The Potential Health Hazard of Tartrazine and Levels of Hyperactivity, Anxiety-Like Symptoms, Depression and Anti-social behaviour in Rats

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Abstract: The current research aimed to determine the influence of different doses of exposure to tartrazine on levels of hyperactivity, anxiety, depression and anti-social behaviours in rats. Forty five weanling male Wistar rats were randomly assigned into 3 groups of 15, divided on 2 replicates and administered our treatment daily in drinking water at different concentrations; 0, 1% and 2.5% for a 16 weeks period. Different animal models of anxiety; open field, elevated plus maze and dark-light transition tests were employed in our study. Tests for depression as well as social interaction were also used. Tartrazine-treated rats showed hyperactivity in open field test presented by increased horizontal locomotion. Anxiogenic effect of tartrazine was evidently observed during open field, elevated plus-maze and dark-light transition tests. Furthermore, tartrazine intake significantly promoted depression as expressed by prolonged immobilization during forced swim test. Impairment in social interaction test was also detected signifying the relevance of administered dose especially on numbers of bouts of social contacts. This study provides sufficient scientific evidence that a causal link truly exists between tartrazine and inflection of hyperactivity, anxiety and depression-like behaviours in rats and points to the hazardous impact of tartrazine on public health.

[Mervat M. Kamel, Heba S. El-lethey. The Potential Health Hazard of Tartrazine and Levels of Hyperactivity, Anxiety-Like Symptoms, Depression and Anti-social behaviour in Rats. Journal of American Science 2011;7(6):1211-1218].(ISSN: 1545-1003). http://www.americanscience.org.

Key words: Food azo-colours tartrazine, hyperactivity, anxiety, depression, social interaction, Wistar rats.

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. Food azo-colours tartrazine is one of the most widely used artificial foods, drugs and cosmetic dyes.

Several human studies have related artificial food colorants such as tartrazine, sunset yellow, carmoisine and ponceau with conduct disorders (Weiss et al., 1980; Schab and Trinh, 2004). Moreover, increasing evidence suggests the potential toxicological risk of tartrazine (Mehedi et al., 2009). In addition, Schauss (1984) and Bateman et al. (2004) concluded general adverse behavioural and psychological effects with artificial food colorants in children.

A number of data has described tartrazine-related hyperactivity in children (Schab and Trinh, 2004; McCann et al., 2007). Moreover, noticeable effect of tartrazine on the behavior of young mice has been reported (Tanaka, 2006; Tanaka et al; 2008). Although several researches have linked tartrazine ingestion to a variety of immunologic responses including anxiety and clinical depression (Rowe and Rowe, 1994), little rigorous research in the field of toxological effects of tartrazine on behaviours relevant for models of CNS disorders, such as anxiety, depression and social behaviour were verified until recently.

Therefore, the goal of this study was to explore the adverse effect of long term exposure to food colorant tartrazine on hyperactivity as well as mood-like behaviours in the form of anxiety, depression and social interaction in rats.

2. Materials and Methods:

2.1. Animals and housing:

Forty five weanling male Wistar rats, weighing approximately 40-50 g were used in this experiment. Animals were obtained from the Unit for Laboratory Animals at Faculty of Veterinary Medicine, Cairo University. They were maintained in plastic cages, with stainless steel wire lids (bedded with wood shavings), on a standard laboratory feed diet. Feed and water were offered ad libitum. Rats were housed at a controlled temperature of 21 ± 1ºC, 60 % humidity and under a 12-h-light:12-h-dark schedule. All efforts were made to minimize the numbers of animals and their suffering in this study through following the guidelines released by Cairo University Policy on Animal Care and Use.

2.2. Administration of tartrazine:

Tartrazine (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 0%, 1% (low dose) and 2.5 % (high dose) (Mehedi et al., 2009). Rats were randomly divided into three groups of 15 animals, each and provided ad libitum access to drinking water containing tartrazine for 16 weeks. The control group received tap water only.

2.3. Behavioural measurements:

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

2.3.1. Open field behaviour test:

The open field test provides simultaneous measures of locomotion, and anxiety (Kelly, 1993; Millan, 2003). 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 centre. The behavioural scores measured in this experiment included total numbers of line crossings, rearing against the wall, grooming, stretch attending posture and fecal boli.

2.3.2. Elevated plus maze test:

The elevated plus-maze was used for testing of anxiety and emotionality. The degree of avoidance of the open arms of the maze has been considered as a measure of strength of fear drive (Trullas and Skolnick, 1993). The apparatus consists of 4 crossed arms, two open arms (50 x 10 x 30 cm) and two closed arms (50 x 10 x 30 cm). The maze was elevated 65 cm above the floor. The rat was placed in the centre of the maze and the number of entries in open and closed arms, respectively, as well as the time the animal spent in the open and enclosed arms during a period of 5 min test session was recorded (Kierstin, 2003; Walf and Frye, 2007). After each trial the maze was wiped out with a cloth dipped in 70% ethyl alcohol and allowed to dry.

2.3.3. Light-Dark transition task:

The light-dark box apparatus consisted of a light, open topped, opaque, plexiglas box connected to a dark, closed topped, plexiglas box, each compartment measuring (30 x 40 x 40 cm) . The boxes were connected by a small opening that allows the rat to cross between chambers. The rat was placed in the light box, allowed to move freely between the chambers, and its location was recorded for 5 min. The time spent on the light side of this apparatus during the 5 min test session compared to the dark side was recorded and used as an index for anti-anxiety behavior (Hascoet et al., 2001; Walf and Frye, 2005). Light box entry was defined as the rat having all four paws into the light box.

2.3.4. Forced swim test:

Rats were tested in the forced swim test as previously described by Frye and Walf (2002). Rats were placed in cylindrical container (50 x 20 cm) filled with 30 cm of 22^{o}C water. The water level does not allow the rat to rest on its tail, or escape the cylinder by climbing out. The rat was placed in the water for 6 min. The time spent floating (represented immobility) was scored during the last 3 min. The time spent immobile is considered as an index of depression-like behavior in rodents (Sanchez and Meier, 1997).

2.3.5. Social interaction test:

On the day of the experiment, animals were socially isolated in plastic cages measuring (43 x 28 x 15 cm) for 3.5 h prior to the experiment. The task was conducted by placing two animals belonging to the same experimental group, but from different cages, into the test cage for a 15-min period. Tested pairs did not differ in body weight by more than 15 g. The social behaviour was assessed for a pair of animals (Schneider and Przewlocki, 2005). The total time spent in social behaviour and the numbers of social contacts were measured (Niesink and Van Ree, 1989).

2.4. Statistical analysis:

Data for open field, elevated plus-maze, dark-light transition, depression as well as social interaction tests were analyzed by analyses of variance (AVOVA), using the general linear models procedure in SPSS® statistical software (SPSS, 2006). Statistical significance of difference between control and treated groups was determined by post hoc Tukey HSD test. A value of ($P<0.05$) was considered

statistically significant. All data are expressed as mean ± SEM.

3. Results:

3.1. Open field test:

The effect of tartrazine treatment on parameters of open field test was illustrated in Table 1. Rats under tartrazine effect increased significantly ($p < 0.001$) the mean covered distance in the open field test when compared with the control group. An anxiogenic like effect was obtained in tartrazine-ingested rats when compared to their counterparts in controls. Tartrazine-treated individuals presented a significant ($p < 0.05$) increase of rearing in peripheral area of the test. Treatment also significantly ($p < 0.05$) reduced numbers of central squares entered. A significant dose-dependent response was noted for numbers of grooming as well as stretch attending postures in tartrazine-exposed rats in comparison to their control ($p < 0.001$). The highest levels of these behaviours were recorded with high tartrazine dose. Moreover, a marked significant ($p < 0.01$) increase in fecal boli was also observed in rats following tartrazine treatment when compared to animals belonging to control group, regardless of incorporated dose.

3.2. Elevated plus maze:

The effect of tartrazine on measurement of elevated plus maze was demonstrated in Table 2. Regardless of the ingested dose, animals under tartrazine effects significantly ($p<0.05$) diminished the numbers of entries in the open arms of the maze, accompanied with significant increase of this measure in the closed arms. Regarding time spent in the open arms, tartrazine was significantly successful in endorsing an aversive dose-related effect since the shortest time spent in open arm was recorded with high tartrazine group.

3.3. Dark-light transition test:

A similar pattern of effects as those displayed in the elevated plus-maze was obtained in rats submitted to the dark-light transition test (Table 3). Statistical analysis showed that time spent by tartrazine-treated rats in the light compartment was significantly ($p < 0.001$) diminished, while increased in the dark compartment compared to control counterparts.

3.4. Forced swim test:

Immobility time during forced swim test was shown in Table 4. This measure was increased significantly ($p < 0.001$) in rats challenged with high dose of tartrazine compared to control rats.

3.5. Social interaction test:

Measurements of social interaction test in rats were presented in Table 5. Exposure to tartrazine significantly ($p < 0.001$) affected social interaction parameters as indicated by reduced time engaged in social behaviour with decreased numbers of social contacts as well. Although administered dose of tartrazine showed no significant change in time spent in social interaction, a marked significant dose response was noted for social contacts numbers ($p < 0.01$) showing less contacts with higher dose.

Table 1. Effect of tartrazine on the behaviour of rats in the open field test.

	Experimental Groups		
	(C) Group	(Low T) Group	(High T) Group
Total no. of squares crossed	**50.20±2.95^{a}**	**68.87±5.22^{b}**	**79.87±4.33^{b}**
No. of rears in the periphery	**6.40±0.94^{a}**	**9.87±0.97^{ab}**	**10.80±1.27^{b}**
No. of center squares entries	**1.20±0.30^{a}**	**0.67±0.19^{ab}**	**0.27±0.15^{b}**
No. of grooming	**3.13±0.34^{a}**	**7.67±0.40^{b}**	**9.40±0.65^{c}**
No. of stretch attending posture	**1.53±0.34^{a}**	**2.53±0.26^{a}**	**5.93±0.59^{b}**
No. of fecal boli	**2.07±0.42^{a}**	**3.27±0.61^{ab}**	**4.87±0.52^{b}**

(C) Group: Animals received plain water without any treatment and served as a control.
(Low T) Group: Animals received 1% tartrazine.
(High T) Group: Animals received 2.5% tartrazine.
$^{a-c}$Values within row with unlike superscripts differ significantly **($p<0.05$)**, according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

Table 2. Effect of tartrazine on the behaviour of rats during the elevated plus maze test.

	Experimental Groups		
	(C) Group	(Low T) Group	(High T) Group
No. of entries (open arm)	**4.20±0.44[a]**	**1.87±0.19[b]**	**1.47±0.22[b]**
No. of entries (closed arm)	**3.87±0.32[a]**	**5.33±0.44[b]**	**6.13±0.36[b]**
Time spent (open arm) (s)	**71.33±6.23[a]**	**54.27±4.06[b]**	**27.40±3.97[c]**
Time spent (closed arm) (s)	**180.27±12.32[a]**	**196.73±6.91[a]**	**205.80±7.59[a]**

(C) Group: Animals received plain water without any treatment and served as a control.
(Low T) Group: Animals received 1% tartrazine.
(High T) Group: Animals received 2.5% tartrazine.
[a-c]Values within row with unlike superscripts differ significantly **($p<0.05$)**, according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

Table 3. Effect of tartrazine on the behaviour of rats during the Dark-light transition test

	Experimental Groups		
	(C) Group	(Low T) Group	(High T) Group
Time spent (light compartment) (s)	**227.33±9.49[a]**	**109.00±10.21[b]**	**107.60±9.58 [b]**
Time spent (dark compartment) (s)	**72.67±9.49[a]**	**191.00±10.21[b]**	**192.40±9.58[b]**

(C) Group: Animals received plain water without any treatment and served as a control.
(Low T) Group: Animals received 1% tartrazine.
(High T) Group: Animals received 2.5% tartrazine.
[a-c]Values within row with unlike superscripts differ significantly **($p<0.05$)**, according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

Table 4. Effect of tartrazine on the behaviour of rats during the forced swim test.

	Experimental Groups		
	(C) Group	(Low T) Group	(High T) Group
Immobility time (s)	**39.60±3.02[a]**	**48.40±3.42[a]**	**83.47±5.48[b]**

(C) Group: Animals received plain water without any treatment and served as a control.
(Low T) Group: Animals received 1% tartrazine.
(High T) Group: Animals received 2.5% tartrazine.
[a-c]Values within row with unlike superscripts differ significantly **($p<0.05$)**, according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

Table 5. Effect of tartrazine on the behaviour of rats in the social interaction test.

	Experimental Groups		
	(C) Group	(Low T) Group	(High T) Group
Time spent in social interaction (s)	**370.67±11.78[a]**	**216.20±7.66[b]**	**224.80±9.00[b]**
No. of social contacts	**81.47±2.12[a]**	**53.33±4.72[b]**	**36.80±2.19[c]**

(C) Group: Animals received plain water without any treatment and served as a control.
(Low T) Group: Animals received 1% tartrazine.
(High T) Group: Animals received 2.5% tartrazine.
[a-c]Values within row with unlike superscripts differ significantly **($p<0.05$)**, according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

3. Discussion:

The present study elucidated the effect of tartrazine on open field locomotor activity in adult male Wistar rats. Tartrazine-treated animals significantly displayed higher levels of ambulation, a measure of hyperactivity, as indicated by increased numbers of crossing squares. Our findings are in accordance with previous scientific research reporting an association between behavioural deficits in young children in form of overactive, impulsive and inattentive behaviour and synthetic food colours (Boris and Mandel, 1994; Overmeyer and Taylor, 1999; Schab and Trinh, 2004; McCann et al., 2007). In contrast to our results, Tanaka (2006) demonstrated no link between tartrazine and hyperactivity in mice offspring. This discrepancy in results might be attributable to the different inoculated doses. Tartrazine-induced hyperactivity might be explained on the basis of zinc chelating property of tartrazine. Zinc depletion was found to be one of the potential causes of childhood hyperactivity following exposure to tartrazine (Ward et al., 1990). 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 (Silbergeld and Anderson, 1882; Mailman and Lewis, 1983).

Anxiety in rats can be measured by behavioral reactivity to non-social or social stressors (Kim et al., 2004). These behaviors were compared by performing the open-field, elevated plus maze and the light-dark transition tests (non-social) as well as social interaction test (social). With regard to the present study, it is important to note that most of the behavioural models cited above have mainly been used in the studies on the neurobiological mechanisms implicated in the production of fear and anxiety elicited in animals exposed to aversive situations (Rodgers, 1997; Rodgers and Dalvi, 1997; Menard and Treit, 1999).

In our study, tartrazine caused a significant increase in the anxiety levels of rats in all three anxiety models employed. Regarding anxiety measurement in the open field test; numbers of rearing against the wall, central squares entered, grooming, stretch attending posture (risk assessment) as well as fecal boli, all parameters were greatly influenced by ingestion of tartrazine. Here, tartrazine prominently increased rearing activity regardless of the incorporated dose in rats. Rearing response against periphery has been proved to reflect higher levels of anxiety in rats (Anderson and Hughes, 2008). Again regardless of the administered dose, tartrazine-exposed rats showed increased entries of central squares in the open field. Frequent entries of central squares have been reported to indicate curious animal with lower levels of anxiety (Frye et al., 2000). Supporting evidence for highly anxious rats in the current study derived from increased grooming activity. Moreover, expression of grooming behaviour was greatly affected by the administered dose where high doses of tartrazine were accompanied by increased grooming responses. Grooming has been validated as indicator of anxiety (Negishi et al., 2005). Risk assessment in the form of stretch attending posture was also found to increase after tartrazine exposure in rats. Higher frequency of these postures was reported to indicate anxious state of individuals (Blanchard et al., 2001). Where fecal boli were shown to be a sensitive measure for anxiety state of animals (Singer et al., 2005; Reolon et al., 2006), present administration of tartrazine was shown to enhance defecation. In light of the above mentioned observations, exposure to tartrazine was noted to induce anxiety state in rats. After ingestion of tartrazine, humans were proved to develop anxiety (Rowe and Rowe, 1994; Ansari and Mosayebzadeh, 2011).

Because of rats' innate fear of height and openness, rats tend to remain longer in the enclosed arm (Treit et al., 1993). Data derived from elevated plus maze further affirmed the previously observed effect of tartrazine on anxiety-related behaviours during open field test. Regardless of administered dose, decreased visits for open arms of elevated plus maze were recorded in our study after exposure to tartrazine. However, a dose-dependent effect of tartrazine on time spent in the open arms was evidently shown, where the less time was observed with high dose-administered rats indicating that they avoid this aversive region of the maze as reported in other studies (Bhattacharya et al., 1995; Schulteis et al., 1998). Therefore, time elapsed in the open arms might be considered as the more sensitive index for anxiety than number of visits.

The light-dark test has been used to assess the anxiogenic effects of multiple classes of drugs (Jonkman et al., 2005; Kliethermes, 2005). Our measurements of dark-ight transition test also support the former observation of tartrazine-induced anxiety-like responses, where marked diminution in time spent in light compartment was noticed with tartrazine-exposed rats.

The forced swim test measures behavioural despair in rodents, and is generally used to study depression (Raghavendra et al., 2000). Currently, data of forced swim task revealed that tartrazine-exposed animals exhibited higher immobility; an index of depression-like behaviour (Sanchez and Meier, 1997), as a response for increased levels of stress reaction. Further support derived from earlier human study for Southwick et al. (2005), where

elevated cortisol in response to chronic stress was associated with increased manifestations of depression. The role of tartrazine in modulation of depression response has been formerly outlined in children where ingestion of tartrazine showed clinical depression, migraines and sleep disturbance (Rowe and Rowe, 1994). In addition, Novembre et al. (1992) reported two cases of unusual reactions to food additives (Tartrazine and benzoates) involving mainly the central nervous system (headache, migraine, over-activity, learning difficulties and depression).

Since many social disorder models in rodents are linked to human social deficits syndrome, social interaction test has been implemented in the current study. A profound reduction in time engaged in social interaction was observed in this work accompanied with decreased frequency of social contacts following exposure to tartrazine. The most interesting finding was the dose-related reducing effect on bouts of social contacts. These antisocial-related findings confirm our formerly reported results for increased hyperactivity, anxiety and depression in tartrazine-treated rats. Holmes et al. (2001) stated that presence of hyperactivity and inattention are the most highly related predisposing factors for presentation of antisocial behavior. Aberrant social behaviours or low levels of social interaction are symptoms of several psychiatric disorders, including anxiety, depression and social phobias (Crawley, 2007). Where serotonin system is important in the pathophysiology of psychiatric disorders including mood and anxiety, healthy levels of serotonin is essential to promote balanced mood (Millan, 2003; Dayan and Huys, 2008). The hippocampal serotonergic alterations have been reported to play an important role in control of anxiety, depression and other mood disorders (File et al., 1996, 2000). 5-hydroxytryptophan (5-HTP), a substance that is created naturally in the body from the amino acid tryptophan, has been shown to elevate the neurotransmitter serotonin naturally in the brain helping with depression and other mood disorders (Feurte et al., 2001; Moreno et al., 2006). Tartrazine has been found to diminish the ability of vitamin B6 to function in critical biochemical pathways such as tryptophan/serotonin metabolism (Bender, 1999; Meletis, 1999; Russo et al., 2003). This dysfunctional serotonin system might enlighten the noticeable tartrazine-imposed modulation in anxiety and depression disorders. Moreover, the underlying causes of these disorders are complex and may also involve other neurotransmitter systems including the noradrenalin and dopamine systems (D'Aquila et al., 2000; Ressler and Nemeroff, 2000). Serotonin can modulate the dopaminergic and noradrenalin systems, and *vice versa* (Iyer and Bradberry, 1996; Esposito, 2006; Salomon et al., 2006).

In conclusion, the results reported herein potently suggest the relevance of tartrazine in inducing harmful effects especially on behaviours related to anxiety and depression. This study also gives insight into the potential hazard of long term exposure to currently food-permitted colorants with increased incidence of psychological disorders and its co-morbidity impact on human health. Interesting avenues for further research on food colorants should be hearten in order to recognize their unexpected toxic effects and urge for prohibited use of harmful colours to ensure public health.

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5/29/2011