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Mitodepressive Effect of Four Food Additives Using the *Allium Cepa* Assay

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ABSTRACT

The mitodepressive effect of potassium bromate, sodium benzoate, sodium bicarbonate and ammonium bicarbonate were determined using the *Allium cepa* Linn. (the common onion) assay. This model was used to determine the effects of these additives on the mean root length, mitotic index and chromosomal aberrations of onion bulbs using 0.25, 0.5 and 1% (w/v) of each food additive. All additives showed a concentration-dependent toxicity on the roots, interfering with mitotic cell division and causing chromosomal aberrations.

Keywords: ammonium bicarbonate, chromosomal aberration, mitotic cell division, potassium bromate, sodium benzoate, sodium bicarbonate

INTRODUCTION

The photo-genotoxic potential of drugs, foods and cosmetic products has recently become the focus of intense research (Brendler-Schwaab *et al.* 2004). Several pigments which consist of UV- and visible light-absorbing compounds are derived from natural substances and are widely used in Japan as food colourants (Ministry of Health and Welfare 2000). Approximately 70% of the world population consumes food additives one way or the other, everyday (WHO 1996). Food additives are substances added intentionally to preserve, flavor or improve the taste and appearance of food and can also act as antioxidants (Kayraldiz *et al*. 2006). People have expressed concern about the mutagenic and carcinogenic potential of food additives worldwide (Anderson 1996). Hence, the scientific assessment of the genotoxicity of food additives is of utmost importance (Growther *et al.* 2009).

Kotsonis *et al*. (1996) showed that any substance whose dietary concentration is as high as 1 ppm should undergo extensive toxicological screening. There are many controversies regarding the use of potassium bromate (Michael 1999). Our earlier finding using a modified Ames assay also documented the mutagenic potential of potassium bromate (Akintonwa *et al.* 2007), which was detected in urine after metabolism (Yang *et al.* 2008). Food additives, including sodium benzoate, sodium bicarbonate and ammonium bicarbonate are used as preservatives in soft drinks, as a leavening agent in baking and as a raising agent in the food industry, respectively (Akintonwa *et al*. 2007; www.preparedfood.com).

In environmental toxicology research, *Allium cepa* L. has been listed as an example of a plant useful in the screening of mutagens (Stich *et al*. 1975; Nilan 1978; Grant 1982). This test is a very useful tool for evaluating and ranking environmental chemicals with reference to their toxicity (Fiskesjo 1985). The onion root tip is a convenient system (Akintonwa *et al*. 2009) to determine both macroscopic (growth, EC_{50} values) and microscopic parameters (c-mitosis, stickiness, chromosome breaks).

This study aimed to investigate the possible effects of four food additives on mitotic cell division using the *A. cepa* assay.

MATERIALS AND METHODS

Food additives

Potassium bromate, sodium benzoate, sodium bicarbonate and ammonium bicarbonate were obtained from Nomek Chemical Merchants, Oshodi-Apapa Express Way, Lagos, Nigeria.

Allium cepa Linn. assay

The *Allium* test (Fiskesjo 1985) provides a rapid screening procedure for chemicals and environmental agents which may represent environmental hazards. Root growth inhibition and adverse effects on chromosomes provide an indication of likely toxicity.

Healthy, equally-sized common onions (*Allium cepa* L.) were obtained from Bariga local market, Lagos, Nigeria. The dried outer scales were carefully removed leaving the ring of the root primordium intact (Fiskesjo 1985). Five onion bulbs were utilized for testing each of three concentrations (0.25, 0.5 and 1%) of sodium bicarbonate, sodium benzoate and ammonium bicarbonate in water and for 0.125, 0.5 and 1% potassium bromate in water.

Tap water of good quality was used as the negative control. The base of each onion bulb was grown (floated) on each of the concentrations of environmental agents inside a 30-ml beaker containing 30-ml solution of the environmental agents. The beakers were not placed in direct sunlight for 4 days after which root length was measured.

Root growth inhibition test

The toxicity assay was performed as a 96-h semi-static exposure test, and 3 concentrations of the test samples were used. Every 24 h the test solutions were replaced by fresh solutions. The test solutions were placed at room temperature and at the end of the exposure period, the length of root bundles was measured and means ± S.E were calculated.

Microscopic analysis

An optical microscope model 109-L with a theoretical resolution limit of around 0.2 µm was used to analyze processed root tips.

Root tips 10 mm long were cut off and fixed in acid (acetic acid): alcohol (ethyl alcohol) (1: 3) by heating for 5 min at 50°C.

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Research Note

Terminal root tips (1-2 mm) were cut off and gently squashed on slides (Reorder No SF 20501, Surgical Medicals, Middlessex, England) and stained with orcein solution for 10 min. Coverslips were then carefully lowered onto the stained area to avoid air bubbles and slides were carefully dampened with the use of a filter paper to remove excess stain. Coverslips were fixed carefully to slides with nail varnish.

The slides were examined under a microscope model 109-L to determine the mitotic index (MI) and to detect chromosomal aberrations. MI (Fiskesjo 1985) was determined by counting all stages of mitotic cells from 1000 cells:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells analyzed}} \times 1000$$

The slides were thoroughly examined and the first 100 metaphase, anaphase and teleophase cells were scored for aberrations. Following ANOVA, a *t*-test was used to compare the significant differences between means of treatment and control onions at $P<0.05$.

RESULTS

Tables 1-3 show the cytotoxic and root growth inhibitory effects of various concentrations of food additives on *A. cepa.* Ammonium bicarbonate (EC_{50} = 0.01) at 0.25% increased root growth by 0.2 ± 0.0% more than the control and an MI = 10. However, 0.5 and 1% of this food additive only increased root growth of 0.1 ± 0.14 with a zero MI (turbagenic effect). Sodium benzoate inhibited root growth at all three concentrations. Furthermore, sodium bicarbonate (EC_{50} = 0.1) showed a concentration-dependent decrease in root length and MI. Ammonium bicarbonate and sodium bicarbonate showed stickiness, bridges and fragment chromosomal aberrations.

Potassium bromate (EC_{50} = 0.05) at 0.125% produced a 0.76 ± 0.08% increase in average root length and an MI = 46 (**Table 4**). Only stickiness and chromosomal aberrations were detected. Roots did not grow at higher concentrations (0.5 and 1.0%).

Fig. 1 shows the normal stages of mitotic cell division. At Interphase, the chromosomes are not visible and they become visible at Prophase. The chromosomes align at the equator in Metaphase and they begin to move to the poles in Anaphase. The cell membrane breaks and divides into two cells in Teleophase.

Fig. 2 shows the various aberrations observed in *A. cepa* root cells. Most of the aberrations were observed at Anaphase. Sodium bicarbonate, ammonium bicarbonate and potassium bromate showed some of the chromosomes to be linked together instead of separating to the poles forming bridges and fragments. There was also a lag observed in the chromosomal migration to the poles.

Fig. 3 shows both the control and treated groups of *A. cepa* root growth. In the control more roots sprouted and roots were longer than the treatment groups.

DISCUSSION

Due to changes in life-style there is increasing demand for safer and more convenient food. Kida (2004) showed the relationship between environmental agents, including food additives, and mutagenicity. Our study used the *A. cepa* assay to investigate the genotoxic potential of four food additives: potassium bromate, sodium bicarbonate, sodium benzoate and ammonium bicarbonate. This model showed

Table 1 Effects of various concentrations of ammonium bicarbonate on the cytology and root growth of *Allium cepa.*

Treatment	Phenotypic indices			Chromosomal aberrations			
conc. (%)	No. of dividing cells	Mitotic index	Mean root length ± SE	Stickiness	Multipolar anaphase	Bridges and fragments	C-mitosis	Vagrant
0 (control)	52	126	4.45 ± 0.35	0	0	0	0	0
0.25	5	10	0.2 ± 0 a	1	0	3	0	1
0.5	0	0	0.1 ± 0.14 a	0	0	0	0	0
1	0	0	0.1 ± 0.14 a	0	0	0	0	0

EC_{50} = 0.1; a indicates a significant percentage increase at $P < 0.05$ using the *t*-test compared with the control.

Table 2 Effects of various concentrations of sodium benzoate on the cytology and root growth of *Allium cepa.*

Treatment	Phenotypic indices			Chromosomal aberrations			
Conc. (%)	No. of dividing cells	Mitotic index	Mean root length ± SE	Stickiness	Multipolar anaphase	Bridges and fragments	C-mitosis	Vagrant
0 (control)	52	126	4.45 ± 0.35	0	0	0	0	0
0.25	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0

a indicates a significant percentage increase at $P < 0.05$ using the *t*-test compared with the control.

Table 3 Effects of various concentrations of sodium bicarbonate on the cytology and root growth of *Allium cepa.*

Treatment	Phenotypic indices			Chromosomal aberrations			
Conc. (%)	No. of dividing cells	Mitotic index	Mean root length ± SE	Stickiness	Multipolar anaphase	Bridges and fragments	C-mitosis	Vagrant
0 (control)	52	126	4.45 ± 0.35	0	0	0	0	0
0.25	63	137	2.4 ± 0.14 a	18	0	2	0	4
0.5	26	72	1.6 ± 0.56 a	6	0	3	0	0
1	25	70	0.25 ± 0.70 a	11	0	0	0	0

EC_{50} = 0.1; a indicates a significant percentage increase at $P < 0.05$ using the *t*-test compared with the control.

Table 4 Effects of various concentrations of potassium bromate on the cytology and root growth of *Allium cepa.*

Treatment	Phenotypic indices			Chromosomal aberrations			
Conc. (%)	No. of dividing cells	Mitotic index	Mean root length ± SE	Stickiness	Multipolar anaphase	Bridges and fragments	C-mitosis	Vagrant
0 (control)	52	126	4.45 ± 0.35	0	0	0	0	0
0.25	23	46	0.76 ± 08 a	23	0	0	0	0
0.5	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0

EC_{50} = 0.5; a indicates a significant percentage increase at $P < 0.05$ using the *t*-test compared with the control.

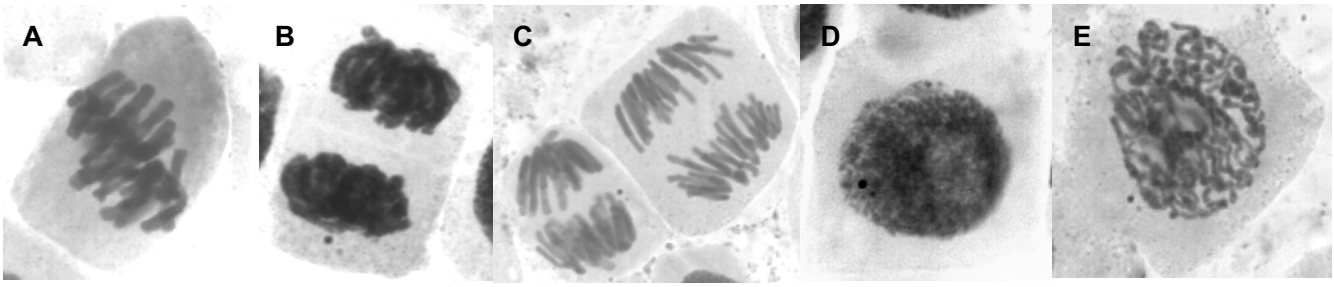

Fig. 1 Stages of normal mitotic division in cells of *Allium cepa* using orcein stain. (**A**) Metaphase, (**B**) teleophase, (**C**) anaphase, (**D**) interphase, (**E**) prophase. (Mag. ×100).

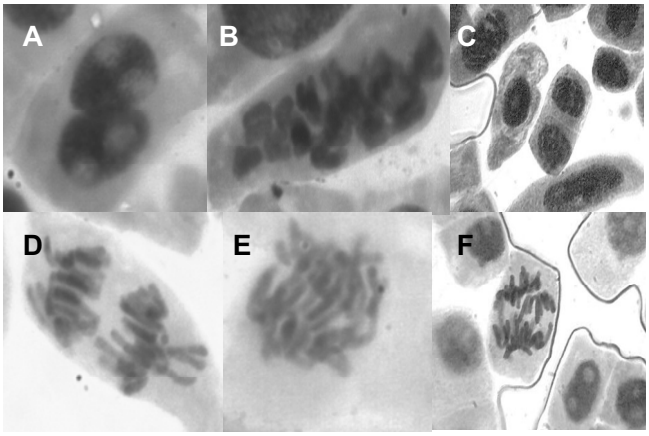

Fig. 2 Aberrations at various stages of mitotic division in cells of *Allium cepa* treated with the suspension of different food additives after staining with orcein. (**A**) Bi-nuclei, (**B**) c-mitosis, (**C**) multipolar anaphase, (**D**) anaphase with laggards, (**E**) bridges and fragments, (**F**) stickiness. (Magnification: ×100).

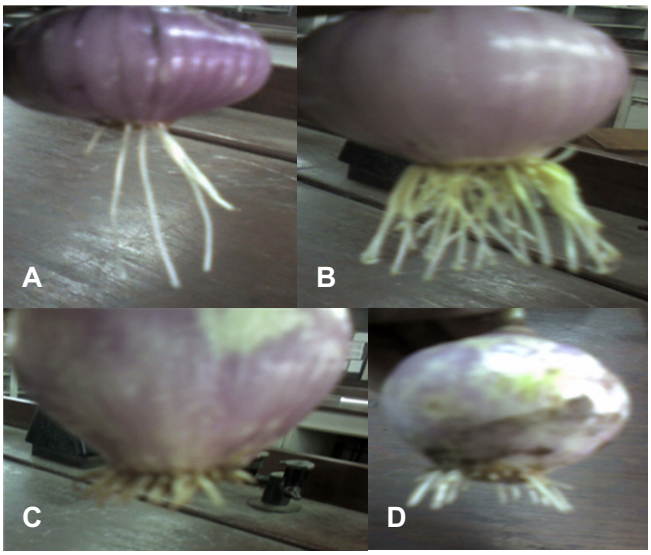

Fig. 3 *Allium cepa* root growth samples of both treated (C, D) and control (A, B) groups.

that potassium bromate produced concentration-dependent root growth inhibitory, mitodepressive and chromosomal aberration effects. Previous studies (Kawachi *et al*. 1980; CSG 1986; Kurokawa *et al*. 1990) used Chinese hamster lung cells and DON-6 cells to show that potassium bromate caused chromosomal aberrations in lung cells. Pottasium bromate was found to interfere with the root growth of *A. cepa.* The concentration dependence root growth inhibitory effects of potassium bromate may be as a result of the toxic effects (Akinboro and Bakare 2007) of potassium bromate on the root of *A. cepa.* Badr (1983) and Inceer *et al*. (2002) using *Allium cepa Linn* assay showed that the mitodepressive effect and chromosomal aberrations may be due to interference with mitotic division and inhibition of spindle formation, respectively.

Furthermore, the *A. cepa* test showed that sodium benzoate completely inhibited the growth of onion at all concentrations tested. However, a lower concentration of sodium benzoate might produce minimal growth with pronounced chromosomal aberrations. Earlier work (Abe and Sasaki 1977; Ishidate *et al*. 1988; Xing and Zhang 1990; Ishidate and Odashima 1997) showed that sodium benzoate produced chromosomal aberrations in human lymphocytes.

In conclusion, the *A. cepa* test could be used to effectively determine the cytotoxic effect of food additives.

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