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Comparative Study of Some Natural and Artificial Food Coloring Agents on Depression, Anxiety and Anti-Social Behavior in Weanling Rats

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Abstract:*Tartrazine (Tar) is a yellow colorant widely used in food products, drugs and cosmetics. The current study evaluates the effect of ingestion of Tar at doses of 1%, 3% of diet on depression, anxiety and antisocial behavior then comparing between its effect and the combined effect of each dose with curcumin (Cur) (natural yellow color). Forced swimming test, open field test and social interaction test were performed to assess the potential hazard of Tar and the anti-depression like effect of Cur in combined treatment. Furthermore, monoamines [dopamine (DA), norepinephrine (NE) and serotonin (5-HT)] were estimated in three different brain areas (frontal cortex, Striatum and hippocampus) of weanling albino rats. Tar intake significantly promoted depression as expressed by prolonged immobilization during forced swimming test. Anxiogenic effect of Tar was evidently observed during open field test and impairment in social interaction test. Tar alone also inhibited neurotransmitters releases while combined treatment of Cur significantly attenuated all the behavioral and biochemical alterations in serum and different brain regions of Tar-treated weanling rats. This study provides evidences that a causal link truly exists between Tar and depression, anxiety and antisocial behavior while Cur possesses potent anxiolytic and antidepressant-like activities, these activities attenuate the potential hazards of Tar.*

Keywords:*color additives, Tartrazine, curcumin, depression, antisocial behavior, anxiety, monoamines.*

I. Introduction

Depression and anxiety disorders are generally classified as separate types of syndromes. It is now clear; however, that depression and anxiety share many overlapping symptoms including fatigue, impaired concentration, irritability, sleep disturbance and somatization in addition to subjective experiences of nervousness, worry and restlessness. They may also share a common pathophysiology[29]. In fact, the key difference between depression and generalized anxiety disorder is whether the patient subjectively has a primarily depressed or anxious mood with many other symptoms being shared. There is abundant evidence for abnormalities of NE, DA and 5-HT neurotransmitter systems in depression and anxiety disorders [22].

This study 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.In addition, authors in [9]reported that the relevance of Tar in inducing harmful effects especially on behaviors related to anxiety and depression. Although several researches have linked Tar ingestion to a variety of immunologic responses including anxiety and clinical depression [25], little rigorous research in the field of toxicological effects of Tar on behaviors relevant for models of central nervous system (CNS) disorders, such as anxiety, depression and social behavior were verified until recently.

On the other hand, Cur is one such molecule that has shown promising efficacy in various animal models of major depression and anxiety. Although the mechanism of the antidepressant effect of curcumin 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 curcumin enhances neurogenesis, notably in the frontal cortex and hippocampal regions of the brain [12].

II. 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 conditions 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 vitamin.

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) [13]. Cur powder 95.02 % Curuminoids was obtained from Sigma chemical Company (Sigma, Aldrich, USA) and suspending in 0.5 % carboxy-methylcellulose just before administration [14].

Ninety six weanling albino rats will randomly assign into 6 groups 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 % Carboxy-methylcellulose.

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

G3, Tartrazine-treated group in high dose (High T): orally administrated high dose (3% of diet) of Tar.

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

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

G6, Cur + high T 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. Forced swim test:

Rats were tested in the forced swim test as previously described by Frye and Walf [7]. Rats were placed in cylindrical container (50 x 20 cm) filled with 30 cm of 22oC 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 [26].

2.3.2. 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 behavior was assessed for a pair of animals [27]. The total time spent in social behavior and the numbers of social contacts were measured [18].

2.3.3. Open field behavior test:

The open field test provides simultaneous measures of anxiety [10, 16]. 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 behavioral scores measured in this experiment rearing into periphery, center square entries and number of fecal boli.

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 and supernatant of homogenates tissues were processed for the biochemical analysis included: monoamines neurotransmitter (norepinephrine, dopamine, serotonin) all were determined by HPLC methods of author of [19].

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 $P < 0.05$.

III. Results

3.1. Forced swimming test:

Treatment of weanling rats orally with 1`%, 3% of Tar caused significant ($p<0.05$) increase in immobility time after force swimming test (fig.1) as compared to the levels of control groups. Administration of high dose of Tar 3% magnified this effect and cause higher significant ($p<0.05$) increase in immobility time as compared to the level of Tar.1% group.

While, Co administration of Cur could attenuate the toxic effect caused by both doses of Tar and the result revealed that the more immobility times which were observed in Tar (1%, 3%) groups were attenuated in both groups treated with Cur (Cur+Tar.1%, Cur+Tar.3%) showing significant ($p<0.05$) decrease as compared to the values of Tar (1%, 3%) groups, respectively.

3.2. Social interaction test:

As depicted in fig.1 the time and number of social contacts recorded in Tar 1%, 3% were strongly reduced throughout the experimental duration *vs.* the value recorded in the control group. The data exhibited dose dependent manner ($p<0.05$) in number of social contacts as compared to the value of Tar. 1% group. The statistical analysis that shown in fig .1 revealed that marked reduction in times and number of social contacts which were observed in (Tar 1%, 3%) were attenuated in all groups treated with Cur.

3.3. Open field test.

As showed in fig. 2 Tar treated individuals presented a significant ($p>0.05$) increase in the number of rearing in peripheral area of the test. Tar treatment also significantly ($p>0.05$) reduced numbers of central squares entered. A significant dose- dependent response was noted for center squares as well as number of rearing towards peripheral in Tar –administrated rats in comparison to their low dose so the highest levels of these behaviors were recorded with high Tar dose. Moreover, a marked significant ($p>0.05$) increase in fecal boli was also observed in rats following Tar treatment when compared to animals belonging to control group.

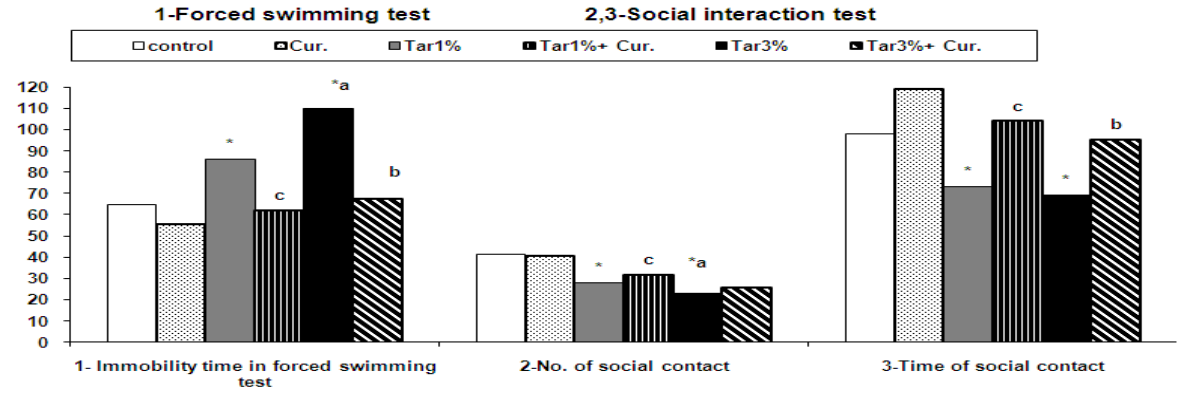
On the other hand, treatments of Cur showed pronounced improve in the number of rearing towards peripheral as compared to their control group. Cur treatment also attenuated the reduction in numbers of central square entered and the increase in fecal boli especially in low dose while in high dose the improvement is slightly observed.

3.4. Neurotransmitter parameters.

In the figs. 3, 4, 5 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%) as compared to its levels of control groups. The data also showed dose dependant 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.

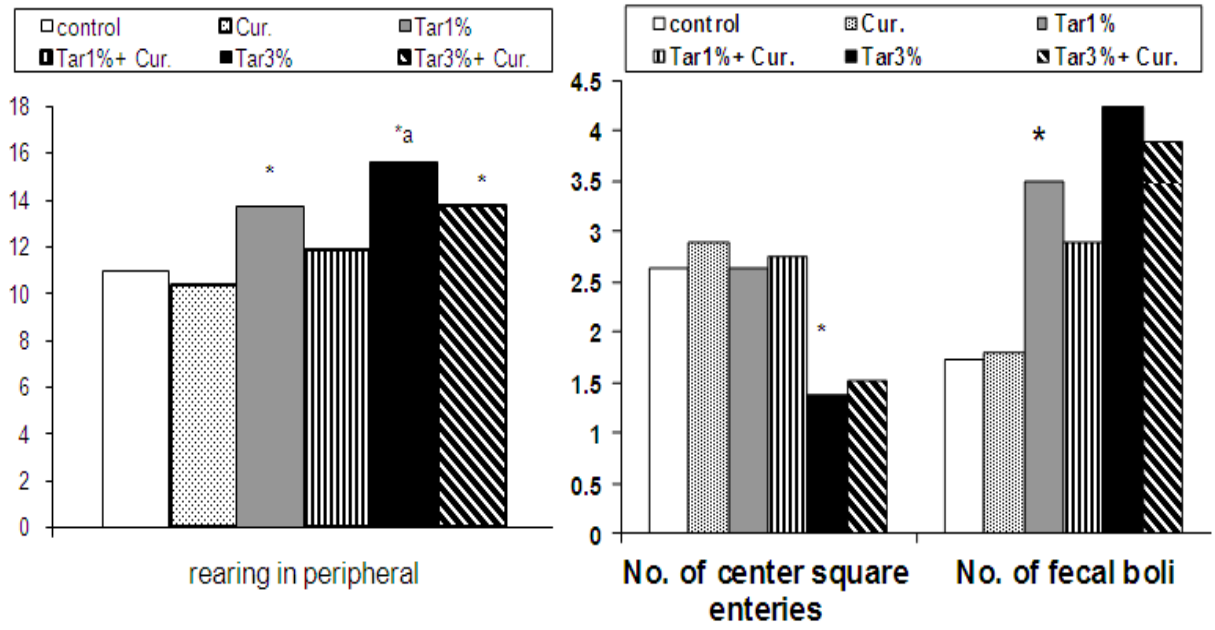
On the other hand, the Cur treated groups showed significant($p<0.05$) improvement in the levels of norepinephrine in the three brain areas and dopamine except in low dose of cortex the improvement is non – significant ($p>0.05$), while Cur significant($p<0.05$) effect in serotonin observed only in cortex and striatum *vs.* the values of Tar (1%, 3%) groups.

Fig.1.Effect of Tar (1%, 3%), Cur (200mg/kg/B.w.) individually and incombination on forced swimming and social interaction tests parameters on weanling rats after 8 weeks of treatment.



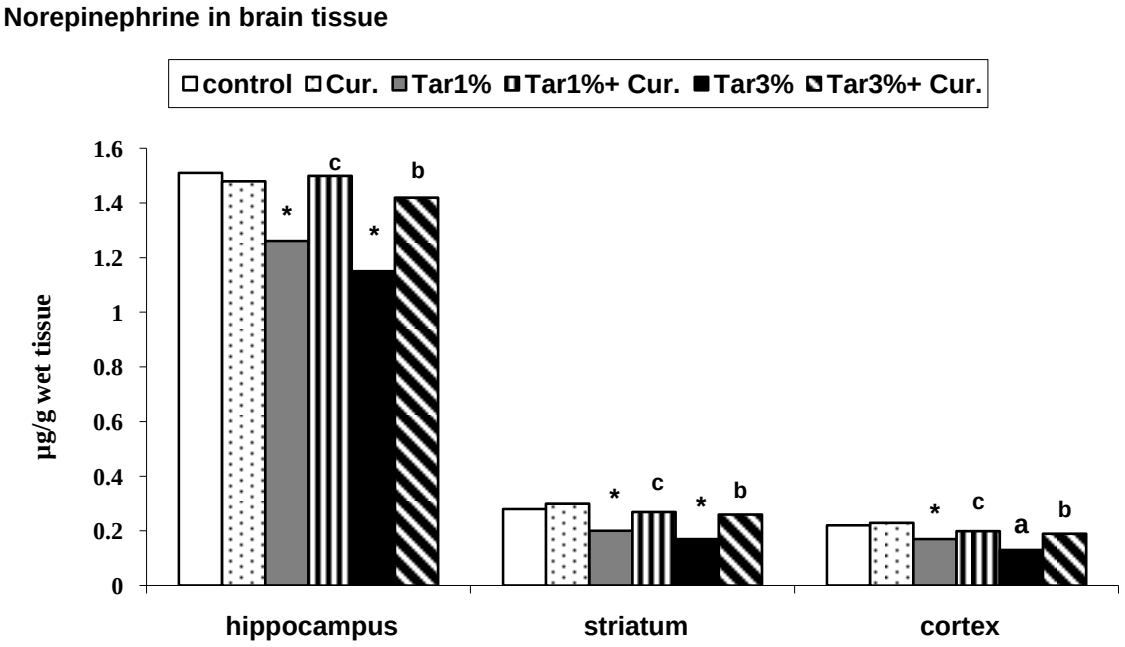
Significant difference from control group *$p<0.05$, Significant difference between Tar3% and Tar1% [a] $p<0.05$, Significant difference between Tar3% and Tar3%+cur [b] $p<0.05$, Significant difference between Tar1% and Tar1%+cur [c] $p<0.05$.

Fig.2. Effect of Tar (1%, 3%), Cur (200mg/kg/B.w.) individually and incombination on open field test parameters on weanling rats after 8 weeks of treatment.



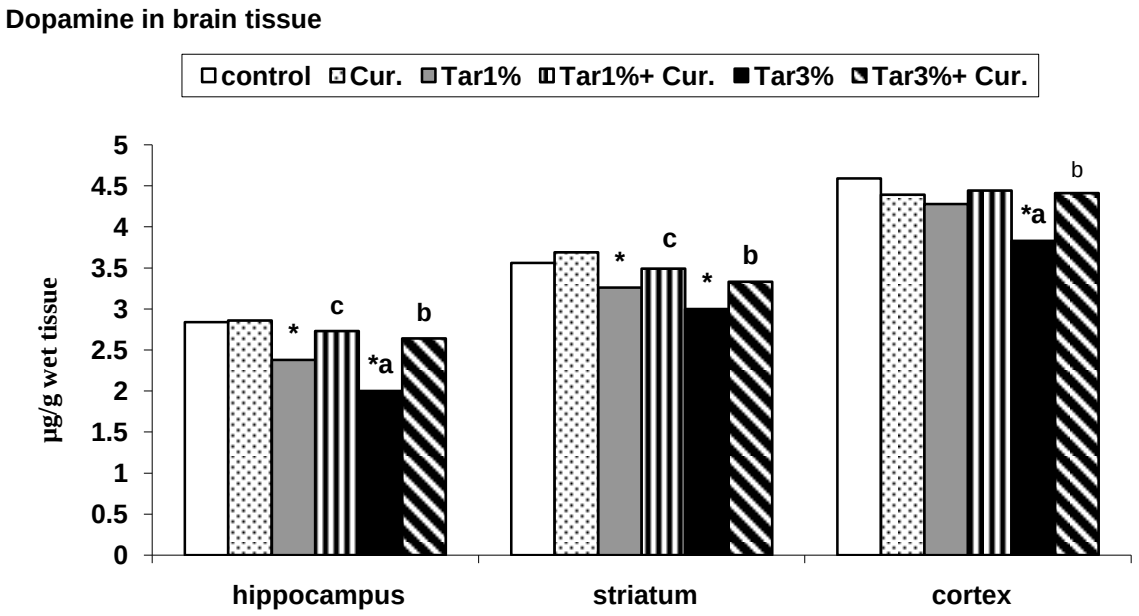
Significant difference from control group *p<0.05, Significant difference between Tar3% and Tar1% [a] p<0.05.

Fig.3. Effect of Tar (1%, 3%), Cur (200mg/kg/B.w.) individually and incombination on norepinephrine (µg/g wet tissue) in different brain tissues of weanling rats after 8 weeks of treatment.



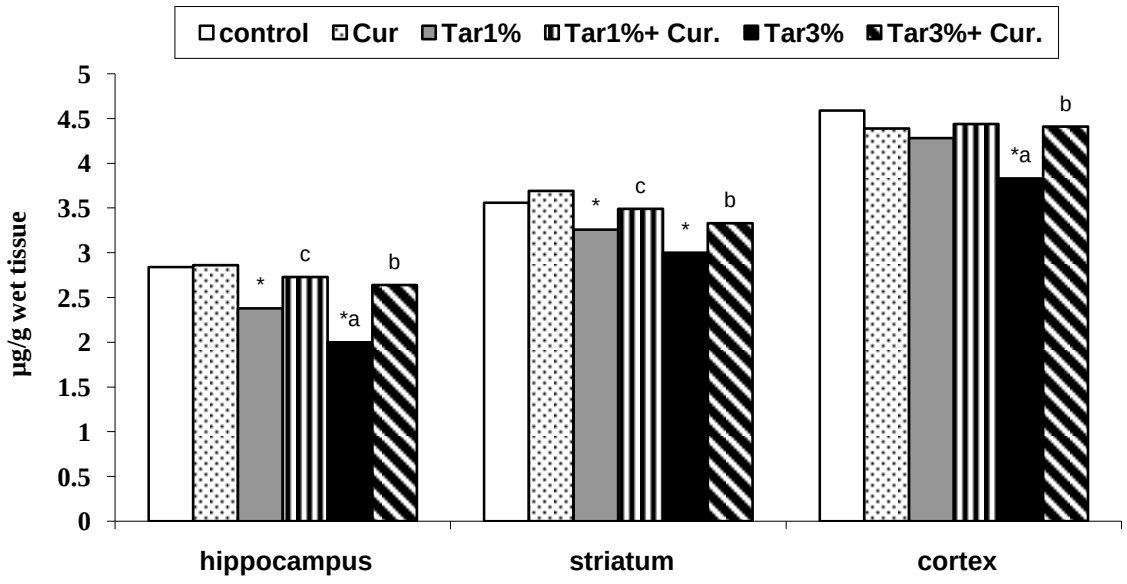
Significant difference from control group *p<0.05, Significant difference between Tar3% and Tar1% [a] p<0.05, Significant difference between Tar3% and Tar3%+cur [b] p<0.05, Significant difference between Tar1% and Tar1%+cur [c] p<0.05.

Fig.4. Effect of Tar (1%, 3%), Cur (200mg/kg/B.w.) individually and incombination on dopamine (µg/g wet tissue) in different brain tissues of weanling rats after 8 weeks of treatment.



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.

Fig.5. Effect of Tartrazine (1%, 3%), Curcumin individually and incombination on serotonin (µg/g wet tissue) in different brain tissues of weanling rats after 8 weeks of treatment.



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.

IV. Discussion

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 [4, 17].Serotonin system is important in the pathophysiology of psychiatric disorders including depression and anxiety, healthy levels of serotonin is essential to promote balanced mood [3, 16]. The hippocampal serotonergic alterations have been reported to play an important role in control of anxiety, depression and other mood disorders [5, 6].

The results of present study recorded that monoamines NE, DA and 5-HT were significantly decreased with the administration of Tar (1%, 3% of diet) in brain frontal cortex, hippocampus and striatum especially high dose. The results are supported by findings from authors of [1] who found that administration of low and high dose of Tar caused significant decrease in norepinephrine, epinephrine and serotonin. In the present study, treatment of weanling rats orally with 1`%, 3% of Tar caused significant increase in the immobility time during forced swimming test as compared to the levels of control group. The forced swimming test measures behavioral despair in rodents and is generally used to study depression [20]. Tar caused also a significant increase in the anxiety levels of rats in all anxiety models employed. The present study elucidated the time and number of social contacts recorded in Tar 1%, 3% treated groups was strongly reduced *vs.* the values recorded in the control groups. Anxiety in rats can be measured by behavioral reactivity to non-social or social stressors [11]. These behaviors were compared by performing the open-field as well as social interaction test. With regards to the present study, it is important to note that most of the behavioral 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 [15, 23, 24]. 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 Tar may be due to decrease in the monoamines levels in brain tissues . The most interesting finding was the dose-related reducing effect on bouts of social contacts.

Regarding anxiety measurements in the open field test; numbers of rearing against the wall, number of central squares entered as well as number of fecal boli all parameters were greatly influenced by ingestion of Tar especially high dose. Here, Tar 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 [2]. Again regardless of the administered dose, Tar-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 [8]. As fecal boli were shown to be a sensitive measure for anxiety state of animals [21, 28], present administration of Tar was shown to enhance defecation.

In the present study, coadministration of Cur (200mg/kg/B.w.) caused marked attenuation effect against behavioral abnormalities such as depressive-like behaviors as well as anxiety in force swimming test, social test and the open field test.

These results confirm the antidepressant and anxiolytic effects of Cur in weanling rats and suggest that these antidepressant and anxiolytic effects may be mediated by actions in the central monoaminergic neurotransmitter systems.

Although the mechanism of the antidepressant and 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 [12].

V. Conclusion

This study provides sufficient scientific evidence that a causal link truly exists between Tartrazine and mood disorder such as depression, anxiety and antisocial behaviour in weanling rats and points to the hazardous impact of Tar on public health. Tar treatment also reduced the monoamines formation that's likely to induce alterations in neurobiological substrates and brain tissue damage, while, combined treatment of Cur ameliorated all the behavioral and biochemical alterations in different brain regions of Tar -treated weanling rats. This could be due to anti-depression and anxiolytic effects of Cur.

So it is 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 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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