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Evaluation the Incidence of Genotoxic Effects of Artificial Food Favoring Additives in Bone Marrow Cells and Spleen Cells in Mice

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Abstract

Genetic material is the most important component of cells because it contains the genetic information; hence any disruption to the structure chromosome of cells could lead to very bad results. Genotoxicity use to evaluate the safety of any chemical compounds on genetic materials. Artificial food flavoring additive are chemical substances to produce specific placebo effects added to foods but impart specific flavor to it.

The present study evaluates the genotoxic effect of artificial food flavoring additive on structure of chromosomes at three different concentrations (50%, 100%and 150%) on both bone marrow cells and spleen cells in mice for fourteen successive days. It was found that artificial food flavoring additive at concentration (50% and 100%) show not significant increase in total chromosomal aberration in both bone marrow cells and spleen cells when compare to negative control ($p>0.05$) meanwhile at concentration 150% it causes a significant increase when compare to negative control ($p<0.05$) .The results have been showed that artificial food flavoring additive had a genotoxic effect at (50%, 100% and 150%).

Keywords: Artificial food flavoring additive, Bone marrow cells, Spleen cells, Genotoxicity, Chromosome

تقييم احتمالية حدوث تشوهات كروموسومية بسبب استخدام الاضافات المنكهة الصناعية في خلايا نخاع العظم وخلايا الطحال في الفئران

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الخلاصة

تعتبر المادة الوراثية من اهم مكونات الخلية وذلك لاحتوائها على المعلومات الوراثية للخلية ولذا اي تعرض سئ لهذة المادة يكون لة عواقب وخيمة على الخلية . فحص السمية الجينية واحد من اهم الفحوصات التي تستعمل لمعرفة تاثير المواد الكيمائية على الشكل العام للكروموسومات. تعتبر المنكهات الصناعية واحدة من اكثر المركبات استعمالا بالوقت الحالي حيث تضيف نكهة مميزة للطعام بدون التاثير على خواصة العامة . الهدف من البحث هو معرفة التاثيرات السمية الجينية للمنكهات الصناعية على الشكل العام للكروموسوم. حيث تم تحضير ثلاثة تراكيز مختلفة من هذة المنكهات الصناعية (٥٠% .١٠٠% و ١٥٠%) وتم اعطاؤها لمدة اربعة عشر يوما للفئران وفي اليوم الخامس عشر تم استخلاص خلايا نخاع العظم وخلايا الطحال لفحص التاثيرات السمية الجينية. .وقد أظهرت االنتائج ان الاضافات المنكهة بتراكيز (٥٠%و١٠٠%) تسبب زيادة غير معنوية للمجموع التكسرات الكوموسومية لخلايا نخاع العظم وخلايا الطحال عند مقارنتها بمحموعة السيطرة السالبة($p>.05$) وان تركيز ١٥٠% بسبب زيادة معنوية للمجموع التكسرات الكوموسومية لخلايا نخاع العظم وخلايا الطحال عند مقارنتها بمحموعة السيطرة السالبة ($p<0.05$). وبذلك يمكن ان نستنتج ان الاضافات المنكهة الصناعية لديها تاثير سام على المادة الوراثية وان هذي التاثيرات السامة تزداد مع زيادة التركيز .

الكلمات المفتاحية:الاضافات المكنهة الصناعية , خلايا نخاع العظم, خلايا الطحال , السمية الجينية , الكوموسومات .

Introduction

Artificial food flavoring additive is chemical substances to produce specific flavor when added to foods [(1)]. In the past, different techniques have been used to preserve foods and make them more desirable by addition different natural compounds. In the present days, there is huge orientation for use different types of food additive to the foods [(2)]. Food additives and their metabolites are subjected to different types of toxicological analysis before marketing [(3)].

A previous study shows that about 75% of the Western diet is made up of different types of processed foods; it has been show that the individual consume 8-10 pounds of food additives per year [(4)]. Unfortunately, the children considered as the higher consumer for the food with these food additive [(5)]. Children are higher consumer for calories compared to adults because their activity is higher as compare to adult [(6)]. Again, the blood-brain barrier, being poorly developed early in life, which in turn affect the blood flow as well as permitting a toxic substance to passively cross into the central nervous system [(7)]. There are very little studies for the evaluation of this compound to the human compared to the animal studies [(8-9)]. Genotoxicity is destruction in the genetic materials within the cell which may lead to bad consequences like mutagenesis or cancer development [(10)]. The DNA in a human cell undergoes several thousand to a million damaging events in every day which most of them are detected by repair system [(11)].

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Any DNA mutations inherit if the DNA damage is not repaired by repair system before mitosis [12]. Cell division is a process in all living organisms. During cell division, eukaryotic cell is divided; DNA replication and cell growth take place in a coordinated way to ensure correct division and formation of progeny cells containing intact genomes [13, 14]. At the end of Eukaryotic cells division cycle the cells that generate either another copy of themselves or to generate gametes (sex cells) [15].

Materials and Methods

Artificial food flavoring additive has been bought from the Iraqi market.

Preparation of solutions for different artificial flavoring additive concentration.

Three different concentrations of artificial flavoring additive [16]

1. 50% artificial flavoring additive solution.

Prepared by dissolving 50 grams of Artificial flavoring additive in sufficient amount of distilled water to complete the volume to 100 ml of .Then, the solution is mixed by a vortex.

2. 100% artificial flavoring additive solution.

Prepared by dissolving 100 grams of Artificial flavoring additive in sufficient amount of distilled water to complete the volume to 100 ml of .Then, the solution is mixed by a vortex**.**

3. 150% artificial flavoring additive solution.

Prepared by dissolving 150 grams of Artificial flavoring additive in sufficient amount of distilled water to complete the volume to 100 ml of .Then the solution is mixed by a vortex.

Experimental model

Sixty Albino Swiss mice (*Mus musculus*) were used for two experiments. They were supplied by Animal house of Tikrit University. Their weights were 23-27 gram. They were divided into five groups; each was kept in a separate plastic cage. The animals were maintained at a temperature of 23 – 25°C, and they had free excess to food (standard pellets) and *ad libitum* of water**.** The animals were divided into five groups (six mice of each) as follow:

Group1: Mice were treated with distilled water. This group was served as negative control the dose was given orally for fourteen successive day days.

Group2: Mice were treated with a single dose (20mg/kg) of methotrexate giving intraperitoneally. This group was served as a positive control.

Group3: Mice were orally treated with (50%) of artificial food flavoring additive for fourteen successive days.

Group4: Mice were orally treated with (100%) of artificial food flavoring additive for fourteen successive days.

Group5: Mice were orally treated with (150%) of artificial food flavoring additive for fourteen successive days.

The different concentration of artificial food additive was given orally instead of drinking water. Mice were sacrificed by (spinal dislocation). Samples of bone marrow cells and spleen cells were taken and genotoxic analyses were carried out as described later [17].

Evaluation of genotoxicity

After fourteen days of treatment, all animals were injected intraperitoneally with 1mg/kg colchicine, and then two hours later they are sacrificed by cervical dislocation. Bone marrow samples were aspirated from the femur bone and processed using aseptic technique and Spleen cells samples for evaluation of mitotic index, but only bone marrow cells have been used for evaluation of micronucleus appearance [18].

Statistical Analysis

All the results were expressed as mean± standard deviation (m±STD). The data were analyzed by utilizing a computerized statistical package for the social sciences (Microsoft office excel 2010) program. Unpaired student t-test was performed for each group pair includes a comparison between negative control and tests groups after fourteen days of treatment. *P*-values< 0.05 were considered to be statistically significant. Two factors with no replication ANOVA test have been performed for each group pair includes a comparison between different test groups after fourteen days of treatment. *P*-values< 0.05 were considered to be statistically significant.

Results

In table (1),in bone marrow cells , artificial food flavoring additive at concentration 50% show not significant increase in total chromosomal aberration , chromosomal break, chromosomal gap and ring chromosome when compared to negative control *(p>0.05)* the rest individual chromosomal aberration show a significant increase at same concentration when compare to negative control *(p<0.05)*. At concentration 100% artificial food flavoring additive show non-significant increase in total chromosomal aberration, chromosomal break and ring chromosome when compared to negative control*(p>0.05)* , the rest individual chromosomal aberration show a significant increase at same concentration when compare to negative control *(p<0.05).*At concentration 150% artificial food flavoring additive show significant increase in all individual and total chromosomal aberration except ring chromosome when compare to negative control *(p>0.05).*

Artificial food flavoring additive at concentrations (50% and 100%) show a significant decrease in , chromosomal break, chromosomal gap , chromatid break, chromatid gap , acentric chromosome , dicentric chromosome, when compare to positive control *(p<0.05)* meanwhile Artificial food flavoring additive at concentration (150%) show non-significant decrease in, chromosomal break, chromosomal gap, chromatid break, chromatid gap , acentric chromosome , dicentric chromosome, when compare to positive control (*p>0.05)*. Artificial food flavoring additive show non-significant decrease in total chromosomal aberration at concentrations (50%, 100% and 150%) when compared to positive control (*p>0.05)*. Artificial food flavoring additive show significant differences in total chromosomal aberration and individual chromosomal aberrations at concentrations (50%, 100% and 150%) when compare between each other (*p>0.05)*.

In table (2),in spleen cells , artificial food flavoring additive at concentration 50% show non-significant increase in total chromosomal aberration , acentric chromosome, dicentric chromosomal and ring chromosome when compared to negative control *(p>0.05)* the rest individual chromosomal aberration show a significant increase at same concentration when compare to negative control *(p<0.05)*. At concentration 100% artificial food flavoring additive show not significant increase in total chromosomal aberration and ring chromosome when compare to negative control *(p>0.05)* , the rest individual chromosomal aberration show a significant increase at same concentration when compared to negative control *(p<0.05)*.at concentration 150% artificial food flavoring additive show significant increase in all individual and total chromosomal aberration except ring chromosome when compare to negative control *(p<0.05)*. Artificial food flavoring additive at concentrations (50% and 100%) show significant decrease in chromosomal gap , chromatid break, chromatid gap , acentric chromosome and dicentric chromosome, when compare to positive control *(p<0.05)* meanwhile Artificial food flavoring additive at concentration (150%) show non-significant decrease in individual and total chromosomal aberrations when compare to positive control (*p>0.05)*.

Artificial food flavoring additive at three different concentrations show significant differences in all individual and total chromosomal aberration except ring chromosome, chromosomal break and chromosomal gaps when compare among each other's *(p<0.05)*.

Table 1. Individual and total chromosomal aberrations in bone marrow cells after exposure to different concentrations of artificial food flavoring additive in mice

Bone marrow cells	Chromatid Break	Chromatid Gap	Deletion	Dicentric Chromosome	Acentric Chromosome	Ring Chromosome	Chromosome Breaks	Chromosome Gap	Total Chromosomal Aberration
Distilled Water (Negative control)	0.062± 0.008	0.064± 0.005	0.238 ± 0.027	0.190± 0.012	0.210± 0.007	0.036± 0.011	0.078 ± 0.008	0.056± 0.011	0.934± 0.081
Methotrexate(MTX) (positive control) 20mg/kg	0.196± 0.021*^{a}	0.218± 0.013*^{a}	0.356 ± 0.015 *^{a}	0.612± 0.019*^{a}	0.866± 0.035*^{a}	0.056± 0.017 ^{a}	0.130 ± 0.012 *^{a}	0.142± 0.036*^{a}	2.518± 0.273*^{a}
Artificial flavoring additive at concentration 50%	0.094± 0.015*^{Ab}	0.094± 0.021*^{Ab}	0.280± 0.022*^{Ab}	0.228± 0.015*^{Ab}	0.244± 0.025*^{Ab}	0.030± 0.020 ^{Ab}	0.084 ± 0.015 ^{Ab}	0.070± 0.019 ^{Ab}	1.14± 0.087 ^{Aa}
Artificial flavoring additive at concentration 100%	0.106± 0.021*^{Bc}	0.108± 0.016*^{Bc}	0.314± 0.018*^{Bc}	0.296± 0.046*^{Bc}	0.278± 0.026*^{Bc}	0.041± 0.015 ^{Ba}	0.090 ± 0.043 ^{Ba}	0.100± 0.025*^{Ba}	1.454± 0.100 ^{Ba}
Artificial flavoring additive at concentration 150% 150mg/kg	0.210± 0.016*^{Ca}	0.236± 0.021*^{Ca}	0.376± 0.017*^{Ca}	0.450± 0.142*^{Ca}	0.798± 0.076*^{Ca}	0.084± 0.043 ^{Ca}	0.126 ± 0.025 *^{Ca}	0.126± 0.021*^{Ca}	2.15± 0.088*^{Ca}

- Data are expressed as mean±S.D; n=6 animals in each group;
- *significantly different compared to distilled water (negative control) (P<0.05);
- Values with non-identical small letters superscripts (a,b,c) consider significant different when compared with methotrexate (positive control) (P<0.05).
- Values with non-identical capital letters superscripts (A,B,C) Significant different when compared between tests groups (P<0.05).

Table 2. Individual and Total chromosomal aberrations in spleen cells after exposure to different concentrations of artificial food flavoring additive in mice .

Spleen cells	Chromatid Break	Chromatid Gap	Deletion	Dicentric Chromosome	Acentric Chromosome	Ring Chromosome	Chromosome Breaks	Chromosome gap	Total Chromosomal Aberration
Distilled Water (Negative control)	0.046± 0.009	0.062± 0.001	0.210 ± 0.016	0.182± 0.008	0.200± 0.016	0.024± 0.006	0.060 ± 0.012	0.044± 0.011	0.826± 0.077
Methotrexate(MTX) (positive control) 20mg/kg	0.200± 0.016*[a]	0.198± 0.026*[a]	0.294 ± 0.027 *[a]	0.490± 0.016*[a]	0.752± 0.059*[a]	0.050± 0.007*[a]	0.118 ± 0.022 *[a]	0.134± 0.023*[a]	2.259± 0.180*[a]
Artificial flavoring additive at concentration 50%	0.072± 0.018*[Ab]	0.072± 0.008*[Ab]	0.252± 0.030*[Ab]	0.192± 0.008 [Ab]	0.218± 0.008 [Ab]	0.025± 0.017 [Ab]	0.078 ± 0.008 *[Ab]	0.064± 0.013*[Ab]	0.964± 0.086 [Ab]
Artificial flavoring additive at concentration 100%	0.086± 0.011*[Bc]	0.094± 0.009*[Bc]	0.288± 0.013*[Ba]	0.282± 0.053*[Bc]	0.270± 0.026*[Bc]	0.033± 0.016 [Aa]	0.086 ± 0.052 *[Aa]	0.088± 0.045 *[Aa]	1.35± 0.097 [Ba]
Artificial flavoring additive at concentration 150% 150mg/kg	0.202± 0.016*[Ca]	0.218± 0.013*[Ca]	0.316± 0.015*[Ca]	0.430± 0.055*[Ca]	0.768± 0.072*[Ca]	0.044± 0.024 [Aa]	0.104 ± 0.089 *[Aa]	0.114± 0.021*[Aa]	1.994± 0.090*[Ca]

- Data are expressed as mean±SD; n=6 animals in each group;
- *significantly different compared to distilled water (negative control) (P<0.05);
- Values with non-identical small letters superscripts (a,b,c) consider significant different when compared with methotrexate (positive control) (P<0.05).
- Values with non-identical capital letters superscripts (A, B, C) Significant different when compared between tests groups (P<0.05).

Discussion

The artificial food additive contains different chemicals substances considered as the major active chemical that responsible for the imparting specific flavor that given, the major active substances are (Monosodium Glutamate, Disodium Inosinate and Disodium Guanylate).

Monosodium Glutamate (MSG) is used in the food industry as a flavor enhancer which gives umami taste [19]. A previous studies have examined the toxicity of monosodium glutamate, it have been found that the use of this chemical associated with the induction of oxidative stress in different experimental animals after giving of chronic doses of monosodium glutamate [20]. Glutamic acid has been proposed as one of the amino acids using by the body during gluconeogenesis [21]. Increased influx of substances into the cells has been associated with increase the incidence of oxidative stress [22]. This has been corroborated in more recent reports in which hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, promote reactive oxygen species (ROS) accumulation that, in turn, promote cellular damage and contribute to many complications can be development and progression. ROS can directly damage lipids, proteins or DNA and modulate intracellular signaling pathways, such as mitogen-activated protein kinases and redox-sensitive transcription factors causing changes in protein expression and, therefore, irreversible oxidative modifications[23-24]. Hyperglycemia , induced by monosodium glutamate , is also known to increase the incidence of glucose auto-oxidation and labile glycation or intracellular activation of the polyol pathway which in turn it induce an oxidative degradation of the glycated protein which lead to increase of reactive oxygen species formations [25].

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell. ROS have been classified as harmful by-products of the normal aerobic metabolism process of the mitochondria and the increase of its production associated with large variety of diseases besides these harmful effects, if the production of ROS is under control, it plays physiological roles especially in cell signaling and regulating cell redox homeostasis [26]. One of the most dangerous deleterious effect of ROS is the disruption of cell division by interfering with many cellular components especially the genetic materials, when the body was unable to regulate high levels of ROS leading to many diseases characterized by both neurodegeneration and bone marrow failure as well as cancer [27], other previous finding showed that the overproduction of reactive oxygen species lead to chromosomal instability, which in turn increase the incidence of cancer and aging [28].

Disodium Inosinate and Disodium Guanylate, It is typically sold in a 50:50 mixture of the two ribosides. This combination with glutamates which imparts the umami [29].These two chemical substances have been estimated for the ability of induction of chromosomal aberration, the test was achieved in *vitro* using a Chinese hamster fibroblast cell line they found that both of these compounds cause a chromosomal aberration in these cell lines [30].

Conclusions

The results showed that artificial food flavoring additive had a genotoxic effect at (50%, 100% and 150%).

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