# EFFECT OF SYNTHETIC COLORING DYE ERYTHROSINE (E127) IN RATS

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## ABSTRACT

Food additives are substances used in food industry in order to improve the food's taste, appearance by preserving its flavor and preventing it from souring. Food additives are added to the most junk and fast foods, especially food for kids. Erythrosine B (ErB) is a cherry-pink food colorant with a polyiodinated xanthene structure. It is unique in this class approved by the US Food and Drug Administration (FDA) and is widely used in foods, drugs and cosmetics. Although its utilization is permitted, ErB has been described as having an influence on childhood behavior and interfering with thyroid function due to the high iodine content. This study was designed to investigate the toxic effect of two doses (0.08g/kg diet, and 0.4g/kg diet) of ErB as synthetic colors on the balance of neurotransmitter in 30 treated male rats compared with the 15 control male rats. Significant decrease ($p<0.01$) was obtained in Dopamine, gamma amino butyric acid (GABA), Norepinephrine (NE), Acetyl choline esterase (AchE), glutathione reductase (GSH) and serotonin when treated with low and high dose ErB compared with the control group. Significant increase ($p<0.01$) in MDA and Glutamate in low and high dose that may explain the abnormal behavior of treated rats.

**Keywords:** Neurotoxicity, Coloring dye, Erythrosine B, oxidative stress.

## INTRODUCTION

Artificial food colors have been utilized over the century for esthetic that make the foods attractive and stimulate appetite, however, in developing countries there has been a sharp increase in the use of synthetic food coloring agents. In the past years there is an uncontrolled use of synthetic color particularly in food mostly consumed by children (Khiralla *et al*., 2015).

Processed foods usually contain additives of some sort. Some of these additives can cause behavior and attention problems. Prevalence of food additive intolerance in children is estimated at 1 to 2% and is mainly found in atopic children in whom the additive aggravates the existing hypersensitivity to some other substances (Brito *et al*., 2008).

Many causal hypotheses for childhood hyperactivity have been addressed in both medical and psychiatric literature including; genetic factors, implications of the central nervous system dysfunction, improper embryological development or subtle chromosomal irregularities, birth complications, and unforeseen interactions in a human body (Tanaka *et al*., 2008).

Neurotransmitters are the chemical messengers of the nervous system, essential for relaying signals within the brain and communicating with all organ systems of the body. Disruptions in neurotransmission have been implicated in a long list of clinical conditions, ranging from Alzheimer’s disease to Attention Deficit Hyperactivity Diseases (ADHD). (Ailts *et al*., 2007).

Erythrosine 127 or Red 3 is a cherry-pink coal tar-based food dye. It has been found to cause all possible clinical forms of allergic reactions, sensitivity to light and also learning difficulties, increase thyroid hormone levels and lead to hyperthyroidism. (Neeta, 2018)

Erythrosine has been found to act as a potent neuro competitive dopamine inhibitor of dopamine uptake by nerve endings when exposed in vitro on a rat brain. Other studies showed that erythrosine can act as an inhibitor also on other neurotransmitters, resulting in an increased concentration of neurotransmitters near the receptors, thus functionally augmenting the synaptic neurotransmission. There is some evidence that a reduced dopamine turnover may lead to childhood hyperactivity. Similar findings have been linked with a reduction of noradrenaline (Wróblewska, 2009).

The aim of the present study is to investigate whether prolonged administration of the artificial dye Erb (commonly known as Red 3) has a significant effect on the levels of different types of neurotransmitters

## MATERIALS AND METHODS

This study included 45 male Albino strain rats (120 – 150 ± 2g, 10 weeks age). From animal house at Medical Research Center (MRC), Rats were divided equally into three groups (control, treated groups with low dose of ErB 0.08g/kg diet and high dose of ErB 0.4g/kg diet groups) for oral administration with ErB daily for 90 days. (ErB was purchased from Sigma–Aldrich).

Samples collected from the eye plexuses of animals by fine capillary glass tubes and placed immediately on ice. The serum was separated after centrifugation for 10 min at 3000 rpm and kept at –20 ºC until analysis.

Serum was used for the following measurement; determine the concentrations of monoamines and amino acids using high performance liquid chromatography (HPLC) system, Agilent technologies 1100 series, equipped with a quaternary pump (Quat pump, G131A model) according to (Hussein *et al*., 2012). Determine the concentration of (MDA) by measuring acid-reactive substances react with thiobarbituric acid to produce a red-colored complex having peak absorbance at 532 nm. (Oberley *et al*., 1988), reduced glutathione (GSH) was determined according to the method of Ellman 1959. Also concentration of acetyl cholinesterase activity (AChE) was determined according to the method described by Ellman 1959. (Noradrenalin, Dopamine, Serotonin, Glutamate and GABA HPLC standards were purchased from Sigma Aldrich Chemicals Company St. Louis USA. All other chemicals of HPLC grade were purchased from Sigma).

## STATISTICAL ANALYSIS

Results were expressed as mean ± SE. One way analysis of variance (ANOVA) followed by post hoc least significant difference analysis (LSD) were performed using the statistical package for Social Science (IBM SPSS) version 23 to compare different groups. Person correlation coefficients were used to assess the correlation between parameters estimated in serum of rats. The confidence interval was set to 95% and the margin of error accepted was set to 5%.

## RESULTS

As illustrated in table (1), Oral intake of ErB at low and high dose (0.08g/kg diet, and 0.4g/kg diet). It was clear that neurotransmitter levels were changes in both treated groups compared with data obtained from control group. Results indicating that ErB low dose group and high dose group recorded highly significant decrease in the level of Dopamine, Serotonin, GABA, NE (p< 0.001) compare with the control group .Meanwhile, a significant increase in Glutamate in both groups (p< 0.001) compare with the control group.

**Table (1):** Effect of oral administration of Erythrosine B on neurotransmitter in serum of male rats

| | | Control group | Low dose | High dose | Test value• | P-value | Sig. |
|---|---|---|---|---|---|---|---|
| | | **No. = 15** | **No. = 15** | **No. = 15** | | | |
| Dopamine | Mean±SD | 25.33 ±1.76 | 24.07±9.57 | 17.80 ± 2.37 | 7.308 | 0.002 | HS |
| | Range (µg/ml) | 23 – 30 | 19 – 58 | 13 – 21 | | | |
| Norepinepherin | Mean±SD | 464.53 ± 32.64 | 287.80 ± 64.79 | 195.40 ± 23.41 | 144.794 | 0.000 | HS |
| | Range (µg/ml) | 420 – 543 | 215 – 390 | 150 – 260 | | | |
| Seratonin | Mean±SD | 5.47 ± 1.06 | 5.20 ± 0.68 | 3.40 ± 0.74 | 26.776 | 0.000 | HS |
| | Range (µg/ml) | 4 – 8 | 4 – 6 | 2 – 5 | | | |
| GAPA | Mean±SD | 6.27 ± 1.28 | 3.47 ± 0.64 | 2.13 ± 0.52 | 86.535 | 0.000 | HS |
| | Range (µg/ml) | 5 – 9 | 3 – 5 | 1 – 3 | | | |
| Glutamate | Mean±SD | 57.07 ± 4.68 | 61.60 ± 1.76 | 65.53 ± 2.45 | 26.042 | 0.000 | HS |
| | Range (µg/ml) | 41 – 60 | 58 – 65 | 60 – 70 | | | |

* P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

Table (2) showed that the effect of oral intake of ErB at low and high doses in levels of GSH, MDA, AchE parameters. It is clear that low and high dose of ErB recorded highly significant decrease in the level of GSH, AchE, (p< 0.001) compared with control group .Meanwhile, a significant increase in MDA in both groups (p< 0.001) compared with the control group.

**Table (2):** Effect of oral administration of ErB on GSH, MDA, AchE in serum of male rats

| | | Control group No. = 15 | Low dose No. = 15 | High dose No. = 15 | Test value• | P-value | Sig. |
|---|---|---|---|---|---|---|---|
| GSH | Mean±SD | 7.93 ± 0.59 | 5.80 ± 0.56 | 3.60 ± 0.74 | 174.669 | 0.000 | HS |
| | Range (μmol/ml) | 7 – 9 | 5 – 7 | 3 – 5 | | | |
| AchE | Mean±SD Range (μ/l) | 599.00 ± 83.33 457-800 | 434.47 ± 71.17 310-580 | 282.80 ± 53.13 204-390 | 75.879 | 0.000 | HS |
| MDA | Mean±SD | 457 ± 800 | 310 ± 580 | 204 ± 390 | 117.750 | 0.000 | HS |
| | Range (nmol/ml) | 12 – 16 | 15 – 20 | 20 – 25 | | | |

P-value >0.05: Non significant (NS);

P-value <0.05: Significant (S);

P-value< 0.01: highly significant (HS)

Person correlation referred that an absolute value of 1.0 indicates a perfect linear relationship. Therefore, table (3) shows a highly significant correlation between seratonin with both AchE and GSH as well as GSH and GABA with AchE ($p< 0.001$, $r= 0.651 – 0.819$). Negative sign of correlation coefficient indicates a reverse direction of relationship (one variable tends to increase the other decrease) Therefore, a reverse correlation in high & low dose of ErB between the level of, dopamine, NE, serotonin, AchE, GABA and GSH corresponding by the level of MDA ($P<0.001$; $r=0.58-0.72$).

**Table (3):** Correlation coefficient between AchE, GSH, MDA level with different parameters in two groups (low & high)

| | **AchE** | | **GSH** | | **MDA** | |
|---|---|---|---|---|---|---|
| | **R** | **P-value** | **R** | **P-value** | **R** | **P-value** |
| Dopamine | 0.437* | 0.026 | 0.574** | 0.002 | -0.587** | 0.002 |
| Norepinepherin | 0.424* | 0.031 | 0.819** | 0.000 | -0.633** | 0.001 |
| Seratonin | 0.772** | 0.000 | 0.651** | 0.000 | -0.708** | 0.000 |
| AchE | - | - | 0.691** | 0.000 | -0.592** | 0.001 |
| Glutamate | -0.500** | 0.009 | -0.577** | 0.002 | 0.564** | 0.003 |
| GABA | 0.649** | 0.000 | 0.661** | 0.000 | -0.724** | 0.000 |
| GSH | 0.691** | 0.000 | - | - | -0.641** | 0.000 |
| MDA | -0.592** | 0.001 | -0.641** | 0.000 | - | - |

*low significant. **slightly significant *** highly significant.

There is a highly significant correlation (table 4) between dopamine with both noradrenaline and GABA main while GABA with both serotonin, norepinephrine & dopamine (p< 0.001, r= 0.612 – 0.750). A reverse correlation coefficient was recorded between GABA & glutamate at high & low dose of ErB where the level of GABA decreased and the level of glutamate increased.

**Table (4):** Correlation coefficient of neurotransmitters in low and high doses of ErB dye

| **Low ErB dose** | **Dopamine** | | **Norepinephrine** | | **Serotonin** | | **Glutamate** | | **GAPA** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **High ErB dose** | **R** | **P-value** | **r** | **P-value** | **r** | **P-value** | **r** | **P-value** | **r** | **P-value** |
| Dopamine | - | - | .750** | 0.000 | 0.301 | 0.135 | -.489* | 0.011 | .630** | 0.001 |
| Norepinepherin | .750** | 0.000 | - | - | .477* | 0.014 | -.464* | 0.017 | .612** | 0.001 |
| Seratonin | 0.301 | 0.135 | .477* | 0.014 | - | - | -.477* | 0.014 | .612** | 0.001 |
| Glutamate | -0.489* | 0.011 | -.464-* | 0.017 | -.477-* | 0.014 | - | - | -.632** | 0.001 |
| GAPA | .630** | 0.001 | .612** | 0.001 | .612** | 0.001 | -.632** | 0.001 | - | - |

## DISCUSSIONS

ErB are used as a food dye in dietary food products, cosmetics and oral drugs. Harmful effects caused by the excessive use of ErB are hyperactivity, various allergic reactions, lesions and tumors, genotoxicity, mutagenicity, (Ruchika *et al*., 2013)

Our results showed that exposure to ErB (approximately 0.08g/kg diet, and 0.4g/kg diet) cause neurotransmitter imbalance this result was concordance with previously published data (McCann *et al*., 2007).

In the current study we highlighted on a neurotoxic effect of ErB where there is a decrease in level of Dopamine, Serotonin, NE,GABA,GSH level and increase in Glutamate, MDA that revealed a relation between food additives and neurotoxicity which accomplished with the same result of Mohamed *et al*., 2017. In more recent study ErB was evaluated for potential neurotoxic effect, where it showed a significant decrease in GABA, Dopamine, Serotonin level as neurotransmitter in brain (Verlaet *et al*., 2018).

Dopamine was decreased in this study as a direct effector on neurotransmitters by intake of diet containing ErB dye. Therefore, it was found a potent noncompetitive inhibitor of dopamine uptake by nerve endings prepared from rat brain and exposed in vitro to the dye for five minutes. Other studies showed that this compound inhibits uptake of many other neurotransmitter and precursors (Tuula, 1994). Inhibition of uptake results in increased concentrations or prolonged presence of neurotransmitters near their receptors, and synaptic transmission is functionally augmented. (Wróblewska, 2009). There is an evidence that a reduced dopamine turnover may lead to a reduction of NE.

Dopamine synthesized from tyrosine which catabolized and converted to NE (Cass, 1993). In present study, there is a significant decrease in NE, which is a major neurotransmitter that regulates several behavioral activities. Depleted levels of NE overall alters working memory and attention regulation. (Arnsten, 2000). No significant differences were obtained between DA and NE concentrations in serum of ErB group compared with the control by study of Khirala *et al*., 2015 which in contrast with this study.

In light of the study prepared by Ellen et al., 1983; who stated the ErB effect on dopamine and glutamate biochemistry in synaptosomes prepared from rat brain. The dye inhibited dopamine uptake by synaptosomes, a process important in the activation of dopaminergically mediated neurotransmission. The present result explained that ErB was found to stimulate the uptake of glutamate by synaptosomes, which in agreement with increasing glutamate in this study. Glutamate is the major excitatory neurotransmitter in the central nervous system and must be tightly regulated for proper neural signaling to occur (Danbolt, 2001).

The current study showed that GABA decreased which in contrast with Patricia et al., 1984 who found that ErB induced release of GABA in rat.

Decreased in serotonin level have been seen in this study in all groups treated by ErB compared with the control, and they may be a result of aggressive and fighting behavior, in agreement with the previous reports by (Comai, 2016).

In a study of (Dalal and Poddar, 2009) administration of high dose of ErB to adults male rats decrease serotonin level .Meanwhile, administration of low dose had no effect on level of serotonin.

The important role of oxidative stress in the pathogenesis of a range of neurotoxicity is established (Praticò, 2008). Our study showed MDA levels were increased while GSH levels were decreased in the serum of rats, but limited studies demonstrated opposing results in the disorder animal models (Shoonees *et al*., 2012; Verlaet *et al*., 2018).

Decreased levels of GSH and higher levels of MDA indicate the presence of oxidative stress as a result of utilization of GSH in conjugation with foreign compounds entering the body as ErB dye (Abdel Wahab and Moram, 2012).

Horton *et al*., (2012), Suarez *et al*., (2012) hypothesized that lower AChE activity would be associated with lower neurodevelopment .Our findings support the hypothesis that AchE inhibition is an important mechanism of neurotoxicity which accomplished by (Richardson *et al*., 2005). Therefore, low Acetylcholine esterase activity was associated with administration of ErB to male adult rats.

## CONCLUSION

Finally, it could be concluded that administration of these food dye resulted in various alterations of antioxidant system and neurotransmitter imbalance which lead to numerous neurotoxic effect. Therefore, it is advisable to limit the use of these food colorant or food additives especially those used for children.

Harmful exposure to food additives like as ErB, may lead to neurological disorders. Therefore, it has been suggested that further investigations are required to find the dual neuroprotective – neurotoxic mechanisms of ErB.

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# تأثير الصبغات الصناعية الملونة على الفئران (الأرثروسين)

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## المستخلص

المواد المضافه الى الأطعمه هي المواد المستخدمة في صناعة الأغذية من أجل تحسين مذاق الطعام ومظهره من خلال الحفاظ على النكهة ومنعها من الفساد. تضاف هذه المواد الى معظم الوجبات السريعه وخاصه وجبات الأطفال. ماده الأريثروسين هى ماده تعطى اللون الأحمر ومسموح بها من إداره الغذاء والدواء الأمريكيه وتستخدم على نطاق واسع في الأطعمة والأدوية ومستحضرات التجميل. وبالرغم من أنها من المواد المسموح بها إلا أن لها تأثير على سلوك الأطفال وتتداخل مع وظيفة الغدة الدرقية بسبب ارتفاع محتوى اليود. هذه الدراسة صُممت لفحص ودراسه التأثير السام لتركيزين من ماده الإريثروسين (٠,٠٨ جم / كجم، و ٠,٤ جرام / كجم) كماده من الملونات الصناعيه وتأثيرها على توازن الناقلات العصبيه فى الدم. هذه الدراسه تمت على ٣٠ عينه من ذكور الفئران مقارنة ب ١٥عينه من ذكور الفئران كعينه ضابطه. وكان معدل الانخفاض ($p<0.01$) في الدوبامين، وحمض جاما أمينو بيوتيريك أسيد، والنوربينفرين، وأستيل الكولين، والجلوتاثيون والسيروتونين عند معاملتهم بجرعة منخفضة وأخرى عالية من الأريثروسين مقارنة بالمجموعه الضابطه. وكان هناك معدل زياده كبيرة ($p<0.01$) في المالوندهيد والجلوتامات بجرعات منخفضة وعالية قد تفسر السلوك غير الطبيعي للفئران المعالجة.

**الكلمات الدالة:** السمية العصبية، الألوان الصناعية، الأرثروسين.