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

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Highlights

- **This study has introduced the role of root tips of garlic as a cheap alternative plant test system for monitoring the toxic effects of additives in common food.**
- **Local data are needed regarding the risks of using this well-known additive and more studies are needed to help researchers in general in understanding this aspect.**

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ABSTRACT

The current study aimed at evaluating the potential cytotoxicity and genotoxicity of different treatments of potassium bromate ($KBrO_3$) using *Allium sativum* bioassay. 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 2,6 and 24 hour incubation times. Our results showed that $KBrO_3$ significantly inhibited the mitotic index (MI) compared to the control in a time and concentration-dependent manner. $KBrO_3$ slightly increased the percentage of abnormal cells such as binucleate, nuclear bud, micronuclei, enlargement and elongated cells. The current result indicates that, broad uses of food additive should be assessed because of genotoxic impacts on public health. Thus, continued efforts are needed to judge the potential toxic effects of these chemicals on other living systems like human to ensure the survival of all forms of life.

1. Introduction

Food additives are chemical substances that are added in very small quantities to the processed food in order to enhance its flavor and to prolong the shelf life of the food products. By inhibiting the development of food-spoiling microorganisms including yeasts, moulds and bacteria. There has been an enormous increase in the use of additives for diverse purposes as coloring, flavoring and drying agents, such as sweeteners, preservatives, stabilizers and thickeners. Potassium bromate is one of the most widely used food additives, commonly added as a raising agent in bread and as a flavor enhancer in food products in many parts of the world as. Potassium bromate is a chemical additive mixed in flour to improve the dough, rising the volume of the bread and hold its shape. It represents a health risk to a huge number of consumers that are exposed due to the daily intake of bakery edibles containing this chemical substance as an additive (Chipman *et al*., 1998; EGVM, 2002; Humans, Cancer, & Organization, 1986).

Potassium bromate is not naturally occurring and can be produced by passing bromine into a solution of potassium hydroxide (Humans *et al*., 1986). $KBrO_3$ is not a natural water contaminant. It is produced as an ozonation by-product generated as a result of the ozonation of water containing bromides. (Ueno *et al.,* 2000). 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 the residual amount will remain (Gibreel, 2008). This residual amount of potassium bromate is undesirable. 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(Atkins, 1993). The Food and Drug Administration (FDA) indicates that 20 parts per billion or less of potassium bromate is safe (Campbell, 2006). Potassium bromate in bread and baked food as low 5ppb (ng/g) can be detected using liquid chromatography (Himata *et al.*, 2000). With the great increase in the use of food additives, a significant proportion of scientific data has emerged highlighting the potential risks of food additive intolerance causing various physical and mental disorders in children. This bioaccumulation if continued for a long time can cause acute cytotoxicity and therefore, 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 (Bakare, 2001). Different biological assays were used to investigate the genotoxic effects of some chemical, *Allium sativum* (Garlic) is commonly used as a suitable genetic model for cytological studies. In general, *Allium*test is used in laboratories to assess cytogenetic and genotoxic effects of harmful chemical substances by evaluating

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chromosomal aberrations and mitotic activity of root tip cells exposed to these substances (Ateeq *et al,* 2002; Bakare, 2001; Fiskesjö, 1988; C. Sharma, 1983).

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, the main objectives of this study were to evaluate the genotoxicity of $KBrO_3$, using a chromosomal abnormality assay and to determine their toxic effect on the mitotic index of *Allium sativum*.

2. Material and methods

2.1 Growing Plants and Treatments

Healthy and equal-sized *A. sativum*, (garlic) bulbs of 1.5 to 2.0 cm in diameter and weighing 20-30 g were purchased from the local market. Bulbs were washed with tap water, and dry scales and old roots were removed. These bulbs were then suspended in small jars containing distilled water for 2-3 days at room temperature to allow roots to grow. The experiment was conducted in three independent replicates and water in the jars was replaced with fresh water every 2 hours. Fresh Allium roots (1-2 cm in length) were then divided into three groups, Another group was treated with only $KBrO_3$ dissolved in distilled water at concentrations of 3.0, 5.0, 7.0 and 9.0 g/L. Treated and control samples were collected after 2,6 and 24 hours.

The root tips were fixed in Carnoy,s solution (ethanol: chloroform: acetic acid, 6:3:1 v/v) for 24 hours at room temperature, after that hydrolyzed in drops of 1N HCL at 60°C for 12 min, moved to clean tubes containing 1% aceto-carmin stain for at least 1 hour. The root tips were then squashed on microscope slides for cytological analysis. Three slides were prepared for each treatment and five random fields of view were selected to determine the mitotic index (MI) and chromosomal aberrations. The slides were examined under Olympus microscope and images were taken with the attached digital camera. MIs were calculated for both treated and untreated samples using the formula (Grant, 1982):

$$MI(\%) = \frac{\text{number of cells in mitosis}}{\text{total number of cells counted (around 1000 cells)}} \times 100$$

2.3 Statistical Analysis

The mitotic index values of both control and treated samples were compared using Sigma Stats.3 software (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analysis using one-way ANOVA test and the level of significance was established at 0.05. For each treatment, root tips were collected from three different bulbs (3 replicates).

3. Results

The present study showed that 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 a significant reduction in MI of root tip cells (Table 1). Increasing concentrations of $KBrO_3$ significantly inhibited mitotic figures in treated samples in a dose-dependent manner. This decrease was found statistically significant in all the doses and incubation periods as compared to the control (p<0.5) (Fig. 2). The 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. Generally, $KBrO_3$ induced several chromosomal aberrations at various stages of the cell cycle. These aberrations include chromosomal bridges, breaks, fragments, sticky chromosomes, lagging chromosomes, etc. (Fig. 1). The most frequent aberrations noticed were binucleate, nuclear bud and micronuclei. Two additional morphological abnormalities i.e. enlarged and elongated cells were observed in samples treated with 7 and 9g/L of $KBrO_3$ (Fig. 1).

The decrease in MI and imbalance in the frequency of the different mitotic stages were dose-and time-dependent at all concentrations and treatment periods used (Fig. 2). The mitotic index reached a minimum value of (0.8) after 24hrs treatment with the 9g/L concentration of $KBrO_3$ compared with the control value (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 9g/L of $KBrO_3$. Also, there is a significant decrease was observed in the mitotic index for all treatments at exposure time 6 and 24h 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≤0.05. The complete inhibition was observed particularly at 24 h treatment period for both T3 and T4 concentrations.

With the increase in concentrations of $KBrO_3$ as well the duration of the incubation period, the frequencies of mitotic stages decreased significantly (Fig. 2), with a large number of the cells in prophase and the least cells at anaphase (Table 1). However, it appears that $KBrO_3$ negatively impacts the percentages of cells in the various stages of mitosis at higher tested concentrations. Interestingly, differences in the number of cells were observed in each stage of the cell cycle at T3 (7 g/L) as shown in Table 1.

After the 24 h prolonged treatment in T3 (7 g/L) has already been shown in Table 1, 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 6 h 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) (Table 1).

The chromosomal aberrations observed in the current study were visualized in all stages of the cell cycle: interphase, prophase, metaphase, anaphase, and telophase. The cells in interphase and prophase showed binucleus and micronuclei (MNs) at the treatments with T5 and T7 we observed stickiness chromosomes at metaphase, and chromosomal breaks, fragments and bridges at anaphases and telophases. The major form of nuclear aberration recorded in all stages was binuclei, which showed comparatively higher than that of the other observed chromosomal aberrations.

Table 1

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

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

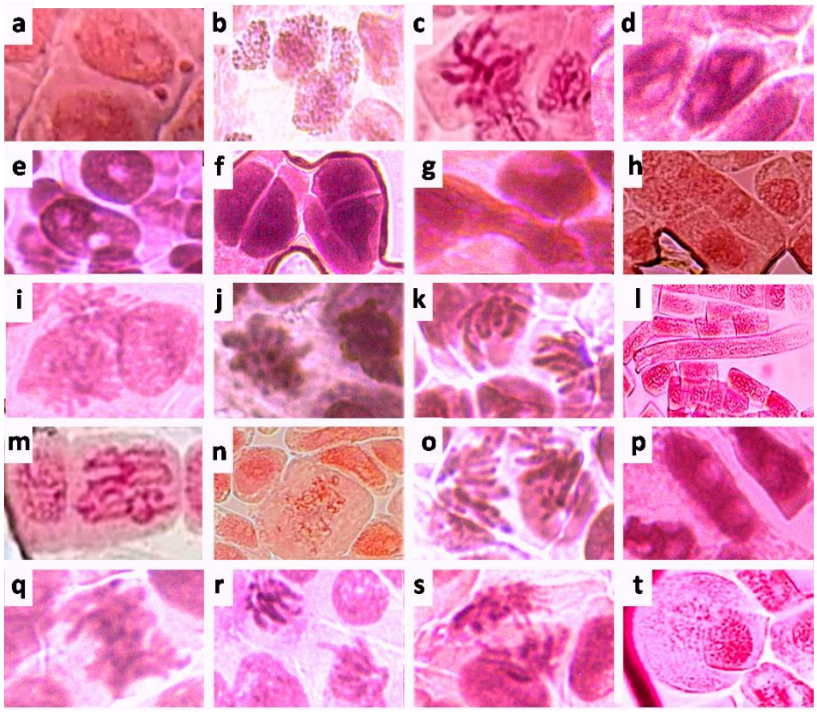

Fig. 1. Different Chromosomal Aberrations induced by $KBrO_3$ in mitotic stages of Allium sativum: a) Micronuclei at interphase, b) Granular prophases, c) Sticky metaphase, d) multiple nuclear lesions, e-f) Two binucleated interphase cells, g-h) Nuclear buds, i) Star anaphase, j) Chained Anaphase showing Pulverization, l) elongated interphase cells m) Disturbed metaphase, n) Giant cells with a fragmented nucleus, morphological changes 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.

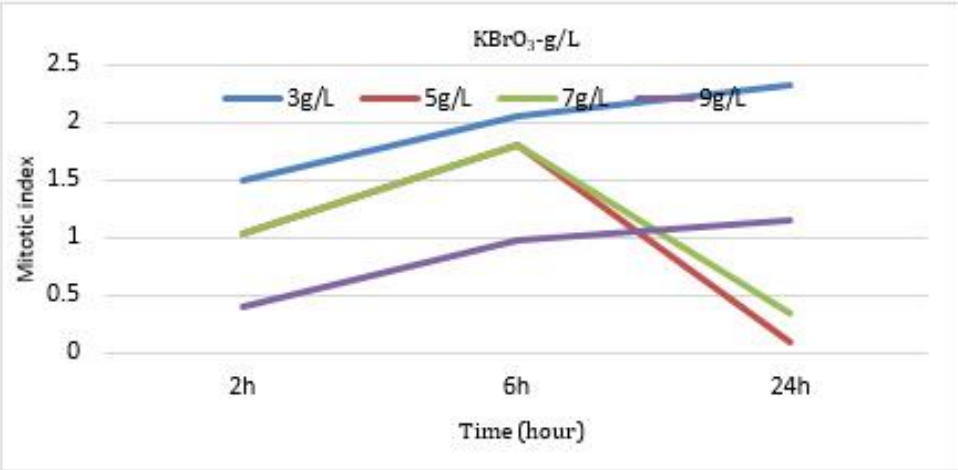

Fig. 2. Comparison of the effects of $KBrO_3$ on the MIs of *A. sativum L*root tips at different concentrations and different time points. Data were performed using SPSS computing software, results with P<0.05 were considered statistically significant. The values are the mean ± S.D. of three independent experiments.

4. Discussion

The *Allium* bioassay has been extensively used throughout the world in assessing the mutagenic activity of chemicals substances (Iqbal *et al*., 2019; Kannangara & Pathiratne, 2015). Owing to the large size of the monocentric *Allium* chromosomes, it represents a model test plant for the detection of chromosomal damage when exposed to harmful compounds (Mishra *et al*., 2013; Shahid *et al.*, 2017). The mitotic index is a reliable indicator to estimate the potential cytotoxicity and genotoxicity by microscopically analyzing cellular division rates in root tips (Fernandes, Mazzeo, & Marin-Morales, 2007; Marcano, *et al*., 2004). Genotoxic agents are generally screened by assessing their inhibitory effects on root tip cells of test plants (Migid, *et al*., 2007). High MIs are indicative of the induction of cell division whereas low MIs may be correlated with the negative impact of tested agents on the growth and development of treated organisms (Ciğerci, *et al.*, 2015; Migid *et al*., 2007).

The results of the current investigation are summarized in Tables 1. The mitotic indices shown in the table reflect the frequency of cell division rates. One of the important findings of this study is the significant reduction in mitotic indices (MI) at all concentrations and treatment periods in a dose-dependent manner. The inhibition of the cellular cycle by the decline of the MI indicates the occurrence of a cytotoxic effect. Similar observations have been made by other researchers on the effects of different food coloring on MIs using *Allium* test (Dwivedi & Kumar, 2015; Gomes, *et al*., 2013). 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 thus resulting in an insignificant reduction in mitotic figures. The effects of mutagenic agents on MI were reported previously, using *A. cepa* bioassay (Marcano *et al*., 2004; Mustafa & Suna Arikan, 2008; Srivastava & Mishra, 2009; Türkoğlu, 2015). Also in human peripheral lymphocytes reported that $KBrO_3$ induced chromosomal abnormality and significantly inhibited cell division rate (Kaya & Topaktaş, 2007). Dramatically reduced in MI could be explained as results of the mitodepressive effect of chemical agents. using these chemicals may disturb the normal process cell cycle resulting in a decrease in cell division rates (Sharma & Vig, 2012). In addition, the DNA synthesis it may be inhibited due to G1 arrest of the cell cycle. that most probably happened due to inhibition of energy synthesis from the functioning of the ATP complex synthesis center (Majewska *et al*., 2003; Sudhakar, KN, & Venu, 2001). The significant reduction of MI in our study may be linked with the previously mentioned reasons. Further, the mitotic index of treated Garlic mitotic cells in our study ranged from 1.5 % to 0.2 %. A mitotic index of less than 22% is recorded to be lethal to the organism (Mustafa & Suna Arikan, 2008). Therefore, the mitotic indices recorded in the present study can be considered in the lethal range and may indicate a high genotoxic effect $KBrO_3$ to human health. 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, chromosome laggard(s), stickiness, and Anaphase Bridge. Other anomalies such as binucleate cell, unequal separation, and fragmentation are also observed thereby influencing the mitotic frequencies inducing chromosomal aberrations and resulting in the formation of micronuclei in tests conducted with *Allium*. 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.

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 Mamur *et al.,* (2010), 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, were observed mostly at interphase and prophases stages. Binucleated cells are cells with two main nuclei, similar in shape, in contact with each other (Torres-Bugarín, *et al*., 2014). Binucleated cells could be an indication of the 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 were also observed by (Borah & Talukdar, 2002; Gömürgen, *et a*l., 2005). 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 a small genetic material protuberance formed at the terminal part of the nucleus without a clear separation (Bolognesi *et al*., 2013). Earlier studies also demonstrated similar abnormalities in plant cells exposed to other food additives (Gömürgen *et al*., 2005; Khatab and Elhaddad, 2015; Türkoğlu, 2007).

5. Conclusion

$KBrO_3$ was found to be genotoxic at all tested concentrations due to significant inhibition of MIs and induction of chromosomal aberrations in *Allium* test. Our results revealed that plant test systems are a reliable genetic model that can be effectively used to screen chemical mutagens to detect genotoxicity due to their sensitivity. These plant systems are also the best models to monitor of genotoxicity in an organism caused by polluted atmosphere, water and harmful chemical compounds. 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 regions where it is widely used.

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