

Cytotoxicity of *Aloe vera* gel extracts on *Allium cepa* root tip cells

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Abstract: *Aloe vera* L. is a valuable medicinal plant and is currently used in pharmaceutical, cosmetic, and food industries worldwide. In the present study, the effect of various concentrations of *Aloe vera* leaf gel extracts was investigated on mitotic and phase indexes of *Allium cepa* L. root tip cells for 24 and 48 h durations. The EC₅₀ value of gel extracts was found to be 20% and was used to determine the experimental concentrations. The results indicated that the mitotic index and root growth rate of *A. cepa* were considerably decreased in comparison to the control. We found that the cytotoxic effect of *A. vera* gel extracts depends on the concentration rather than the exposure time. Even the low doses caused a considerable decrease in root growth rate. The lowest mitotic index value was found to be 3.72% at 40% gel extract treatment for 48 h duration. Average prophase index during 24 and 48 h applications was found to be 53.80% and 56.48%, respectively. We conclude that *A. vera* gel extracts have a cytotoxic effect on the root tip cells of *A. cepa*.

Key words: Mitotic index, phase index, *Allium cepa*, *Aloe vera*, cytotoxicity

Aloe vera jel ekstraktlarının *Allium cepa* kök ucu hücreleri üzerindeki sitotoksik etkisi

Özet: *Aloe vera* L. dünyanın her yerinde ilaç, kozmetik ve gıda endüstrisinde kullanılan önemli bir bitkidir. Bu çalışmada *Aloe vera* yaprağından elde edilen jel ekstraktlarının *Allium cepa* L. kök hücrelerinin mitotik ve faz indeksleri üzerindeki etkisi 24 ve 48 saat süreyle incelenmiştir. Jel ekstraktlarının EC₅₀ değeri % 20 olarak bulunmuş olup deneysel konsantrasyon değerlerinin hesaplanmasında kullanılmıştır. Yapılan çalışmada *A. cepa* kök hücrelerindeki mitotik indeks ve faz indekslerinin kontrole göre belirgin bir şekilde azaldığı görülmüştür. *A. vera* jel ekstraktlarının sitotoksik etkisinin zamandan daha çok doza bağlı olduğu görülmüştür. Düşük dozlarda bile kök büyümesinde kontrole göre önemli azalma görülmüştür. 48 saatlik sürede gerçekleştirilen % 40'luk jel ekstrakt uygulamasında en düşük mitotik indeks değeri % 3,72 olarak bulunmuştur. 24 ve 48 saatlik uygulamalarda ortalama profaz indeksi sırasıyla % 53,80 ve 56,48 olarak bulunmuştur. Bu sonuçlara göre *A. vera* jel ekstraktlarının *A. cepa* kök hücrelerinde sitotoksik etki gösterdiği anlaşılmaktadır.

Anahtar sözcükler: Mitotik indeks, faz indeksi, *Allium cepa*, *Aloe vera*, sitotoksosite

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Introduction

It is well known that the use of plants as a therapeutic material due to their chemical substances of medicinal value is common all over the world (Prabhu et al., 2011). *Aloe vera* L. (*Aloe barbadensis* Miller) has been used for its curative and therapeutic properties ranging from dermatitis to cancer for centuries. It continues to be used even though therapeutic effects have not been correlated well with individual components of *A. vera* (Choi & Chung, 2003; Habeeb et al., 2007; Palanikumar & Panneerselvam, 2010).

Solid material of *A. vera* leaves consists of a range of compounds including vitamins, minerals, enzymes, polysaccharides, phenolic compounds, and organic acids (Boudreau & Beland, 2006). This heterogeneous composition of *A. vera* pulp may contribute to the diverse pharmacological and therapeutic activities that have been observed for *Aloe* L. gel products (Talmadge et al., 2004). It has been claimed that the polysaccharides in *A. vera* gel have therapeutic properties such as immune stimulation; anti-inflammatory effects; wound healing; promotion of radiation damage repair; anti-bacterial, anti-viral, anti-fungal, anti-diabetic, and anti-neoplastic activities; stimulation of haematopoiesis; and antioxidant effects (Reynolds & Dweck, 1999; Ni & Tizard, 2004; Talmadge et al., 2004). Different studies indicated anti-tumour activity for *A. vera* gel in terms of reduced tumour burden, tumour shrinkage, tumour necrosis, and prolonged survival rates. In addition to these effects, *A. vera* gel was also shown to have chemo-preventative and anti-genotoxic effects on benzo (α) pyrene-DNA adducts (Boudreau & Beland, 2006). Stimulation of immune response is one mechanism of action that was proposed for these anti-cancer effects of *Aloe* polysaccharides (Steenkamp & Stewart, 2007).

Allium L. is the largest genus of petaloid monocotyledons, containing hundreds of species naturally distributed in temperate climates of the northern hemisphere (Koçyiğit & Özhatay, 2010). The *Allium* L. test has important advantages (Rank, 2003; Kuras et al., 2006) and has been used for many years in investigating physical and chemical mutagenesis, pollutant agents, plant extracts, and similar active material's cytogenetic effects in mitotic cell division. It is stated that the *Allium* test exhibits similar results

with mammalian test systems (El-Shabbaby et al., 2003; Teixeira et al., 2003). The present study was designed to examine the effect of leaf gel extracts of *A. vera* on mitotic cell division in root tip cells of *Allium cepa* L., commonly known as onion.

Materials and methods

The preparation of test materials

Aloe vera plants for extraction were obtained from Dr. Ragıp Esener (Palmiye Merkezi, P.K. 33, 48800, Köyceğiz, Muğla, Turkey). *A. vera* leaves (longer than 60 cm) were washed, dried, and sliced into 2 equal parts in a longitudinal section. Leaf gel was scraped off with the help of a spoon and diluted with an equal amount of dH₂O. The mixture was homogenized with an electric mixer for 2 min and stored at +4 °C overnight. The mixture was then filtered and 50% leaf gel extract was prepared. Dry *A. cepa* bulbs 1.5 to 2.2 cm in diameter were rinsed with tap water and the outer scales were removed. Old root remnants were removed with great care in order not to destroy the root primordia. Since the *A. cepa* cell cycle is completed in 24 h (Rank, 2003), the application process was carried out at 24 and 48 h for investigating the mitotic and phase indexes in 2 consecutive cell cycle periods. For the first 24 h, the onions were grown in dH₂O, after which actively dividing root tip cells of *A. cepa* were exposed to 2%, 5%, 10%, 20%, and 40% of *A. vera* leaf gel extracts for 48 h.

EC₅₀ value and cytotoxicity test

EC₅₀ is defined as the concentration that produces a 50% decrease in root growth rate. The *Allium* root growth inhibition test was carried out for determining the EC₅₀ value of *A. vera* gel extracts (Rank, 2003). *Allium cepa* bulbs were germinated in distilled water at room temperature (21 ± 2 °C) with 15 h light/9 h dark photoperiod for 24 h. After the roots were homogeneously grown, 6 seedlings per treatment were exposed to different concentrations (5%, 10%, 15%, 20%, 25%, and 30%) of *A. vera* gel extracts for 96 h. Test solutions and dH₂O were changed every 24 h. The best developed 10 roots of each onion in each group were measured and mean root length calculated. EC₅₀ value was taken into consideration for determining the concentration of gel extracts.

The experimental concentrations were composed of 200% of EC_{50} as the highest concentration followed by 100%, 50%, 25%, and 10% of the EC_{50} .

Fixation, hydrolysis, and dyeing

The root tip cells were fixed, stained, and examined using a compound microscope. The treated roots were rinsed in distilled water and cut into segments of 1-2 cm length from the tips and fixed in pure glacial acetic acid (45%) for 30 min at room temperature before rinsed twice in ethanol (70%) for 5 min. Then the specimens were transferred into cases containing 70% ethanol and sealed with stretch film and kept at +4 °C until use. The root tips were hydrolyzed in 1 N HCl for 3 min at room temperature and stained with 2% aceto-orcein solution for 30-60 min (Howell et al., 2007).

Microscopic investigation

Five preparations were arranged for every group in mitotic index and phase index applications. Specimens were transferred from dye to a microscope slide and a drop of acetic acid (45%) was added. Root tips (1-2 mm) were cut into tiny pieces and covered with a cover-glass. The cells were subsequently squashed by knocking with a blunt end of a pencil and pressing slightly down with the thumb. Excess liquid was sucked up by a piece of blotting paper. Mitotic index was calculated as percentage of dividing cells. Slides were scanned to investigate the different stages of mitosis. Approximately 5000 cells were scanned for each group of onion.

Statistical analysis

Data gathered from the experiment were analysed using one-way analysis of variance. Duncan's multiple range tests was then used to determine which mean values were different at the 5% level of significance (Duncan, 1955). Correlation and linear regression analysis were used to evaluate the relationships of gel extract concentrations and time periods with mitotic index values. All data were analysed using SPSS 15.0 (SPSS, Chicago, IL, USA).

Results and discussion

The cytotoxicity assays were carried out with 5 different concentrations. The EC_{50} value of our extract was found to be 20% and used to determine

the mitotic index and phase indexes (Figure 1). The experimental concentrations consisted of the EC_{50} value followed by 200%, 50%, 25%, and 10% of the EC_{50} value (Table 1).

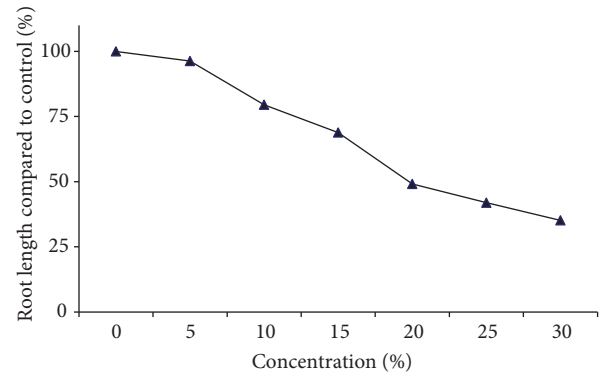


Figure 1. Root growth inhibition of *Allium cepa* exposed to *Aloe vera* gel extracts.

The mitotic index of *A. cepa* root tip cells treated with increasing concentrations of *A. vera* gel extracts was significantly decreased in comparison to the control group ($r = -0.902$; $P < 0.01$) (Table 1). We observed that the effect of increases in gel extract concentrations was more pronounced than that of implementation period, probably due to the short durations of the mitotic phases. For example, while the mitotic index during 24 h application of 20% gel extract was 6.86%, it was 6.54% with only a 4.7% decrease for 48 h at the same concentration ($P > 0.05$) (Table 1). Palanikumar et al. (2011) reported that aloin, the active component of *A. vera*, was clastogenic in both a dose- and time-dependent manner. Our results showed that when the concentration doubled from 20% to 40%, mitotic index rate decreased from 6.86% to 4.14% with an approximately 40% decrease in 24 h. Similarly, a 43% decrease was observed for 48 h duration (Table 1). Marcano et al. (2004) observed similar results on *A. cepa* cells when they applied the maleic hydrazide in 10^{-3} M and 10^{-6} M for 2 and 24 h periods, respectively. Smaka-Kincl et al. (1996) reported that the decrease in mitotic index was the result of cytotoxic effects. The cytotoxic threshold was estimated as the concentration causing 50% mitodepression compared to the control (Sharma, 1983). We observed that the mitotic index was lower than the cytotoxic limiting value at the highest gel

Table 1. Effect of *Aloe vera* gel extracts on mitotic index of *Allium cepa* root tip cells.

Applied concentration (%)	24 h Application mitotic index (%) (\pm SE)	48 h Application mitotic index (%) (\pm SE)
0	9.29 \pm 0.51 a**	9.07 \pm 0.23 a
2	9.31 \pm 0.46 a	8.88 \pm 0.52 a
5	8.74 \pm 0.34 a	8.82 \pm 0.30 ab
10	7.97 \pm 0.42 b	7.09 \pm 0.45 b
20	6.86 \pm 0.36 b	6.54 \pm 0.44 b
40	4.14 \pm 0.28 c	3.72 \pm 0.35 c

* SE = Standard error

** The differences between the means followed by the same letter within the same column are not significant according to Duncan's multiple comparison test ($P < 0.05$).

extract concentration (40%) in both 24 and 48 h periods (Table 1). However, the cytotoxic effect of *A. vera* gel extract treatments was evident as observed by a significant decrease in the mitotic index. Our observations support the antiproliferative effects of *A. vera* gel extracts reported by Danhof and McAnally (1983), and Avila et al. (1997). Schulze and Kirscher (1996) stated that the decrease in the mitotic index was the result of the suppressive effect of some plant gel extracts on DNA and nucleoprotein synthesis. El-Ghamery et al. (2000) explained the mitodepressive effect as the blockage of the G₁ phase and consequently the depression of DNA synthesis. When the mitotic index of test organisms dropped below 22% of the control, it caused a lethal effect (Antonsie-wiez, 1990). In the current study, a lethal effect was not observed, because, among all the applied gel extract concentrations and periods, the lowest mitotic index value was only 3.72% at the highest gel extract concentration for 48 h duration. This value was approximately 41% of the control (Table 1). It can be concluded that, together with the increase in *A. vera* gel extract concentrations, the mitotic index of *A. cepa* root tip cells decreased due to the blocking effect of some components of extract in the G1 phase or DNA synthesis inhibition in the S phase as stated by El-Ghamery et al. (2000).

We observed that the prophase index increased significantly with increasing concentrations ($P < 0.05$) (Figures 2, 3). A strong positive correlation was evident between concentration increase and

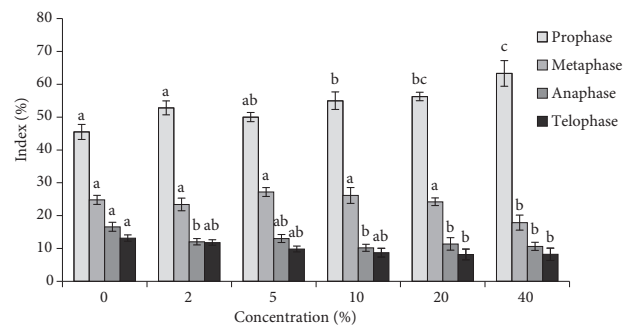


Figure 2. Index rates of mitotic phases at root tip cells of *Allium cepa* during 24 h gel extract applications. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different according to Duncan's multiple comparison test ($P < 0.05$).

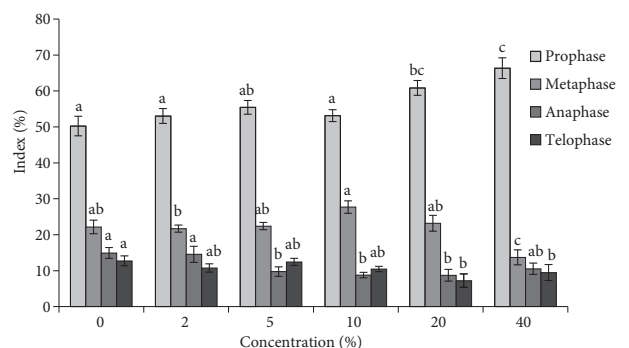


Figure 3. Index rates of mitotic phases at root tip cells of *Allium cepa* during 48 h gel extract applications. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different according to Duncan's multiple comparison test ($P < 0.05$).

prophase index ($r = 0.700$, $P < 0.05$). However, a very weak correlation was observed between the prophase index and implementation period ($r = 0.188$, $P > 0.05$). We also observed that the metaphase index generally stayed constant (except at 40%), and anaphase and telophase indexes showed a decrease for both 24 and 48 h periods (Figures 2, 3). A weak negative correlation was observed between gel extract concentrations and metaphase ($r = -0.499$, $P < 0.05$), anaphase ($r = -0.328$, $P < 0.05$), and telophase indexes ($r = -0.379$, $P < 0.05$). Similarly, a very weak negative correlation was observed between time periods and metaphase ($r = -0.212$, $P > 0.05$), anaphase ($r = -0.132$, $P > 0.05$), and telophase ($r = -0.149$, $P > 0.05$) indexes. The results of our study are in agreement with the study reported by Scolnic and Halazonetis (2000), who stated that a control point between prophase and metaphase, called Chfr, prevents the entry into anaphase in cells treated with chemicals. It can also be deduced that prophase build up is the result of prevention or delay of spindle fibre formation. All our findings are in agreement with the studies reported by Shulze and Kirscher (1996), and El-Ghamery et al. (2000).

Our results showed that all tested concentrations of *A. vera* gel extracts, except for 5%, caused a significant inhibition in mean root growth rate ($r = -0.946$; $P < 0.05$) (Table 2). In the control, mean root length was 5.18 cm; however, after application of 30% gel extract concentration the length was

estimated to be 1.82 cm with a 65% decrease (Table 2). It was reported that the number of dividing cells in *A. cepa* decreased parallel to the inhibition in root growth rate (Fiskesjo, 1997; Babatunde & Bakare, 2006; Çelik & Aslantürk, 2010). In a similar way, our results supported these data and showed that root growth rate decreased in parallel to the increasing concentrations of gel extracts.

The results of this study indicate that the cytotoxic effect of *A. vera* gel extracts depend on their concentration rather than the time period, with even the low doses demonstrating a considerable rate of inhibition in root growth rate. Moreover, the detractive effect of *A. vera* extracts on mitotic index of *A. cepa* show that it has a cytotoxic effect on root tip cells. It is known that the *Allium* test shows a good correlation with mammalian test systems (El-Shabbaby, 2003; Teixeira et al., 2003). For this reason it is possible that *A. vera* plants can have a therapeutic effect to destroy the cancerous cells as reported by Winters (1981) and Danhof and McAnally (1983). However, further studies on active components and their effects on cell divisions are needed.

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Table 2. Effect of *Aloe vera* gel extracts on *Allium cepa* root growth.

Applied concentration (%)	Mean root length (cm) (\pm SE*)	Root length compared to control (%)	Decrease compared to control (%)
0	5.18 \pm 0.11 a**	100.00	-
5	4.98 \pm 0.17 a	96.29	3.71
10	4.12 \pm 0.21 b	79.52	20.48
15	3.56 \pm 0.15 c	68.86	31.14
20	2.54 \pm 0.14 d	49.11	50.89
25	2.17 \pm 0.09 de	41.92	58.08
30	1.82 \pm 0.07 e	35.16	64.84

* SE = Standard error

** The differences between the means followed by the same letter are not significant according to Duncan's multiple comparison test ($P < 0.05$).

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