# Effects of some synthetic coloring additives on DNA damage and chromosomal aberrations of rats

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## ABSTRACT

*Today, food colorants additives are randomly used in many foods. However, their use in food is still controversial. It causes and will cause severe tension to the consumers as the general health sensitivity of people increases. The present study on rats aimed to investigating the genotoxic effects induced by tartrazine and chocolate brown as synthetic food coloring agents. Such effect might explain hyperactivity of children that was claimed to be associated with consumption of food colorants. The rats were divided into five equal groups, each composed of 4 rats, as follows: The 1st group ($G_1$) served as untreated control. The 2nd ($G_2$) and 3rd ($G_3$) groups were orally treated with a daily dose of tartrazine 7.5 and 15 mg/kg b.wt., respectively. The 4th ($G_4$) and 5th ($G_5$) groups were orally treated with a daily dose of chocolate brown 0.15 and 0.3 mg/kg b.wt., respectively for 7 weeks. Two rats from each of the experimental groups were fasted and sacrificed under anesthesia. Our results revealed that tartrazine and chocolate brown caused DNA liver and kidney damage as detected by comet assay. Chromosome ring and end to end association were the most common abnormalities observed on bone marrow cells of treated rats. The results indicated that some of the colorants used have a retard destructive effect on some vital organ functions. Therefore, large quantities and/or long periods of colorants administration should not be used as additive in man's diet or as a drink. Hence, these colorants should restrictly be used in nutritional therapy. And more caring must be done to prevent our children from eating or drinking large amount of food containing these colorants or for long period.*

***Key word:*** *Comet assay, chromosomal aberrations, DNA damage, tartrazine, chocolate brown.*

## INTRODUCTION

A wide range of food additives, running into more than 2,500 items are used for various purposes, including preservation, coloring and increase nutrient value (Toledo, 1999). Some food additives, however, have been prohibited from use because of their toxicity. These additives proved to induce DNA damage in bacteria, fungi, insects and mammalian cells *in vivo* and *in vitro*. They also cause chromosomal aberrations in mammalian cells, including human cells (IARC, 1983). The individual response varies not only according to dose, age, gender, nutritional status and genetic factors, but also according to long term exposure to low doses (Sasaki *et al.*, 2002).

Several metabolites of these substances, such as nitrous compounds, have been found to be carcinogens. The Food Drug administration (FDA) established acceptable daily intake (ADI) for tartrazine of 7.5 mg/kg/day (Walton *et al.*, 1999). Whereas ,

the ADI for chocolate brown is 0.15 mg/kg/ day (FDA, 1985). Tartrazine is an azobenzene artificial yellow, because it is a nitrous derivative (azo class) it is reduced in the organism to an aromatic amine which is highly sensitizing. Its main metabolite identified to date is sulfanylic acid (Chung *et al.*, 1992**).** Tartrazine has been implicated as the food additive most often responsible for allergic reactions, having thus been targeted by the scientific community. Some countries such as Sweden, Switzerland and Norway have withdrawn tartrazine on the grounds of its anaphylactic potential (Wüthrich, 1993**).**

The alkaline comet assay is a rapid, simple, sensitive and versatile technique, and can be used to investigate the genotoxicity of industrial chemical, biocides, agrochemicals and pharmaceuticals (Tice 1995 and Singh 2000). The technique is capable of detecting a wide variety of DNA damage and lesions such as DNA single strand breaks, double strand breaks, base damage (Tice *et al.*, 2000). The basic of the comet assay is the migration of DNA in agarose matrix under electrophoretic conditions. When viewed under microscope, a cell has the appearance of a comet with a head (the nuclear region) and a tail containing DNA fragments or strands migration in the direction of the anode (Duez *et al.*, 2003). The comet assay can be applied to any tissue in the given *in vivo* model, provided that a single cell/ nucleus suspension can be obtained. Therefore, the comet assay has potential advantages over other *in vivo* genotoxicity test models. Merk and Speit (1999) and Sasaki *et al.* (2002) using the comet assay showed that tartrazine starting at 10 mg /kg b.w., induced dose related DNA damage in the rats colon after oral administration.

Tartrazine induced chromosomal aberrations and sister chromatid exchanges (SCEs) in mice and rat bone marrow cells and showed a significant increase in chromosomal aberrations in the higher concentrations (Giri *et al.*, 1990). El-saadany (1991) studied the effect of synthetic chocolate brown additive administered to healthy adult male albino rats. He found that food additives significantly increased serum protein, RNA, while DNA and T3 hormone were insignificantly elevated. Aboel-Zahab *et al.* (1997) found that, adult male albino rats fed on diets with chocolate colors agents for 60 day periods, had significantly decreased rat body weight, serum cholesterol and HDL-cholesterol fraction, while liver enzymes (S.GOT, S.GPT and alkaline phosphatase), total protein and globulin fractions were significantly elevated.

As the food colorants are widely used in our food and little is known about their genotoxicological effects, the present study was designed to investigate *in vivo* the genotoxic effects on the liver and kidney cells of rats induced by tartrazine and chocolate brown as synthetic food coloring agents.

## MATERIALS AND METHODS

### Food colors additives

Tartrazine yellow 700 and chocolate brown E102 were obtained from the local market and were mixed with diet and drink water of albino Wistar rats.

### Selection of doses

Tartrazine and chocolate brown were administered orally according to Walton *et al*. (1999**)** and FDA (1985).

### Animals and treatments

Twenty albino male Wistar rats weighing 250-280 gram were obtained from the Animal House of the Faculty of Science, Fayoum University, Egypt. All animals were subjected to one week acclimatization before the start of experimental procedures and were equally divided into 5 groups, approximately equal in weight, and the animals were kept in cages

(four animals / cage). Group one ($G_1$) served as control, which was provided normal diet and water *ad libitum*. While, groups 2($G_2$) and 3($G_3$) were given 7.5 and 15mg/kg/b.w./day from tartrazine mixed with diet and water *ad libitum,* respectively. Groups 4 ($G_4$) and 5 ($G_5$) were given 0.15 and 0.3 mg/kg b.w / day from chocolate brown E102 mixed with diet and water *ad libitum,* respectively. The animals were weighed at the initial and final experiment period. The animals were sacrificed after 7 weeks.

On day 50 of the dosing period, were killed by decapitation. Different organs namely the heart, liver, spleen, kidneys and testes were carefully dissected out and weighed (absolute organ weight). The relative organ weight of each animal was then calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \text{X100}$$

**DNA damage detected by the comet assay**

**Sampling**

The liver and kidney were rapidly removed and quickly minced, suspended in chilled homogenizing buffer (pH7.5) 0.075 M NaCl and 0.024 M $Na_2$ EDTA, and then gently homogenised in ice using homogeniser, (Sasaki *et al*., 1997**).**

**Comet assay**

The comet assay used in the present study was applied under alkaline conditions, using ordinary microscope slides, as previously described by Tice *et al*. (2000**).** The nuclei were obtained and slides prepared from nuclei isolated by homogenisation (Sasaki *et. al.,* 1997). Electrophoresis was conducted at 4 ºC in the dark for 15 min at 25 V and approximately 250 mA. The slides were stained by 50 µl of 20 µ g /ml ethidium bromide. About 50 nuclei per slides were examined and photographed at 200X magnifycations with the aid of a fluorescence microscope, using the public domain software for image analysis based on the NIH image system (Helma and Uhi, 2000). For the migration, the difference between the length of whole comet (length) and the diameter of the head (diameter) were measured for 50 nuclei per organ per rat. Mean length of DNA migration from each animal for an organ was compared.

**Calculation of results**

The DNA damage was quantified by measuring the displacement between the genetic material of the nucleus (Comet head) and the resulting (tail). Tail moment and tail DNA % are the two most commonly used parameters to analyze Comet assay results

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$$\text{Tail DNA\%} = \frac{\text{Tail DNA intensity}}{\text{Cell DNA intensity}} \text{X100}$$

Tail moment = Tail DNA % X Length of tail

**Chromosomal aberrations**

At the end of experimental period (50 days), two animals of each of the five groups were injected intraperitoneal (i.p.) with colchicine (4mg/kg) 1 hour prior to sacrifice by decapitation. The bone marrow cells were collected from the femora according to (Khuda *et al.*, 2002**)**. Slides were stained with 10% phosphate buffered Giemsa (pH7.0) for 10 min. Around 100 metaphases were studied from each animal and the different types of chromosomal aberrations were scored. The results of chromosomal aberrations were analysed.

**Statistical analysis of the data**

Body weight, body weight gain, chromosomal aberrations and relative organ weight data were analysed for homogeneity of variance using the General Linear Model procedure of statistical analysis system SPSS (1997**)**. Means of treatments were compared using Duncan multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

**Body weight gain and relative weight organs of male rats**

The results revealing the mean of body weight, body weight gain and relative weight of organs are presented in Tables (1) and (2), respectively. The differences in mean body weight gain between control and treated groups (7.5mg/kg tartrazine and 0.15 and 0.3 mg/kg chocolate brown) were non- significant. On the other hand, gain in body weight was slightly retarded in rats fed on diets containing 15 mg/kg b.w / day of tartrazin (Table 1). The present finding indicates that the high concentration of tartrazine in diets induced a retardation in growth. These results are in agreement with Ershoff (1977**)**. In explanation for this retardation of body weight gain for the rats treated with the highest concentration of tartrazine could be the rejection of the diet due to its palatability or to the higher concentration of potential energy sources contained in the diet (Alves de Lima *et al.*, 2003**)**.

***Table (1): The body weight and body weight gain of male fed diets containing different concentrations of tartrazine and chocolate brown (Mean ± SE).***

| Treatments | Initial weight (g) | Final weight (g) | Weight gain (g) |
|---|---|---|---|
| Control | 255.5 ±13.1 | 314.0 ± 24.0 | 58.5 ± 11.48 |
| 7.5mg/kg tartrazine | 279.0 ± 4.5 | 323.5 ± 9.5 | 44.5 ± 6.3 |
| 15 mg/kg tartrazine | 257.5 ± 10.5 | 295.5 ± 22.2 | 38.0 ± 12.25 |
| 0.15 mg/kg chocolate brown | 269.0 ± 19.8 | 342.25 ± 23.9 | 73.75 ± 9.41 |
| 0.3 mg/kg chocolate brown | 256.0 ±5.6 | 308.75 ± 8.6 | 52.75 ± 3.22 |

***Table (2): The relative organ weights for male rats fed diets containing different concentrations of tartrazine and chocolate brown colors (Mean ± SE).***

| Treatments | Heart | Liver | Kidneys | Spleen | Testis |
|---|---|---|---|---|---|
| Control | 0.256±0.00 $^{C}$ | 3.123±0.03 $^{A}$ | 0.652±0.01 $^{A}$ | 0. 251± 0.00 $^{C}$ | 1.036±0.01 |
| 7.5mg/kg tartrazine | 0.305±0.00 $^{AB}$ | 3.055±0.01 $^{AB}$ | 0.541±0.01 $^{C}$ | 0.2755±0.00 $^{BC}$ | 0.958±0.00 |
| 15 mg/kg tartrazine | 0.263 ±0.01 $^{C}$ | 2.927± 0.01 $^{B}$ | 0.641±0.01 $^{A}$ | 0.292±0.01 $^{B}$ | 0.924±0.12 |
| 0.15mg/kg chocolate brown | 0.316 ± 0.00 $^{A}$ | 2.742±0.06 $^{C}$ | 0.662±0.00 $^{A}$ | 0.202±0.02 $^{C}$ | 1.056±0.00 |
| 0.3 mg/kg chocolate brown | 0.288 ±0.00 $^{B}$ | 2.68±0.03 $^{C}$ | 0.584±0.01 $^{B}$ | 0.3317±0.01 $^{A}$ | 0.921±0.06 |

A, B.C significant at $p \leq 0.01$

A significant increase was observed in the relative weight of heart at 7.5 mg/kg tartrazine and 0.15 and 0.3 mg/kg chocolate brown. However, the high level of tartrazine and the two levels of chocolate brown produced a significant decrease in the relative weight of the liver (Table 2). Also, there was an increase in spleen relative weight of groups treated with the high level of tartrazine and chocolate brown but there was no effect on testis relative weight was observed.

**DNA damage detected by the comet assay**

The migration of DNA from the organs examined for each treatment group is shown in Figs. (1 and 2) and the results are summarized in Table (3). An increase in DNA damage was indicated by an increase in migration length of the stained DNA as shown in Table (3). Increased DNA migration indicates the induction of DNA strand breaks and / or alkali-labile sites (ALS). The percentage of DNA in the tail is the most appropriate parameter to analyze induced DNA damage (De-Boeck *et al.*, 2000**).** Fig. (1) shows the level of DNA comet tail length in rat liver obtained from treated groups and control. Photo (LE) reveals liver rats in control rats. Photos (LA and LB) show the DNA damage detected by comet assay after 7 weeks feeding on 7.5 mg/kg and 15 mg/kg tartrazine, respectively. Whereas photos (LC and LD) show the DNA damage in liver of rats fed on 0.15 mg/kg and 0.3 mg/kg chocolate brown, respectively. Our results indicated that, the levels of DNA degradation (comet tail length) were dose- dependent.

***Table (3): Score of DNA damage in cells from different organs of male rat fed diet containing different concentrations of tartrazine and chocolate brown colors.***

| Treatments | Tail length (µm) | | DNA% | |
|---|---|---|---|---|
| | Liver | Kidney | Liver | Kidney |
| Control | 2.39 | 2.06 | 1.49 | 1.67 |
| 7.5mg/kg tartrazine | 3.03 | 2.41 | 2.46 | 1.38 |
| 15 mg/kg tartrazine | 5.76 | 3.98 | 4.68 | 3.23 |
| 0.15 mg/kg chocolate brown | 3.88 | 2.28 | 3.15 | 1.92 |
| 0.3 mg/kg chocolate brown | 4.30 | 3.35 | 3.49 | 2.72 |

As shown in Fig. (2) the obtained results displayed elevation of DNA comet tail length in kidneys of rats (KA and KB) for treated groups with 15mg and 0.3 mg / kg b.w / day of tartrazine and brown chocolate, respectively compared with control (KC). Tartrazine and chocolate brown induced liver and kidney DNA damage at various doses ( Figs. 1and 2) .Our results showed that tartrazine and chocolate brown are genotoxic in rats. A number of azo compounds are mutagenic in assay if chemical reduction or microsomal activation or both, are induced (Chung and Cerniglia, 1992). Orally administered tartrazine and chocolate brown are poorly absorbed to be metabolized so that tartrazine can be reduced *in vivo* when given orally but not when given by intraperitoneal injection. It must be concluded therefore, that reduction of this compound is carried out by the gastro-intestinal flora (Radomski and Mellinger, 1962). The carboxyl group in tartrazine was responsible for the blocking of biliary excretion (Ryan and Wright, 1962**).** When they reach to colon they can undergo extensive reduction by microflora, and the metabolites can be rapidly absorbed, it is also possible for the dyes to be reduced to free aromatic amins by mammalian azo reductase in the liver (**C**hung *et al.*, 1992).

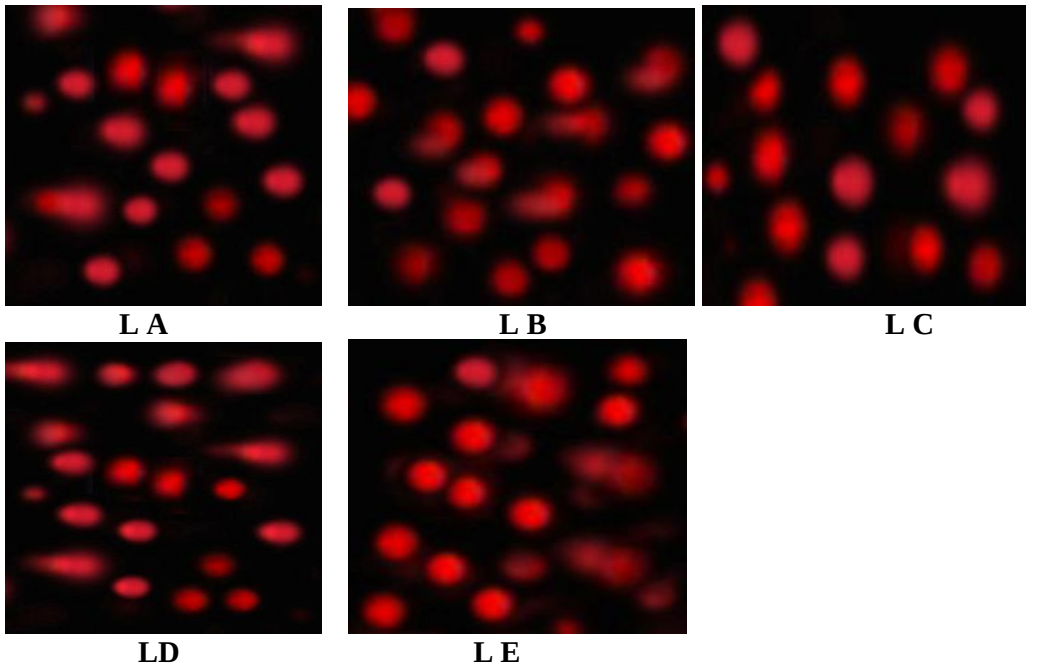


*Fig. (1): Photomicrographs representating DNA damage (Comet assay) in liver of rats fed diet containing tartrazine and chocolate brown for 7 weeks (LA-LD). Panel LA and LB: liver cells arising from male rat treated with 7.5 and 15mg/kg b.w. tartrazine, respectively; Panel LC and LD: liver cells arising from male rat treated with 0.15 and 0.3mg/kg b.w. chocolate brown ,respectively, Panel LE: liver cells of rat fed normal diet (Control).*

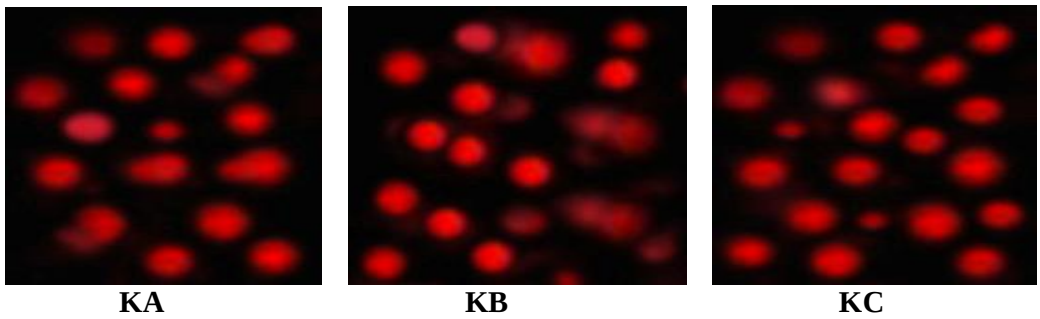


*Fig. (2): Photomicrographs representating DNA damage (Comet assay) in kidney of rats fed diet containing tartrazine and chocolate brown for 7 weeks (KA-KC). Panel KA and KB:Kidney cells arising from male rat treated with 15 and 0.3mg/kg b.w. tartrazine and chocolate brown respectively; Panal KC: kidney cells of rat fed normal diet (control).*

Then the genotoxicity of food color additives are dependent on their conversion to reactive metabolites, the activation is accomplished by acetyltransferases, which are widely distributed in animals (King *et al.,* 1997**)**. Because the activation process of these compounds in animals is complex, Salmonella tests with $S_9$ might not efficiently detect mammalian genotoxicity. The *in vivo* comet assay, which is a fast, simple and sensitive genotoxicological technique for measuring DNA damage in an individual cell type of animal or plant origin. The comet assay would be a more effective tool for detecting the genotoxicity of these food additives. The alkaline comet assay is becoming established as a genotoxicity test with many fold applications *in vitro* and *in vivo* (Speit *et al.,* 1999**).**

There is a relationship between DNA damage migration and alkaline condition (Miyamae *et al.,* 1997). They demonstrated that the alkaline condition is an important factor for the alkaline single-cell gel electrophoresis assay to detect the genotoxic effects of chemicals. Alkaline electrophoresis at pH 12.1 did not show an increase of migration of DNA, whereas at pH 12.6 or higher, alkaline –labile sites are quickly transformed to strand breaks (Tice *et al.,* 2000). In addition, DNA double strand breaks are considered to be closely related to cell death because it is suggested that they can lead directly to chromosome aberrations and the loss of genetic material (Myllyperkio *et al.,* 1999). Thus the DNA damage induced by food dyes may have been due to adduct formation, resulting in alkaline- labile sites.

**Chromosomal aberrations**

The data on the types of chromosome aberrations (CA) are given in (Table 4). Chromosome aberrations were observed like chromatid gap, chromosome ring, chromatid break, centromeric attenuation and centric fusion. Also numerical aberration such as polyploidy was recorded. Samples of the most frequent type of abnormality observed were given in Fig. (3). All the concentrations of tested food colors induced mitotic division abnormalities, but these abnormalities except end to end association weren't significant when compared to control. In the present study chromosomes ring and end to end association were the most common abnormalities that were observed on bone marrow cells of treated rats (Table 4). The increase in the frequency in chromosomal aberrations was dose-dependent in both tartrazine and chocolate brown. The production of (CA) is a complex cellular process. According to the prevailing theories, structural (CA) result from (i) direct DNA breakage, (ii) replication on a damaged DNA template, (iii) inhibition of DNA synthesis, and other mechanisms such as topoisomerase II inhibition. Although polyploidy was not frequent abnormality, such abnormalities showed that this chemical most probably inhibited the function of DNA topoisomerase II on DNA. Since this enzyme especially plays an important role in the mitotic chromosome segregation after DNA replication, if such an error has existed in event, aberrant mitosis such as polyploidy occurs (Cortes and Pastor, 2003)**.** Other food additives such as maltitol and sodium metabisulfite induced (CAs) in human lymphocytes as well (Canimoglu and Rencuzogullari, 2006)**.** Many researchers observed end to end association and ring chromosome as chromosomal aberrations. Patterson and Butler (1982) indicated that tartrazine (FD&C Yellow No.5) induced centromeric attenuation and dicenteric chromosomes in fibroblast cells of *Muntiacus muntjac in vitro*. In the studies of Ali *et al.* (1998 and 2001), carmoisine induced chromosomal aberrations including end to end association on the bone marrow cells. Yahagi

*et al.* (1988) found several azo dyes and their derivatives to be mutagenic or carcinogenic and suggested that these effects may involve modification of the DNA.

Chromosomal aberration analysis of animals as well as comet assay are used as the most useful assays to detect the potential genotocicity of chemicals (Blasczyk *et al.*, 2003). Abnormalities which were observed in the present study may be due to the accumulation effect of the dye since the animals received the dye for 50 consecutive days.

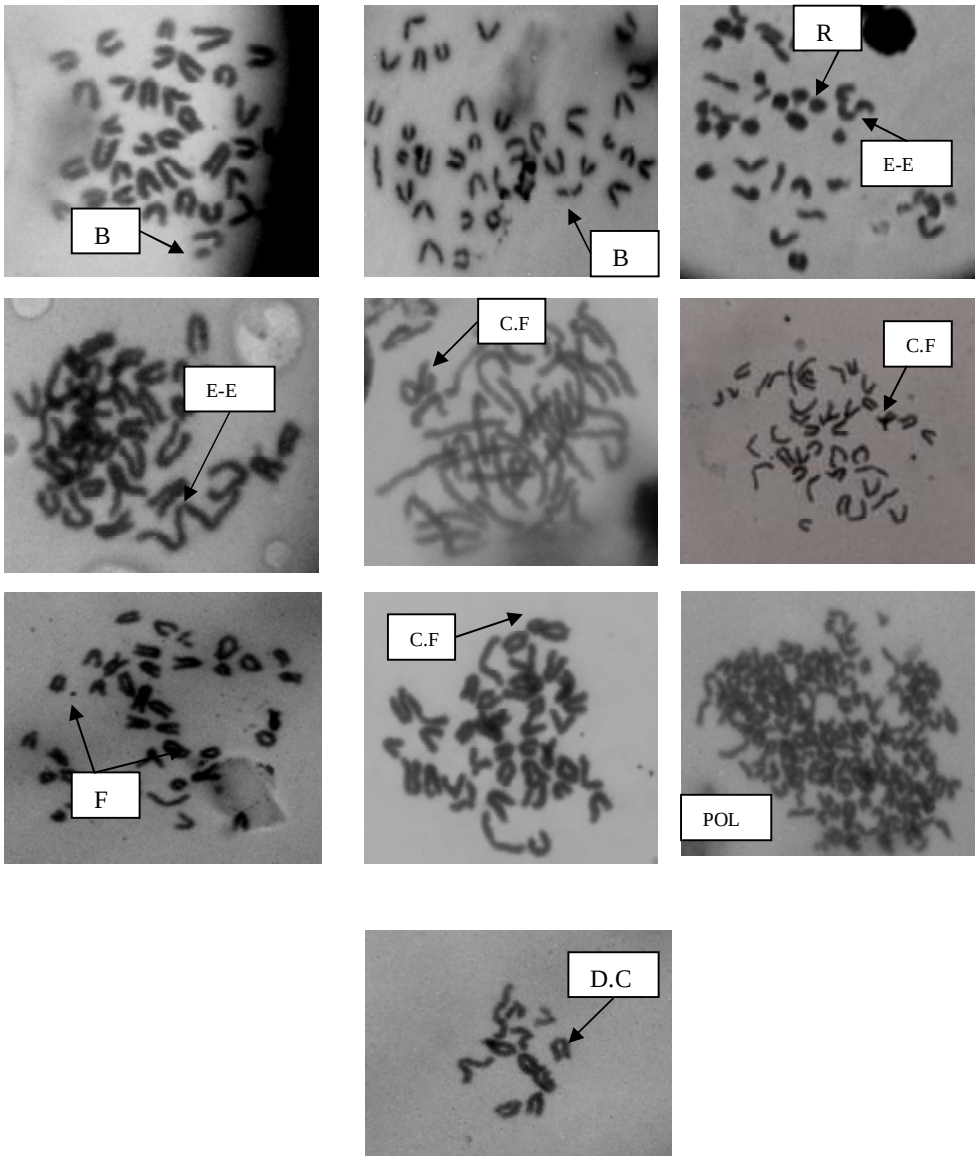


***Fig. (3):Photomicrographs of chromosomal aberrations in bone marrow cells of male mice induced by added food additive colors(tartrazine and chocolate brown) to rats diet. C.F.: Centric fusion , E.E. : End to end association,B: Chromatid break, D.C.: Dicentric, F.: Fragment, R.: Chromosome ring and POL: Polyploidy.***

***Table (4): Different chromosomal aberrations in bone marrow cells detected due to different doses of tartrazine and chocolate brown in rats diet and water drink.***

| Treatment | Dose mg/kg b.w. | No.of metaphase examined | Average mean of aberrant metaphases | Average means structural aberration (± SE.) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Ring | C.F. | E.E. | Break | Frag. |
| Control | 0 | 200 | 4.0 ±2.0 | 2.0±1.0 | 0.0 ± 0.0 | 1.5 ± 0.5 $^{bc}$ | 0.5 ± 0.5 | 0.0± 0.0 |
| Tartrazine | 7.5 | 200 | 11.5 ±1.5 | 6.5 ± 1.5 | 1.0 ± 0.0 | 3.5 ±0.5 $^{a}$ | 0.5 ± 0.5 | 0.0±0.0 |
| | 15 | 200 | 15 ± 3.0 | 8.5 ± 2.0 | 1.5 ± 0.5 | 2.5 ± 0.5 $^{ab}$ | 1.5 ± 0.5 | 1.0±0.0 |
| Chocolate | 0.15 | 200 | 7.0 ±1.0 | 5.5 ± 0.5 | 0.0 ± 0.0 | 0.5±0.5 $^{c}$ | 0.5 ± 0.5 | 0.5± 0.5 |
| brown | 0.30 | 200 | 11.5 ±2.5 | 7.5 ± 1.5 | 0.5 ± 0.5 | 2.0 ± 0.0 $^{abc}$ | 0.5 ± 0.5 | 1.0±0.0 |

C.F.: Centric fusion , E.E. : End to end association, Break: Chromatid break, Del.: Deletion, Frag: Fragment

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**الملخص العربي**

## دراسة تأثير بعض الالوان الاصطناعيه المضافة للاغذيه على الكروموسومات والمادة الوراثية ( د ن أ ) فى الجرزان البيضاء

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أصبح الاستخدام غير المرشد للالوان الاصطناعية المضافة للاغذية خطرا يهدد صحة الانسان وخاصة الاطفال، و من هذه المواد االترترازين (Tartrazine) و بنى الشيكولاتة حيث يتم اضافة هذه الالوان لبعض الاغذية لتحسين اللون والطعم ويضاف بتركيزات اعلى من التركيز الموصى به من قبل منظمة FDA .ولذلك نعنى بهذه الدراسة على الجرذان الي معرفة التأثيرات الوراثية التى تحدثها الالوان الاصطناعية . قسمت الجرذان إلى خمس مجموعات، كل مجموعة تشمل اربعة جرذان كما يلى: المجموعة الأولى كمجموعة ضابطة. المجموعة الثانية والثالثة تمت تغذيتها بالفم بجرعة يومية 7.5و 15 ملىجرام /كجم وزن حى من الترترازين على التوالى. أما المجموعة الرابعة والخامسه فقد تمت تغذيتها بالفم بجرعة يومية 0.15 و0.3 ملىجرام /كجم وزن حى من بنى الشيكولاته على التوالى لمدة 7 أسابيع. تم أخذ جرذان من كل مجموعة و تم تصويمها و ذبحها تحت تأثير مخدر خفيف. و قد أظهرت الدراسة بتقنية (Comet assay) ان الجرعات العالية من الالوان الاصطناعية المضافة لعلائق الجرذان لها تاثيرات معنوية فى حدوث تحلل للـDNA لخلايا الكبد والكلى لكلا اللونين ،كذلك وجدت اختلافات معنوية فى عدد الكروموسومات الشاذة ونوعيتها للمجاميع المعاملة مقارنة بالمجموعة الضابطة.ولهذا لا يفضل تناول كميات كبيرة ولمدة طويله من الالوان الاصطناعية والتى تضاف للطعام وللشراب وبخاصة للاطفال.