Effect of synthetic colorants (Sunset yellow and Ponceau 4R) in some biochemical and histopathological parameters of albino rats

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Abstract

Color additives are used for a wide variety of purposes and in great amount. However, the sharp increase in the use of synthetic food colorants in the past few years and additionally there is an uncontrolled use of synthetic color. The present investigation is planned to illustrate the effects of two synthetic color additives (Sunset yellow and Ponceau 4R) for biochemical and histopathologically on liver and kidney of rats. Thirty adult Wistar albino rats were randomly divided into three equal groups as follows; Animals in control group (CG) served as the control group received distilled water, Sunset yellow group (SG) received Sunset yellow (2g/kg bw) dissolved in distilled water, Ponceau group (PG) receive Ponceau 4R (4g/kg bw) dissolved in distilled water, treatments done for 45 days. Then the blood sample was collected from heart puncture to estimate serum urea, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels and the liver and kidney were removed for histopathological study. The results showed a significant increase ($P < 0.05$) in serum urea concentration (mg/dl) in SG and PG compared with CG, also a significant increase in AST and ALT compared with CG. The histological examination showed a fatty degeneration of the liver in (SG) while kidney showed (nephritis) meanwhile (PG) showed vacuolar degeneration with congestion in central vein of liver the kidney showed tubular degeneration. Therefore, synthetic colorants administration should not be used for large amount or for long period in man's diet or drink.

Key words: colorants, biochemical, histopathological, rats.

تأثير الملونات الصناعية (Sunset yellow and Ponceau 4R) على بعض المعايير الكيموحيوية والنسجية المرضية في الجرذان

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الخلاصة

تم استخدام المضافات الملونة بشكل واسع ولاغراض عديدة وبكميات كبيرة، إن الزيادة الحادة في استخدام الملونات الغذائية في السنين الأخيرة وبشكل غير متوازن وبخاصة الملونات الغذائية ذات المنشأ الصناعي. لقد صممت هذه التجربة لمعرفة مدى تأثير بعض الملونات الغذائية الصناعية (Sunset yellow and Ponceau 4R) على وظيفة والتركيب النسجي للكبد والكلى. تم أخذ 30 جرذاً وزعت بشكل عشوائي ومتساو إلى ثلاث مجاميع المجموعة الأولى (CG) مجموعة سيطرة جرعة بماء مقطر، المجموعة الثانية (SG) جرعت بالمادة اللونية الصفراء Sunset yellow (2g/kg bw) ومجموعة الثالثة (PG) جرعت بالمادة اللونية الحمراء Ponceau 4R (4g/kg bw) ومجموعة سيطرة جرعة بماء مقطر. استمرت التجربة لمدة 45 يوماً ثم تم جمع الدم لأجراء الفحوصات الكيموحيوية والتي شملت يوريا الدم، فحص الانزيم الناقل الأميني (ALT) و (AST)، كما تم أخذ الكبد والكلى لأجراء الفحوصات النسجية المرضية. أظهرت النتائج ارتفاع معنوي في تراكيز يوريا الدم و الانزيم الناقل الأميني (ALT) و (AST) في المجموعتين المعاملتان (SG & PG) بالمقارنة مع مجموعة السيطرة (CG)، كما وأظهر الفحص النسجي في مجموعة (SG) أضرار الكبد تنكساً دهنياً أما الكلى أوضحت ارتشاحاً للخلايا الالتهابية في متن الكلى، أما الفحص النسجي المرضي لمجموعة (PG) أضرار الكبد تنكس خلوي مع احتقان الاوردة المركزية أما الكلى أظهرت تنكس في النبيبات الكلوية. إن الملونات الغذائية ذات المصدر الصناعي يجب عدم الاكثار منها أو اخذها بصورة مستمرة ولمدد طويلة في الطعام أو الشراب.

الكلمات المفتاحية: الملونات الصناعية، المعايير الكيموحيوية، النسجية المرضية، الجرذان.

Introduction

Color additives are used for a wide variety of purposes in foods, drugs and cosmetics (1). The wide range of food additives, running into more than 25000 items used to preserve, dye or enhance foods (2 and 3). In Iraq there has been a sharp increase in the use of synthetic food colorants in the past few years and additionally there is an uncontrolled use of synthetic color particularly in food mostly consumed by children. Natural and synthetic color additives were used extensively (4). Many azo compounds are genotoxic in short-term tests and carcinogenic in laboratory animals (5 and 6). Suggested that food additives can be toxic, causing hyperactivity in sensitive children. Despite the importance of food colorants, there is an ever growing concern about the adverse effects of synthetic food colorants on human health (7). Most of food additives used in growing countries are not permissible (8). azo dye that is used to color bakery goods, cereals, beverages, dessert powders, candies, gelatin desserts, sausage, and numerous other foods, as well as cosmetics and drugs (9). Food colorants such as sunset yellow and ponceau 4R are azo dyes (used all over the world in great amounts) which are derived from chemical substances (10). Sunset yellow or sunset yellow is a synthetic azo dye, manufactured from aromatic hydrocarbons from petroleum. When added to foods, it is denoted by E110 (11). Ponceau 4R (E 124) it is also an azo dye has at least 115 synonyms in use, the most commonly used synonyms in published literature are Ponceau 4R, New Coccine Food Red 102 and Coccine red. Ponceau 4R is soluble in water and slightly soluble in ethanol (12). In 2001, Tsuda *et al.* reported that food colorant induce colon DNA damage at a very low dose in mice. More attention must be focused on the physiological and pathological effects of color additives for safety purposes such as identification of pharmaceuticals (14). The present investigation is planned to compare and illustrate the effects of two synthetic color additives on liver and kidney of rats.

Materials and methods

Animals

30 Male and female Wistar rats weighing between 170 and 200 g were housed in a controlled room with a 12 h light-dark cycle and temperature of $22 \pm 2^\circ\text{C}$ (Animal house colony of Embryo Research and Infertility Treatment Institute – Al-Nahrain University). They were kept in transparent plastic cages with free access to water and dry rat pellets feeds.

Chemicals

Sunset yellow (E110) and Ponceau 4R (E102) were purchased from Roha/deychem PVT (India).

Experimental design

The animals were divided into 3 groups each group containing 10 animals each:- Sunset yellow group (SG), Ponceau group (PG) and Control group (CG). The animals in SG were treated with sunset yellow receive half LD50 (2g/kg bw) dissolved in distilled water according to study done by Lu and Lavallée (1964) also Sasaki *et al.* (2002). The animals in PG were treated with Ponceau 4R receive half LD50 (4g/kg bw) dissolved in distilled water, according to study done by Gaunt *et al.* (1967). For the control group (CG) it was administered with distilled water only. All groups treated by daily oral gavage for 45 days. The animals were observed daily for general conditions. They were weighed once every ten days during the administration period, to calculate the dose. At the end of experiment, all rats were deprived of food, but not water, overnight and then blood samples were collected via the heart for serum biochemistry. Then animals were sacrificed.

Biochemical analysis

Blood samples were taken from heart of rats under ether anesthesia by inhalation at the end of the study. After centrifugation at 3000 rpm for 15 min, serum was separated. Serum samples were analyzed for determination of the levels of urea and for the measurement of enzyme activities of aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined spectrophotometrically by using commercial kits, according to the BioLinear chemicals kits company (SPAIN). All

biochemical tests will done by Unico spectrophotometer (Germany).

Histopathology study

At the end of the treatment, the liver and kidney of different groups were removed and fixed in 10% neutral buffered formalin. Paraffin sections of 5 micron thick, were routinely stained with haematoxylin and eosin (H&E) (17) and examined under in a light microscope (CYAN, China).

Statistical analysis

Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results were expressed as mean $\pm$ S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses (18).

Results

The statistical analysis for Serum urea concentration revealed that the SG and PG showed a significant increase ($P < 0.05$) in serum urea concentration (41.0 ± 6.1 and 39.8 ± 4.2 mg/dl) respectively compared with CG (28.0 ± 3.9 mg/dl). Mean while the Serum AST concentration (mg/dl) revealed that the SG and PG showed a significant increase ($P < 0.05$) (59.9 ± 3.2 and 59.7 ± 3.1 mg/dl) compared with CG (46.5 ± 4.5 mg/dl). Also SG and PG was showed significant ($P < 0.05$) increased in ALT concentration at (29.3 ± 4.7 and 22.9 ± 1.3 mg/dl) as table (1).

Table (1) Evaluation of serum level with different parameter.

Group	Urea(mg/dl)	AST(mg/dl)	ALT(mg/dl)
SG	41.0 ± 6.1 b	59.9 ± 3.2 B	29.3 ± 4.7 b
PG	39.8 ± 4.2 a	59.7 ± 3.1 A	22.9 ± 1.3 a
CG	28.0 ± 3.9 a	46.5 ± 4.5 A	9.0 ± 3.1 a

CG = Control Group. SG= Sunset yellow Group. PG=Ponceau Group .Values are expressed as mean $\pm$ SE. n=10/group. The letters denote significant difference ($P < 0.05$).

The results of histological examination . In(CG) no structural changes were identified by histopathology in the liver, kidneys. The histopathological studies of (SG) showed fatty degeneration of the liver (Fig.1), while kidney showed inflammatory cells infiltration in the glomerula and interstitial tissue (nephritis) (Fig.2). Meanwhile the histopathological studies of

(PG) showed vacuolar degeneration with congestion in central vein of liver (fig.3), the kidney showed tubular degeneration (fig.4).

Fig.(1) Liver of SG shows fatty degeneration of hepatocytes (→) (H&E 400 X).

Fig.(2) Kidney of SG shows inflammatory cell infiltration (→) (H&E 400 X).

Fig.(3) Liver of PG shows vacuolar degeneration (→) with congestion (→) (H&E 400 X).

Fig.(4) Kidney of PG shows vacuolar degeneration (→) (H&E 400 X).

Discussion

The study showed increase in serum urea levels significantly ($P < 0.05$) in SG and PG as compared to CG. These results are in agreement with Varley (1976) who decided that the blood urea can be increased in all forms of kidney diseases such as hydronephrosis congenital cystic kidney, renal tuberculosis, condition in which deposition of calcium occurs as hypervitaminosis D. The data obtained revealed a significant increase in AST and ALT activities in treated groups these result may be due to the haptic potency of these colors resulting in destructive changes in the hepatic cells. The colors were administered orally and, hence, they reach the liver first through the portal vein, or may be due to alteration in permeability of cell membrane, increasing the synthesis of the enzyme or decreasing rate of degradation of enzyme (19). The effect of the colorants on the liver

is in accordance with Abdel-Rahim *et al.* (1987a, b and 1989), Ibrahim *et al.* (1988), Gaunt *et al.* (1972) and Abou El-Zahab *et al.* (1997) who recorded a pronounced increase of serum and liver transaminases activity in rats ingested synthetic colorants. The pathological change in liver in all treated groups may occur due to toxic and oxidative injury (26; 27 and 28). The cellular infiltrations in kidney of SG group which may be due to allergies (29) Our findings are also in agreement with Stoews and *et al.* (1973), Atef *et al.* (1991) and Rowe and Rowe, (1994), which decided that sunset has the following side effects artica-ria. The pathological change of kidney in PG group it may be injured by any toxic, metabolic and immunologic mechanism (33 and 34). The toxic irritant substances brought to the kidney by circulatory blood cause degenerative changes in the kidney tissues (35).

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