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Investigation of the Antimitotic and Antimutagenic Effects of Methanolic Extracts of *Pyracantha coccinea*

Pyracantha coccinea'nın Metanol Ekstrelerinin Antimitotik ve Antimutajenik Etkilerinin Araştırılması

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Abstract

In this investigation, the antimitotic and antimutagenic effects of *Pyracantha coccinea* methanol extracts on somatic cells of *Allium cepa* was evaluated. The onion bulbs were applied to 400, 800, 1600, and 3200 µg /mL doses of the extract for 6, 12 and 24 h. Distilled water was utilized as a negative control and ethylmethanesulfonate was utilized as a positive control. The mitotic index mostly declined as increasing the extract concentrations. The mitotic abnormalities were observed as disturbed prophase, stickiness, laggards, chromatid bridges and c-mitosis. Also, micronucleus frequency was calculated in interphase. Thence, the antimitotic and antimutagenic potency of *P. coccinea* methanol extract with various tests were evaluated by using the root tip cells of *A. cepa*, and the use of a defined antimutagenic dose was proposed.

Keywords: *Pyracantha coccinea*, *Allium cepa*, Methanolic extract, Antimitotic, Antimutagenic

Öz

Bu araştırmada, *Pyracantha coccinea* metanol ekstraktlarının *Allium cepa* somatik hücreleri üzerinde antimitotik ve antimutajenik etkileri değerlendirildi. Soğanlara ekstraktın 400, 800, 1600, and 3200 µg/mL dozları 6, 12 ve 24 saat için uygulandı. Saf su negatif kontrol olarak kullanıldı ve etilmetansülfonat bir pozitif kontrol olarak kullanıldı. Mitotik indeks çoğunlukla artan ekstrakt konsantrasyonlarıyla azaldı. Mitotik anormallikler düzensiz profaz, yapışık kromozom, geri kalmış kromozom ve c-mitoz olarak gözlendi. Ayrıca, mikroçekirdek sıklığı interfazda hesaplandı. Böylelikle *P. coccinea* metanol ekstraktlarının antimitotik ve antimutajenik potansiyeli çeşitli testlerle *A. cepa* kök ucu hücrelerinin kullanılmasıyla değerlendirildi ve belirlenen antimutajenik dozun kullanımı önerildi.

Anahtar Kelimeler: *Pyracantha coccinea*, *Allium cepa*, Metanol ekstresi, Antimitotik, Antimutajenik

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1. Introduction

Pyracantha coccinea M.J. Roemer (Rosaceae) is an evergreen tree (Pignatti 1982) and cultivated as an ornamental plant in Turkey. It is generally utilized in alternative treatment aimed at diuretic, cardiac or tonic possessions in its fruits (Kowaleuki and Mrugasiewicz 1971). Earlier surveys indicated that the presence of flavonoids both in aerial and in hypogeal portion is quite diverse (Bilia and Catalano 1991; Bilia and Flamini 1993; Bilia and Morelli 1994).

Plants benefit in following of the toxic influences of various substances on living systems (Singh et al. 2008; Karaismailoğlu 2014) by chromosome abnormalities and micronucleus examination, their most important are *Allium cepa* (Fiskesjö 1985; Karaismailoğlu 2015), *Vicia faba* (Khadra et al. 2012), and *Helianthus annuus* (Kaymak 2005; Karaismailoğlu et al. 2013).

Current revisions have discovered that plants utilized for medicinal aims may induce toxic influences on living systems as utilized in the inappropriate dose (Kayraldız et al. 2010; Ping et al. 2012; Karaismailoğlu 2014). It has been made several investigations about application of *P. coccinea* including determine of free and bound phenolic acids in leaves and fruits (Sokolowska et al. 2009), and determine of the flavonoid distribution in plant at different growth phases (Fico et al. 2000) or study of antioxidant capacities and total phenol contents of methanolic and aquatic extract of this fruit (Vahabi et al. 2014), so far. However, the extract of *P. coccinea* is utilized in

alternative medicine and there is no data including antimitotic and antimutagenic effects on model organisms. The aim of this investigation was to assess the antimitotic and antimutagenic effects of the methanol extract of *Pyracantha coccinea* with utilizing the *Allium cepa* root tips.

2. Materials and Methods

Plant materials were obtained from Alfred Heilbronn Botanical Garden in Istanbul University (Fig. 1). The collected specimens were identified, and stored at the Herbarium of The Department of Biology; Istanbul University (ISTF).

Figure 1. *Pyracantha coccinea* overview and its fruits.

Extraction from dried fruits of *P. coccinea* was done using a technique changed from those of Ping et al. (2012) and Karaismailoğlu (2014). Accordingly, fruits were dehydrated in an oven at 30°C for 5 days and powdered by blender, and extracted by maceration in 200 mL of methanol for 5 days with regular shaking. After, solution was filtered by filter paper. Later, the liquid solvent was volatilized by evaporator under vacuum at 45°C, the dry weight was recorded, and the dry extract relocated to petri dishes and kept at -20°C in a freezer. The output ratio of the obtained extract from the raw material was calculated as nearly 8%.
The exterior scales of *Allium cepa* bulbs were taken without harmful primordia of roots. The bulbs were grown in distilled water at 22°C. When the root lengths of the bulbs were 1-2 cm in length, they were applicated with extracts at 400, 800, 1600, and 3200 µg/mL of *P. coccinae* for 6, 12 and 24 h. Distilled water was utilized as a negative control, however; Ethylmethanesulfonate ($2x10^{-1}$ M) (EM) was utilized as a positive control. The root tips obtained from the controls and the treated bulbs were fixed in ethanol-glacial acetic acid (3:1) and kept at 4°C overnight. Later, they were hydrolyzed in 1 N HCl at 60°C for 10 minutes, and stained with Schiff's reagent for 2 h in the dark (Darlington and La Cour 1976; Karaismailoğlu 2014). Five slides were selected from each treatment group and the mitotic index (MI), micronucleus (MN) in the interphase, and chromosome abnormalities in the dividing cells were examined in cytological analysis.
The values were assessed with utilizing Analysis of Variance (ANOVA) at a P=0.05 importance level with SPSS computer program. The dunnet-t multiple range test was used to define the statistical importance of variances among the means (SPSS 2008). The statistical examines are offered in Tables 1, 2 and 3.

3. Results

The influences on MI and frequency of mitotic phases of the tested concentrations of *P. coccinea* on *A. cepa* root tip cells are given in Table 1. Mostly, the MI reduced meaningfully as compared to negative control (P=0.05). Exceptionally, MI was not importantly different in 400 and 800 µg/mL concentration of *P. coccinea* at all treatment times, as compared to the negative control. Moreover, there were important different in the MI between applied to other concentrations (1600 and 3200 µg/mL), the positive control, and negative control (P=0.05) (Table 1). Furthermore, MI was importantly low at positive control, as compared to *P. coccinea* extract concentrations at 6, 12, and 24 h (Table 1). Additionally, there were statistically meaningful outcomes when the mitotic phase percentages were compared to the negative control in application times, (P=0.05). According to Table 1, virtually all applications impressed meaningfully the mitotic phase percentages. As the ratios of prophase and metaphase expanded, the percentage of the anaphase-telophase declined in the observed cells. Besides, an important increase in percentages of abnormality in the mitotic stages was defined with increasing *P. coccinea* concentrations (Table 1).
Chromosome abnormalities were observed in mitotic phases. The types and rates of chromosome abnormalities induced with applications are shown in Table 2 and Figure 2. *P. coccinea* applications and control groups have caused five aberrations types, which are disturbed prophase, c-mitosis, stickiness, laggards, and chromatid bridges. A remarkable increase in the chromosome abnormality was strong in all treatment groups. *P. coccinea* and EM applications exhibited a meaningfully higher chromosomal abnormality than the negative control, exclusive of 400 and 800 µg/mL applications of *P. coccinea* extract. Particularly, the 3200 µg/mL application was alike to EM (Table 2).

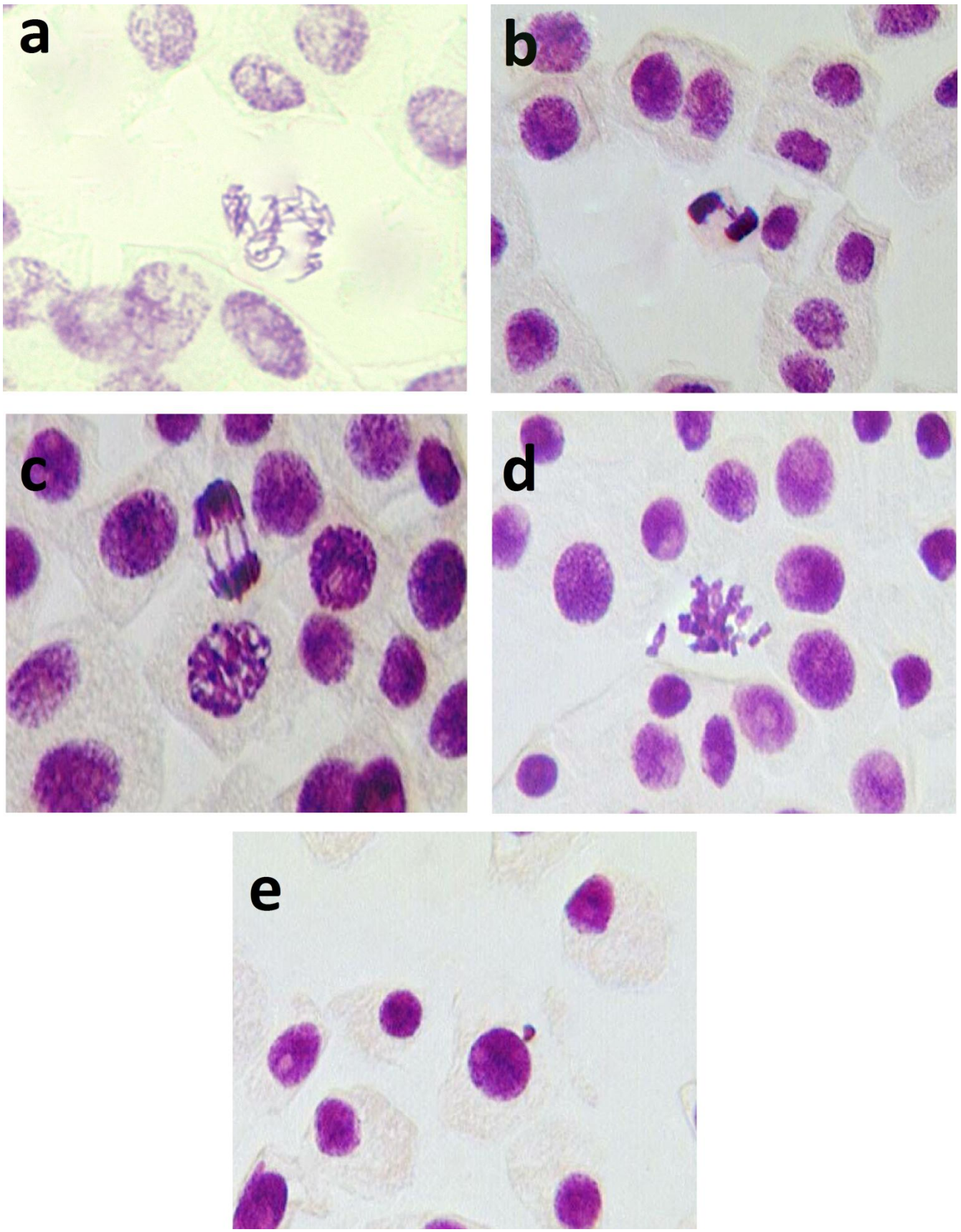

Figure 2. Chromosome aberrations caused by *P. coccinea* methanol extract in root meristem cells of *A. cepa*: **a;** disturbed prophase, **b;** laggards, **c;** chromatid bridge **d;** c-mitosis, **e;** micronucleus.

Table 1. Effects of the tested *P. coccinea* concentrations and controls on mitotic cell division in *A. cepa*.

Time (h)	Concentrations (µg/mL)	Number of the observed cells	MI±SD	Prophase		Metaphase		Ana-Telophase	
				Total	Anormal	Total	Anormal	Total	Anormal
6	Negative control	5000	19.86±0.26	39.76	-	21.67	-	38.57	-
	Positive control	5000	13.62±0.37*	36.51*	8.22*	24.48*	6.57*	39.01*	5.74*
	400	5000	19.91±0.12	33.18*	-	27.09*	-	39.73*	-
	800	5000	19.79±0.24	29.47*	-	31.99*	-	38.54	-
	1600	5000	17.28±0.18*	31.77*	1.89*	32.54*	2.05*	35.69*	2.21*
	3200	5000	15.41±0.27*	30.13*	3.86*	38.16*	4.41*	31.71*	4.27*
12	Negative control	5000	21.29±0.31	36.51	-	28.79	-	34.70	-
	Positive control	5000	13.05±0.24*	36.49	9.18*	30.91*	7.17*	32.60*	5.11*
	400	5000	22.01±0.65	36.44*	-	28.88	-	34.68	-
	800	5000	21.18±0.22	36.52	-	28.75	-	34.73	-
	1600	5000	16.33±0.41*	32.19*	1.54*	28.84	2.43*	38.97*	1.87*
	3200	5000	15.08±0.10*	36.48	4.29*	28.81	4.85*	34.71	5.16*
24	Negative control	5000	22.15±0.29	41.19	-	34.65	-	24.16	-
	Positive control	5000	10.76±0.21*	41.15	10.19*	30.89*	8.73*	27.96*	6.98*
	400	5000	22.22±0.15	39.81*	-	32.04*	-	28.15*	-
	800	5000	22.10±0.08	37.42*	-	34.64	-	27.94*	-
	1600	5000	15.28±0.33*	35.69*	3.44*	30.91*	2.68*	33.40*	2.33*
	3200	5000	14.56±0.17*	33.66*	5.29*	33.09*	6.17*	33.25*	5.13*

*Different from the control p=0.05, MI: mitotic index; SD: standard deviation

The results of the MN tests in *A. cepa* somatic cells exposed to the controls and concentrations of *P. coccinea* are presented in Table 3 and Figure 2. The frequency of MN formation was dependent on application dose, and usually detected as significantly different in all treatments as compare to the negative control (P=0.05), excluding the 400 and 800 µg/mL of *P. coccinea* treatment. Particularly, MN formation frequency at 3200 µg/mL was clearly higher than the other concentrations of *P. coccinea* extract (Table 3).

4. Discussion

The MI may utilize as a bioindicator of cell increase, which measures the percentage of the cells in different mitotic phases (Ping et al. 2012; Karaismailoğlu 2014). Accordingly, the influences on the MI of tested concentrations of *P. coccinea* methanol extract on *A. cepa* somatic cells are presented in Table 1. MI was declined meaningfully with growing *P. coccinea* concentrations as compared to the negative control at each application time (Table 1). Exceptionally, the MI in 400 and 800 µg/mL concentrations of *P. coccinea* showed an increment in accordance with the negative control at all treatment times (P=0.05) (Table 1); however, the 3200 µg/mL of *P. coccinea* has more mitodepressive effect than 1600 µg/mL concentrations in all treatment periods. MI value was the lowest in the positive control applications, it decreased 31.41%, 38.70%, and 51.42% respectively in comparison with the negative control. If decrement in MI is below 22% as compared to the negative control, this situation remarks a lethal influence for living systems (Karaismailoğlu 2014). If reductions are below 50%, this situation signs sublethal effect and stated the toxic limit rate (Sharma 1983; Panda and Sahu 1985). In this work, the highest sublethal effect was determined in the 3200 µg/mL as compared the negative control in all treatment periods. The sublethal effect percentages for 6, 12 and 24 h were defined as 22.40%, 39.16%, and 34.26%, respectively. The obtained results are suitable with outcomes of the earlier works (Çelik and Aslantürk 2009; Kayrayildiz et al. 2010; Ping et al. 2012; Karaismailoglu et al. 2013; Karaismailoğlu 2014). This decrement in MI might be by reason of blocking of cells from S stage to M stage of cell cycle (Smaka-Kincl et al. 1996) or may be because of preventing of DNA synthesis or holding the cell from ingoing mitosis (Sudhakar et al. 2001; Karaismailoglu et al. 2013; Karaismailoğlu 2014, 2015 and 2016) by virtue of *P. coccinea* treatments.

The influences of tested concentrations of *P. coccinea* on cell cycle in *A. cepa* root tip cells are presented in Table 1. Accordingly, there were statistically important results the phase percentages as compared with the negative control in treatment periods (P=0.05). All the treatments

Table 2. Chromosomal abnormalities in the root tips of *A. cepa* exposed to concentrations of *P. coccinea* and controls

Time (h)	Concentrations (µg/mL)	Abnormality percentages					Total Abnormality
		Stickiness	Disturbed prophase	Chromatid bridge	c-mitosis	laggards	
6	Negative control	-	-	-	-	-	-
	Positive control	1.24±0.08*	0.88±0.06*	1.05±0.15*	0.88±0.24*	1.05±0.18*	5.10*
	400	-	-	-	-	-	-
	800	-	-	-	-	-	-
	1600	0.56±0.12*	-	0.22±0.04*	0.36±0.08*	-	1.14*
	3200	-	0.73±0.15*	0.65±0.08*	0.54±0.12*	0.22±0.04*	2.14*
12	Negative control	-	-	-	-	-	-
	Positive control	1.88±0.16*	1.24±0.04*	1.33±0.36*	0.75±0.12*	1.88±0.08*	7.08*
	400	-	-	-	-	-	-
	800	-	-	-	-	-	-
	1600	0.97±0.04*	0.56±0.10*	0.48±0.08*	0.54±0.08*	0.33±0.04*	2.88*
	3200	1.41±0.24*	1.08±0.23*	1.13±0.15*	0.88±0.12*	0.96±0.15*	5.46*
24	Negative control	-	-	-	-	-	-
	Positive control	2.09±0.21*	2.85±0.33*	3.41±0.12*	1.09±0.08*	1.25±0.22*	10.69*
	400	-	-	-	-	-	-
	800	-	-	-	-	-	-
	1600	2.15±0.12*	0.99±0.08*	1.45±0.15*	0.75±0.21*	0.81±0.08*	6.15*
	3200	1.69±0.18*	1.86±0.12*	1.31±0.18*	2.14±0.04*	2.37±0.21*	9.37*

*Different from the control p=0.05

meaningfully influenced the frequency of the mitotic phases (P=0.05). While the percentage of the ana-telophase and metaphase enlarged, the percentage of the prophase reduced in the observed cells. Altering mitotic stages with utilizing *P. coccinea* concentrations may be attributed as hindering of the beginning of prophase and metaphase phases (Karaismailoğlu 2014). Additionally, tested concentrations of *P. coccinea* induced an important increase in the frequencies of aberration in the mitotic stages in the *A. cepa* somatic cells (P=0.05). Parallel outcomes were reported by earlier works (Bolle et al. 2004; Karaismailoğlu 2013).

The influences on the mitotic aberrations and ratios of total aberrations in the *A. cepa* somatic cells exposed to *P. coccinea* concentrations and control groups are indicated in Table 2. Accordingly, the observed chromosomal aberrations like disturbed prophase, c-mitosis, stickiness, laggards and chromatid bridges are shown in Figure 2. The most widespread type of these aberrations was stickiness. This aberration type forms as a result of causing chromatid tip irregularity (Badr 1983). Another remarkable aberration was c-mitosis, which occurs with inhibiting the formation of spindle, like the influences of colchicine (Badr 1983). As well as, chromatid bridges were widespread among of the aberrations. Chromatid bridges were arranged with the breaking and gathering of chromatid (Shehab and Adam 1983). In addition, disturbed prophase was found, it can occur in results of chromatid erosion. Moreover, disturbed prophase, stickiness and chromatid bridge aberration types characterize a permanent and toxic influence (Fiskesjö and Levan 1993).

Micronucleus tests have a key role for monitoring of the toxic influence (Gebel et al. 1997; Karaismailoğlu 2016). Accordingly, the formation of MN and its percentage in applicated groups is shown in Figure 2 and Table 3. MN percentage improved with the increasing tested concentrations in comparison with the negative control, excluding the 400 and 800 mg/mL in all application periods (P=0.05).

Consequently, the *Pyracantha coccinea* methanol extract may present a possible hazard to DNA and cell structure of the applicated *Allium cepa*. It has still utilized in alternative medicine today. If it is utilized in concentrations under 1600 µg/mL, which is toxic limit of *P. coccinea* extract on *A. cepa*, the toxic influences of *P. coccinea* methanol extract on *A. cepa* will be decreased.

Table 3. The effects of *P. coccinea* and controls on the micronucleus assay.

Time (h)	Concentrations (µg/mL)	Micronucleus (%)
6	Negative control	-
	Positive control	3.45±0.23*
	400	-
	800	-
	1600	0.89±0.11*
	3200	1.26±0.33*
12	Negative control	-
	Positive control	3.79±0.29*
	400	-
	800	-
	1600	1.05±0.17*
	3200	1.17±0.30*
24	Negative control	-
	Positive control	4.19±0.15*
	400	-
	800	-
	1600	1.21±0.04*
	3200	1.44±0.08*

*Different from the control p=0.05

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