



Effect of Ferrous Gluconate on Chromosomal Abnormality Index of *Allium Cepa* Root Tip

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ABSTRACT

In completed research, ferrous gluconate -a food additive- used to preserve black color to prevent discoloration during storage in ripe black olives, and *Allium cepa* L. species. *A. cepa* L. roots were treated with different doses of ferrous gluconate. The effective concentration EC₅₀ (0.068 g/l) was determined. *A. cepa* root tips were treated with EC₅₀/2 (0.034 g/l), EC₅₀ (0.068 g/l), 2XEC₅₀ (0.136 g/l) dose for 24, 48, 72 hours, and afterward, the root tips were prepared for observation under the light microscope according to the method of preparing mitotic preparation. Chromosomal abnormality index (CAI) and genotoxic effect of ferrous gluconate in *A. cepa* root tip cells were determined. Repeated measurement ANOVA and TUKEY multiple comparison tests were used to investigate the effect of time and dose together on genotoxicity. C-mitosis, polyploidy, polar shifting in anaphase, polar shifting in telophase, equatorial plate shifting, laggard chromosome was observed by microscope. The highest CAI (70.16±4.85) was observed at 72h for 2XEC₅₀ dose. Chromosomal aberration is also observed in control group. While the most common chromosomal aberration is determined as C-mitosis; The least observed chromosomal aberration is determined as polyploidy. Research results revealed that ferrous gluconate has a genotoxic effect on the root tip of *A. cepa*.

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Introduction

Significant increases were observed consuming of food preservatives in the 20th century with the increase in food production (Altuğ, 2001). Food preservatives are defined as substances used to effect properties of food as requested. Natural preservatives have been used since the past; artificial preservatives have been used which reduce the fee with the development of technology and accelerating of production in recent years (Gürsoy, 2001). These are the application forms of food additives that create the desired effect, such as preventing the bad taste of the food or the production of faulty products, etc (Altuğ, 2001). It is important to consider that even if the food additives used are not harmful to health, it may cause damage to tissues and accumulate in the body over time. So, these food preservatives might threaten human health as indirect or direct. Because of the risks, clastogenic, mutagenic, aneugenic effects of various substances including food preservatives are investigated by *in vivo* and *in vitro* tests. Many studies were indicated a positive correlation between the mutagenic effects of chemicals and the risk of cancer (Kaderlik et. al., 1992; van Sittert et. al., 2000; Moore and

Chen, 2006; Maertens et. al., 2008; Prado-Ochoa et. al., 2020).

The color of black olives is not permanent, it is gradually disappearing after oxidation and over the life of the packaged product. Iron salts (ferrous gluconate, ferrous sulphate, ferrous lactate) are used to prevent this degradation (Cruess, 1962). Ferrous gluconate (E579) is a food additive which is well soluble in water (Hurrell, 1997) and it consists of iron and glucose. This food additive is used to preserve the black color after the darkening step in order to prevent color degradation during storage of ripe black olives (Garcia et al., 1986; Anonymous, 1995). Plant test systems have a common usage in evaluating the genetic aberrations caused by different chemicals. *A. cepa* test is being used by many explorers as bioindicators in effect assessment of chemicals (Bagatini et al., 2009; Leme and Marin-Morales, 2009). *Allium cepa* test can predict possible damage to the DNA of eukaryotes, therefore *Allium cepa* test is valuable (Tedesco and Laughinghouse, 2012). It was indicated that the *A. cepa* test correlates well with mammalian test systems (Fiskesjö, 1985).

The decreasing mitotic index indicates inhibition of the cell cycle and decreased proliferative of the cells (Gökalp Muranlı, 2006).

In this study, genotoxicity caused by ferrous gluconate in the mitotic division cells in *A. cepa* L. ($2n=16$) root tips were researched. At the beginning, the EC_{50} value was determined, afterward, ferrous gluconate was applied to the root tips for 24, 48, 72 hours at $EC_{50}/2$, EC_{50} , $2XEC_{50}$ concentration. Thus, genotoxic effect of ferrous gluconate was evaluated.

Materials and Methods

Material

In study, *A. cepa* L. ($2n=16$) was used. Ferrous gluconate (E579) used as a food additive was supplied from Cesa Chemical Industry Trade Limited Company.

Method

Treatment of Root Tips with EC_{50} Concentrations

In the first place, *A. cepa* L. root tips were applied with different concentrations prepared by dissolving ferrous gluconate in water (0.16- 0.20- 0.30- 0.35- 0.40- 0.50- 0.60- 0.70 g/l). Root tip lengths were measured and EC_{50} concentration was determined. (Fiskesjö, 1993). *A. cepa* L. roots were treated with $EC_{50}/2$ (0.034 g/l), EC_{50} (0.068 g/l), $2XEC_{50}$ (0.136 g/l) concentrations for 24, 48, 72 h.

Preparation of Mitotic Preparations

At the end of the application period, root tips were cut and put into the farmer's fixative. The root tips were washed with water and hydrolyzed as expressed by Souguir et al. (2008). Samples were stained with 2% acetocarmine (w/v). For all treatment groups, at least 5000 cells were counted. Prophase, metaphase, anaphase and telophase cells were observed by the light microscope and root tips were evaluated.

Chromosomal abnormality index (CAI) was calculated by the formula $CAI = \text{Number of cells with chromosomal abnormalities} / \text{total cells number}$.

Statistical Analysis

Repeated Measurement ANOVA and The TUKEY Multiple Comparison Tests were used.

Results and Discussion

Mitotic phases (prophase, metaphase, anaphase and telophase) and chromosomal aberrations were observed at root tips after treatment of ferrous gluconate at doses of $EC_{50}/2$, EC_{50} , $EC_{50}X2$ to *A. cepa* root tips for 24, 48 and 72 hours. In addition, chromosomal aberration index (%) were given together with the mean and standard errors (Table 1).

It has been determined that the difference between concentrations varies according to the duration of treatment. It was detected that ferrous gluconate causes chromosomal abnormalities such as C-mitosis, equatorial shifting, polar shifting, laggard chromosome, chromosomal fragment and polyploidy in *A. cepa* root tip cells (Figure 1). In terms of chromosomal aberration index, it was determined that the duration of treatment period didn't affect the control group. The highest chromosomal aberration index was determined for $EC_{50}X2$ dose at 72 hours. In this way, it has been shown that ferrous gluconate has genotoxic effect (Table 1).

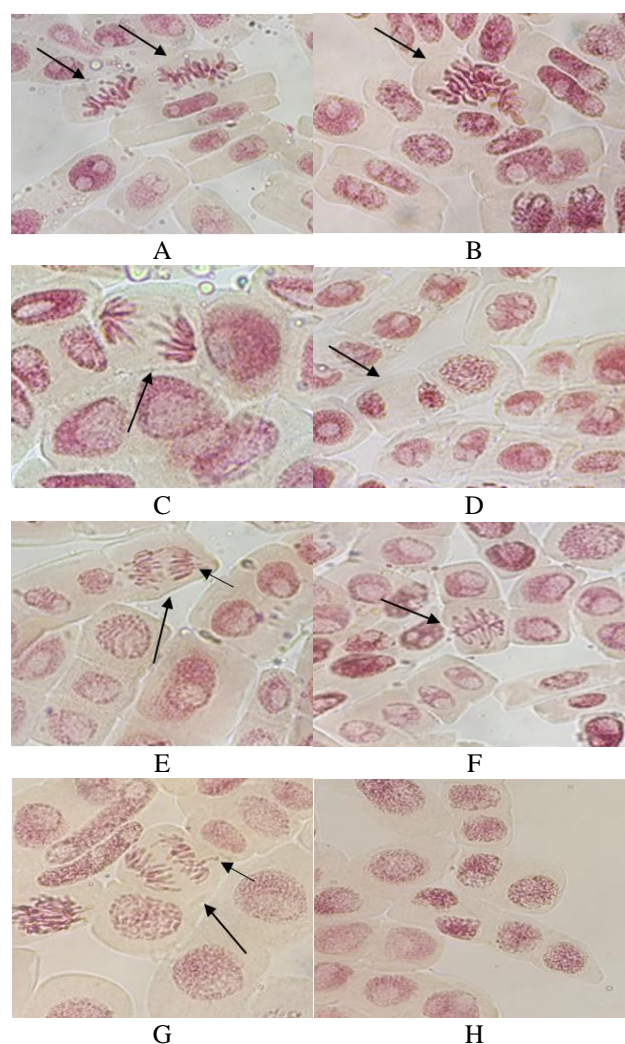


Figure 1. Photographs of chromosomal abnormalities observed in mitotic phases in root tip cells as a result of ferrous gluconate application to root tips of *A. cepa* are shown.

It was determined that usage of orange G and brilliant blue (Kumar and Sing, 2017); sodium saccharin and sodium cyclamate food sweeteners (Oliveira et al. 2017); synthetic sweeteners passion fruit and vanilla (Nunes et al, 2017); tartrazine (Lerda, 2017) was caused chromosomal aberrations due to concentration and increasing application time in root tip cells. Thus, it has been shown that the mentioned food additives have genotoxic effect. It has been shown that doses of cookies and tutti-frutti aroma and combined doses of these sweeteners induce chromosomal abnormalities in a significant number of cells at the root tip of *A. cepa*. Therefore, it was indicated the cookie flavor and the combined doses are genotoxic; tutti-frutti aroma showed genotoxic effect. Brilliant blue and sunset yellow have been found to have genotoxic effect (Kuş and Eroğlu, 2015). Tripathy and Rao (2015) found that orange red food colorant caused mitotic aberrations in *A. cepa* L. root tip cells. It was determined that chromosomal aberration index increased at the root tip, when applied with sunset yellow azo dye (Dwivedi and Kumar, 2015); benzoate and boric acid (Kumar and Pandey, 2015); sodium nitrate, butylated hydroxyanisole, sorbic acid, butylated hydroxytoluene and propyl gallate (Pandey et al., 2014). Pandey et al. (2014) found that the total percentage of chromosomal aberrations increased with increasing dose and treatment time.

Table 1. Chromosome aberration %, total aberration (Mean±Std. Error) in root meristem cells of *A. cepa* after exposure EC₅₀/2, EC₅₀ and EC₅₀X2 ferrous gluconate dose for 24, 48 and 72 h

Treatment period (h)	Dose (g/l)	Chromosome aberrations (%)						Total aberration % (Mean±Std. Error)
		C-Mit	E.PI	Sh	PI	Sh	Lag	
24	Control	30	21.67	10	18.33	0	0	1.482±0.462 ^{Ca}
	EC50/2	38.62	24.99	33.34	29.7	1.51	0	13.717±0.576 ^{Bc}
	EC50	42.99	14.63	22.42	16.45	0	0	20.546±0.683 ^{BAa}
	EC50X2	9	16.53	23.12	14.59	0	0	26.524±0.787 ^{Aa}
48	Control	42	20	0	18	0	0	1.286±0.496 ^{Ca}
	EC50/2	41.65	15.16	20.25	22.02	0	0	33.99±1.28 ^{Bb}
	EC50	43.48	15.77	33.95	19.39	0	0	41.14±2.52 ^{BCb}
	EC50X2	32.43	19.44	31.22	13.13	3.11	2.1	45.973±0.593 ^{Ab}
72	Control	33.33	0	0	6.67	0	0	0.486±0.359 ^{Ca}
	EC50/2	40.26	14.82	21.47	17.93	1.51	0	51.603±0.792 ^{Ba}
	EC50	40.54	13.73	32.31	8.2	4.95	1.2	53.46±6.12 ^{Ba}
	EC50X2	20.04	19.06	34.71	26.41	1.38	0	70.16±4.85 ^{Aa}

Note 1. The difference between the doses indicated in different capital letters during the same period is important. Note 2. The difference between treatment period indicated in different small letters at the same dose is important.

It was determined that tartrazine and sunset yellow (Dwivedi and Kumar, 2017); monosodium glutamate (Adeyemo and Farinmade, 2013); sunset yellow, bordeaux, tartrazine (Gomes and Oliveira, 2013); monosodium glutamate, monopotassium glutamate, calcium glutamate, monoammonium glutamate, magnesium diglutamate flavor enhancers (Türkoğlu, 2013); potassium metabisulfite and potassium nitrate (Gömürgen, 2005) increased chromosomal aberrations. It was reported that trisodium phosphate, disodium phosphate and monosodium phosphate (Türkoğlu, 2009), potassium propionate, calcium propionate and sodium propionate (Türkoğlu, 2008); boric acid, sodium citrate, sodium benzoate, potassium citrate and citric acid (Türkoğlu, 2007) inhibited cell division. In addition to this, it was reported that those food preservatives increased chromosome aberrations. In parallel with these studies, it was determined that chromosomal aberration index increased due to increasing in dose and treatment period by treating different concentration of ferrous gluconate at different periods. Similar to these researches, it was reported that it has been observed chromosomal aberrations such as C-mitosis (Nunes et al., 2017; Kumar and Pandey, 2015; Türkoğlu, 2013, 2009, 2007; 2008; Dwivedi and Kumar, 2007; Gömürgen, 2004), laggard chromosome (Dwivedi and Kumar, 2015, 2017; Kumar and Pandey, 2015; Türkoğlu, 2013, 2008, 2007) in mitotic cells. In addition to these aberrations, some other aberrations (polar shifting, equatorial table shifting etc.) were detected in mitotic cells in this research.

Conclusion

In the result of this research, it was determined that ferrous gluconate may cause genotoxicity to living organisms when exposed to more than certain doses and periods. This effect occurs by inhibiting mitosis and causing chromosomal abnormalities. Genotoxic effects determined by *A. cepa* test can be generalized to all living organisms. Therefore, it should be noted that the use of ferrous gluconate in foods may have toxic effects on humans over certain doses and treatment periods.

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