Potassium Sorbate Induces Oxidative Stress and Genotoxicity in Human Lymphocytes

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Abstract

Potassium sorbate, a potassium salt of sorbic acid, has been used as food preservative and antimicrobial agent. Some observations in rat and hamster cells suggest its toxicity. However, observations in human cells are limiting. Therefore, it has been intended to investigate impacts of potassium sorbate on human lymphocytes. The lymphocytes were cultured and treated with 0.5, 1.0, 1.5 and 2.0 mg/ml of potassium sorbate for 24 and 48 h. Evaluations of its impacts were done through the MTT assay, analysis of chromosomal aberrations, formation of micronucleus and activities of superoxide dismutase. The results revealed that potassium sorbate induced oxidative stress, genotoxicity, damages to chromosome, formation of micronucleus in human lymphocytes.

Keywords : *Potassium sorbate, Genotoxicity, Oxidative stress, Lymphocytes.*

Introduction

Food additive, substance or mixture of substances, is added to basic components of food in a scientifically controlled amount. It is used to color, preserve, blend, thicken or flavor foods. It also prolongs the shelf-life of products by protecting them from deterioration caused by micro-organisms. However, some of the food additives have been reported to cause sensitization, inflammation of tissues. They also act as potential risk factors of several chronic diseases and activator of inflammatory pathways. [1]

Potassium sorbate (E202) is the food preservative. It is the potassium salt of 2,4-hexadienoic acid (sorbic acid). It is used as antimicrobial and fungi static agent in cigarettes, cheeses, fishes, baked goods, syrups and jams. It has been proposed as a preservative for bacon, as an additive in conjunction with nitrite and ascorbate or erythorbate. It is also used as a preservative for dehydrated foods like jerky and dried fruit. It is commonly used in wine production because it stops the yeast from continuing to ferment in the bottles. [2] Many beauty products are prone to mold growth and use the preservative such as potassium sorbate to extend the life of skin and haircare products. Taking too much potassium sorbate preservative over a long period of time could lead to symptoms such as nausea, vomiting and diarrhea. The weak genotoxic effect was observed in Chinese hamster cells at 3-4 mg/ml concentration, with chromosome aberrations test and in human lymphocytes at 4 and 8 mM. [3, 4] Contrary to the other findings, many studies have reported that potassium sorbate is not genotoxic in different rat organs at 2000. mg/kg concentration with comet test and Hela cells. [5, 6]

Antioxidant enzymes are proteins involved in the catalytic transformation of reactive oxygen species and their by-products into stable nontoxic molecules. Their functions are the most important defense mechanisms against oxidative stress-induced cell damage. [7]

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Superoxide dismutase (SOD, EC 1.15.1.1) is one of the most important antioxidative enzymes in nearly all living cells exposed to oxygen. Three forms of superoxide dismutase are present in humans, in all other mammals, and most chordates. [8]

The effects of food additive on antioxidative enzyme and oxidative stress were reported. The reports suggest significant elevation in MDA and reduction in GSH levels in mice administrated with sodium benzoate for 4 weeks and reduction in GSH and antioxidant enzymes in monosodium glutamate (MSG) treated Wister rats. [9, 10]

In vitro methods are useful for assessing the safety of chemicals including food additives. They also serve as indicators for specific toxic effects of discrete molecules and substances. Increased consumption of food preservatives causes some health problems such as asthma, headaches, migraines, urticaria and genotoxic effects. [11]

Potassium sorbate is the food preservative and there are controversial genotoxicity and/or toxicity data about this preservative. At present, there is no published data on the induction of oxidative stress by potassium sorbate in lymphocytes. Therefore, this report presents its evaluation on human lymphocytes.

For these reasons, the aim of this study was to further evaluate the potential genotoxic activity, oxidative stress induction, and safety of potassium sorbate in human lymphocytes.

Materials and Methods

Lymphocyte cultures

Human lymphocyte cell line (ATCC® PCS-800-013TM) was cultured in RPMI medium containing 10% fetal bovine serum (Invitrogen, USA.), antibiotics and phytohaemagglutinin M. The human lymphocyte cultures were incubated in a 5% CO_2 incubator at 37ºC. The cells were incubated with potassium sorbate (Sigma-aldrich, USA.) at the concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml for 24 and 48 h. There was no addition of potassium sorbate in the control experiments.

Cytotoxic study of potassium sorbate in lymphocytes by MTT assay

Genotoxic study of potassium sorbate in lymphocytes

Chromosomal aberrations

Lymphocytes ($1x10^6$ cells/ml) were incubated in RPMI 1640 medium (containing fetal bovine serum, antibiotics and phytohaemagglutinin M at 37ºC for 72 h. The cells were treated with potassium sorbate with different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) for 24 and 48 h. Colchicines 0.06 µg/ml was added 1 h prior to the harvesting of the culture.

To collect the cells, the cultures were centrifuged (216 g for 15 min), treated with hypotonic 0.075 M KCl solution for 30 min at 37ºC and then fixed in methanol and acetic acid fixative in a 3:1 ratio for 20 min, at room temperature. They were then treated with the fixative twice. Finally, metaphase spreads were prepared by dropping the concentrated cell suspension onto slides.

For the chromosomal aberration assay, the slides were stained with 5% Giemsa (pH = 6.8) prepared in sorensen buffer solution, for 20-25 min, washed with distilled water, dried at room temperature and mounted. [13] The chromosomal aberrations were observed from 100 well-spread metaphases for each potassium sorbate concentrations. Mean of the numbers of metaphase chromosome (each metaphase chromosome contains 46 chromosomes) was calculated.

Micronucleus assay

Potassium sorbate with different concentrations were added in lymphocyte cultures for 24 and 48 h. At 44 h, after the start of the culture, cytochalasin B (3µg/ml) was added to arrest cytokinesis. Cells were harvested after 28 h, treated with a hypotonic solution (0.075 M KCl) and fixed with methanol and glacial acetic acid in a 3:1 ratio v/v, supplemented with formaldehyde, according to Palus et al. with some modifications. The slides were air-dried and stained with 5% Giemsa. Micronucleus was scored from 1000 binucleated cells per potassium sorbate concentration under microscope. [14]

Assay of SOD activity in lymphocytes treated with potassium sorbate

Lymphocytes ($1x10^6$ cells/ml) were treated with potassium sorbate at the concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml for 24 and 48 h. Then, the SOD activity

was measured by Superoxide Dismutase (SOD) Activity Kit (Biovision).

Statistics Analysis

For the percentage of the numbers of metaphase chromosome and micronucleus cells, the results were expressed as mean±SE (standard error). SOD activity was also expressed as mean±SE. The differences among the potassium sorbate-treated and the control groups were analyzed by ANOVA and $p< 0.05$ was regarded as statistically significant.

Results

Cytotoxic effect of potassium sorbate on lymphocytes

The results of MTT assay showed that the potassium sorbate affected viability of lymphocytes ($p<0.05$) at all concentrations (0.5 to 2.0 mg/ml) at 24 and 48 h. Lymphocyte viability was decreased when the concentration of potassium sorbate increased. After 0.5 mg effect was almost similar up to 2 mg but 24 h (Figure 1) was more effective than 48 h incubation time. The IC_{50} value at 24 h incubation time was 0.79 mg/ml and IC_{50} value at 48 h incubation time was 0.94 mg/ml.

Genotoxic effect of potassium sorbate on chromosome aberration and micronucleus induction

At 24 and 48 h incubation time, the potassium sorbate significantly reduced numbers of metaphase chromosome in a dose dependent manner ($p<0.05$) (Figure 2). The effect was concentration dependent in both incubation times. The sister chromatid separation and chromosome break were also observed (Figure 3).

The induction ($p<0.05$) of micronucleus was also observed in lymphocytes at all concentrations (0.5 to 2.0 mg/ml) of potassium sorbate both at 24 and 48 h incubation time (Figure 4 and Figure 5).

Effect of potassium sorbate on SOD activity in lymphocytes

The potassium sorbate affected activity of SOD in lymphocytes at 24 and 48 h incubation time. The concentration 1.0-2.0 mg/ml of potassium sorbate affected SOD activity. The effect was more after 24 h incubation time than the percentage activity inhibition of SOD after 48 h incubation time. At 48 h, the percentage of SOD activity inhibition was decreased when the concentration of potassium sorbate increased ($p<0.05$) (Figure 6).

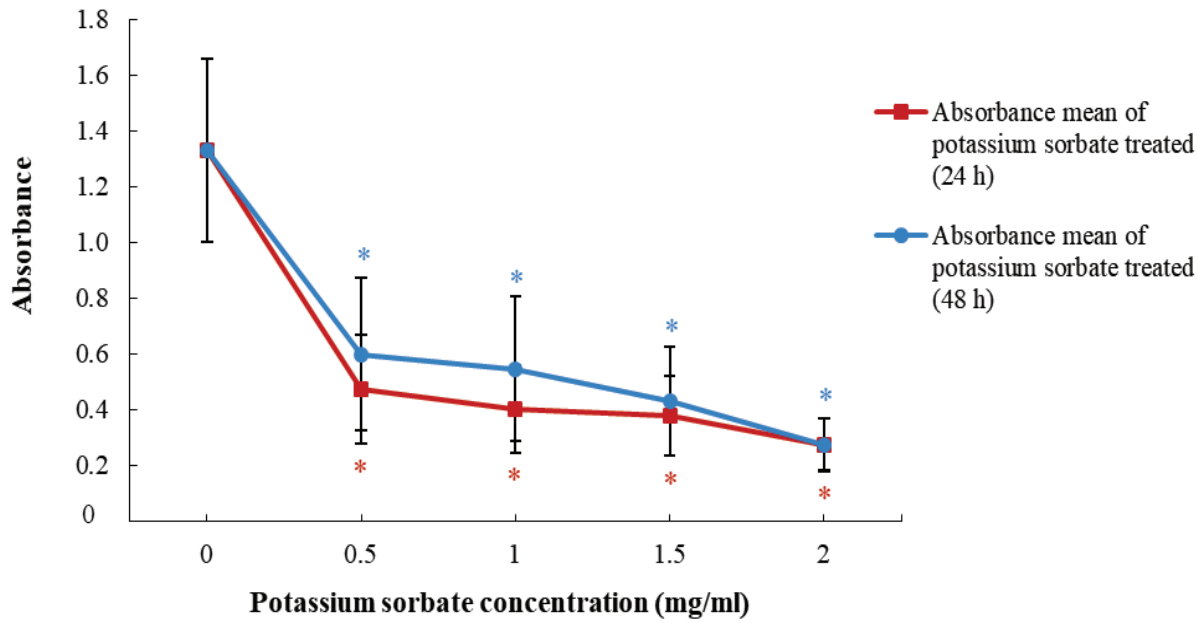

Figure 1 The MTT assay (measured absorbance at 570 nm) showed effects of potassium sorbate on the lymphocyte viability at 24 and 48 h incubation time (n = 3).

The value of absorbance depended upon viability of lymphocytes or the numbers of living lymphocytes. The lymphocyte viability was decreased when the potassium sorbate concentration increased.

* $p<0.05$, p value compared with control group.

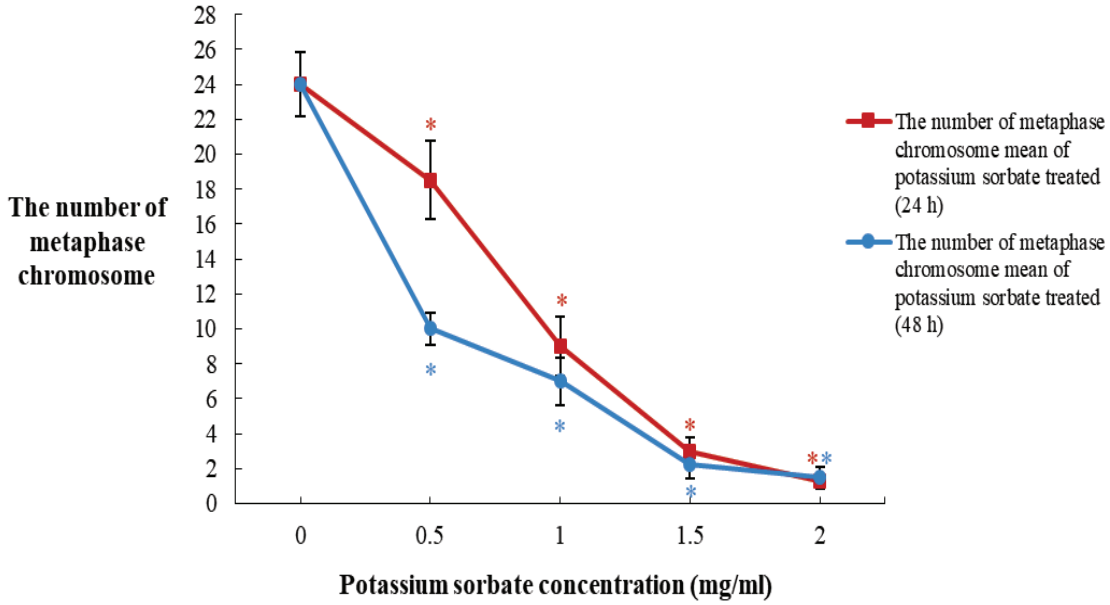

Figure 2 Effects of potassium sorbate on metaphase chromosomes at 24 h and 48 h incubation time (n = 3).

* $p<0.05$, p value compared with control group.

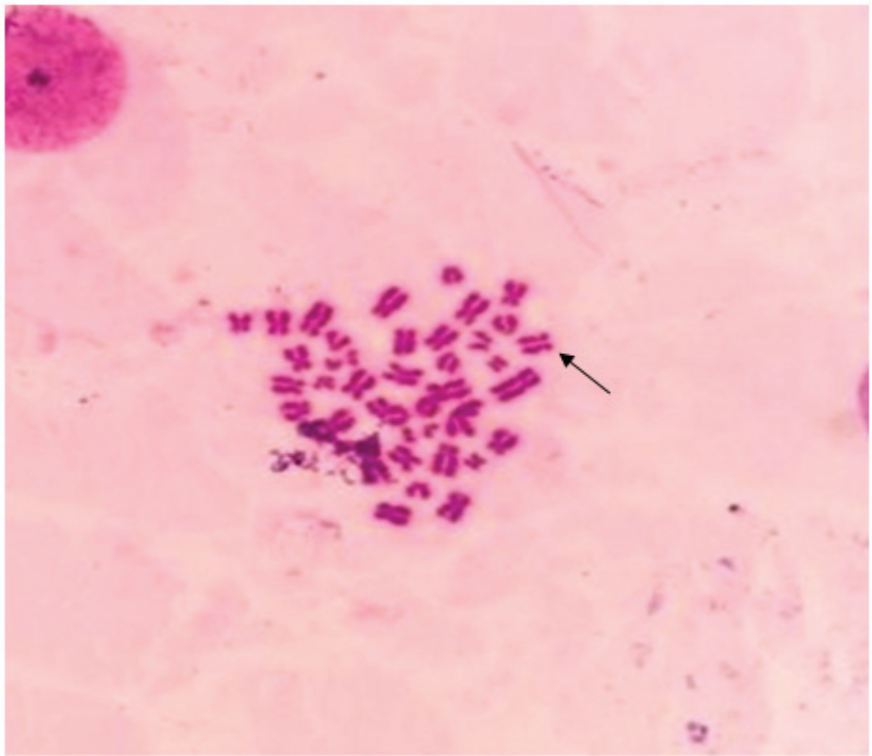

Figure 3 Sister chromatid separation (the arrow) caused by potassium sorbate.

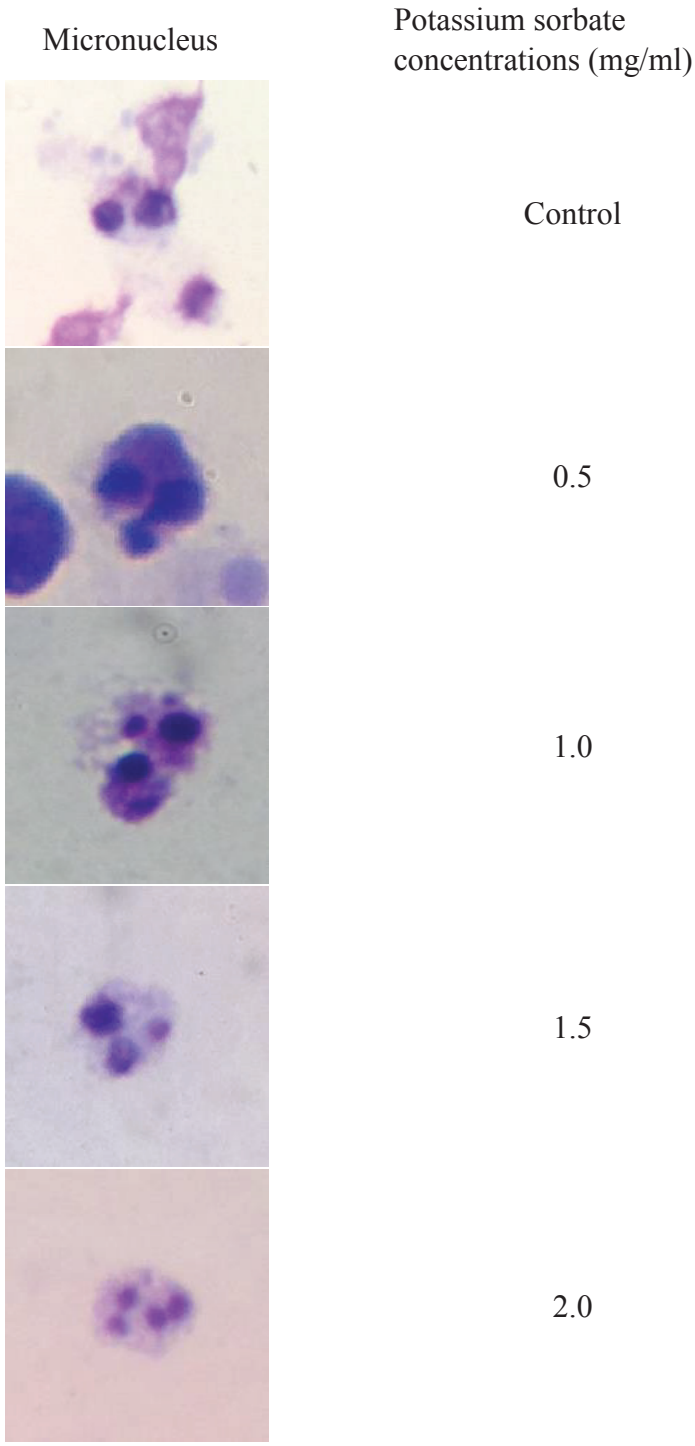

Figure 4 Micronucleus formation induced by the potassium sorbate concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml at 24 h incubation time.

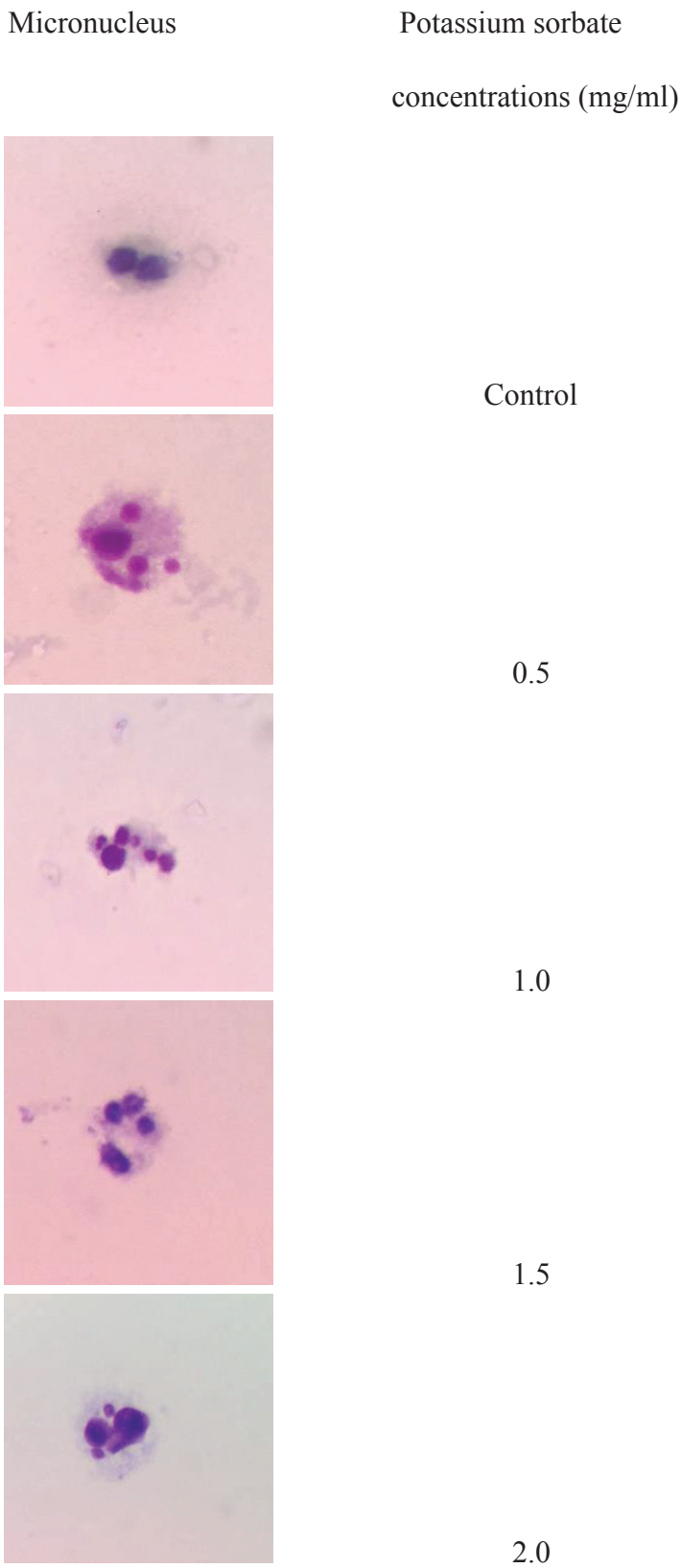

Figure 5 Micronucleus formation induced by the potassium sorbate concentrations of 0.5, 1.0, 1.5 and 2.0 mg/ml at 48 h incubation time.

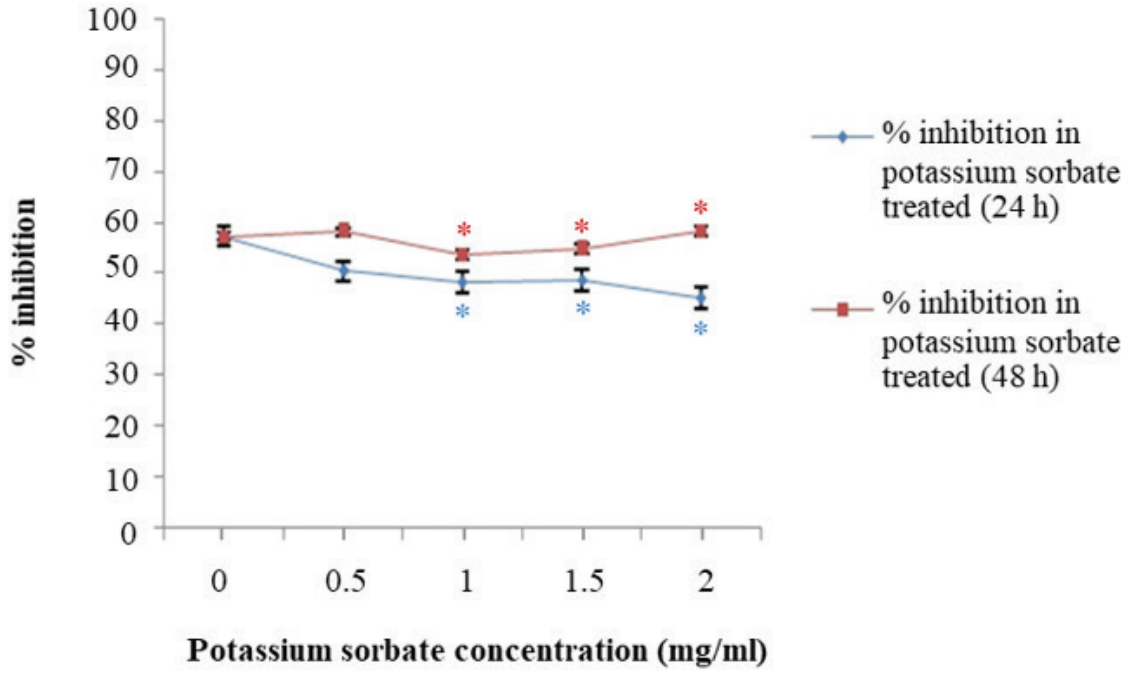

Figure 6 Effects of potassium sorbate on the percentage activity inhibition of SOD in lymphocytes at 24 and 48 h incubation time (n = 3).

* $p<0.05$, p value compared with control group.

Discussion

The damage to genome has been used as a biomarker of carcinogenesis. The status of micronucleus (microscopically visible round to oval cytoplasmic chromatin mass next to the nucleus) has been observed useful tool for measuring genotoxicity in vitro cultures. [15] This assay detects both clastogenicity (chromosome breakage) and aneugenicity (chromosome lagging due to dysfunction of mitotic apparatus). [16] An increased micronucleus also implies risk of cancer in human.

The report on potassium sorbate presented negative results in vivo. [17] Potassium sorbate did not show any adverse genetic effects in the hamster embryo fibroblast micronucleus assay or in the cell transformation test. [18] Sorbic acid and potassium sorbate were less genotoxic than the sodium sorbate. [19, 20, 21] On the other hand, potassium sorbate was found to cause sister chromatid exchanges in Chinese hamster cells. [22]

The mechanism of toxicity of sorbic acid and its salts are probably related to the alkylating activity. Sorbic acid and sorbate showed alkylating activity on the nucleophile 4-(p-nitrobenzyl) pyridine (NBP), which is used as a trap for alkylating agents having nucleophilic characteristics similar to DNA bases. [23] Alkylating agents can damage DNA by attaching the alkyl groups to the DNA bases or mispairing of the nucleotides or formation of cross-bridges, bonds between the atoms in the DNA. [24] Alkylating agents cause gross mitotic abnormalities and can effect gene mutations. This study indicated that potassium sorbate is a genotoxic agent. It clearly reveals the cytotoxic and genotoxic effects of potassium sorbate in cultured human lymphocytes. It significantly reduced the numbers of metaphase chromosome during the 24 h and 48 h incubation periods, in a concentration-dependent manner. Sister chromatid separation and chromosome break were recorded in these studied concentrations. It caused micronucleus formation in a concentration-dependent manner. The potassium sorbate concentrations (1.0-2.0 mg/ml) reduced SOD activity which indicates impact on enzymes of oxidative stress management. The results also indicate that the

impact was maximum within 24 h. As compared to SOD activities observed with same concentrations of potassium sorbate at 48 h, it is presumed that the cells tend to recover due to its defense. It is supported by increased activity of SOD after 48 h.

Several reports explain that potassium sorbate did not show any adverse effect on toxicity testing for DNA profile. [17] The reports are also contradictory. However, potassium sorbate has been used as food preservative. Our observations are in agreement with most of the reports that impact of potassium sorbate is less on SOD but genotoxic. Our observations in human lymphocytes suggest that potassium sorbate is toxic to cause chromosomal aberrations, formation of micronuclei and damage to oxidative stress management system.

Conclusion

Potassium sorbate which is commonly used in food as preservative is toxic to cause chromosomal aberrations, formation of micronuclei but shows mild damage to oxidative stress management system. Potassium sorbate may cause a wide range of long term health problems and side effects. The usage of food preservatives demands more awareness and surveillance.

Ethical Clearance : Not required

Conflict of Interest : Nil

Source of Funding : Self

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