ORIGINAL ARTICLE

CYTOTOXIC AND GENOTOXIC EFFECTS OF TRIACETIN (GLYCEROL TRIACETATE) ON *ALLIUM CEPA* ROOT TIP

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ABSTRACT

Background: Genotoxic and cytotoxic effects of triacetin (E1518) (glycerol triacetate) food additive in *Allium cepa* root tip cells were researched. **Objectives:** The aim of this study is to investigate whether triacetin food additive has genotoxic and cytotoxic effects on *Allium cepa* root tip cells. **Methods**: Root tips were treated with different doses of triacetin (0.020, 0.025, 0.030, 0.035, 0.040, 0.048, 0.050, 0.060, 0.065, 0.75 g/l). In order to observe the effect of doses on root tip length, EC50 (effective concentration) was determined as 0.050 g/l by measuring root tips. Theroot tips were then kept at doses of $EC_{50}/2$ (0.025 g / l), EC_{50} (0.050 g / l), $2XEC_{50}$ (0.100 g / l) for 24, 48, 72 hours. Mitotic cells were observed with a light microscope. At least 5000 cells were counted for each group. Cells in interphase, prophase, metaphase, anaphase and telophase were examined. **Results:** Mitotic index (MI) and chromosomal anomalyindex (CAI) were determined. Repeatedly measured ANOVA and TUKEY multiple comparison test was applied. When evaluated statistically, it was found that the difference between doses varies according to the time applied. In terms of MI and CAI, it was demonstrated that the mean of the control treatment was considerably higher in all treatment times compared to other doses treated and decreased with increasing dose concentration. As a result of treatment with $EC_{50}X2$ dose, it was determined that it decreased statistically due to the increase in treatment time**. Conclusion:** Triacetin was found to reduce cell division in root tips compared to the control group. Thus, it has been demonstrated that triacetin has cytotoxic and genotoxic effects. It was found that cytotoxic and genotoxic effects of triacetin increased depending on the time of treatment and dose, and thus, the mitotic index decreased and the chromosomal anomaly index increased.

Keywords: *Allium cepa test system, triacetin, genotoxic, cytotoxic*

1. INTRODUCTION

Nowadays important increasing has been observed in the using of food additives. Food additives are defined as substances used to effect the properties of foods in the desired way. These features include flavor, storage life, appearance and texture [1]. Food additives is added to foods in order to prevent spoilage of foods during the preparation of foods, increase nutritional value, taste and structure. Although in the past, natural additives have been used, in nowadays artificial additives have been used which accelerate production and reduce the cost [2].

To use of food in order to mask bad quality food or to avoid faulty product acquisition, to misrepresent food, to improper food production and to deceive the consumer, to decrease the nutritive value of the product, to use more than the technical amount to create the desired effect, food additives are illegal forms of application [1]. When food additives are used illegally, toxic and allergic reactions can be seen among people [3]. It is significant that food additives may cause damage to cells and accumulate over time and therefore threaten human health. Because mutagenic, clastogenic, aneugenic effects of several including food additives are researched by *in vitro* and *in vivo* test systems. Many researches have found a positive correlation between the risk of cancer and the mutagenic effects of these food additives [4].

Triacetin (glyceryl triacetate, glicerol triacetate) (GTA) is a liquid soluble in water, biodegradable in activated sludge and is capable of forming a homogeneous mixture of alcohols, aromatic hydrocarbons and diethyl ether [5]. Triacetin is used as flavor solver [6]. It is commercially produced from acetic acid and glycerol. Triacetin has no adverse health effects expected from inhalation. When ingested in large doses, it may cause a gastro-intestinal upset. No adverse effects expected on skin contact. No adverse effects expected on eye contact. However, it may cause irritation, redness, and pain [7]. Triacetin is adequately used in chewing gums and all other nutrients. There is only limitation in tahini halva [8].

Plant test systems have a widespread use in assessing the possible genetic damage caused by different food additives. Mant researchers have been used Allium cepa test system as bioindicators in effect appraisal of these food additives [9, 10]. Because, *Allium cepa* test system is very sensitive to detect these substances that cause chromosome changing. This test is significant, because *Allium cepa* can grow in direct interaction with the material

researched cytotoxicity and genotoxicity and *Allium cepa* test is an *in vivo* model that can predict possible damage to the DNA of eukaryotes [11]. *Allium cepa* test has shown a particularly good correlation between mammalian testing systems [12].

Mitotic index (MI) is evaluated cytotoxicity for living organisms [13]. Reduced mitotic index shows inhibition of cell cycle progression or loss of proliferative capacity [14]. The percentage of mitotic index is expressed by the following formula. MI (%) = The cell number in mitosis/Total cell count X 100 [12-15]. To detect the dose to be used to determine the mitotic index, the root growth inhibition test is performed and the appropriate concentration range is determined. For this, the effective dose value (EC_{50}), which causes 50% reduction in root length relative to the control, should be determined [16, 17]. The chromosomal abnormality index (CAI) is established in the appreciating of genotoxicity. The chromosomal abnormality index is calculated according to the following formula. CAI = The number of chromosomally abnormal cells/ Total cell count X 100 [12-15].

In this study, the cytotoxicity and genotoxicity caused by triacetin (E1518) in the mitotic cycle of *Allium cepa* root tips (2n= 16) were established. Firstly, the EC_{50} value was determined. Then triacetin was implemented to the roots for 24, 48, 72 hours at $EC_{50}/2$, EC_{50}, $2XEC_{50}$ concentration.

2. MATERIAL AND METHOD

2.1. Plant Material

In this reserach, *A. cepa* (2n=16) was used as plant material and triacetin (E1518) was used as food aditive. Triacetin was bought from Cesa Chemical Industry Trade Limited Company.

2.2. Method

2.2.1. Determination of EC_{50} value

A. cepa root tips have been treated with different doses of triacetin (0.020, 0.025, 0.030, 0.035, 0.040, 0.048, 0.050, 0.060, 0.065, 0.75 g/l). Triacetin has been prepared by dissolving in water. Thus, EC_{50} value was determined [18]. *A. cepa* root tips were treated with $EC_{50}/2$ (0.025 g/l), EC_{50} (0.050 g/l), $2XEC_{50}$ (0.100 g/l) doses for 24, 48, 72 hrs. when they reached 1.5-2 cm in 5 days.

2.2.2. Determination of MI and CAI

A. cepa root tips were cut at the end of the treatment period and placed in dark at 4°C into farmer fixative containing ethanol: glacial acetic acid (3:1). The root tips were washed with distilled water and hydrolyzed with 1N HCl at 60°C for 10 min [19]. Roots were stained with %2 acetocarmine (w/v). In the preparation of the slides, one root tip was used for each slide. For each groups, at least 1000 cells were counted in each slide. For the control and treatment groups, at least 5000 cells were counted. Interphase, prophase, metaphase and telophase cells were observed by light microscopy on 1000X objective.

Cytotoxic effect was specified by mitotic index (MI). Mitotic index is calculated by the formula MI=Divided cell count/Totall cell numberX100. The cells in mitotic division and their division stages were specified.
Mitotic stages of mitosis cells and mitotic abnormalities observed in these stages were counted and stages were determined. Thus, chromosomal anomaly index (CAI) was calculated. Chromosomal anomaly index was calculated by the formula CAI=Number of cells with chromosomal abnormalities/total cell number.

2.3. Statistical Analysis

ANOVA with a two-factor repetitive measurement was used in which one of the factors included repeated measures (time). Repeated Measurement ANOVA was used to examine the effects of time and dose on the genotoxic and cytotoxic effect. the difference caused by which subgroup was determined by The TUKEY Multiple Comparison Test.

3. RESULTS AND DISCUSSION

In this research, cytotoxic and genotoxic effects of triacetin on A. cepa has been determined. For this reason, mitotic index and chromosomal anomaly index have been calculated. It has been determined that the mitotic index decreases with increasing dose and increasing duration of treatment. The chromosomal anomaly index was found to increase with increasing dose and increasing duration of treatment. The results of the statistical evaluation of genotoxic and cytotoxic effects are shown in Table 1. The mitotic phases observed at the root tips of the control group are

interphase, prophase, metaphase, anaphase, telophase. The chromosomal anomalies observed at the root tips are C-mitosis, polar shifting, laggard chromosome (Figure 1).

Table 1: Mitotix phases (%), mitotix index %, chromosome aberrations %, total aberrations

Treatment period (h)	Dose (g/l)	Mitotic Phases (%)				Mitotic index % (Mean±Std. Error)	Chromosome aberrations (%)					Total aberration % (Mean±Std. Error)
		Prophase	Metaphase	Anaphase	Telophase		C-Mit	E.Pl Sh	Pl Sh	Lag	Polip	
24	Control	65,76	23,76	19,69	11,18	21,34± 1,03Aa	23	32,64	15,43	21,7	0	0,387±0,278Cb
	EC50/2	56,59	21,46	22,32	14,01	14,121±0,83Ba	51,59	27,87	27,57	18,45	0	19,398±0,739Bc
	EC50	46,8	18,13	17,57	14,83	8,023±0,219Ca	21,95	23,53	15,65	16,78	15,23	23,218±0,198BAbc
	EC50X2	55,67	25,32	18,89	19,12	6,127±0,536Da	13	19,75	23,07	27,89	0	29,231±0,820Ac
48	Control	41,8	32,2	25,76	7,24	19,153±0,127Aa	52	0,00	31,03	15,45	0	1,182±0,280Ca
	EC50/2	43,83	25,42	17,85	14,43	9,762±0,172Bb	49,63	18,09	18,01	25,04	0	43,198±0,281Bb
	EC50	50,7	32,42	19,48	16,59	5,537±0,390Cb	67,49	14,91	21,43	19,2	0	35,109±0,532ABb
	EC50X2	45,64	24,06	18,65	9,76	5,156±0,516Ca	38,44	17,48	12,16	16,09	16,35	38,829±0,230Ab
72	Control	56,76	15,04	16,55	19,33	16,526±0,012Aa	35,56	12,05	32,12	6,9	0	1,620±0,328Ca
	EC50/2	45,67	26,12	19,23	16,58	5,090±0,061Bc	41,87	18,22	23,05	7,08	5,52	61,179±0,435Ba
	EC50	47,69	27,57	22,54	16,13	2,001±0,062Cc	43,51	15,75	19,57	14,98	0	74,928±0,08Ba
	EC50X2	43,89	29,62	35,73	13,75	1,020±0,451Cb	21,18	16,98	26,14	27,81	0	83,298±0,291ABa

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

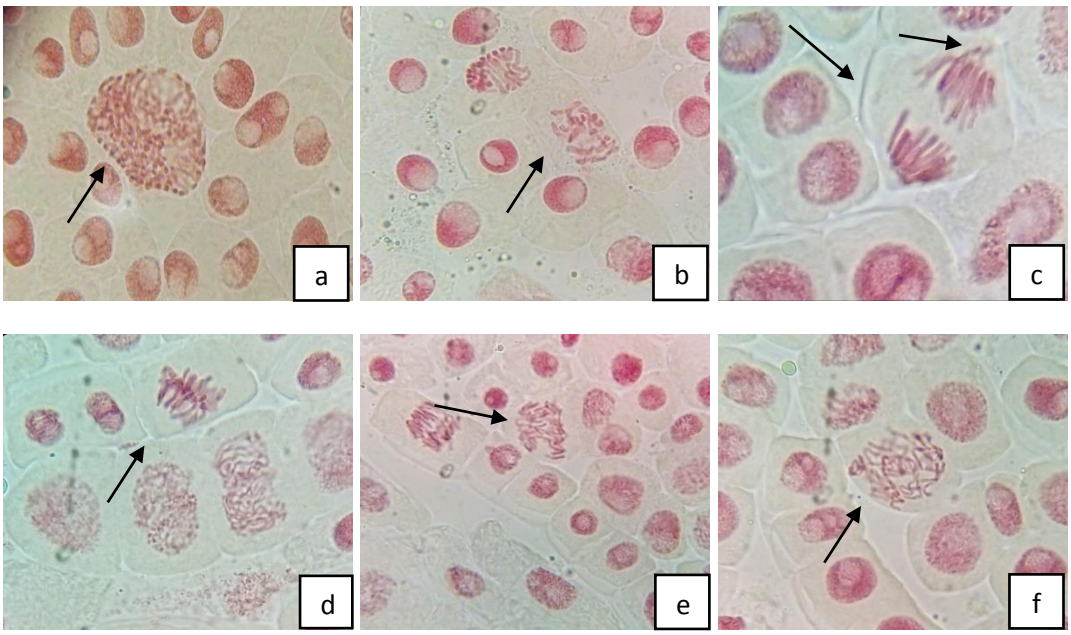

Figure 1: **a**) Poliploidy, **b**) C-mitosis, **c**) Polar shifting in anaphase and laggard chromosome, **d**) equatorial plate shifting , **e**) Polar shifting in anaphase, **f**) C-mitosis

When monosodium phosphate, disodium phosphate and trisodium phosphate [20]; sodium propionate, calcium propionate, potassium propionate [21]; sodium benzoate, boric acid, citric acid, potassium citrate, sodium citrate [22] are applied to the root tips with different concentrations and different treatment times, the mitotic index value is reported to decrease with increasing concentration and increasing duration of treatment. It has also been reported to increase chromosomal anomalies. Thus, it has been specified that it has cytotoxic and genotoxic effects. It has been determined that sunset yellow and brillant blue food colorants have a genotoxic and cytotoxic effect [23] and Ponceau 4R food coloring is cytotoxic [22]. Pandey et al., (2014) determined that the total percentage of anomalies increased with increasing concentration and application time. Tripathy and Rao (2015) found that orange red food coloring causes mitotic anomalies in *A. cepa* L. stem tip cells. These results are parallel with this research results.

4. CONCLUSION

Excessive using of food additives may cause allergic reactions, toxicite, etc. It has been detected by this research results that triacetin food addivite, is used more than specific dose and exposure time, is to be a cytotoxic and genotoxic effects in *Allium cepa* root tip cells. So, using of triacetin in foods should be take care. This research results might generalize to human research for genotoxicity and cytotoxicity.

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