

G6, Cur + higher Tar treated group: orally administrated cur (200mg/kg/B.w.) plus 3% of tar.

2.3. Behavioral measurements

Behavioral tests were performed in the first half of light phase of the light/dark cycle. All behaviors were scored by a single trained observer unfamiliar with treated animals. Hand operated counters and stop watches were used to score animals' behavior. Behavioral tests were separated by at least 24 h from each other and executed in the same order presented below.

2.3.1. Open field behavior test

The open field test provides simultaneous measures of locomotion [30]. The open field used was a square wooden arena measured (90 x 90 x 25cm).The wood of the apparatus is covered with a plastic laminate (Formica), which prevents absorption of fluids (urine of rats). The floor was divided by black lines into 36 small squares (15 x 15cm). The open field maze was cleaned between each rat using 70% ethyl alcohol to avoid odor cues. The rats were carried to the test room in their home cages and tested once at a time for 5 minutes each. Rats were handled by the base of their tails at all times. Rats were taken from their home cages and placed

randomly into one of the four corners of the open field facing the center. The behavioral scores measured, in this experiment, total numbers of line crossings.

2.3.2. Morris Water Maze Tank

The water maze consisted of a white circular galvanized tank (its size was 150 cm diameter and 60 cm height) filled with opaque tap water made by adding dry milk powder to water at the temperature of 27 C. Four locations around the edge of the pool were defined as start points, and these divided the pool into four equal quadrants. A circular escape platform 15 cm in diameter was placed 2 cm below the surface of the water in the middle of one of the four quadrants of the pool. A video camera suspended from the bracket above the middle of the tank permitted the observer to monitor the animal's behavior on a monitor. Animals were tested on three daily trials, each trial separated by 2 min, for three consecutive days. Animals were placed into the tank, facing the wall of the pool, and were allowed to circumnavigate the pool in search of the escape platform for a maximum of 90 s. On each day, the start points used for each trial varied in a pseudo random sequence such that no two trials on the same day commenced from the same start point.

The time (latency) to reach the escape platform was recorded, and the animals were permitted 30 s to rest on the platform before removal from the tank. If an animal failed to locate the platform within 90 s, it was guided to the platform by the experimenter, placed on it for 30 s and assigned a latency score of 90 s for that trial. A single probe trail was done on the final test days in which the platform was removed and animals were allowed to swim freely for 90 s. The number of times the animals spent in where the platform had been located was recorded [12].

2.4. Biochemical measurements

At the end of the treatment schedule, rats were sacrificed; brain tissues were removed and were homogenates in 3 different areas (cortex, hippocampus and striatum regions) in iced 70% methanol. Blood samples were collected then separated serum and supernatant of homogenates tissues were processed for the biochemical analysis included: oxidative stress **(**MDA, NO, GSH, GSSG), monoamines neurotransmitter (norepinephrine, dopamine, serotonin) all were determined by HPLC methods of [29, 26, 22, 37] respectively.

2.5. Statistical analysis

The statistical analysis was done by using SPSS v. 22. Results are expressed as means ± SE. Differences between groups were analyzed by one-way ANOVA, P-values are considered significant when $P < 0.05$.

3. Results

3.1. Open field test

The effect of Tar treatment on parameters of open field test was illustrated in Table.1. Rats under tar (1%, 3%) effect increased significantly ($p<0.05$) the mean covered distance in the open field test when compared with the control group.A significant ($p<0.05$) dose- dependent response was noted for total numbersin Tar –

administrated rats in comparison to their low dose so the highest levels of these behaviors were recorded with higher Tar dose.

On the other hand, treatment of cur (200 mg/kg.bw.) showed pronounced improvement in the number of total squaresin both doses dose of Taras compared to Tar-treated groups.

3.2. Learning and memory impairment in Morris water maze.

Data in table 2, Figure1 showed that Tar administration increased significantly($p<0.05$) the latency time required for rats to find the hidden platform in water maze task as compared to the normal control value in the 1st, 2nd and 3rd days of trails. The data exhibited dose and time dependent manner effect in which the dose 3% showed the maximum effect and the 3rd day showed the lowest effect. On the other hand, treatment with Cur (200 mg/kg.bw.) caused significant($p<0.05$) decrease in latency time as compared to Tar- treated groups at 2nd and 3rd day of trails at both doses.

Prop test:

The data in table 3, Figure 2 showed that time in the platform zone was significantly($p<0.05$) reduced in Tar-treated groups as compared to control groups. A significantly($p<0.05$) does- dependent response was noted for both doses of Tar. While, Cur (200 mg/kg.bw.) treated rats showed significant($p<0.05$) increase in time spent in a platform zone as compared to tar 1%, 3% of diet.

3.3. Oxidative stress parameters.

As depicted in Table. 4, 5 treatment of weanling rats orally with 1`%, 3% of tar alone disturb oxidative stress marker by causing significant ($p<0.05$) increase in serum NO and serum and tissues MDA and GSSG while it caused significant decrease in serum and tissues GSH as compared to the levels of control groups. The data also exhibited dose dependent manner that showed higher significant ($p<0.05$) increase in the levels of serum of NO, GSSG (in serum and all tissues), MDA (in serum and cortex) and GSH (in serum and hippocampus) as compared to the level of tar.1% group.

On the other hand, the statistical analyses revealed that the higher levels in serum NO and in serum and tissues MDA, GSSG which were observed in Tar (1%, 3% of diet) groups were attenuated in both groups treated with Cur (200 mg/kg.bw.) (Cur+tar.1%, Cur+tar.3%) showing significant($p<0.05$) decrease except in serum GSSG the improvement is non-significant as compared to Tar 1%, 3%, respectively. while the marked reduction in levels of GSH in serum and brain tissues which caused in rats treated with Tar 1%, 3% were attenuated significantly ($p<0.05$) in all groups treated with cur (200mg/kg/B.w.) .

3.4. Neurotransmitter parameters.

In the Table (6) the data showed highly significant ($p<0.05$) decrease in the level of norepinephrine, dopamine and serotonin in three brain areas (striatum, cortex and hippocampus) of rats treated with Tar (1%, 3% of diet)

as compared to the levels of control groups. The data also showed dose dependent effect and this effect was significant ($p<0.05$) in dopamine (hippocampus and cortex) and in norepinephrine cortex only as compared to the level of tar.1% group.

On the other hand, the cur treated groups showed significant ($p<0.05$) improvement in the levels of norepinephrine in the three brain areas, also Cur (200mg/kg/B.w.) significantly ($p<0.05$) improved the dopamine levels in all areas except in low dose of cortex the improvement is non-significant, while the improvement effect of cur in serotonin level observed only in cortex and striatum *vs*. the values of Tar (1%, 3% of diet) groups.

Table (1): Effect of tar (1%, 3% of diet)), Cur (200mg/kg/B.w.) individually and incombination on the number of total squares in open field test of weanling rats after 8 weeks of treatment.

Groups / Time	Control	Cur	Tar1%	Tar1% + Cur.	Tar3%	Tar3% + Cur.
8 weeks	43.00 ± 1.97	40.00 ± 1.83	52.75 ± 3.54*	42.50 ± 2.99 [c]	61.75 ± 1.85* [a]	46.63 ± 2.24 [b]

Significant difference from control group *$p<0.05$. Significant difference between Tar3% and Tar1% [a]$p<0.05$. Significant difference between Tar3% and Tar3%+cur [b]$p<0.05$.

Table (2): Effect of Tar (1%, 3% of diet), Cur (200mg/kg/B.w.) individually and in combination on escape latency time of weanling rats after 8 weeks of treatment.

	Control	Cur	Tar1%	Tar1% + Cur.	Tar3%	Tar3% + Cur.
1st day	42.92 ± 3.65	40.93 ± 2.50	49.58 ±3.15*	46.74 ± 2.04	54.86 ± 2.91*	49.19 ± 2.50*
2nd day	29.24 ± 3.42	23.10 ± 1.59	41.66 ± 3.7*	27.71 ± 3.24[c]	47.67 ± 3.08*	32.63 ± 1.96[b]
3rd day	20.08 ± 2.24	18.99 ± 1.39	34.96 ± 2.8*	24.12 ± 2.16	46.23 ± 2.5*	25.7 ± 2.29

Significant difference from control group *$p<0.05$, Significant difference between Tar3% and Tar1% [a] $p<0.05$,

Significant difference between Tar3% and Tar3%+cur [b] $p<0.05$, Significant difference between Tar1% and Tar1%+cur [c] $p<0.05$.

Table (3): Effect of Tar (1%, 3% of diet), Cur (200mg/kg/B.w.) individually and in combination on time spent in platform zone of weanling rats after 8 weeks of treatment.

Groups Duration	Control	Cur	Tar1%	Tar1%+Cur.	Tar3%	Tar3%+Cur.
8 weeks	30.13 ± 2.23	29.38 ± 1.58	22.75 ± 1.46*	26.75 ± 1.85	17.13 ± 1.35*[a]	25.94 ± 1.25[b]

Significant difference from control group *$p<0.05$, Significant difference between Tar3% and Tar1% [a] $p<0.05$, Significant difference between Tar3% and Tar3%+cur [b] $p<0.05$.

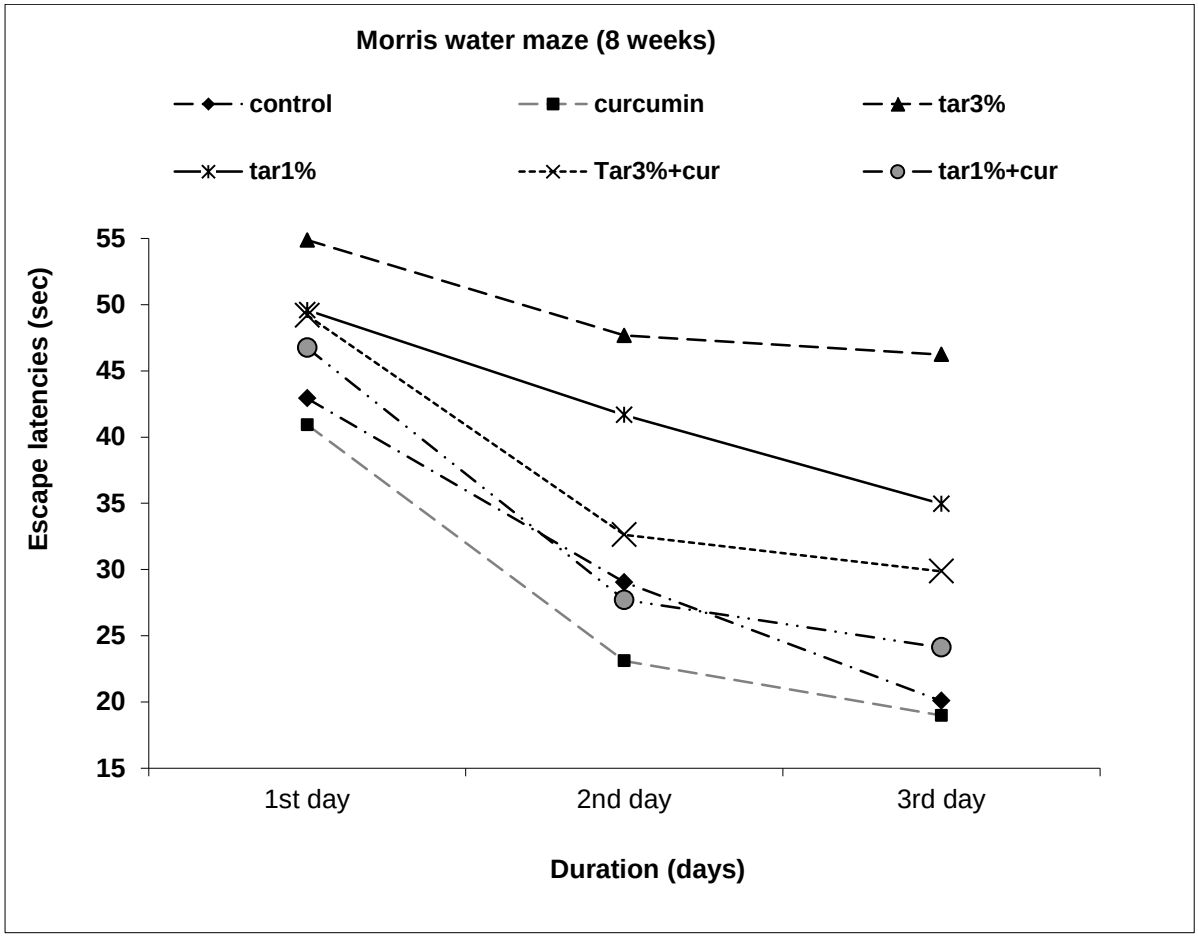

Figure (1): Effect of Tar (1%, 3%of diet), Cur (200mg/kg/B.w.) individually and in combination on escape latency time of weanling rats after 8 weeks of treatment.

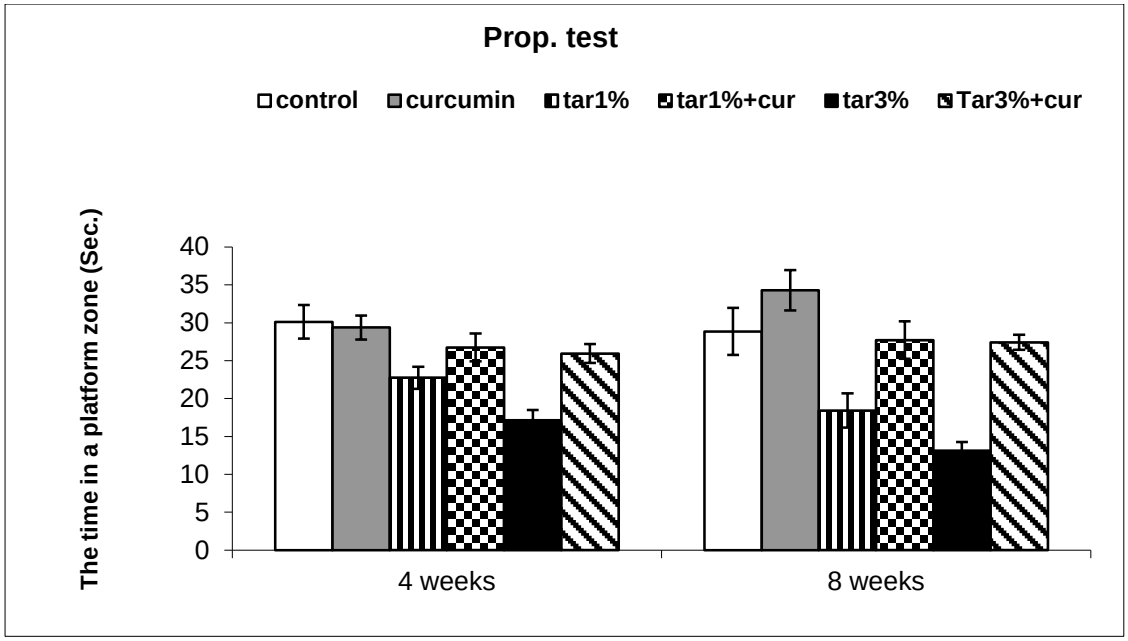

Figure (2): Effect of Tar (1%, 3%of diet), Cur (200mg/kg/B.w.) individually and incombination on time spent in platform zone of weanling rats after 4 weeks of treatment.

Table (4): Effect of Tar (1%, 3% of diet), Cur (200mg/kg/B.w.) individually and in combination on serum (NO, MDA, GSH, GSSG) of weanling rats after 4 and 8 weeks of treatment.

Groups	NO (%)	MDA µmol/ml	GSH nmol/dl	GSSG nmol/100 ml
Control	0.99 ± 0.03	0.70 ± 0.01	84.38 ± 3.10	2.39 ± 0.10
Cur	1.02 ±0.03	0.67 ± 0.02	99.60 ± 4.71	2.63 ± 0.06
Tar 1%	1.24 ±0.04*	1.14 ± 0.02*	70.08 ± 1.87*	2.89 ± 0.17*
Cur + Tar 1%	0.92 ±0.02 [c]	0.75 ± 0.02[c]	85.57 ±1.88 [c]	2.70 ± 0.09
Tar 3%	1.52 ±0.08* [a]	1.26 ± 0.03* [a]	62.20 ± 1.67*	3.28 ± 0.13*[a]
Cur + Tar 3%	0.90 ±0.02 [b]	0.74 ± 0.03[b]	83.37 ± 2.45 [b]	2.98 ± 0.04*

Significant difference from control group *p<0.05, Significant difference between Tar3% and Tar1% [a] p<0.05, Significant difference between Tar3% and Tar3%+cur [b] p<0.05.

Table (5): Effect of Tar (1%, 3%of diet), Cur (200mg/kg/B.w.) individually and in combination on the levels of MDA, GSH and GSSG in different brain areas (striatum, cortex and hippocampus) of weanling rats after 8 weeks of treatment

	***MDA(* nmol/g.tisse)**			***GSH(* µmol/g. tissue*)***			***GSSG(* µmol/g. tissue*)***		
	COX	**H.P.**	**STR**	**COX**	**H.P.**	**STR**	**COX**	**H.P.**	**STR**
Control	36.75 ± 2.66	44.69 ±0.57	41.34 ±2.76	0.64 ± 0.01	0.93 ± 0.01	0.55 ± 0.01	16.54 ± 0.45	29.97 ± 0.70	17.63 ± 0.51
Cur	36.77 ± 1.56	32.38 ± 0.82	34.78 ±1.21	0.68 ± 0.01	1.03 ± 0.02	0.57 ±0.01	16.89 ± 0.47	29.91 ± 0.31	17.80 ± 0.22
Tar 1%	57.33 ±1.45*	64.51 ± 0.57*	47.66 ±2.62	0.51 ±0.02*	0.79 ± 0.01*	0.46 ± 0.01*	19.45 ±0.55*	39.45 ± 0.80*	21.77 ± 0.67*
Cur + Tar 1%	47.85 ±1.10^{*c}	57.68 ±1.06^{*c}	37.29 ±2.58^{c}	0.61 ± 0.01^{c}	0.89 ± 0.01^{c}	0.55 ± 0.03^{c}	17.32 ±0.31^{c}	30.03 ± 0.46^{c}	18.23 ± 0.36^{c}
Tar 3%	69.99 ±1.91*^{a}	72.04 ± 1.10*	55.00 ±1.73*	0.48 ±0.01*	0.61 ±0.02*^{a}	0.39 ± 0.01*	22.81 ±0.68*^{a}	48.33 ±0.84*^{a}	36.54 ± 1.06*^{a}
Cur + Tar 3%	55.69 ±1.14*^{b}	59.43 ±0.93*^{b}	46.02 ±1.25^{*b}	0.59 ± 0.02^{b}	0.71 ±0.03*^{b}	0.50 ± 0.04^{b}	17.43 ±0.54^{b}	33.20 ±0.50*^{b}	18.71 ± 0.81^{b}

Significant difference from control group *p<0.05, Significant difference between Tar3% and Tar1% ^{a} p<0.05, Significant difference between Tar3% and Tar3%+cur ^{b} p<0.05.

4. Discussion

Considerable number of previous studies suggests a link between the repeated or long-term ingestion of artificial food colors and onset of behavioral hyperactivity [7, 31]. Tar- administration induced neurotoxicity; deficits in learning and memory also caused brain tissue damage in animals [51]. Antioxidant system is involved in the defense system against free radical mediated tissue or cellular damage [15]. In the present study, oral administration of Tar (1%, 3%) increase nitric oxide (NO) and malondialdhyde (MDA) levels as a product of lipid peroxidation occurs by the ROS action on lipids of cellular membrane, the role of free radicals and nitric oxide (NO) in the neurotoxicity of environmental chemicals and in the pathogenesis of neurodegenerative

diseases is well accepted [11, 48, 45, 16]. Nitric oxide and related reactive nitrogen oxide species (RNOS) mediate intricate physiological and pathophysiological effects in the central nervous system. Depending on environmental conditions, NO and RNOS can initiate and mediate neuroprotection or neurotoxicity either exclusively or synergistically with other effectors [25]. On the other hand, the Tar treatment caused suppressed the activity of antioxidant enzymes (GSH) in serum and brain tissues especially at higher dose when the need of them was increased while the level of GSSG increased as a result of oxidation of reduced glutathione to protect the cells from damage by the toxic materials and free radicals, so GSH depleted as a result of dye toxicity. Because of food dye Tar is from the group of azo dye food colorants, they are metabolized into aromatic amine by intestinal flora and the formed aromatic amines can generate reactive oxygen species as part of their metabolism, by interaction of these amino groups with nitrate or nitrate containing foods [14].

Table (6): Effect of Tar (1%, 3%of diet), Cur (200mg/kg/B.w.) individually and in combination on the levels of norepinephrine, dopamine and serotonin in different brain areas (striatum, cortex and hippocampus) of weanling rats after 8 weeks of treatment

parameters	***Norepinephrine(µg/g.tissue)***			***Dopamine (µg/g.tissue)***			***Serotonin(µg/g.tissue)***		
Brain area	**COX**	**H.P.**	**STR**	**COX**	**H.P.**	**STR**	**COX**	**H.P.**	**STR**
Control	0.22 ±0.006	1.51 ±0.15	0.28 ±0.013	4.59 ±0.09	2.84 ±0.10	3.56 ±0.10	2.85 ±0.08	3.86 ±0.15	3.85 ±0.09
Cur	0.23 ±0.007	1.48 ±0.09	0.30 ±0.012	4.39 ±0.11	2.86 ±0.11	3.69 ±0.08	2.86 ±0.09	3.87 ±0.09	3.92 ±0.15
Tar1%	0.17 ±0.003*	1.26 ±0.03*	0.20 ±0.007	4.28 ±0.15	2.38 ±0.07*	3.26 ±0.08*	2.00 ±0.07*	3.37 ±0.18*	3.05 ±0.15*
Cur+Tar1%	0.20 ±0.003^{c}	1.50 ±0.13^{c}	0.27 ±0.01^{c}	4.44 ±0.17	2.73 ±0.08^{c}	3.39 ± 0.14	2.96 ±0.09^{c}	3.45 ±0.15*	3.85 ±0.17^{c}
Tar 3%	0.13 ±0.007*^{a}	1.15 ±0.06*	0.17 ±0.011*	3.83 ±0.24*^{a}	2.00 ±0.08*^{a}	3.0 ±0.10*	1.94 ±0.05*	3.52 ±0.17*	2.97 ±0.16*
Cur+Tar3%	0.19 ±0.003^{b}	1.42 ±0.06^{b}	0.26 ±0.007^{b}	4.41 ± 0.20^{b}	2.64 ±0.08^{b}	3.33 ±0.12^{b}	2.64 ±0.08^{b}	3.73 ±0.11	3.52 ±0.11^{b}

Significant difference from control group *p<0.05, Significant difference between Tar3% and Tar1% ^{a} p<0.05, Significant difference between Tar3% and Tar3%+cur ^{b} p<0.05.

As a result of the ROS formation the antioxidant defense mechanism of the cells began to be consumed to

prevent the cell death by these toxic radicals [23].Also, the result ofpresent study recorded that monoamines norepinephrine (NE), dopamine (DA) and serotonin (5-HT) were significantly decreased with the administration of Tar in brain frontal cortex, hippocampus and striatum especially high dose. 5-hydroxytryptophan (5-HTP), a substance that is created naturally in the body from the amino acid tryptophan, is involved in elevating the neurotransmitter serotonin naturally in the brain [1, 19] the dysfunctional serotonin system might involve other neurotransmitter systems including the epinephrine system [27]. Also, the food colors induced lowering of monoamine content in the brain of rats may be further explained by that reported by author in [21] who mentioned that the adverse effect of food additives on hyperactivity symptoms was moderated by histamine degradation gene polymorphisms HNMT (histamine N- methyletransferase). Since HNMT polymorphisms impair histamine clearance [4] and there is evidence that food additive challenge triggers histamine release [2]. The activity of central histamine H2 receptores have been shown to affect inhibition learning, to increase hyperactivity levels in mouse models, and to promote dopamine release in the frontal cortex [13]. Tart-treated animals significantly displayed higher levels of ambulation, a measure of hyperactivity, as indicated by increased numbers of crossing squares in open field [31]. These findings are in accordance with previous scientific research reporting an association between behavioral deficits in young children in form of overactive, impulsive and inattentive behavior and synthetic food colors [6, 8, 43]. In addition, the formerly reported implication of azo dyes in motor system affection in mammals through dopamine pathways might further clarify the current noticeable hyperactivity in rats [31]. Other neurobehavioral and neurochemical studies had also suggested that brain serotonin modulates the activity of neural circuits and thereby plays a central role in several behavioral/motor activities [20, 24]. Serotonin system also is important in the pathophysiology of psychiatric disorders including mood and anxiety, therefore healthy levels of serotonin is essential to promote balanced mood [35]. Tar has been found to diminish the ability of vitamin B6 to function in critical biochemical pathways such as tryptophan/serotonin metabolism [5, 44]. Serotonin can modulate the dopaminergic and norepinephrine systems, and *vice versa* [9, 27]. On the other hand, The Morris water maze test allows the parallel measurement of responses related to both conditioned and unconditioned fear in the same subject. It also permits simultaneous assessment of memory and learning of these behaviors [17, 38].

In the present study, the effects of Tar on learning and memory were examined in weanling rats by the Morris water maze test. The results indicated that, However, chronic oral administration of Tar significantly increased the escape latency as compared with the control groups the dose levels of Tar in the present study produced adverse effects in learning and memory functions in animals. The mechanisms might be attributed to promoting lipid peroxidation products and reactive oxygen species, inhibiting endogenous antioxidant defense enzymes and the brain tissue damage [51]. The dose levels of Tar in study produced a few adverse effects in learning and memory functions in animals [51]. On the other hand, the present study, focused attention on three brain regions the frontal cortex, the hippocampus and the striatum. These brain regions are important because they are involved in important behavioral functions, such as emotion, motivation, learning and memory, all of which may be related to the expression of depression [49].

On the other hand, Cur acts as a free radical scavenger and antioxidant, inhibiting lipid peroxidation [10] oxidative DNA damage and anti-inflammatory properties [34, 3]. In the present study, inhibition in the level of NO in the serum of rat simultaneously treated with Tar and Cur could be associated with scavenging activity of

Cur. On the other hand, elevation of MDA, GSSG levels and the depletion in GSH level in serum and brain tissues that observed in Tar treated groups in recent study is attenuated in combined treatment of Cur and Tar groups. These effects may reflect the ability of Cur to enhance the scavenging and inactivation of H_2O_2 and hydroxyl radicals. In addition, Cur may serve as a chelator and directly bind to Fe^{2+}, which catalyzes formation of free radicals via the Fenton reactions [52, 53]. Results of the present study also indicated that Cur treatment restored monoamines. Administration of Cur (200 mg/kg/ B.W.) showed attenuation against the toxic effect of Tar 1%, 3% in (striatum, cortex & hippocampus) especially in high dose. In agreement with the present study, Cur increased levels of catecholamine and their metabolites and 5-HT and decreased levels of NO in corpus striatum, frontal cortex and hippocampus as compared to rats treated. Although the mechanism for such changes leading to neuroprotection is difficult to explain at present, it could be due to the antioxidant, and anti-inflammatory effect of Cur [3, 40]. These findings are interesting and suggest that Tar-induced changes in brain biogenic amines and NO could be protected by Cur. Coadministration of Cur (200 mg/kg/ B.W.) also caused marked attenuation effect against behavioral abnormalities such as hyperactivity in the open field and deficient in memory and learning in Morris water maze that caused by both doses of Tar.

Although the mechanism of the mood modulating effects of Cur is not fully understood, it is hypothesized to act through inhibiting the monoamine oxidase enzyme and modulating the release of serotonin and dopamine. Moreover, evidences have shown that Cur enhances neurogenesis, notably in the frontal cortex and hippocampal regions of the brain [42]. This result is in agreement with author in [39] who revealed that treatment of Cur (200 mg/kg/ B.W.) significantly inhibited the hyperactivity observed in the open field test parameters like high ambulation and Cur was effective in preventing memory decline observed in Morris water maze tasks index in Aluminum-intoxicated rats. Cur ability to modulate oxidative stress could be responsible for inhibiting the memory decline observed in Morris water maze tasks.

5. Conclusion

This study provides sufficient scientific probability that a causal link truly exists between Tar and inflection of hyperactivity and depletion in learning and memory in weanling rats and points to the hazardous impact of Tar on public health, Tar treatment also inducing oxidative stress, inhibiting endogenous antioxidant defense enzymes and reduction of the monoamines formation that's likely to induce alterations in neurobiological substrates and brain tissue damage. While, combined treatment of Cur (200 mg/kg/ B.W.) ameliorated all the behavioral and biochemical alterations in serum and different brain regions of Tar-treated weanling rats this could be due to anti-oxidant, anti- inflammatory effects of Cur.

So its recommended that it is necessary to restrict the use of artificial dyes and to use a diet free of artificial food coloring especially for children or try to using Cur (natural food coloring) instead of Tar (artificial food coloring) or at least in combined with Tar to protect against Tar neurobehavioral toxicity especially in children.

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References

[1] **B.B. Aggarwal, and B. Sung,** "Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets". *Trends in Pharmacological Sciences*, vol 30(2) p.p. 85–94, 2009.

[2] **B.J. Kaplan,** ''Food additives and behavior: first genetic insights". *Am J Psychiatry*, vol. 167(9), p.p.1023-5, 2010.

[3] **C. Buhrmann, A. Mobasheri, F. Busch, C. Aldinger, R. Stahlmann, A. Montaseri and Shakibaei, M.** "Cur modulates nuclear factor kappaB (NF-kappaB)-mediated inflammation in human tenocytes in vitro: role of the phosphatidylinositol 3-kinase/Akt pathway. *J BiolChem*, vol. 12; 286(32), p.p. 28556-66, 2011.

[4] **C.V. Preuss, T.C. Wood, C.L. Szumlanski, R.B. Raftogianis, D.M. Otterness, B. Girard and et al** "Human histamine N methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity". *MolPharmacol*, vol. 53, p.p. 708-717,1998.

[5] **D. Bender** ''Non-nutritional uses of vitamin B6". *Br J Nutr*, vol. 81, p.p.7-20, 1999.

[6] **D. McCann, A. Barrett, A. Cooper, D. Crumpler, L. Dalen, K. Grimshaw and et al** ''Food additives and hyperactive behavior in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial". *Lancet*, vol. 370(9598), p.p. 1560-7**,** 2007.

[7] **D.J. Stein, J. Fan, J. Fossella, and V.A. Russell** "Inattention and hyperactivity-impulsivity: psychobiological and evolutionary underpinnings of ADHD". *CNS Spectr*, vol. 12(3), p.p. 193-6, 2007.

[8] **D. Schab, and N. Trinh** ''Do artificial food colours promote hyperactivity in children with hyperactive syndromes? A meta analysis of double-blind placebo-controlled trials". *J Dev BehavPediatr*, vol. 25, p.p. 423-434, 2004.

[9] **E. Esposito** ''Serotonin-dopamine interaction as a focus of novel antidepressant drugs". *Curr DrugTargets*, vol 7(2), p.p. 177-85,2006.

[10] **E. Molina-Jijón, E. Tapia, C. Zazueta, M.d. El Hafidi, Z. L Zatarain-Barrón, R. Hernández-Pando and et al** '' Cur prevents Cr (VI)-induced renal oxidant damage by a mitochondrial pathway".*Free Radical Biology & Medicine*, vol. 51, p.p. 1543–1557, 2011.

[11] **E.O. Koylu, M. Kanit, D. Taskiran, T. Dagci, B. Balkan, and S. Pogun** ''Effects of nitric oxide synthase inhibition on spatial discrimination learning and central DA2 and mACh receptors. *PharmacolBiochemBehav*, vol. 81(1), p.p. 32-40, 2005.

[12] **F. Guangqin, F. Chang, L. Yu, W. Chunhong, Y. Ji, L. Wei, F. Jiangao, S. Xianglin and B. Yongyi**" Selection of Nutrients for Prevention or Amelioration of Lead-Induced Learning and Memory Impairment in Rats". *Ann. Hyg*, vol. 53, p.p. 341-351, 2009.

[13] **G.B. Fox,; T.A. Esbenshade, J.B. Pan, R.J. Radek, K.M. Krueger, B.B. Yao and et al** ''Pharmacological properties of ABT-239 [4-(2-{2-[(2R)-2-Methyl-pyrrolidinyl]ethyl}-benzofuran-5-yl) benzonitrile]: II. Neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H3 receptor antagonist". *J PharmacolExpTher*, vol.313 (1), p.p.176-90, 2005.

[14] **I.L. Moutinho, L.C. Bertges, and R.V. Assis,** ''Prolonged use of the food dye Tar (FD&C yellow

no.5) and its effects on the gastric mucosa of Wistar rats". *Braz J Biol*, vol. 67(1), p.p.141-5, 2007.

[15] **I.M. Mourad, and N.A. Noor** ''Aspartame (a widely used artificial sweetener) and oxidative stress in the rat cerebral cortex". *Int. J. Pharm. Biomed. Sci*, vol.2, p.p. 4–10, 2011.

[16] **I. Stevanovic, A.Jelenković, I.Stevanović, D.Pavlović, G. Bjelaković, and T. Jevtović-Stoimenov** ''Spermidine influence on the nitric oxide synthase and arginase activity relationship during experimentally induced seizures". *J Basic ClinPhysiolPharmacol*, vol. 21(2), p.p.169-85, 2010.

[17] **J. Harro, R. Häidkind, M. Harro, A.R. Modiri, P.G. Gillberg, R. Pähkla and et al ():** Chronic mild unpredictable stress after noradrenergic denervation: attenuation of behavioral and biochemical effects of DSP-4 treatment. *EurNeuropsychopharmacol*, vol.10 (1), p.p. 5-16, **1999**.

[18] **J. Mehla, K. H. Reeta, P. Gupta, and Y. K. Gupta** '' Protective effect of Cur against seizures and cognitive impairment in a pentylenetetrazole-kindled epileptic rat model". *Life Sci*, vol. 87(19-22), p.p. 596-603, 2010.

[19] **J. Moreno, M. Campos, C. Lara, G. Lopez, L. Pavon, M. Hernadez, and et al** '' Tryptophan and serotonin in blood and platelets of depressed patients: effect of an antidepressant treatment". *Salud Mental*, vol. 29(4), p.p. 1-8, 2007.

[20] **J.F. Pflieger, F. Clarac, and L. Vinay** ''Postural modifications and neuronal excitability changes induced by short-term serotonin depletion during neonatal development in the rat". *J. Neurosci.*, vol. 22, p.p. 5108–5117, 2002.

[21] **J. Stevenson, E. Sonuga-Barke, D. McCann, K. Grimshaw, K.M. Parker, M.J. Rose-Zerilli, J.W. Holloway and J. O. Warner** "The role of histamine degradation gene polymorphisms in moderating the effects of food additives on children's ADHD symptoms". *Am J Psychiatry*, vol. 167(9), p.p.1108-15, 2010.

[22] **J. Jayatilleke, and S. Shaw** ''A high performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples". *Anal. Biochem*, vol. 214(2), p.p. 452-457,1993.

[23] **K.A. Amin, H. Abdel Hameid, and A.H. AbdElsttar** ''Effect of food azo dyes Tar and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats" *Food and Chemical Toxicology*, vol. 48, p.p. 2994-2999, 2010.

[24] **K.D. Alex, and E.A. Pehek,** "Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission" *Pharmacology & Therapeutics*, vol.113 (2), p.p. 296–320,2007.

[25] **K.M. Boje** ''Nitric oxide neurotoxicity in neuron-degenerative diseases". *Front Bio sci, vol.* 1; 9, p.p. 763-76, 2004.

[26] **L. N. Papadoyannis, V. F. Samanidou, and Ch. C. Nitsos** '' Simultaneous determination of nitrite and nitrate in drinking water and human serum by high performance anion-exchange chromatography and UV detection". *J. Liq. Chrom. Rel. Technol*, vol. 22(13), p.p. 2023 – 2041, 1999.

[27] **L. Salomon, C. Lanteri, J. Glowinski, and J. Tassin** ''Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons". *Proc Natl AcadSci.USA*, vol. 103, p.p. 7476-7481, 2006.

[28] **M. Bishnoi, K. Chopra, and S.K. Kulkarni** ''Protective effect of Cur, the active principle of turmeric (Curcuma longa) in haloperidol-induced orofacial dyskinesia and associated behavioral, biochemical and neurochemical changes in rat brain". *PharmacolBiochemBehav*, vol. 88(4), p.p.511-22,2008

[29] **M. Karatep**: Simulatenous determination of ascorbic acid and free malondialdehyde in human serum by HPLC-UV. *Chromatographic Line*, vol. 12, p.p.362-365**,** 2004.

[30] **M. Millan ''** The neurobiology and control of anxious states". *ProgNeurobiol*, vol. 70, p.p. 83-244,2003**.**

[31] **M.M. Kamel and M.H. El-lethey ''** The Potential Health Hazard of Tar and Levels of Hyperactivity, Anxiety-Like Symptoms, Depression and Anti-social behavior in Rats". *Journal of American Science*, vol.7 (6), p.p. 1211-1218**,** 2011.

[32] **M.O Elhkim, F. Héraud, N.Bemrah, F. Gauchard, T. Lorino, C. Lambré, et al** "New considerations regarding the risk assessment on Tar An update toxicological assessment, intolerance reactions and maximum theoretical daily intake in France". *RegulToxicolPharmacol*, vol 47(3), p.p.308-16, 2007.

[33] **N. Mehedi, S. Ainad-Tabet, N. Mokrane, S. Addou, C. Zaoui, O. Kheroua, and I. Saidi** "Reproductive toxicology of Tar (FD and C Yellow No. 5) in Swiss albino mice". *Am J PharmacolToxicol*, vol. 4(4), p.p.130-135, 2009.

[34] **P. Basnet, and N. Skalko-Basnet ''** Cur: an anti-inflammatory molecule from a curry spice on the path to cancer treatment" *Molecules*, vol. 16(6), p.p. 4567-98, 2011.

[35] **P. Dayan, and Q. J. Huys, ''** Serotonin, inhibition, and negative mood". *PLoSComputBiol*, vol.4(2):e4, 2008

[36] **P. K. Shukla, V. K. Khanna, M.Y. Khan, and R.C. Srimal ''**Protective effect of Curcumin against lead neurotoxicity in rat". *Hum ExpToxicol*, vol. 22, p.p. 653-658,2003.

[37] **P. Pagel, J. Blome, and H. U. Wolf ''**High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease". *Journal of Chromatography*, xztrdfsc 746: 297-304, **2000**.

[38] **P.A. Gargiulo, M.B. Viana, F.G. Graeff, M.A. Silva, and C. Tomaz ''**Effects of anxiety and memory of systemic and intra-amygdala injection of 5-HT3 receptor antagonist BRL 46470A". *Neuropsychobiology*,vol.33(4), p.p. 189-95, 1996.

[39] **P. Sethi, A.Jyoti, E.Hussain and D.Sharma ''**Curcumin attenuates aluminium-induced functional neurotoxicity in rats. *Pharmacology, Biochemistry and Behavior*, vol. 93, p.p. 31–39, 2009.

[40] **R.S. Yadav, M.L. Sankhwar, R.K. Shukla, R. Chandra, A.B. Pant, F. Islam, and V.K. Khanna ''**Attenuation of arsenic neurotoxicity by Cur in rats". *ToxicolApplPharmacol*, Vol. 240(3), p.p. 367-76, 2009.

[41] **S. Daniel, J.L. Limson, A. Dairam, G.M. Watkins, and S. Daya. ''**Through metal binding, Cur protects against lead- and cadmium-induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain". *J InorgBiochem*, vol. 98(2), p.p. 266-75, 2004.

[42] **S. Kulkarni, A. Dhir, and K.K. Akula ''**Potentials of Cur as an antidepressant". *Scientific World Journal*, vol. 9, p.p. 1233-41, 2009.

[43] **S. Overmeyer, and E. Taylor ''** Annotation: principles of treatment for hyperkinetic disorders: practice approaches for the UK". *J Child Psychol Psychiatry*, vol. 40, p.p. 1147-1157, 1999.

[44] **S. Russo, I. Kema, M. Fokkema, and J. Boon ''** Tryptophan as a link between psychopathology and somatic states". *Psychosom Med*, vol. 65, p.p. 665-671, 2003.

[45] **S.J. Flora, K. Bhatt, and A. Mehta ''** Arsenic moiety in gallium arsenide is responsible for neuronal apoptosis and behavioral alterations in rats". *ToxicolApplPharmacol*, vol. 240(2), p.p.236-44,2009.

[46] **T. Tanaka, O. Takahashi, S. Oishi, and A. Ogata ''**Effects of Tar on exploratory behavior in a three-gneration toxicity study in mice". *Reproductive Toxicology*, vol. 26(2), p.p.156–163,2008.

[47] **T. Tanaka ''**Reproductive and neurobehavioral toxicity study of Tar administered to mice in the diet". *Food ChemToxicol*, vol. 44(2), p.p. 179-187,2006.

[48] **V. A. Gomes, A. Casella-Filho, A.C. Chagas, and J.E. Tanus-Santos ''** Enhanced concentrations of relevant markers of nitric oxide formation after exercise training in patients with metabolic syndrome". *Nitric Oxide.* Vol. 19(4), p.p. 345-50,2008.

[49] **V. Butterweck, T. Böckers, B. Korte, W. Wittkowski, and H. Winterhoff,** "Long-term effects of St. John's wort and hypericin on monoamine levels in rat hypothalamus and hippocampus". *Brain Res*, vol. 930(1-2), p.p. 21-9, 2002.

[50] **V. P. Menon and A. R. Sudheer ''**antioxidant and anti-inflammatory properties of Cur. The molecular targets and therapeutic uses of Cur in health and disease". *Advances in experimental medicine and biology.* Vol. 595, p.p. 105-125, 2007.

[51] **Y. Gao, C. Li, J. Shen, H. Yin, X. An, and H. Jin''** Effect of Food Azo Dye Tar on Learning and Memory Functions in Mice and Rats, and the Possible Mechanisms Involved". *Journal of Food Science*, vol. 76, p.p. T125–T129, 2011.

[52] **Y. Jiao, J. Wilkinson, P. E. Christine, J. L. Buss, W. Wang, R. Planalp, and et al** "Iron chelation in the biological activity of Cur". *Free RadicBiol Med*, vol.40(7), p.p.1152-60, 2006.

[53] **Y. Jiao, J. Wilkinson, X. Di, W. Wang, H. Hatcher, N.D. Kock, and et al ''**Cur, a cancer chemopreventive and chemotherapeutic agent, is a biologically active iron chelator". *Blood.* 113(2):462-9,2009