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ORIGINAL ARTICLE

INVESTIGATION OF CYTOTOXIC AND GENOTOXIC EFFECTS OF *ECBALLIUM ELATERIUM* JUICE BASED ON *ALLIUM* TEST

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SUMMARY

The genus Ecballium only comprises the Ecballium elaterium (EE) (L.) A.Rich species which is a wild medicinal plant found in the Mediterranean region. EE fruit juice is widely used in Turkish folk medicine for the relief of sinusitis and for several illnesses. Up to date, there has been no report on the genotoxicity of EE fruit juice. Thus, the aim of this study was to investigate the potential genotoxic effects of EE fruit juice using the Allium test system. Allium cepa (A. cepa) bulbs were treated with four concentrations (10 ml/L, 20 ml/L, 50 ml/L and undiluted) of EE fruit juice for 72 h and tap water (pH 7.3) was used as a control. The results showed significant dose-dependent ($P < 0.05$) inhibition of root growth and mitodepressive effects on cell division in A. cepa root tip cells after the EE fruit juice treatments. Also, EE fruit juice significantly increased the dose-dependent frequency of chromosome aberrations (breaks, stickiness and pole deviations) in root tip cells and micronucleus formations. There was no dividing cell in the undiluted EE fruit juice treated group, but there were pyknotic/apoptotic cells with varying frequency.

Key words: *Allium cepa* - Cytotoxic effect - *Ecballium elaterium* - Genotoxic effect - Medicinal plant

INTRODUCTION

The pharmacologic activity of plants and herbs in teas, syrups, cataplasm and tinctures is well established in popular medicine. Although plant extracts have been used in the treatment of diseases according to knowledge accumulated over centuries, scientific research has shown that some substances present in these medicinal plants are potentially toxic and carcinogenic (1). Investigation of traditional medicinal plants is thus valuable on two levels: as a source of potential chemotherapeutic drugs and as a measure of safety for the continued use of such medicinal plants (2). Plant species represent a great source of biologically active compounds, the effects of which on heritable material are mostly unknown. However, the present widespread use of essential oils in pharmaceutical products and the industry (antiseptics, soaps, deodorants, flavors and dentistry products) as well as of aqueous extracts in traditional medicine would seem to necessitate research on their cytotoxicity and genotoxicity (3).

Turkey has one of the highest levels of biodiversity and potential genetic resources in terms of medicinal plants that can be used as primary sources for the manufacture of synthetic pharmaceuticals. Among the plants used in Turkey as popular medicine, *Ecballium elaterium* (EE) (L.) A. Rich (Cucurbitaceae), also known as the 'squirting cucumber', is recommended for chronic sinusitis or rhinosinusitis jaundice, nocturia, lumbago and otalgia (4). It is used in the treatment of liver cirrhosis as well as for other conditions that are thought to be inflammatory by nature, including rheumatism and infections. Also, EE roots are used as analgesics and in the treatment of hemorrhoids (4).

EE fruit juice contains proteins, lipids, sugars and glycoproteins (5). Cucurbitacin (Cuc) B, D, E, I, L and R, cucurbitacin derivatives, such as glycosyl cucurbitacins and hexanorcucurbitacins (6–8), and triterpenoid glycosides (9) have been identified in the juice.

The toxic and beneficial effects of dried (elaterium) or fresh juice from EE fruit have been reported, including analgesic, antipyretic and antiinflammatory effects (10, 11), agglutination of erythrocytes and change in heart rate (12). Nasal aspiration of EE was associated with toxic aspects such as uvular angioedema (13), irritation of mucous membranes, drooling, dysphagia and vomiting (14). When used without diluting, the juice of this plant is highly toxic.

Plant extracts with mutagenic, cytotoxic and genotoxic potentials produce effects such as DNA fragmentation, induction of chromosome aberrations, inhibition of cellular division and arrest of the cel-

lular cycle, that can be cytologically and genotoxically detected (15, 16). Among the bioassays developed for detection of mutagenicity, genotoxicity, cytotoxicity and clastogenicity due to various plant extracts, plant systems have proven to be sensitive, cheap and effective (17).

The *Allium cepa* (*A. cepa*) test also makes it possible to evaluate different endpoints. Among these endpoints, the analysis of anatomical (root growth, deformity, twist) and microscopic parameters (chromosome abnormalities, altered mitotic index [MI], and micronucleus [MN] formation) have been the most widely used to detect cytotoxicity and genotoxicity throughout the years. Moreover, the *A. cepa* test system provides important information to evaluate the action mechanisms of an agent and its effects on the genetic material (clastogenic and/or aneugenic effects) (18, 19).

The aim of this study was to investigate the cytotoxic and genotoxic effects of aqueous extracts from EE fruit juice used on *A. cepa* root tip meristematic cells.

MATERIALS AND METHODS

In this study, *A. cepa* was used as a test system and EE fruit juice was used as a test substance.

Collection of *Ecballium elaterium*

EE fruit was collected in September-October 2006 from the vicinities of Söke-Kusadasi, Aydin, Turkey, in the Eastern Mediterranean region. A voucher specimen was deposited in the herbarium (AYDN 881) of the Faculty of Arts and Science, University of Adnan Menderes.

Preparation of extracts

The fruits were sterilized with 70% ethanol and squashed in sterile dishes to obtain the fruit juice. The extract was filtered in sterile conditions; the fruit juice was then diluted with sterile water (10 ml/L, 20 ml/L and 50 ml/L concentrations, respectively) before being added to test tubes. The fruit extracts were prepared freshly before treatment.

Allium Test

The *Allium* test was carried out as described by Fiskesjö G. (20). For this purpose, commercial small onion bulbs (*A. cepa*) were obtained from the local market in Aydýn, Turkey. The onions were not treated with any growth inhibitors. Before use, the loose scales were carefully removed and the dry bottom plates were scraped away without damaging the root. Ten onion bulbs were used for each treatment. The onions were treated with four concentrations (10 ml/L, 20 ml/L, 50 ml/L and undiluted) of EE juice for 72 h. The roots grown in tap water (pH 7.3) were used as the control group and were handled similarly throughout the experiment. The EE fruit juice samples and tap water were changed everyday. The test tubes were kept in an incubator at 22 ºC. After 72 h, the roots were counted and the length of each onion was measured. Prior to that, the root tips were immediately placed in 3:1 (v/v) alcohol-acetic acid for 24 h. After placing them in the alcohol-acid solution, slides were prepared for examination using five root tips from each bulb. The root tips were hydrolyzed in 1N hydrochloric acid (HCl) at room temperature for 2 minutes followed by staining in aceto-orcein stain. Root tips were then squashed in a 2% aceto-orcein solution. The slides were examined microscopically (Olymphus BX51) and were photographed.

The following parameters were used to determine cytotoxicity and genotoxicity: (i) MI, calculated as the percent ratio of dividing cells and total number of cells observed (a total of five slides per onion); (ii) chromosome aberrations, characterized and classified as fragments, stickiness and polar deviation in three randomly picked zones, and MN were scored in interphase cells per 1,000 cells (onion in each group which included ten onions).

Statistical Analysis

The mean values ± SD were calculated for each concentration group and for the control group. For the determination of significance among the calculated mean values, the OneWay ANOVA test was applied ($P < 0.05$).

RESULTS

The average root lengths and numbers for the control group and EE fruit juice treatment groups are summarized in Table 1. The results obtained for undiluted (100%) EE fruit juice apparently indicated that the roots grew less when treated with various concentrations of EE fruit juice (39 ± 0.40, 1.11 ± 0.44 and 0.70 ± 0.18, respectively) than in the control group (3.79 ± 0.331) (Table 1). Inhibition of root growth was concentration-dependent and was statistically significant ($P < 0.05$) at the test concentrations. For all EE fruit juice samples, restricted root growth implying toxicity was noted.

Mitotic activity, expressed as MI, was the first parameter for the cytotoxicity evaluation of EE fruit juice. The cytotoxicity level can be determined by the decreased rate of MI. In Table 2, MI values are given for control and for each treatment concentration. It is evident that EE fruit juice reduced MI compared with the control group (Table 2). There was a rapid decrease in the mitotic index with increasing concentrations of EE fruit juice. The mitotic index was also positively correlated to root length, which decreased with increasing concentrations of EE fruit juice. No dividing cells were found in the undiluted EE juice treatment group (Table 2). Strong inhibition of mitosis by EE fruit juice at diluted and undiluted concentrations showed evidence of cytotoxicity. The reduction in the number of dividing cells in the roots shows the cytotoxic effects of the substances that are found in EE fruit juice.

Table I. *Effects of Ecballium elaterium (EE) juice on root growth of Allium cepa after 72 hours.*

Concentrations	Average Root Number ± SD	Average Root Length (cm) ± SD
Control	34.9 ± 7.23	3.79 ± 0.33
Ecb_1	34.4 ± 7.76	1.39 ± 0.40*
Ecb_2	35.7 ± 6.25	1.11 ± 0.44*
Ecb_3	29.0 ± 5.89	0.70 ± 0.18*
Ecb_4	9.3 ± 5.14*	0.14 ± 0.06*

Ecb_1: EE juice at 10 ml/L concentration; Ecb_2: EE juice at 20 ml/L concentration; Ecb_3: EE juice at 50 ml/L concentration; Ecb_4: undiluted EE juice; *$P < 0.05$ in One Way ANOVA.

Table II. *Effects of Ecballium elaterium (EE) juice on number of total and dividing cells and mitotic index values of Allium cepa root tips cells.*

Concentrations	Total Cells	Dividing Cells	Mitotic index (MI) ± SD
Control	50000	6314	12.63 ± 0.45
Ecb_1	50000	4362	8.72 ± 1.55*
Ecb_2	50000	2440	4.88 ± 0.41*
Ecb_3	50000	1300	2.60 ± 1.29*
Ecb_4	50000	2	0.00 ± 0.06*

Ecb_1: EE juice at 10ml/L concentration; Ecb_2: EE juice at 20ml/L concentration; Ecb_3: EE juice at 50ml/L concentration; Ecb_4: undiluted EE juice; *P < 0.05 in One Way ANOVA.

Table 3 presents the percentage of aberrant cells in dividing cells. In meristematic cells of *A. cepa* treated with diluted and undiluted EE fruit juice, total chromosomal aberration (CA) frequencies were found to be higher than in the control group and all the differences were statistically significant ($P < 0.05$). Total CAs showed a significant dose-dependent ($P < 0.05$) increase in the groups treated with 10 ml/L, 20 ml/L and 50 ml/L EE juice compared with the control group. CA was not found in the group treated with undiluted EE fruit juice since undiluted EE fruit juice causes a drastic reduction in dividing cells. The results of this investigation show a clastogenic effect of EE fruit juice, which can be understood from the induction of CAS at the concentrations used. Mitotic and chromosomal aberrations in terms of breaks, stickiness, polar deviations and MN for each group were counted by microscopic observation of each slide (Fig. 1).

Micronucleus (MNC) formation in 1,000 cells per slide in extract treatments was compared with control (Table 3). In the meristematic cells of *A. cepa* treated with diluted and undiluted EE fruit juice, MNC frequencies were found to be higher than in the control group and all the differences were statistically significant ($P < 0.005$) compared with the control group. The differences became significant ($P < 0.005$) beginning with the 50 ml/L and undiluted EE juice treatment groups for which MNC values were 5.10 ± 2.58 and 13.24 ± 6.4, respectively ($P < 0.05$).

Also, pyknotic and/or apoptotic cells (Fig. 1 e-f) were detected in the group treated with undiluted EE fruit juice. Our cytogenetic results also revealed changes in cytoplasm and damaged nuclei and cells. The cells survived but they were slightly misshaped after swelling up, becoming rounder rather than square in outline, especially after the EE fruit juice treatment.

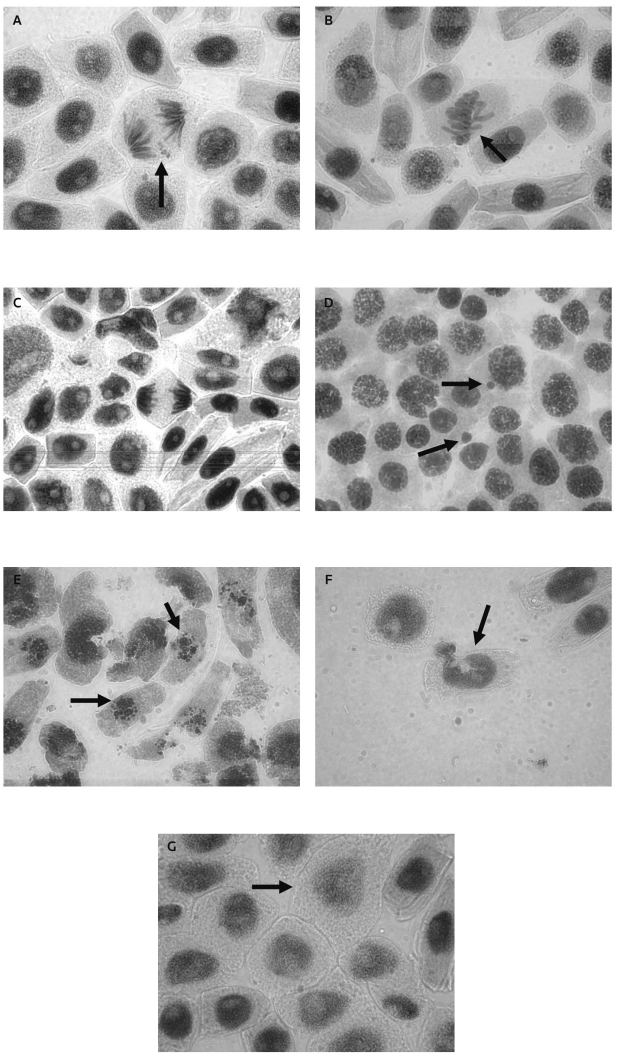

Figure 1. Mitotic and chromosomal aberrations after *Ecballium elaterium* fruit juice treatment in *Allium cepa* root tip meristematic cells visualized with light microscopy. (a) fragments; (b) stickiness; (c) polar deviation; (d) micronuclei; (e) apoptotic cells; (f) cell with damaged membrane and nucleus; (g) normal and swelling cells.

Table III. *Frequency of chromosomal aberrations; Pyknotic/apoptotic cells and ‰ MNC in Allium cepa after Ecballium elaterium (EE) juice treatment.*

Concentrations	Dividing Cells	Breaks (%) ± SD	Stickiness (%) ± SD	Polar Deviations (%) ± SD	Aberrant Cells (%) ± SD	Pyknotic/Apoptotic cells (%) ± SD	‰ MNC (Micronuclei) ± SD
Control	6314	––	0.986 ± 0.28	1.391 ± 0.25	2.377 ± 0.35	––	0.72 ± 0.21
Ecb_1	4362	––	3.954 ± 1.25*	3.371 ± 1.28*	7.325 ± 2.50*	0.10 ± 0.06	1.76 ± 0.30
Ecb_2	2440	––	6.739 ± 0.77*	6.739 ± 1.13*	13.474 ± 1.66*	0.74 ± 0.14	2.58 ± 0.18
Ecb_3	1300	0.798 ± 0.81*	14.401 ± 3.02*	16.116 ± 2.81*	31.320 ± 4.76*	0,63 ± 0.32	5.10 ± 2.58*
Ecb_4	2	0.159 ± 0.47	––	––	––	14.88 ± 5.40*	13.24 ± 6.47*

Ecb_1: EE juice at 10 ml/L concentration; Ecb_2: EE juice at 20 ml/ L concentration; Ecb_3: EE juice at 50 ml/L concentration; Ecb_4: undiluted EE juice; * $P < 0.05$ in One Way ANOVA.

DISCUSSION

In the present study, the potential genotoxicity of EE fruit juice was evaluated with the *A. cepa* test by analyzing two macroscopic parameters - root length and root number - and microscopic parameters, such as MI, chromosomal aberration and MNC frequencies in *A. cepa* cells. Plant root growth inhibition has been considered to be a toxicity indicator since it may result from some inhibition of cell division (21, 22). The change in MI assesses the altered frequency of cell division and is an indication that cell proliferation is affected (23). Our results did show differences in terms of root growth inhibition in *A. cepa* treated with EE fruit juice compared with the control group. EE fruit juice treatment caused a concentration-dependent decrease in root growth. In addition, considering the decrease in MI in *A. cepa* roots treated with EE fruit juice, a cytotoxic effect by substances interfering with the cell cycle may be considered. Inhibition of root growth and the reduction in the number of dividing cells in treatment groups suggest that EE fruit juice has a mitodepressive effect on cell division in *A. cepa* root tip meristematic cells. This hypothesis is in agreement with published data that have revealed a deep mitodepressive effect promoted in *A. cepa* by cytotoxic substances such as heavy metals, polycyclic hydrocarbons, herbicides, various plant extracts, industrial and domestic discharges and other drugs (24-27). The mitodepressive effects of some plant extracts, such as the ability to block the synthesis of DNA and nucleus proteins, have been reported previously (28, 29). They may not even allow initiation of biosynthesis and such an action occurring in the interphase nucleus, apart from influencing the ultimate structure of the chromosome during cell division, could also cause a reduction in the number of other stages (30).

In this study, EE fruit juice decreased the mitotic index of *A. cepa* root tip cells. This decrease was significant for all treatment concentrations (10 ml/L, 20 ml/L, 50 ml/L and undiluted EE fruit juice) when compared with the control group. These results show that all treatment concentrations of EE were cytotoxic on *A. cepa* root tip cells. In particular, the MI value of the undiluted EE fruit juice treatment group was almost zero (Table 2).

The mitotic index (MI) measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be considered as cellular death or a delay in cell proliferation kinetics (31). There are several possible mechanisms to explain a chemically decreased mitotic index in plant cells. The first is that a decrease in MI could be due to G1 blocking, thus suppressing DNA synthesis (32). The second possible mechanism is G2 blocking, which prevents the cell from entering mitosis (33). The decrease in the mitotic index might have been achieved by inhibition of DNA synthesis at the S-phase (34). Taking into account the above results, the reduction in MI value in *A. cepa* roots treated with EE fruit juice most probably arises from disturbances in the cell cycle and the chromatin dysfunction induced by interactions between the chemical component of EE fruit juice and DNA. We used the crude extract of EE fruit juice, which includes a mixture of many chemical compounds which can synergistically affect each other.

EE is a local medicinal plant which stores several compounds that fall under the name: cucurbitacins. Cucurbitacin E (CuE) is a cytotoxic tetracyclic triterpenoid extracted from dried EE fruit juice. Attard et al. (34) previously proved that CuE possesses cytotoxic properties by producing a cell kill exponential curve on treated ovarian cancer cells (OV-95-CC3). Cucurbitacins have been shown to enhance the pinocytic process and bind to the glucocorticoid receptor. Overexpression of these receptors enhances the binding activity of cucurbitacins and could account for their cytotoxicity and antitumor activity, but they are not cytotoxic to fibroblasts (L929) in vitro. Basaran et al. (35) reported that EE extracts were not mutagenic in *Salmonella typhimurium* TA98 and TA100 strains. However, they increased the DNA fractions in the COMET assay. Also, Rencüzogullari et al. (36) investigated the mutagenic and antimutagenic effects of EE juice in human peripheral lymphocytes. They reported that EE fruit juice has no antimutagenic effect, while it has mutagenic and cytotoxic effects on human peripheral lymphocytes. There are several reports on allergic complications caused by EE fruit juice in humans (27, 28, 36).

The present investigation was conducted using chromosome aberrations, detecting clastogenic activity qualitatively and quantitatively in an *Allium* test system in order to assess the genotoxic potential of EE fruit juice. EE fruit juice induced a significant increase in total CAs in treated root tip cells at all concentrations when compared with controls. This increase was significant for all treatment concentrations. EE fruit juice induced fragments, stickiness and polar deviations, as well as structure aberrations in metaphase cells in this study. Chromosomal aberrations occur due to lesions in both DNA and chromosomal and spindle protein causing genetic damage. This caused drastic changes in chromatin, spindle apparatus and centromeres, leading to impairment of chromosome alignment onto metaphase plate, abnormal spindle orientation and abnormal chromosomes were found to be due to the altered quality and quantity of kinetochore heterochromatin (37). Chromosome stickiness is caused probably by immediate reactions with DNA during its inhibition period, causing DNA-DNA or DNA-protein cross linking (38, 39), or by reactions with the liposomal system altering the physicochemical properties of nucleic acids and/or nucleoproteins (40) and causing liquification of the chromatin material (41). Fiskesjö G. (23) reported that sticky chromosomes indicate highly toxic chemical effects that are usually not reversible and will probably lead to cell death. These results were reported by many investigators following treatment with some plant extracts (42, 43). The present data show an increase in the percentage of aberrant cells (Table 3), suggesting a strong interaction between the active principles of EE fruit juice extract and DNA, which could be responsible for the observed genotoxicity.

In addition to the types of chromosomal anomalies induced in dividing cells, MNC in interphase cells (Fig. 1d) have been recorded. The frequencies of MNC increased dramatically after treatment with the highest concentration (undiluted fruit juice, 13.24 ± 6.47) (Table 3). The induction of micronuclei is usually the outcome of chromosome breaks/fragments or spindle poisoning which is an anomalous disjunction of chromosomes at the anaphase stage of the cell cycle (44). Micronuclei may also originate from lagging chromosomes or chromosome fragments in a preceding mitosis. The lagging chromosome(s) may be lost or may form a nuclear membrane around itself thereby forming a micronucleus (42). Therefore, any substance able to promote micronuclei formation is said to be clastogenic or aneugenic (45).

In our study, the cells with pyknotic/apoptotic characters were also detected in the group treated with EE juice. The pyknotic/apoptotic cell values in the 10 ml/L, 20 ml/L and 50 ml/L treatment groups were 0.10 ± 0.06, 0.74 ± 0.14, and 0.63 ± 0.32, respectively. However, the percentage of these cells in the undiluted EE juice treatment group was significantly higher than in the other treatment groups (14.88 ± 5.40). Pyknotic/apoptotic cells were not found in the control group. Apoptosis is a genetically controlled form of cell suicide which is also involved in the specific elimination of damaged cells. The cytotoxic activity exerted by EE fruit juice, partly due to apoptosis, apparently induced deformations of cell membrane and nuclei. It is possible that substances with high concentrations of EE fruit juice cause irreparable DNA damage and inhibition of mitotic events resulted in cell death.

Initial studies reported that CuE from EE fruit juice had a toxic effect on cancer cells in vitro, inhibiting cell proliferation and producing morphological changes indicative of apoptosis. In this study, the cytogenetic results also revealed changes in cytoplasm. The cell survived, its shape varied slightly, it swelled up, becoming rounder rather than being square in outline, especially in the 50 ml/L EE juice treatment (Fig. 1g). In the undiluted EE fruit juice treatment group, an abnormal appearance of nucleus material extruded from the cell membrane in many interphase cells, damaged cells and nuclei (1 g) were observed. This effect may be due to necrosis. The appearance of necrotic cells may also result from incomplete apoptosis. The extract probably induces apoptosis and the cells enter necrosis at a later stage, or this result may be connected with a more complex effect of the compounds of the extract, reflected by changes in the percentage of cells at particular stages of the cell cycle after treatment with the extract (46).

In conclusion, results of this study are important since they suggest EE fruit juice has cytotoxic and genotoxic effects. Undiluted EE fruit juice especially induced structural CAs at a higher frequency than diluted EE fruit juice. Accordingly, it can be concluded from this report and our results that EE fruit juice has cytotoxic and genotoxic effects on *A. cepa* root tip cells. The determination of genetic biomarkers would help estimate the potential toxicity of medicinal herbs in order to regulate medicinal plant consumption, which would be an important measure of public health protection. Thus, caution regarding the indiscriminate use of medicinal plants by the population remains a necessity.

DISCLOSURE

The authors state no conflicts of interest.

REFERENCES

1. De Sa Ferreira, I.C.F., Ferraro Vargas, V.M. *Mutagenicity of medicinal plant extracts in Salmonella/microsome assay.* Phytother Res 1999, 13: 397–400.
2. Verscheave, L., Kestens, V., Taylor, J.L.S., Elgorashi, E.E., Maes, A., Van Puyvelde, L., De Kimpe, N., Van Staden, J. *Investigation of the anti-mutagenic effects of selected South African medicinal plant extracts.* Toxicol In Vitro 2004, 18: 29–35.
3. Barnes, J. *Pharmacovigilance of herbal medicines: a UK perspective*. Drug Saf 2003, 26: 829–51.
4. Shabbar, I.M.S. *How to elicit the genotoxic effect of aqueous extract of Ecballium elaterium using micronucleus assay and DNA single strand break techniques*. Internet J Health 2007, 6(2): 1-8.
5. Khalil, A.M., Qaoud, K.M. *Toxicity and partial characterization of Ecballium elaterium fruit juice*. Int J Pharm 1993, 31: 135–41.
6. Attard, E.G., Sciluna-Spiteri, A. *Ecballium elaterium: An in vitro source of cucurbitacins.* Fitoterapia 2001, 72: 46–53.
7. Rao, M.M., Meshulam, H., Lavie, D. *The constituents of Ecballium elaterium L. Part XXIII. Cucurbitacins and hexanorcucurbitacin*s. J Chem Soc Perkin I 1974, 2552–6.
8. Chen, J.C., Chiu, M.H., Nie, R.L., Cordell, G.A., Qiu, S.X. *Cucurbitacins and cucurbitane glycosides: Structures and biological activities*. Nat Prod Rep 2005, 22: 386–99.
9. Chakravarty, A.K., Sarkar, T., Das, B., Masuda, K., Shiojima, K. *Triterpenoids glycosides from fruits of Ecballium elaterium.* J Indian Chem Soc 1997, 74:858–63.
10. Yesilada, E., Tanaka, S., Sezik, E., Tabata, M. *Isolation of anti-inflammatory principle from the fruit juice of Ecballium elaterium*. J Nat Prod 1988, 51: 504–8.
11. Agil, M.A., Risco, S., Miro, M., Navarro, M.C., Ocete, M.A., Jiménez, J. *Analgesic and antipyretic effects of Ecballium elaterium (L.) A. Richard. extract in rodents*. Phytother Res 1995, 9:135–8.
12. Khatib, S.Y., Mahmoud, I., Hasan, Z.A. *Effects of crude Ecballium elaterium juice on isolated rabbit heart*. Int J Pharm 1993, 31: 259–68.
13. Eray, O., Tuncok, Y., Eray, E., Gunerli, A., Guven, H. *Severe uvular angioedema caused by intranasal administration of Ecballium elaterium*. Vet Hum Toxicol 1999, 41: 376–8.
14. Raikhlin-Eisenkraft, B., Bentur, Y. *Ecballium elaterium (squirting cucumber)–remedy or poison?* J Toxicol Clin Toxicol 2000, 38: 305–8.
15. Grant, W.F. Mutat Res 1999, 624: 107-12. In: Sang, N., Li, G., Xin, X. *Municipal Landfill leachate induces cytogenetic damage in root tips of Hordeum vulgare.* Ecotoxicol Environ Saf 2006, 63: 469-73.
16. Evseeva, TI., Geras'kin, S.A. Shuktomova, I.I. *Genotoxicity and toxicity assay of water sampled from a radium production industry storage cell territory by means of Allium test*. J Environ Radioact 2003, 68(3): 235-48.
17. Majer, B.J., Grummt, T., Uhl, M., Knasmüller, S. *Use of plant bioassays for the detection of genotoxins in the aquatic environment*. Acta Hydrochim Hydrobiol 2005, 33(1): 45-55.
18. Barbério, A., Barros, L., Voltolini, J.C., Mello, M.L.S. *Evaluaion of the cytotoxic and genotoxicpotential of water from the river Paraíba do Sul, in Brazil, with the Allium cepa L. test*. Braz J Biol 2009, 69(3): 837-42.
19. Leme, D.M., Marin-Morales, M.A. *Allium cepa test in environmental monitoring: A review on its application*. Mutat Res 2009, 682: 71-81.
20. Fiskesjö, G. *Allium test on river water from Braan and Sexan before and after closure of a chemical factory*. Ambio 1985, 43: 803-8.
21. Odeigah, O., Nurudeen, O., Amund, O.O. *Genotoxicity of oil field wastewater in Nigeria.* Hereditas 1997, 26(29): 161-7.
22. Marcano, L., Carruyo, I., Campo, AD., Montiel, X. *Cytotoxicity and mode of action of maleic hydrazide in root tips of Allium cepa L*. Environ Res 2004, 94(2): 221-6.
23. Fiskesjö, G. *Benzo[a]pyrene and N-methyl-N'-nitro-N-nitrosoguanidine in the Allium test*. Hereditas 1981a, 95(1): 155-62.
24. Liu, D., Jiang, W., Li, M. *Effects of trivalent and hexavalent chromium on root growth and cell division of Allium cepa*. Hereditas 1992, 117(1): 23-9.
25. Bakare, A.A., Wale-Adeyemo, A.R. *The mutagenic and cytotoxic effects of leachates from domestic solid wastes and Aba-Eku landfll, Nigeria on Allium cepa*. Nat Environ Pollut Tech 2004, 3(4): 455-62.

26. Fernandes, T.C.C., Mazzeo, D.E.C. Marin-Morales, MA. *Mechanism of micronuclei formation in polyploidizated cells of Allium cepa exposed to trifuralin herbicide*. Pestic Biochem Physiol 2002, 88(3): 252-9.
27. Mercykutly, V.C., Stephen, J. *Adriamycyn induced genetic toxicity as demonstrated by Allium cepa test*. Cytologia 1980, 45: 769-77.
28. Schulze, E., Kirscher, S. *Microtubule dynamics in interphase cells*. J Cell Biol 1986, 102: 1020-31.
29. Akinboro, A., Bakare, A.A. *Genotoxic effects of aqueous extracts of five medicinal plants on Allium cepa Linn.* J Ethnopharmacol 2007, 112: 470-5.
30. Rojas, E., Herrera, L.A., Sordo, M., Gonsebatt, M.E., Montero, R., Rodriguez, R., Ostrosky-Wegman, P. *Mitotic index and cell proliferation kinetics for the identification of antineoplastic activity*. Anti-Cancer Drugs 1993, 4: 637-40.
31. Schneiderman, M.H., Dewey, W.P., Highfield, D.P. *Inhibition of DNA synthesis in synchonised chinese hamster cells treated in G_1 with cyclohexamid*. Exp Cell Res 1971, 67: 146.
32. Van't Hof, J. *The action of IAA and kinetine on the mitotic cycle of proliferative and stationary phase excised root meristems*. Exp Cell Res 1968, 51: 167.
33. Gliñska, S., Bartczak, M., Oleksiak, S., Wolska, A., Gabara, B., Posmyk, M., Janas, K. *Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of Allium cepa L. roots treated with heavy metals*. Ecotoxicol Environ Saf 2007, 68: 343-50.
34. Attard, E., Scicluna-Spiteri, A., Brincat, M., Cuschieri, A. *The effects of Cucurbitacin E on the proliferation of prostate and breast cancer cell lines, and peripheral T-lymphocytes.* Maltese Fourth Med School Conf 1999, R034: 145.
35. Basaran,A.A., You,T.W., Plewa,MJ., Anderson,D. *An investigation of some Turkish herbal medicines in Salmonella typhimurium and in the COMET Assay in human lymphocytes.* Teratogen Carcinogen Mutagen 1996, 16: 125-38.
36. Rencüzogullari, E., Ýla, H.B., Kayraldiz, A., Diler, S.B., Yavuz,, A., Arslan, M., Funda Kaya, F., Topaktaþ, M. *The mutagenic and antimutagenic effects of Ecballium elaterium fruit juice in human peripheral lymphocytes*. Russ J Genet 2006, 42(6): 623-7.
37. Jannifer, A., Graves, M., Zelesco, P.A. *Chromosome segregation from cell hybridis. Does segregation result from asynchronous centromere separation*. Genome, 30-124.
38. Ashour, F.M. *A study of some aspects of chemically-induced chromosomal aberrations in plant cells.* Ph.D., Fac Sci, Alexandria University, Egypt, 1988.
39. Amin, A.W. *Cytogenetic impact of the Molluscicide Bayluscide on plants.*Ph.D., Fac Sci, Alexandria University, Egypt, 1991.
40. Mertz, W. *Chromatin occurrence and function in biological system*. Physiol Rev 1969, 49: 163.
41. Adam, Z.M., Farah, O.R. *Cytological effect of water extracts of Cymbogon proxmus (Halfa barr) on Vicia faba*. Cytologia 1989, 54: 498-92.
42. Aþkýn Çelik, T., Aslantürk, Ö.S. *Cytotoxic and genotoxic effects of Lavandula stoechas aqueous extracts*. Biologia 2007, 62(3): 292-6.
43. Soliman, M.I. *Genotoxicity testing of neem plant (Azadirachta indica A. Juss.) using the Allium cepa chromosome aberration assay.* Online Journal of Biological Sciences 2001, 1(11): 1021-7.
44. Yi, H.L., Meng, Z.Q. *Genotoxicity of hydrated sulfur dioxide on root tips of Allium sativum and Vicia faba.* Mutat Res 2003, 537: 109-14.
45. Attard, E., Scicluna-Spiteri, A., Grixti, M., Cuschieri, A. *The cytotoxic activity of cucurbitacin E and busulphan on ovarian and stomach cancer cells in vitro: A comparative study.* Xjenza 1996, 1: 29-34.
46. Majevska, A., Wolska, E., Furmanova, M., Urbánska, N., Pietrosiuk, A., Zobel, A., Kuraœ, M. *Antimitotic effect, G2/M accumulation, chromosomal and ultrastructure changes in meristematic cells of Allium cepa root tips treated with the extract from Rhodiola rosea rhizomes.* Caryologia 2003, 56: 333-47.

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