THE EFFECTS OF DIETARY ARTIFICIAL COLORS ON EXPERIMENTAL RATS

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ABSTRACT

Recently the use of synthetic food coloring additives was increased and the levels of human exposure to such agents are very broad, thus feeding over long periods may continually possess potential hazards to the human health. Evaluation of the toxic effects of synthetic dyes Brilliant Blue, Tartrazine and Carmoisine were tested in rats by measuring their actions on renal, hepatic function, and body-weight gain. Rats were fed synthetic dyes supplemented diet, daily for 60 days orally in two doses, one low and the other high dose followed by serum sample collection for determination of urea, creatinine, uric acid, ALT, AST, ALP, glucose cholesterol, triglycerides and estimation of hemoglobin conc.

Our data showed a significant increase in cholesterol, triglycerides, ALT, AST, in addition to serum urea and creatinine levels in treated rats, while, they recorded a significant decrease in percentage of body weight change, and this significant change were more apparent in high doses than low doses.

Keywords: Food coloring additives, Brilliant Blue, Tartrazine, Carmoisine

INTRODUCTION

Food additives are used for various purposes, including preservation, coloring or sweetening. The wide range of food additives, running into more than 2500 items used to preserve, dye or enhance foods are a consequence of industrialization and the development of food processing technology. Most coloring agents are used to improve the overall attractiveness of food. A

number of natural and synthetic additives are used to color foods. Although synthetic coloring agents are continued to be used extensively, there has been a concomitant significant increasing interest concerning the using of natural colorants. Shadia,2010.

The Egyptian famous food additives which are used as coloring substances are Tartrazine and CarmoisineAmin, *et al*., 2010.

Many products contain Tartrazine like foods cotton candy, soft drinks, flavored chips, cereals, cake mixes, some of non-food products include Tartrazine such as soaps, cosmetics, shampoos and other hair products, also some medical preparations contain Tartrazine such as vitamins, antacids, medicinal capsules and certain prescription drugs. Walton *et al*., 1999.

Carmoisine present in food like jams, preserves, yoghurts, jellies, breadcrumbs, and cheesecake mixes. It is also present in oral Dene mouthwash. Amin *et al.*, 2010.

Brilliant Blue, is a water-soluble coloring used in many baked goods, beverages, dessert powders, candies, cereals, drugs, and other products.Mahmood,2005.

Several studies have incriminated synthetic colorants to cause some adverse effects to human health.

MATERIALS AND METHODS

Materials:

A) Chemicals: Tartrazine a yellow color substance known as E102Walton, *et al*., 1999.Carmoisine a red color substance known as E122 or Food Red

3Amin *et al*., 2010.

Brilliant blue FCF, color index no. 42090, E 133Neveen, 2006

Tartrazine, Carmoisine and Brilliant blue were in a solid state so we prepared two solutions of each substance (one low and the other high concentration) by dissolving the solid in distilled water, low doses of Tartrazine, Carmoisine and Brilliant blue were 0.5%, 0.25 % and 0.75gm %of diet respectively while high doses were 1%, 0.5% and 1.5 gm % of diet respectively according to Minister of Health and Population Decree No. 411for the year 1997.

B-Animals: A total of 56 young malealbino rats (Sprague Dawley strain) weighting about 45– 70 g were used in the present study. They were obtained from National nutrition institute, Cairo, Egypt. Animals were kept under observation for about 2 days before the onset of the experiment to exclude any intercurrent infection. They were maintained in stainless steel cages at normal atmospheric temperature of 27 ± 5 ^{o}Cas well as under good ventilation. This study was approved by the Research Ethics Committee at National Nutrition Institute (NNI).

C-Experimental design

The rats were divided into 7 groups each of 8 ratsdivided as follows:

Group 1:Control group fed on standard diet was prepared according to Reeves et al., 1993.

Group 2: Fed on standard diet +Low dose of Tartrazine (E102) 0.5%.

Group 3: Fed on standard diet +High dose of Tartrazine(E102) 1%.

Group 4: Fed on standard diet + Low dose of Carmoisine (E122) 0.25%.

Group 5: Fed on standard diet + High dose of Carmoisine (E122) 0.5 %.

Group 6: Fed on standard diet + Low dose of brilliant blue (E133) 0.75%.

Group 7: Fed on standard diet+ High dose of brilliant blue (E133) 1.5%.

During the conditioning rats were weighted twice weekly and their food intake were calculated.The experimental lasted for 8 weeks, food intake and body weights (wt.) were recorded twice a week. Wastes collected after 8 weeks from beginning of experimental.

At the end of experimental period, blood samples were collected from the eye plexuses of animals by a fine capillary glass tubes and placed immediately on ice. Blood serum samples were collected into dry clean centrifuge tubes; the serum was separated after centrifugation for 10 min at 3000 rpm and kept at –20 CO until biochemical analysis. The whole blood were collected on ethylene diamine tetra acetic acid (EDTA) tubes for immediate hemoglobin analysis.

D-Biological evaluation: The total food consumption of experimental period (8 weeks) was calculated, body weight gain (BWG) and feed efficiency ratio (FER) were determined according to Hsu *et al.,* 1978.

E-Biochemical analysis

E.1. Liver functions: Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activates were measured according to Reitman and frankel 1957. and Alkaline phosphatase (ALP) activates were measured according to Guder *et al*., 2001.

E.2. Kidney functions:

Serum creatinine was determined according to Bartles *et al*.,1972.

Serum Urea was determined according to Thomas *et al*., 2009.

Serum uric acid was determined according to Young, 2000.

E.3. Hemoglobin (HB) was determined according to Drabkin, 1949.

E.4:. Glucose was determined according to Snacks *et al.*, 2000.

E.5: Determination of cholesterol and triglycerides had been determined according to Guder *et al*., 2001.

F-Statistical Analysis: Results are expressed as Mean ± SD the difference among groups where analyzed by analysis of variance (T.Test). The analyses were carried out using statistical package for the social science SPSS.Version (18) Computer Programs.

RESULTS

Table (1): Effect of food colorants concentration on body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) in rats.

Groups / Parameters	Control Group 1	Lower dose			Higher dose		
		Tartrazine Group 2	Carmoisine Group 4	Brilliant blue Group 6	Tartrazine Group 3	Carmoisine Group 5	Brilliant blue Group 7
BWG (gm)	58.3±10.5	62.8±5.3	51.3±5.8	48.0±7.0	64.3±5.4	43.3±11.5*	53.3±9.9
FI (gm)	712.7 ± 41.4	794.3 ± 45.20	914.0 ± 162.4	819.5 ± 302.3	749.2 ± 92.1	679.7 ± 208.8	637.2 ± 145.4
FER(gm)	0.08± 0.01	0.08± 0.01	0.06±0.01**	0.06± 0.02	0.09± 0.01	0.07± 0.01**	0.08± 0.01

All data presented as mean ± standard deviation.

Significance p-value <0.05 .* **highly significance p-value < 0.01.

Data in table (1) revealed that rats fed on low dose of Carmoisine and low dose of brilliant blue had decrease BWG compared to control group but the difference was not significant. It was also noticed that high dose of Carmoisine and high dose of brilliant blue had decrease BWG compared to

control group but the difference was significant with high dose of Carmoisine and no significance with high dose of brilliant blue.

Concerning food intake, results showed that rats fed on low dose of Tartrazine, Carmoisine and Brilliant Blue had led to increased food intake compared to control group the differences were not significant. While food intake decrease in high doses of Carmoisine and brilliant blue groups compared to control group but the difference were not significant. As regards to feed efficiency ratio (FER), results showed that rats fed on low and high doses of Carmoisine highly significance $p \leq 0.01$ compared to control group. But there were no any significance differences among other groups.

Table(2): Effect of food colorants concentration on liver function in male albino rats

Groups / Parameters	Control Group 1	Lower dose			Higher dose		
		Tartrazine Group 2	Carmoisine Group 4	Brilliant blue Group 6	Tartrazine Group 3	Carmoisine Group5	Brilliant blue Group7
AST (U/L)	141.2 ± 14.7	129.8±6.8	175.3±13.5*	148.3 ± 8.3**	149.2±37	153.7 ± 30.8	139.8±40.0
ALT (U/L)	52.9 ± 4.4	62.4 ± 11.7	107.6 ± 30.7**	91.8 ± 12.9**	63.2 ± 22.0	95.9 ± 25.2*	85.7±15.2**
ALP (mg dl)	189.0 ± 28.2	295.0±54.6**	242.5±50.4*	299± 37.8**	277.5±43.2**	256.7±55.7**	298 ± 32.8**

All data presented as mean ± standard deviation.

*Significance p-value <0.05 **highly significance p-value < 0.01.

Data in table (2) revealed a marked increase ($P < 0.05$) in the serum AST level of treated group with low dose of group (4) compared to control group. Also resulted a highly significant ($P < 0.01$) with low dose of group (6) compared to control group. And resulted no significance with other groups

compared to control group. ALT conc. in serum showed a significant increase in group (5) while a highly significant increase in groups (4), (6), and (7)Compared to control group.

Regarding ALP, the results indicated a significant increase in group (4) while a highly significant increase in groups (2, 3, 5, 6& 7) compared to control group

Table(3): Effect of food colorants concentration on kidney function in male albino rats

Groups / Parameters	Control Group 1	Lower dose			Higher dose		
		Tartrazine Group 2	Carmoisine Group 4	Brilliant blue Group 6	Tartrazine Group 3	Carmoisine Group 5	Brilliant blue Group 7
Urea (mg/dl)	23.5± 1.2	36.5±9.5**	25.1 ± 3.9	22.0 ± 5.8	30.4 ± 3.8**	30.5 ± 5.4**	40.2 ± 8.7**
Creatinine (mg/dl)	0.4 ± 0.1	0.8 ± 0.1**	0.8 ± 0.2**	0.7 ± 0.1**	0.6 ± 0.1**	0.7 ±0.1**	1.0 ± 0.1**
Uric Acid (mg/dl)	1.5 ± 0.2	2.0 ± 0.3**	2.1 ± 0.2**	1.6 ± 0.2	2.0 ± 0.3**	1.5 ± 0.5	1.4 ± 0.1

All data presented as mean ± standard deviation.

*Significance p-value<0.05 . **highly significance p-value< 0.01.

Table (3), Showed a highly significant increase ($p < 0.01$) in serum urea, in groups treated with synthetic color of low dose of Tartrazine, and high doses of Tartrazine, Carmoisine. And brilliant blue comparedto the control group. No significance was found among other groups.

Regarding creatinine conc. in serum showed a highly significant increase in all groups compared to control group.

The results of uric acid conc. in serum indicate a highly significant increase of groups (2, 3 & 4) compared to control group.

Table(4): Effect of food colorants concentration on hemoglobin and glucose in male albino rats

Groups / Parameter	Control Group 2	Lower dose			Higher dose		
		Tartrazine Group 2	Carmoisine Group 4	Brilliant blue Group 6	Tartrazine Group 3	Carmoisine Group 5	Brilliant blue Group 7
HB	12.8 ± 1.3	12.2 ± 0.4	12.0 ± 0.7	10.2 ± 1.9	10.8 ± 1.1*	11.7 ± 0.7	11.4 ± 1.5
Glucose	84.9 ± 9.7	89.5 ± 24.4	74.0 ± 9.4	81.4 ± 19.0	84.1 ± 3.4	77.4 ± 12.3	108.7 ± 13.4**

All data presented as mean ± standard deviation.

* Significance p-value <0.05 . **highly significance p-value < 0.01.

The data represented in table (4) revealed a significant decrease of ($p<0.05$) on HB in group (3) compared to control group while no significance in other groups. The results of Glucose conc. in serum indicate a highly significant increase ($p<0.01$) of glucose concentration was showed in group (7) compared to control group and no significant other groups.

Table(5): Effect of food colorants concentration on lipid profile in male albino rats:

Groups / Parameters	Control Group 1	Lower dose			Higher dose		
		Tartrazine Group 2	Carmoisine Group 4	Brilliant blue Group 6	Tartrazine Group 3	Carmoisine Group 5	Brilliant blue Group 7
Cholesterol	56.4 ± 7.0	58.8 ± 6.0	76.3 ±19.6*	80.2 ± 17.6*	69.7 ± 17.9	78.0 ± 16.5**	69.2 ± 8.6**
Triglyceride	67.8 ± 10.5	73.3 ± 11.1	81.3 ±10.2*	87.3 ± 17.2*	66.9 ± 14.6	91.9 ± 15.2**	85.1 ± 9.5**

All data presented as mean ± standard deviation.

*Significance p-value<0.05 . **highly significance p-value< 0.01.

The Table (5) indicates that a significant increase in cholesterol and triglycerides of Carmoisine and Brilliant Blue in both low and high doses when compared to control value .While no significant of Tartrazine in both doses (low and high) when compared to control group.

DISCUSSION

In this study some trials were adopted to throw a light on the side toxic effects and biochemical changes in some constituents in serum of experimental rats treated with 3 compounds (each of low and high doses) that are commonly used in Egyptian field of food additives. We considered low dose (double of ADI) because our young children in Egypt can consume a double of ADI (or more) daily in several products without control, in addition we used the high dose (a much higher than ADI) to evaluate the toxicity and health hazards of these additives on biochemical assay. High dose of Carmoisine showed a significant decrease in body-weight gain after 2 month while non significance in the percentage of body weight change of rats after treatment with 0.5% of diet & 1 % of diet tartrazine, 0.25 % of Carmoisine, and 0.75 %, 1.5 % of diet brilliant blue for 8 weeks as compared with the control rats. These observations were in agreement with Shadia, 2010. This effect in growth is thought to be due to a reduced availability of nutrients caused by the rapid transit of food colorants through the upper segments of the gastrointestinal tract Aritsuka et al., 1989; and Takeda et al., 1992 and to colorant's inhibitory effects on the processes of digestion and absorption.

However, there are many reports on weight loss in Carmoisine administered experimental animals Abu El- Zahab *et al.* 1997; El-Shamy *et al*., 1999; Marie *et al*., 1999; and Helal *et al*., 2000. Meanwhile, Osman *et al*.,1995 found that the synthetic food colorants caused a significant increase in the body weight gain of mice until the fourth month, followed by a significant decrease.

The present study revealed that a marked rats consuming low dose of Carmoisine and Brilliant blue exhibited a significant increase in serum AST and ALT compared to control group. On the other hand showed an insignificant change of AST activity of high dose of Carmoisine and Brilliant blue. These changes in liver function attributed to hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood. Elevated levels of the transaminases can signal hepatic disease, muscular dystrophy, and organ damage. Thus serum aminotransferases activities are known as toxicity markers in the study of hepatotoxicity caused by Chemicals Govindwar and Dalvi,1990.

Abdel-Rahim *et al.,* 1989 found a significant increase in both serum AST and ALT of rats fed on chocolate brown HT for three months, and they attributed these changes in liver function to hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood.

Furthermore, Abu El-Zahab *et al.,* 1997 investigated the effect of three different synthetic chocolate colorant agents in rats whose diets were supplemented with chocolate colors A and B (Sunset yellow, Tartrazine,

Carmoisine and brilliant blue in varying concentration, which revealed a significant increase in serum aspartate and alanine transaminases (AST and ALT respectively). Attia *et al.*, 2005 showed significant increase in the activity of the serum enzymes ALT and AST of benzene sulfonic acid sodium treated rats, it may be taken as a good index for disturbance in the liver function.

On the other hand Borzelleca and Hallagan, 1988 and Ford *et al.*, 1987 stated that Tartrazine and Carmoisine caused insignificant changes in rat serum AST and ALT. Yet these contradictory results were recorded after long-term (1-2years) toxicity studies which may indicate an adaptation mechanism on the part of the liver. While, Salah, 1994 found that the synthetic food colorants inhibited the activity of AST.

Regarding ALP, the results indicated a significant increase in low dose of Carmoisine and a highly significant increase in all groups compared to control group.

A rise in the alkaline phosphatase (ALP) occurs with all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system such as hyperparathyroidism, rickets and osteomalacia, as well as fractures and malignant tumors.

In the same aspect, Attia *et al*., 2005 recorded a significant increase in the activity of the serum alkaline phosphatase (ALP). The increase in ALP activity is attributed to early cholestasis liver damage which primary effects the liver parenchyma and is a key for an early diagnosis of infiltrative diseases El-Elaimy and El-Nabi, 1990. However, the increased serum activity of ALP is not specific only for liver tissues but also many other tissues may

be affected especially the gastrointestinal tract, the intestinal microvilli membrane is rich in ALP Young *et al*., 1981, Mahmood *et al*., 2005,El-Shamy et al.,1999observed a significant increase in serum ALP in rats treated with a green-coloring dye.

Also, Abu El-Zahab *et al*.,1997 investigated the effect of chocolate colors (sunset yellow, Tartrazine, Carmoisine and brilliant blue in varying concentrations), which revealed a significant increase in serum alkaline phosphatase (ALP). On the other hand Borzelleca and Hallagan 1988 and Ford *et al*., 1987stated that Tartrazine and Carmoisine caused insignificant changes in rat serum ALP.

These results are in accordance with Sharma *et al*., 2006 who found that the two doses of Tomato Red (blend of Carmoisine and Ponceau 4R) showed a significant increase in alkaline phosphatase activity when Swiss albino mice consumed these colorants for 21 days as short term or 42 days as long term. The present findings are in agreement with Helal *et al*., 2000 who found that oral administration of synthetic or natural colorants induced a marked increase in the serum AST and ALT level of all treated groups after 30 days of treatment.

Our study demonstrated that the daily intake for 60 days of Tartrazine, Carmoisine and Brilliant blue either low or high doses exhibited a significant increase in serum creatinine, urea and uric acid concentration when compared with control rats, while high dose of Tartrazine, Carmoisine and Brilliant blue exhibited a significant increase more than low dose in serum creatinine level (Table 3). Our results are in agreement with Helal *et al*., 2000 who found that

a significant elevation in serum creatinine and urea in rats consumed a synthetic or natural food colorants after 30 days of treatment Furthermore, the present findings are in accordance with data reported by Ashour and Abdel Aziz, 2009 who observed a significant elevation in serum creatinine and urea level of rats dosed with organic azo dye (fast green) orally for 35 days. We believe that the significant elevation in urea and creatinine levels is closely related to the impairment of renal function. These results are in agreement with Varely, 1987 who determined that the blood urea can be increased in all forms of kidney diseases such as hydro nephrosis congenital cystic, kidney renal tuberculosis, condition in which deposition of calcium occurs as hypervitaminosis D.

Further, Attia *et al.,* 2005reported a significant increase in the concentrations of serum creatinine and uric acid after seven weeks administration of BSAto rats was observed.

While, Chambers et al., 1966 and Carpanini *et al.,* 1978 found that chocolate brown HT had no effect on renal function after both short-term and long – term toxicity studies in rats. Also, Ford *et al*, 1987 found insignificant changes in blood urea in rats fed on Carmoisine. Besides, Abu El-Zahab *et al*., 1997found that blood urea and serum creatinine in rats supplemented with synthetic food colors remained unchanged throughout the experiment.

The increase in serum cholesterol, and triglyceride levels obtained in this study are in accordance with results reported by previous studies Abou El-Zahab *et al*., 199); Himri *et al*., 2011who observed significant increases in serum total lipids, cholesterol and triglycerides in rats whose diets were supplemented with some food colorants in varying concentrations.

The current results of this study are in a contrary with Sharma *et al*., 2006 who reported that two doses of tomato red (blend of Carmoisine and ponceau 4R) showed a significant decrease in serum total cholesterol and triglycerides when Swiss albino mice consumed these colorants for 21 days as short term or 42 days as long term. Also, these results are in opposite with those reported by Ashour and Abdel Aziz, 2009 who noticed a significant reduction in serum total cholesterol and triglycerides level when food color azo dye (fast green) was consumed orally to male albino rats for 35 days.

Results of the present investigation revealed that food colorants caused a high significant decline in hemoglobin content agreement with the present work, Shadia *et al*.,2010 demonstrated a reduction of hemoglobin when Tartrazine was administered to the diet of mice. Furthermore, by long-term feeding study on Red 2 G dye at the dose of 130 mg/kg b.wt. /day in the mouse and 32 mg/kg b.wt. /day in the rat, the spleen showed enlargement with an increased deposition of iron. In the mouse, accelerated erythropoiesis was observed and the rats showed necrosis of elastic. Above 0.5% of dye in the diet, adverse effects were observed in the spleen, liver and bone marrow. Heinz body formation in the erythrocytes was also observed in both species of rodents (rats& mice) WHO,1981. In The same aspect, Abu El-Zahab *et al.,* 1997 mentioned that rats supplemented with sunset yellow, Carmoisine and brilliant blue for 60 days exhibited a significant decrease in hemoglobin content as well as red cell count. These changes induced by food colorants may be due to the prevention of red blood cell synthesis via inhibition of erythropoiesis in the bone marrow.

On the other hand, Shaker *et al*., 1989 noted an increase in hematological content of rats received 0.1% chocolate brown color (0.1% w/w) consisting of Tartrazine, NovalCoccine, Carmoisine and Indigocarcarmine.

While, Hooson *et al*., 1975 found that indigo carmine had no effect on the total erythrocytes count. Moreover, Ford *et al.,* 1987 stated that Carmoisine (given in high doses for 6 months) did not cause any changes in the hematological investigations of rats.

Also, Borzelleca &Hallagan 1988 using Tartrazine in high doses and long terms on rats revealed insignificant effects on the hematological parameters of these animals.

The present study, exhibited no significant change in serum glucose of color foods compared to control group Table (4) with short term 2 months show a highly significance in fasting serum glucose fed on low or high doses from Tartrazine ,Carmoisine and brilliant blue. The present study are in a contrary with Amin *et al*.,2010 who demonstrated that high dose of synthetic color (Tartrazine at 500 mg/kg b.wt) or low dose of Tartrazine (15 mg/kg b.wt) caused no significant increase in serum glucose concentration. The present study are in agreement with Himri *et al.,* 2011 who found that a significant increase in serum glucose concentration when administration synthetic color (Tartrazine and sulfonic acid) at low and high dose compared to control group.

The elevation of glucose level can be explained by stimulation of glycogenolysis and gluconeogenesis by the liver with a temporarily loss of endocrine functions of pancreas leading to hyperglycemia Al-Shinnawy, 20009.

CONCLUSION

Food azo dyes like Tartrazine, Carmoisine and brilliant blue can affect adversely and alter biochemical markers in vital organs e.g. liver and kidney not only at higher doses but also at low doses. Tartrazine and Carmoisine not only cause changes in hepatic and renal parameters but also their effect become more risky at higher doses because they can induce oxidative stress by formation of free radicals. Therefore, it is necessary to create consumer awareness regarding the ill effects of these food azo dyes and mention the type and concentration of each material added to food.

Based on our results, we believe that more extensive assessment of food additives in current use is warranted

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تأثير الألوان الصناعية الغذائية على حيوانات التجارب

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المستخلص

تجعل الألوان الصناعية الأطعمة أكثر جاذبية وإقبالاً للأطفال، ولكن تساهم الألوان الصناعية في فرط النشاط لدى الأطفال، كما تساهم في إحداث إضطرابات بصرية وإضطرابات في التعليم، تلف الأعصاب وقد تكون مسببة للسرطان.

تم تقييم التأثيرات السامة للأصباغ الاصطناعية (بريلانت بلو وتارترازين وكارمويزين) علي الجرذان وذلك لقياس تأثيرهم علي وظائف الكلي والكبد وزيادة وزن الجسم.

وتم تغذية الفئران بالأصباغ الاصطناعية يوميا لمدة ٦٠ يوماً عن طريق الفم في جرعيتن (جرعة منخفضة وجرعة أخري عالية) ثم يلي ذلك جمع عينات المصل لتحديد اليوريا والكرياتينين ALT,AST فوسفاتيز قلوي، الكوليسترول في الدم، الدهون الثلاثية وتقدير الهيموجلوبين والجلوكوز.

وأظهرت النتائج زيادة معنوية في الكوليسترول والدهون الثلاثية و ALT,AST بالإضافة إلي مستويات اليوريا والكرياتينين في الجرذان المعاملة بينما سجلت انخفاض معنوي في نسبة تغير وزن الجسم وكان هذا التغير الكبير أكثر وضوحاً في الجرعات العالية عن الجرعات المنخفضة.