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Review Paper

A review on *in vitro* genotoxic effects of food Preservatives on Human Lymphocytes

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Abstract

Food Preservatives or other food additives are use to protect food from spoilage. Preservatives have hazards effects on human's organs and also have effects on individual cell. The different concentration of preservatives affects the chromosomes which lead to chromosomal breakage. Human lymphocytes are used for Genotoxic study of preservatives. Micronucleus test, Sister Chromatid Exchanges and Chromosomal aberration studies are used. Lymphocytes cell culture carried out with 4-5 different concentrations of preservatives and results of different studies were comparing with control values. 24 and 48 hrs. treatments of preservatives are given to lymphocytes and from their results the range of concentration are prepared which cannot be hazards for human's daily intake. This concentration range of preservatives can be later on approved by Food and Drug Administration.

Keywords: Genotoxicity, Food preservatives, Human Lymphocytes, Chromosomal aberration, Sister chromatid exchanges, Micronucleus.

Introduction

Food preservatives and other additives play an important role in present day's fast life food supplements. Preservatives and other additives is any chemical compound which is not the part of main food components, and can be added into food as per prefix allowable limit. These substances are added at the time of production or during processing, treatment, packaging, transportation or storage of food. Food additives other than preservatives are generally used for modify the food i.e. blending, thickening, flavoring, and to give different colors to food. Some of the additives also used in pharmaceuticals products[1].

Preservatives are the substances which prevent the decomposition of any type of microbial growth like fungal or bacterial growth or undesirable chemical changes[2].

Food and Drug Administration [FDA] regulates the concentration of additives which can be used in foods or other goods to ensure safety and reduce the overconsumption of hazards substances[3].

Food safety cannot be a newer concept of the modern era, it can be seen in all over the history of humankind development. In most of the developed countries, the problems were upraised after the 2nd world war. The spoilage of food was being a commonest problem and it can occur mostly due to activities of microbes.

Both the natural and chemical method of food preservation have been common for past 1000 to 8000 years[4]. In second half of 1988, the European Community formed a directives which can established the list of food additives with their usable amount[5]. The uses of the preservatives can be modulated by legislation across the European Union by forming certain directive[6].

The uses of preservatives and other food additives can be increased from past few years. In a present day's about 75% of the western diet can be made up of different processed foods, which contain a smaller part of additives. It can be determined that, the single person can consume an average of 8-10 Ibs of additives every year. The over consumption of food additives can cause adverse effects like, urticaria, eczema, angioedema, dermatitis, exfoliative, irritable bowel syndrome, nausea, vomiting, diarrhea, hyperactivity, rhinitis, migraine, bronchospams, anaphylaxis, and other behavioral disorders[3].

High doses of Preservatives and other additives have Hazards or toxic effects on animal/Human body. It can also have the direct effect on cell and chromatin materials. Many studies can be evaluated for genotoxicity of preservatives and other additives. In Tokyo 1976, Researchers were tested 134 Compounds. These compounds were mostly food additives, some of medical drug, pesticides, and another chemicals which can have most common use in laboratories or in industries. From 134 about 63 compounds were negative [N-Group], at high dose of these compounds cell were about to be killed, no significant increases of chromosomal aberration were observed.

17 compounds were rated as suspicious [S-Group]. These were not ranked as negative, because they produced more than 5.0% of chromosomal aberrations. 54 compounds were ranked as positive [P-Group]. These were strongly produce chromosomal aberration[7].

Sodium Benzoate [E211], Potassium Benzoate [E212], Sodium Sorbate [E201], Potassium Sorbate [E202] are most commonly used preservatives. Which can be used in Beverages like carbonated and non-carbonated, Cider, Margarine, syrup, Fruit juices, Fruit jam, Fruit butter, Pickles, and in some medicines[8]. Genotoxic effects of these preservatives on chromosome are mainly studied by some common parameters like Micronucleus technique, Sister Chromatid exchange, Chromosomal breakage and Comet assay.

FDA approved different concentrations of preservatives or food additives which can be used. Benzoic acid used upto 0.1%, Sodium benzoate less than 0.1%, Sodium Sorbate less than 0.3%, Potassium Sorbate less than 0.3%, Potassium benzoate upto 0.1%, and Sorbic Acid less than 0.2%.

Materials and methods

The Genotoxic study of preservatives and other food additives were carried out using Human Lymphocytes. In some of the other studies researchers can also used CHL-Chinese hamster fibroblast cell line[7], and Human Erythrocytes[9].

In 2008, P. Mpountoukas et al. were studied the Genotoxic effect of three commonly used preservatives Sodium benzoate, Potassium Sorbate, Potassium nitrate on human lymphocytes[1]. They were collected the blood sample from six normal persons, 2 male and 4 female; 20-30 age group; non-smoker and were not take any kind of drug for medical and other purpose. They were used the parameter Sister Chromatid exchange (SCEs) for study. For culture they were added 11 drops of blood in 5 ml of chromosome medium B (Biochrom 0303 H) and add 5-bromodeoxyuridine 5 µg/ml. They can use the different concentration 0.02, 0.2, 2, 4, 8 mM of Sodium benzoate, Potassium Sorbate, Potassium nitrate at the beginning of culture. Incubation can be done at 37ºC for 72 hrs in dark environment. The dark environment protects the Brdu from photolysis. 0.3 µg of Colchicine were added at last 2 hrs of incubation. After harvesting chromosome were prepared with modified Fluorescence plus Giemsa (FPG) Technique[10]. They were evaluated the statistical data analysis by ANOVA procedure and use Duncan test for pair-wise comparisons[11,12].

Researchers carried out a study to know the Genotoxic effects of benzoic acid in Human peripheral blood lymphocytes[13]. They were studied Chromosomal aberration (CA), Sister chromatid exchanges (SCEs), and Micronucleus test (MN test). They obtained blood from two individuals of 24-25 age; non-smoker; not exposed to any drug or mutagenic agent for last two years; and also not exposed to any ionizing agent for last 6 months. For SCEs they were took 0.2 ml of blood and add into 2.5 ml of Chromosome Medium B (Biochrom 5025) prespplemented with Brdu 10 µg/ml. 0.06 µg/ml of Colchicine were added during last two hrs. of incubation. Incubation at 37ºC for 72 hrs, cell harvested by centrifugation for 10 min at 216x g. After harvesting treatment of hypotonic solution of 0.075 M KCl were given for 30 min at 37ºC, then again centrifuged and fixed in fixative 3:1 Methanol:Acetic acid for 20 min, repeated 3 times. For CAs study, slides were stained with Giemsa and For SCEs FPG (Fluorescence plus Giemsa) stain technique were used[14]. 100 metaphase plate scored for CAs per donor and number of SCEs were count in 25 cells per donor about 1000 cells were determined from each donor for Mitotic index (MI) and for replication index (RI) 100 cells per donor were scored. For MN analysis they can add 5.2 µg/ml Cytochalasin B at 44 hr of incubation to block the process of cytokinesis. About 1000 binucleated cells can determined for counting micronucleus per donor. Here, they can took four different concentration of benzoic acid 50, 100, 200, 500 µg/ml and give 24 and 48 hrs treatment.

In 2009, another study carried out by researcher to know the Genotoxic effects of Potassium Sorbate in Human lymphocytes[15]. They were used all three parameters CAs, SCEs, and MN which was used in study of Benzoic Acid[13], the method used were also similar, but here the concentration of Potassium Sorbate was 125, 250, 500, 1000 µg/ml. They were carried out Comet assay according to the method of Singh *et al*[16]. For statistical analysis of SCEs and Comet assay they were used t-test, and for abnormal cells, CAs per cell, SCEs, mean MN and DNA damage they were used concentration-response relationships which can determine from correlation and regression coefficients.

In 2010, Researchers carried out Genotoxic studies of two food preservatives Sodium Benzoate and Potassium Benzoate[17]. The blood sample carried out from two healthy individual of age 25; non-smoker; no medication for last 3 weeks; no any radiological examination within last 3 months. For CAs and SCEs test they were used the methods of Evans and Perry and Thompson, with some modifications according to Yüzbaşioğlu's method[18]. For staining of SCEs assay with Giemsa were done by Speit and Houpter's method[14] with some modifications according to Mamur's method[15]. Preparation of Micronucleus was done according to Fenech[19] and Palus *et al.*[20]. Concentration of Sodium Benzoate was 6.25, 12.5, 25, 50, and 100 µg/ml, and concentration of Potassium Benzoate was 62.5, 125, 250, 500, and 1000 µg/ml.

In 2011, Genotoxicity testing carried out by a researchers in which the effects of Sodium Sorbate a Food Preservative studied in Human lymphocytes[21]. The Sodium Sorbate was prepared from sorbic acid according to Schiffmann and Schlatter method[22], with some modifications according to Schlatter's method[23]. They were used four different concentrations of Sodium Sorbate 100, 200, 400 and 800 µg/ml.

They were used CAs, SCEs, MN, and Comet assay techniques for study. Slides of SCEs were stained as per the method used by Speit and Houpter. Micronucleus assay was performed as per method used by Palus *et al.*[20], some modification can be done with the method used by Mamur[15]. Comet assay performed as per method of Singh *et al*[16] with some modifications according to Mamur's method[15].

In 2014, Researcher carried out a study to know the effects of Sodium Benzoate preservative in Human lymphocytes[2]. Four different concentration of Sodium Benzoate were used 0.5, 1.0, 1.5, and 2.0 mg/ml and two treatments were given, of 24 hr and 48 hr. RPMI media with 10% fetal bovine serum, antibiotics, and phytohemagglutinin M was used for culture. Incubation can be done in 5% CO_2 incubator at 37ºC. Micronucleus test performed as per Fenech and Palus' method[19],[20]. For Chromosome study, Chromosomes were stained by Giemsa[24].

Discussion

Chromosomal Aberration, Sister Chromatid exchanges and Micronucleus are the most useful assays to detect the Genotoxic effects of different chemicals[1,2,13,15,17,18]. Almost all researchers can get the positive result of above assays in compare to control.

P. Mpountoukas et al. concluded that the potassium sorbate was significantly increased the sister chromatid exchanges per plate in two higher doses 4mM and 8 mM ($p < 0.01$) in compare to the control group. And also caused more significant ($p < 0.01$) cell division delay. Sodium benzoate also show same result as potassium sorbate significantly ($p < 0.01$) increased sister chromatid exchanges per plate and caused significant ($p < 0.01$) cell division delay in 4 mM and 8 mM dose. 0.02, 0.2 and 2 mM doses also caused significant cell division delay in compare to control and higher doses. None of the doses of potassium nitrate can be increased the sister chromatid exchanges in compare to control only 0.02 mM dose can caused cell cycle delay ($p < 0.05$) in compare to 4 mM dose of potassium nitrate[1].

Yılmaz et al.[13] concluded that the benzoic acid was significantly increse chromosomal aberration in all of the test concentration in compare to negative control. But the chromosomal aberration induction was lower than the positive control. Benzoic acid also significantly increased the sister chromatid exchenges per cell in all of the test concentration in compare to negative control, and decrese the mitotic index.

Induction of chromosomal aberration and sister chromatid exchanges were dose dependent in both 24 and 48 hr. treatment.

In 2009, S. Mamur et. al concluded that the potassium sorbate was significantly increased the chromosomal aberration in three concentration 200, 500, and 1000 µg/ml for 24 hours of treatment and in two concentration 500 and 1000 µg/ml for 48 hours of concentration. Except 125 µg/ml of 24 hours treatment group, all the concentration in both 24 and 48 hours of treatment group significantly increassed the sister chromatid exchanges in compare to the negative control. potassium sorbate was not significantly induse micronucleus formation except 500 and 1000 µg/ml of 48 hour treatment group[15].

In 2010, N. Zengin et al. concluded that the sodium benzoate and potassium benzoate were significantly incresed the chromosomal aberration per cell in all concentration (SB: 6.25, 12.5, 25, 50, 100 µg/ml; PB: 62.5, 125, 250, 500, 1000 µg/ml) and in all treatment group 24 hr. and 48.

In compare to control group. SB and PB also significantly increasaed the sister chromatid exchanges per cell in all concentration and in all treatment group in compare to control, and cased cell cycle delay. SB an PB also significantly incresed micronucelus formation in almost all the test concentration for 48 hr. treatment in compar to control group[17].

S. Mamur et al. concluded that the sodium sorbate significantly incresed the chromosomal aberration per cell in all test concentration and in both treatment 24 and 48 hr. in compare to negative control, only one exception of 100 µg/ml for 24 hour treatment. Sodium sorbate also significantly incresed sister chromatid exchanges per cell in 400 and 800 µg/ml at both treatment of 24 and 48 hr. in compare to negative control. Sodium sorbate incresed the micronucleus formation in 400 and 800 µg/ml test concentration in compare to negative control[21].

M. Pongsavee concluded that the sodium benzoate significantly incresed micronucelus formation in almost all the test concentration and at both the treatment 24 and 48 hr. in compare to control. Sodium benzoate also caused the sister chromatid sepration and gaps in chromosome at higher concentration 2.0 mg/ml in compare to control[2].

Conclusion

According to all of the above studies, it can be concluded that the preservatives are genotoxic and have the some or more effects on Genome. It can causes genomic instability which is associated with increse rate of cancer[25]. More damaging effects of preservatives can be clarified by further and further sudies. It is necessary to kept all the preservatives under relentless inspection and re-assess whenever needed.

Abbreviations: FDA - Food and Drug Administration, CHL - Chinese Hamster Fibroblast Cell line, CHO-Chinese Hamster Ovarian Cells, MEM - Minimum Essential Medium, CA - Chromosomal Aberration, SCE - Sister Chromatid Exchange, MN - Micronucleus, FPG - Fluorescence Plus Giemsa, SB - Sodium Benzoate, PB - Potassium Benzoate.

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