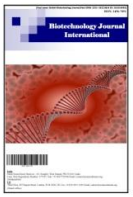

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Genotoxicity Assessment of Potassium Bromate by Means of DNA Image Analysis on the Root Tip Nuclei of *Allium sativum* L.

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HAK and NSE designed the study. Author HAK performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HAK and NSE managed the analyses of the study. Authors HAK, NSE and SE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Allium sativum assay was widely used to assess the compound's cytotoxicity and genotoxicity on plants and animals. Only few studies analyzed the genotoxic effect of potassium bromated ($KBrO_3$) on the DNA content of plant cells. DNA content assay is an efficient test for the measure of chromosomal DNA damages. Based on this approach the effect of $KBrO_3$ on DNA content change was investigated in root tip nuclei of *A. sativum.* Different concentrations of food additives $KBrO_3$. 3 g/l, 5 g/l 7 g/l and 9 g/l % were prepared and treatments were given. *A. sativum* root were incubated for 2, 6 and 24 hours and DNA image analysis of root tip nuclei was performed. The analysis was based on the measurement of the Mean Optical Density (MOD) which represents the cellular DNA content. The results showed that the $KBrO_3$ significantly decreased of DNA content compared to the control at all concentrations and treatment periods in dose-dependent manner. The present study suggests that extensive use of food additives should be banned due to genotoxic effect on living cells. Therefore, there is an urgent need to evaluate potential mutagenic effects of $KBrO_3$ on human.

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1. INTRODUCTION

Historically, the popularity of food additives boomed in the 1500 BC when the use of spices in foods to flavor and make them more appealing became widely known [1]. Potassium bromide ($KBrO_3$) is a potent oxidizing agent used as a food additive and commonly used in the manufacture of flour, cosmetics and a byproduct of drinking water disinfection. Bromate can also produced from hypochlorite solution during electrolysis of bromide [2]. Despite the ban of the use of potassium bromate, its continued use has not been managed. There is considerable debate on the allowable amount of potassium bromate that can be safely added to outused [3].

The daily use and exposure to potassium bromate leads to stimulate the oxidative modification of both proteins and lipids in animal tissues as result of the production of reactive oxygen species in living cells [4]. Sai et al. [5] found that bromate has genotoxic and carcinogenic effect on the tested biological systems. The Food and Agricultural Organization and World Health Organization has been evaluated the maximum level of $KBrO_3$ to be consumed by human about 75 ppm. However, no local data are available regarding allowable amount of potassium bromate in flour and other. More efforts needed to improve our knowledge about these chemicals. Studies have to help our nations in general in understanding the potential toxic effects behind use of food additives. Different short-term bioassays have been used to identify sources of genotoxic hazards including microorganisms, insects, or plants.

Plant systems are inexpensive, highly reliable and quite sensitive to study the cytogenetic and mutagenic effects [6]. Gunther & Nasta [7] showed that 148 chemicals had the potential cytotoxic and genotoxic action when tested through the Allium test. Several studies show that the DNA content measurement is an effective technique to detect genotoxic damage caused by toxic substances [8].

Previous studies suggest the genotoxic effects of $KBrO_3$ leads to a significant increase in the yields of DNA double-strand breaks (DSBs) through DNA damage-dependent and DNA damage-independent mechanisms [8].

Our previous experience with food additives has led us to consider plant root DNA content as a parameter that we could examine for the detection of genotoxic responses to potassium bromate. Similarly, different studies showed that food additives lead to nuclear DNA content decreased in tested root tips nuclei of *Allium sativum.*

In this study, we wanted to focus on the toxic effects of $KBrO_3$ *in vivo* in terms of DNA content changes of the, *Alium sativum* (*A. sativum*) by the total pixel intensity (TPI) of manually defined regions of interest DNA image analysis.

2. MATERIALS AND METHODS

2.1 Growing Plants and Treatments

This works carried out in department of Botany, University of Omer Almukhtar. Clean and healthy bulbs of *A. sativum*, common name (garlic) 2n = 16, purchased from local market. Dry scales of bulbs were removed and placed in small jars with their basal ends dipping in distilled water and germinated at room temperature (25±20 C). Garlic bulbs were then divided to six groups and were treated with different dose of $KBrO_3$ that dissolved in distilled water at concentrations used in of 0.0, 3.0, 5.0, 7.0 and 9.0 g/l. The solutions of $KBrO_3$ were prepared in distilled water. Root tips were collected after 2, 6 and 24 hours incubation period, three replicates for each treatment and controls were used. Root tips were placed in freshly prepared fixative 3:1 (v/v) ethanol alcohol: glacial acetic acid for 24 hours. The root tips were then washed with distilled water several times and stained with 1% acetocarmin using the squash technique.

2.2 Estimation of Relative Nuclear DNA Content

Relative DNA content of *A. sativum* root tip nuclei was measured based on the resulting amount stained nuclei. Images were captured on a Olympus microscope attached with digital camera. On average of 60 2C prophases nuclei and $KBrO_3$ treated nuclei were selected randomly and measured. To process the captured images, ImageJ 1.48 software was used in order to determine the Mean optical intensity (MOI) of manually defined regions of interest (ROI) encompassing root tip cell nuclei, which represents the cellular DNA content. DNA content of prophase stained nuclei was analysed and compared to the nuclear DNA content of

treated root tips nuclei. DNA content of 2 C was measured for control prophase nuclei and used to normalise the DNA content of $KBrO_3$ treated root tips nuclei. Five root tips were analyzed and for each treatment. Around 60nuclei of root tip cells of *A. sativum* from each concentration (i.e. microscopic slide) were measured and each experiment was repeated three times. The methods described in previous works [9].

3. RESULTS

The main finding of the present study is that to test the effect of $KBrO_3$, significantly on the DNA content, when compared to the control root tips nuclei (Fig. 1).

To this aim, the roots were treated with 3.0, 5.0, 7.0 and 9.0 g/L concentrations for incubation times 2, 6 and 24 hrs. The result obtained from control series and processed plants are shown in Fig. 1 as can be seen.

All of the concentrations of $KBrO_3$ used in the present study significantly decreased DNA values in all treatment when compared to control DNA of meristemic nuclei (2C). The data shown that after 2 h treatment period the DNA content had values of 1.9 C compared to 2C in the control (Fig. 1). Similarly, 1.9 C a nuclear DNA content was observed during the next 6h and 24h incubation times. Furthermore, a statistically significant reduction of DNA quantity was seen in root tips nuclei in the T3 (7 g/l) and T4 (9 g/l) of $KBrO_3$ treatments after 2h incubation.

Comparatively, increasing concentrations of $KBrO_3$ (5%, and 7%) have induced the significant reduction ($P< 0.05$) in DNA content in a dose and time-dependent manner (Fig. 1) also shows the exponential relationship between the average of DNA content and the tested concentration of $KBrO_3$. The average of DNA was clearly inhibited when the incubation time was increased to 6h. The proportion of nuclei with lower DNA content clearly increased in the root tip cell at T5 and T7 treatments, respectively.

The lowest DNA values were obtained when the concentration of $KBrO_3$ was increased to the highest dose (9 g/l) in the three tested incubation times, 2, 6 and 24h, all treatment doses were characterized by a significant differences in C-values and were noted for all concentrations compares to the control. The 2h period of incubation $KBrO_3$ stimulated DNA reduction to 1.7 and the value was decreased after 6h period of KBRO3 incubation to reach 1.5C. Comparatively, reduction of DNA content observed during the next 24h of the experiment caused a decrease of DNA to reach 1.4 C. The observation of the study indicated that $KBrO_3$ significantly inhibited nuclear DNA content and in a dose and time-dependent manner.

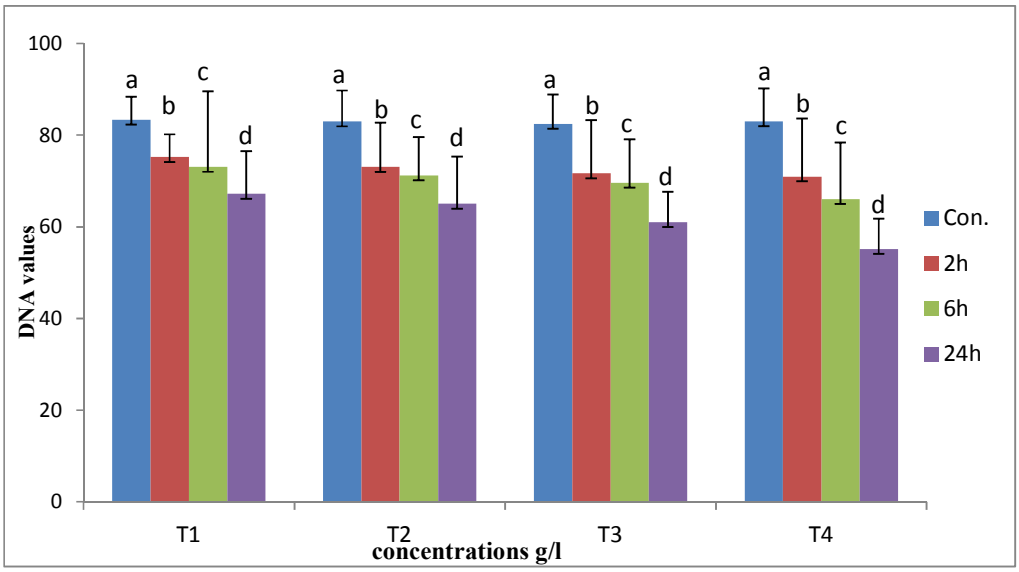

Fig. 1. Relative DNA content measurement expressed as mean optical intensity (MOI) in *Allium sativum* root tips nuclei treated with increasing $KBrO_3$ concentrations at 2, 6 and 24 hours

Each bar represents the MOI of approximately 60 nuclei. The statistical analysis of the results was carried out using the student's t-test and mean values and standard deviations were calculated from three independent replicate. Different letters indicate significant differences ($p < 0.05$)

Table 1. Image analysis of the DNA content of the root tips nuclei of *A. sativum*, Expressed as Mean Optical Density (MOD). se =standard error, n= number of nuclei, C= DNA content

Treatment		n	Mean ± se	C-value
T1	**con**	65	83.33 ±5.02	2.0
	2h	60	75.19 ±7.2	1.8
	6h	69	73.05 ±16.5	1.8
	24h	67	67.17 ±9.35	1.6
T2	**con**	63	82.96 ±6.01	2.0
	2h	67	73.05 ±11.7	1.8
	6h	60	71.22 ±8.37	1.7
	24h	61	65.00 ±10.36	1.6
T3	**con**	52	82.45 ± 5.98	2.0
	2h	67	71.65 ±8.37	1.7
	6h	66	69.58 ±9.57	1.7
	24h	60	60.99 ±6.67	1.5
T4	**con**	66	83.00 ± 6.22	2.0
	2h	60	70.96 ±14.7	1.7
	6h	58	66.05 ±12.3	1.6
	24h	62	55.165 ±6.67	1.4

4. DISCUSSION

The results of the present study demonstrated a clear evidence of the genotoxic and mutagenic effects of $KBrO_3$. As described in the study, root tips of *A. sativum* were exposed to various concentrations of KBrO3 between 3 to 9 g/l for 2, 6 and 24h, and the cells were investigated for the DNA content measurement after each period time. The wide range of observed meristematic nuclei with damaged DNA contents proved that even short term exposure to relatively small doses of $KBrO_3$ significantly decreased the DNA content. The genotoxic action of $KBrO_3$ were seen to be dependent onboth their concentration and the time period, with even the low doses causing a significant rate of decrease of mitotic index and increase in percentage of mitotic abnormalities [10].

Nuclear DNA content significantly decreased at all concentrations and treatment incubation times dose dependent manner. Such inhibition of DNA values was also reported by Türkoğlu [11] using different treatments of six food additives increased concentration and periods of treatment resulted in increased reductions in the amount of DNA. Similar results for these parameters with $KBrO_3$ have been determined using TK6 cell cultures to induced DNA damage, the data in this study showed the DNA damage was dose-response effect [12]. These data are consistent with our results showed the dose-response dependence damage. According to [13], the genotoxicity of $KBrO_3$on DNA damage is believed to begin with induce the 8-OH-dG DNA directly by KBrO3 under cell-free conditions [11]. Using DNA comet assay in different rat organs [14,15] found that the potassium bromate induced DNA damage in the different tested animal tissues.

Njagi and Gopalan [16] also reported that two food preservatives, sodium benzoate and sodium sulphite, inhibit the DNA synthesis in *Vicia faba* root meristems. The reduction of DNA synthesis might be caused by the decreasing ATP level and the pressure from the functioning of the energy production centre.

Fig. 1 and Table 1 showed a significant reduction in DNA synthesis of *A. sativum* root tipnuclei compared to the control. In particular, the obtained results statistically significant decreased in DNA values in all concentrations after exposure to $KBrO_3$. The average of measured DNA was from 2C (control value) to >1.8 C per cell, in general, DNA relatives values ranged from 1.8C to 1.4C in the measured nuclei. When comparing all treated samples from different treatments exhibited lower DNA and this reduced as the concentration increased and the period of treatment prolonged Different studies suggested that that low concentration of sodium metabisulfite inhibited DNA synthesis in root tips nuclei of *A. cepa* L. [17]. According to previous experiments of ours with the food preservative mono sodium glutamate showed different behavior on DNA values of *Allium* root tip nuclei as the DNA content is increased above normal (2C) [9]. We postulate that these differences in terms of onset of action represent the different

lipophilic properties of the two molecules of food additives.

The inhibition of DNA content reflect in the decline of the mitotic index (unpublished data) indicates the occurrence of a genotoxic effect. DNA values decreased with increasing concentrations of $KBrO_3$. Earlier studies on DNA content using other food additives demonstrating the reduced values of DNA compared with control might result of a mitodepressive action prevent the DNA synthesis [18].

5. CONCLUSION

In conclusion, it is clear that $KBrO_3$, which are used frequently in the food industry, has a clear potential genotoxic activity. The Allium assay is sensitive test system for monitoring mutagenic effects. Hence, the Allium test should be considered as a warning and detector that the tested chemical may be a risk to human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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