Mito depressive effect and chromosomal aberrations induced by $KBrO_3$ on *A. sativum L* root tips.

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Abstract

The present study was conducted to evaluate the potential cytotoxicity and genotoxicity of different treatments potassium bromate ($KBrO_3$) using *Allium sativum* bio assay. The roots of *A. sativum* were treated with the concentration of 3 g/L, 5 g/L, 7 g/L and 9 g/L of $KBrO_3$ for 2 hour, 6 hour and 24 hour treatment incubation times. Our results show that $KBrO_3$ significantly inhibited the Mitotic Index (MI) compared to the control in a time and concentration-dependent manner. The decreasing of the mitotic index was dependent on the concentration and incubation time of treatment. $KBrO_3$ slightly increased the percentage of abnormal cells. The present study indicates that extensive use of food additive should be assessed because of genotoxic and cytotoxic impacts on public health. Thus, continued efforts are needed to judge potential toxic effects of these chemicals on other living systems like human to ensure the survival of all forms of life.

Keywords: Treatment, Chemicals, Incubation, Cytotoxicity.

Introduction

Food additives are the substances that are added to food in order to prolong the shelf-life of the food's factories by inhibiting the development of microorganisms such as bacteria and fungi. Some other purposes including coloring, flavoring, sweetening and thickening are also targeted of the food additives. Potassium bromate is one of the chemicals that extensively used as a food additive. Plenty of countries, mainly in South East Asia are using potassium bromate as flavor enhancer in food products. Potassium bromate is a chemical additive mixed in flour to improve the dough, raising the volume of the bread and holds its shape, subsequently; humans are daily exposed to this chemical substance [1]. Potassium bromate it is not a naturally occurring and can be produced by passing bromine into a solution of potassium hydroxide is generated as a contaminant in drinking water due to conversion of bromide found naturally in water to bromate by ozone which is used as disinfectant. Potassium bromate under the right conditions will be completely used up in the baking bread. However, if too much is used, or the bread is not cooked long enough or at high enough temperature, then residual amount will remain. This residual amount of potassium bromate is undesirable [2]. Bromate is an oxidizing improver which acts slowly and throughout the dough fermentation, and has two primary effects. Firstly, it enables loafs with high volume qualities to be produced from the low protein wheat. Secondly, it helps to produce bread with fine crumb structure. Potassium bromate is a highly reactive substance which rapidly breaks down to the inactive bromide during dough fermentation and baking and it was always considered that breakdown was complete. The Food and Drug Administration (FDA) indicate that 20 parts per billion or less of potassium bromate is safe. Potassium bromate in bread and baked food as low 5 ppb (ng/g) can be detected using liquid chromatography. With the great increase in the use of food additives, there also has emerged considerable scientific data linking food additive intolerance with various physical and mental disorders, particularly with childhood period [3]. This bioaccumulation if continued for a long time can become cytotoxic and can offset the biochemical equilibrium of the delicate human system or cause some genomic disruptions in the cells of the human system. The genomic disruptions which are damaging to the DNA could range from point mutation to chromosomal mutations. Different biological assays were used to investigate the genotoxic effects of some chemical, *Allium sativum* (Garlic) is a commonly used as a suitable genetic model for cytological studies. In general, *Allium* test is an efficient cytological model for chromosome aberration and mitotic activity assay of different factors [4]. The goal of this study was to evaluate the cytogenic activity of potassium bromate on *Allium stavium* chromosomes and also assess the recent studies have proven that long-term exposures to food additives might be associated with increases in the rates of some genetic diseases and the development of some types of cancer. In addition to systemic toxicity, the possible genotoxicity of food additives has been investigated in recent years. Thus, in this study we aimed to evaluate the genotoxicity of $KBrO_3$, using chromosomal aberration assay and to determine their effect on the mitotic index of *Allium sativum*.

Materials and Methods

Growing plants and treatments

Dry healthy *A. sativum*, (Garlic) bulbs of 1.5 cm to 2.0 cm in diameter and weighing 20 g-30 g were purchased from local market. Bulbs were washed by using tab water, peeled and then the old roots were removed. They were then placed on top of small jars containing distilled water that have been changed with fresh water every morning to allow root to germinate for three days at room temperature [5]. *Allium* root were then divided to three groups, the first group was treated with only $KBrO_3$ that dissolved in distilled water at concentrations used in foods of 3.0 g/L, 5.0 g/L, 7.0 g/L and 9.0 g/L. Samples were collected after 2 hours, 6 hours and 24 hours, three replicates for each treatment and controls were included [6]. The root tips were fixed in fixative (Ethanol: Chloroform: Acetic acid, 6:3:1 v/v) for 24 hrs at room temperature, hydrolyzed in drops of 1N HCL at 60°C for 12 min, moved to clean tubes for staining in 1% aceto-carmain stain for at least 1 hr [7]. The tips were then squashed on the microscope slides, three slides were made for each treatment and counting was randomly done on five fields to determine the Mitotic Index (MI) and chromosomal aberrations. The mitotic index was calculated for each treatment as the number of cells in mitosis/total number of cells counted (1000 cell) and expressed as percentage. Images were taken under Olympus microscope attached with digital camera [8].

Statistical analysis: Un-paired t-tests were used to compare the mitotic index values of both control and treated samples. For each treatment, root tips were taken from four different bulbs (4 replicates). Data were performed using SPSS computing software, results with $P<0.05$ were considered to be statistically significant.

Results

The present study showed the exposure of *A. sativum* root tip cells to different concentrations (3 g/L 5 g/L, 7 g/L and 9 g/L) of the food additive, $KBrO_3$, resulted in significantly reduction in MI of root tip cells. The observed effect of $KBrO_3$ on MI was dose and is dependently inhibited. This decrease was found statistically significant in all the doses and incubation periods as compared to the control ($P<0.5$). Concentration of 7 g/L and 9 g/L of $KBrO_3$ caused complete inhibition of mitotic activity in garlic root tip cells after 24 h of exposure [9]. Generally, $KBrO_3$ induced several chromosomal aberrations at various stages of cell cycle. These include bridges, breaks, fragments, sticky chromosomes, lagging chromosomes etc. The most frequent aberrations noticed were binucleate, nuclear bud and micronuclei. In additions, two morphological abnormalities, enlargement and elongated cells were found in cells treated with 7 g/L and 9 g/L of $KBrO_3$.

The decreasing in MI and imbalance in the frequency of the different mitotic stages was dose-and time dependent at all concentrations and treatment periods used. The mitotic index reached a minimum value of (0.8) after 24 hrs treatment with the highest concentration of $KBrO_3$ compared with the control value of (27.21 ± 9.3). All the concentrations of $KBrO_3$ used in the study caused changes in the percentage of phase distribution in comparison to the control. For treatments at exposure time 2 h the highest percentage of MI% was (1.5) at 3 g/L when compared to the lowest value which was (0.4%) at 9 g/L of $KBrO_3$. Also there is a significant decrease was observed in the mitotic index for all treatments at exposure time 6 h and 24 h as the lowest value was (0.6) at 7 g/L of, treatments, which suggest that all the concentration is highly effective in the reduction of cell division cycle or mitosis. We noticed that as the incubation period increased the potential effect of increases in $KBrO_3$ concentrations was more pronounced this result was significant at $p \leq 0.05$ [10]. The complete inhibition was observed particularly at 24 h treatment period for both T3 and T4 concentrations [11].

$KBrO_3$ caused a change in the frequencies of different mitotic stages decreased as the concentrations and period of incubation increased, with a large number of the cells in prophase and the least cells at anaphase [12]. However, it appears that $KBrO_3$ does affect the percentages of cells in the various stages of mitosis at higher tested concentrations. Interestingly, differences in number of cells in each stage of cell cycle at T3 (7 g/L) [13].

After the 24 h prolonged treatment in T3 (7 g/L) has already been shown in table, it does lengthen the mitotic cycle or prevents the entry of cells into anaphases. For example, while the number of cells during 24 h treated of T3 (7 g/L) cell was observed at anaphase stages was 34 ± 0.44 compared with 15 ± 0.43 at 6h in the same tested concentration ($P>0.05$), while the percentage of cells in telophase was the lowest among phases in every case similarly, the number of the metaphase stages was generally increased by about 40% at 24 for compared with 6 h treatment period time in T4 (9 g/L) [14].

The chromosomal aberrations observed in the current study were visualized in all stages of the cell cycle: Interphase, prophase, metaphase, anaphase, and telophase [15]. The cells in interphase and prophase showed binucleus and Micro Nuclei (MNs) at the treatments with T5 and T7. We observed stickiness chromosomes at metaphase, and chromosomal breaks, fragments and bridges at anaphases and telophases [16]. The major form of nuclear aberration recorded in all stages was binuclei, showed comparatively higher than that of the other chromosomal aberrations (Table 1 and Figure 1) [17].

Table 1. *Mitodepressive effect by increasing concentrations $KBrO_3$ in Allium sativum root tip cells in different exposure periods (for 6 hrs, 12 hrs and 24 hrs), on number of total cells in mitosis, means of Mitotic Index (MI) and frequency of mitotic phases and stages after treatment. Each number represent $M \pm SD$ for 1000 root tip cells, different letters within the same column refer to significant differences at $p \leq 0.05$.*

Concentration of $KBrO_3$	Duration (Hours)	MI%	Phase index			
			P	M	A	T
Control (0)	2	20.0	2146 ± 14.26	123 ± 2.66	72 ± 0.44	58 ± 0.30
	6	24.4	2636 ± 4.679	136 ± 0.89	95 ± 0.51	60 ± 0.50
	24	27.2	2912 ± 6.98	192 ± 1.7	95 ± 0.87	69 ± 0.67
T1 (3 g/L)	2	1.5	129 ± 0.69	39 ± 0.44	10 ± 0.20	3
	6	2.0	204 ± 1.05	31 ± 0.49	10 ± 0.29	3
	24	2.3	249 ± 1.46	18 ± 0.43	9 ± 0.3	2
T2 (5 g/L)	2	1.1	100 ± 0.6	18 ± 0.33	9 ± 0.4	4
	6	2.0	190 ± 1.31	30 ± 0.58	7 ± 0.3	3
	24	0.2	2	0	2	0
T3 (7 g/L)	2	0.4	32 ± 0.25	15 ± 0.43	7 ± 0.20	2
	6	0.8	44 ± 0.66	34 ± 0.44	1	3
	24	0.6	1	1	0	0
T4 (10 g/L)	2	0.4	28 ± 0.24	19 ± 0.22	1 ± 0.08	2
	6	0.8	46 ± 0.47	50 ± 1.05	2 ± 0.16	2
	24	0.8	1	1	2	0

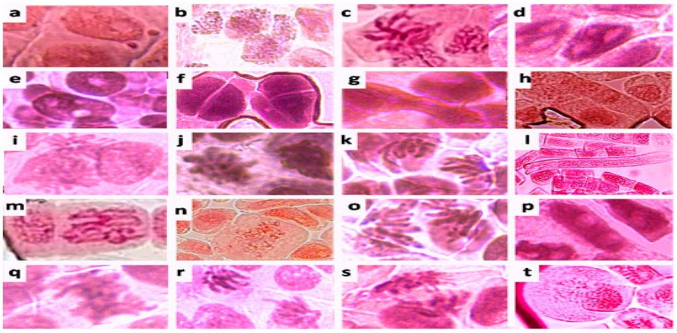

Figure 1. *Different chromosomal aberrations induced by KBrO3 in the root tips of Allium sativum; a) Micronuclei at interphase; b) Granular prophase; c) Sticky metaphase; d) multiple nuclear lesions; e -f) Two binucleated interphase cells; g-h) Nuclear buds; i) Star anaphase; j) Chained anaphase showing pulverization; l) Elongated interphase cells; m) Disturbed metaphase; n) Giant cells with fragmented nucleus, morphological alterations in shape and size of cells; o) Anaphase tripolar; p)Elongated binucleated cells; q) Clumped chromosome; r-s) Anaphase chromosome with bridges; t) Circular cell*

Discussion

The *Allium* bio assay has been extensively used throughout the world in assessing mutagenic activity of chemicals substances [18]. Due to its large monocentric chromosomes in reduced numbers, *Allium*, has been used as suitable test material for the detection of chromosomal damage [19]. The mitotic index is a reliable indicator which allows to estimate the potential genotoxicity through determining cellular division rates [20]. Inhibition of mitotic activities is generally used for screening of genotoxic agents [21]. High MIs it could be resulted of the induction of cell division whereas MIs low may be correlated with the effect of tested agents on the growth and development of treated organisms [22]. The MI reflects the frequency of cell division rates [23]. In this study, $KBrO_3$ significantly decreased the Mitotic Index (MI) at all concentrations and treatment periods [24]. In additions, the decreasing of the MIs was dose-dependent [25]. The inhibition of cellular cycle by the decline of the MI indicates the occurrence of a cytotoxic effect. Similar observations have been made by other researchers on effects of different food coloring on MIs using *Allium* test [26]. The dose-dependent reduction of MIs reviled the potential mutagenic action of $KBrO_3$ in *A. sativum*. In the present study, $KBrO_3$ was shown to have a genotoxic effect result in MI inhibition. The effects of mutagenic agents on MI were reported by many earlier studies using *A. cepa* bioassay. Also in human peripheral lymphocytes reported that $KBrO_3$ induced chromosomal abnormality and significantly inhibited cell division rate. Dramatically reduced in MI could be explained as results of the mito depressive effect of chemical agents. The used chemicals interfere in the normal process cell cycle resulting in decrease in of cell division rates [27]. In addition, the DNA synthesis it may be inhibited due to G1 arrest of cell cycle. That most probably happened due to inhibition of energy synthesis from the functioning of the ATP complex synthesis center. The significant reduction of MI in our study may be linked with the previous mentioned reasons [28]. Further, the mitotic index of treated *A. sativum* root tip cells in the present study ranged from 1.5% to 0.2%. A mitotic index less than 22% is recorded to be lethal to the organism. Therefore, the mitotic indices recorded in the present study can be considered

in the lethal range and may indicate high genotoxic effect $KBrO_3$ to human health [29].

Different types of chromosome aberrations were observed, namely, chromosome unorientation, micronuclei formation, chromatin bridge, and stickiness of chromosomes, at metaphase and diagonal anaphase: Disturbed anaphase-telophase, choromosome laggard(s), stickiness, anaphase bridge(s) [30]. Other anomalies have also been observed such as binucleate cell, unequal separation, and fragmentation can influence the mitotic rates, induce chromosomal aberrations and the formation of micronuclei in tests conducted with *Allium* [31]. Among all the aberrations observed, micronuclei and binucleate were recorded to be the most prominent observed mutation followed by nuclear buds and giant cell formation [32]. In our study, the appearance of cells with micronuclei indicates the mutagenic action of $KBrO_3$ on cell division. According to some authors, such as the occurrence of micronuclei as the best available evidence to evaluate the mutagenic effect of chemicals. The induction of micronuclei in cells, after $KBrO_3$ treatment, was observed mostly at interphase and prophases stages [33]. Binucleated cells are cells with two main nuclei, similar in shape, in contact with each other. Binucleated cells could be an indicative of possible disturbing of normal cell plate formation at cytokinesis or microtubules. Food additives might affect microtubule organizational defects, misaligned or incomplete cell plates formation, and resulting in the inhibition of cytokinesis. Nuclear abnormalities was also observed by exposure to 5 g/L and 7 g/L under long incubation time resulted in nuclear bud formation. The occurrence of nuclear budding was considered as small genetic material protuberance formed at terminal part of nucleus without a clear separation. Earlier studies also demonstrated similar abnormalities in plant cells exposed to other food additives [34].

Conclusion

In conclusion, $KBrO_3$ was found to be genotoxic due to inhibition of MIs and induction of chromosome aberrations in *Allium* test. Our results revealed that plant test systems are reliable genetic model to detect the genotoxicity due to their sensitivity to apply. This investigation is also in agreement with several previous studies suggesting that more care is needed to manage the use of $KBrO_3$ as food additives in our country where it is widely used.

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