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ORIGINAL ARTICLE

Evaluation of Cytotoxicity and Genotoxicity of Aqueous Extract of *Althea kurdica* with *Allium* test

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ABSTRACT
The potential cytotoxic and genotoxic effects of aqueous extracts of Althea kurdica on Allium cepa were evaluated. Data on the effects of the extract on root growth of Allium cepa showed that there was concentration dependent decrease in normal root morphology. There is a linear relationship between macroscopic and microscopic parameters for all the extracts. The purpose of this study was to use Allium cepa root-tip cell test to investigate the cytotoxic and mutagenic potential of aqueous extract of this plant.
Keywords: Althea kurdica , Allium cepa, cytotoxicity, genotoxicity

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Introduction

Plants have been a source of medicine for thousands of years, and phytochemicals continue to play an essential role in medicine [1]. Medicinal plants are used in both the pharmaceutical industry and as alternative non-prescription medicines, although systematic investigations of the therapeutic potential of such species are often lacking. Plant biodiversity is extensive but only 15% to 17% of known plant species have been systematically studied for their medicinal properties [2]. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value that are commonly used to treat a wide range of diseases. However, it has also been pointed out that despite the profound therapeutic advantages possessed by some of the plants, some constituents of medicinal plants have been shown that may be potentially toxic, carcinogenic, teratogenic and mutagenic and their use has been correlated with high rate of tumor formation in some human populations [3, 4, 5, 6, 7, 8, 9].

Iran has one of the highest levels of biodiversity and potential genetic resources in terms of medicinal plants which can serve as primary sources for the manufacture of synthetic pharmaceuticals. Among several medicinal plants used in Iran, *Althea kurdica* is widely used. This specie usually is known in the entire world and grows in the west Iran. They are found on the banks of rivers and in salt marshes, preferring moist, sandy soils. It is a medicinal plant used in traditional medicinal practice and has been reportedly useful in the treatment of many diseases [10, 11]. The decoction from the flower is used in the treatment of anuria, diarrhoea, lithiasis, internal injuries, nerve pain, bee sting and tooth-ache [12]. In addition, the flowers have been used in Iranian folk medicine for treating cystitis, urethritis and urinary gravel as well as bronchitis, respiratory catarrh, irritating coughs [12]. Anticomplement activity [13], Anti-inflammatory activity [14], Antimycobacterial activity effect [15], Antitussive activity [16, 17], Antiviral activity [18], Antiyeast activity, Antibacterial activity [19], Radical scavenging effect of this plant rhizome have been reported [20]. The phytochemical study of this plant demonstrated the presence of quercitrin, a glicosidic flavonoid, and also tanines, saponines, resins and essential oils in the tips, as well as inulin and rutine diterpines, quinine acid, rhamnoides and caffeic acid, chlorogenic and hydrocynamic and their derivates in the roots which has reported antitumour activity [21].

Despite their widespread use, there are no data in the literature, at least of our knowledge, with regard to the cytotoxicity and genotoxicity of this medicinal plant popularly used. The effects of mutagens on eukaryotic nuclei can be assessed cytologically by observing inhibition of cell growth or division, interruption of metaphase or the induction of numerical and structural chromosomal aberrations and changes among sister and other chromatids [22]. Onion root-tip cytotoxicity tests are based on the analysis of various parameters including a typical nucleolus patterns (*e.g.* heteromorphic pairing of nucleoli) and the appearance of micronuclei as a consequence of disordered mitosis and chromosomal breakdown [23]. The purpose of this study was to use *Allium cepa* root-tip cell test to investigate the cytotoxic and mutagenic potential of aqueous extract of this plant.

MATERIAL AND METHODS

Plant collection

The flowers of *A. kurdica* were collected from the campus of Agriculture Department, Bu-Ali Sina University.

Preparation of the aqueous extracts of *A. kurdica* flowers

Plant material (flowers) was dried at room temperature in the dark and ground finely using blender. The powder was placed in small plastic bags (100 g each) and stored at 4◦C until use. Weighted dried ground roots were boiled in distilled water for 10 min and, cooled to room temperature for 20 min. Thereafter, the extract was filtered through a filter paper to remove particulate matter. Stock solution was diluted with distilled water to 12.5, 62.5 and 125 mg/ ml concentrations. Fresh extract was prepared daily for each experiment, just before administration.

Allium cepa assay

Onions bulbs were commercially obtained from a local supermarket. Before use, they were dried and the dried outer scales of the bulbs were removed away without destroying the root primordia. These were used for the bioassay according to standard procedures. For the root growth inhibition, three concentrations of each extracts, viz: 12.5, 65 and 125 mg/ml were considered. A series of six bulbs were placed in tap water for 48h to germinate at room temperature 25°C for each concentration of each extract and the control (tap water). After the newly roots (1-2cm in length) were emerged, then onion roots were treated with the roots extracts for 24h. After end of 24h, several of root tips were then cut from each bulb for chromosomal analysis. Then the bulbs were returned to water for a recovery period of 24 hours. So, Roots were collected three times from each bulb: before treatment (control), after 24h of treatment in aqueous solution (treatment) and after 24h of recovery in water (recovery). To study genotoxic effect, after 24h of exposure for each treatment, several root tips were removed from each concentration, fixed in 3:1 (v/v) ethanol: glacial acetic acid and stored overnight at 4◦C. The next day they were placed in 70% (v/v) aqueous alcohol and refrigerated until used. An average of five slides was made for each bulb using five root tips which hydrolyzed in 1N hydrochloric acid (HCl) for 3 min and microscope slides were prepared by squashing the stained root tips in 0.5% (w/v) toluidine blue. Five slides were prepared per treatment and control, and each slide was examined using Olympus BX51 at a total magnification of 40×10. The following parameters such as the mitotic index (the ratio between the number of mitotic cells and the total number of cells scored and expressed as percentage) [24, 25] and cytogenetic effects were scored in interphase cells per 6000 cells were used for determination of cytotoxicity and genotoxicity. The effect of each sample on the morphology of growing roots was also examined [26].

Statistical analysis

The MSTATc statistical software was used for this analysis. Data on mitotic and phase indices were compared using analysis of variance to confirm the viability of the data and validity of results. Differences between the control and the individual dosage group of each extract were analyzed by means of the Duncan's test of significance at the $P < 0.05$ level.

RESULTS

Physicochemical characterization

The levels of physiochemical parameters (morphology of root tips and colour of root tips) are presented in Table 1. This results show that all tested concentrations of *A. kurdica* flower extract caused significant inhibition in the growth of roots in comparison to control. The inhibition of morphological was greater with increasing concentrations of *A. kurdica* flower extract. Almost all morphological parameters in 12.5 mg/ml concentration were found nearly equal to the control. The roots treated in appeared slightly yellow in the first concentrations and the roots treated in 125 mg/ml concentrations appeared slightly brown in colour and broken tips. At 125 mg/ml flower extract, the roots morphology showed an obvious difference in its appearance in that it turned to brownish in colour and broken tips.

Cytological analysis

When *A. cepa* bulbs were treated with the *A. kurdica* extract, the frequency of meristematic cells was reduced both at high and low concentrations. Table 3 shows the number of interphase and dividing cells along with the mean mitotic index (MI). According to this table, the number of interphase, prophase, metaphase, anaphase and telophase cells is shown in Table 1. For the 125 mg/ml infusion made from *A. kurdica* flowers the number of telophase cells was very low and there were a little prophase and metaphase or telophase cells, while for this concentration, there were no metaphase cells. On the other hand, the extract exhibited a strong depressive effect on the mitosis of *A. cepa* roots.
In the *A. kurdica* extract used, the mitotic index of the control experiment was found to be 5.47%. As for the treatment, at concentration of 12.5, 62.5 and 125 mg/ml the mitotic indices were found to be 2.23, 2.18 and 0.31 respectively. Thus, the mitotic indices decreased considerably when compared to control bulbs, demonstrating a drastic inhibition of cell division (Table 3). This shows a very negative correlation between the concentration of the extract and the mitotic indices in all the mitotic phases. In view of the cytotoxic effect of these concentrations, the bulbs were placed in water for 24 hours after treatment, and partial recovery of cell division was observed (Figure 1).

Table1. Morphological characterization of *A. cepa* root tip cells under treatment concentrations of aqueous extract of

A. kurdica

Morphology / Concentration	Fresh root	Yellow	Dark brown	Broken tips
Control	Yes	Yes	No	No
12.5 mg/ml	Yes	Yes	No	No
65 mg/ml	Yes	Yes	Yes	Partial
125 mg/ml	No	No	Yes	Yes

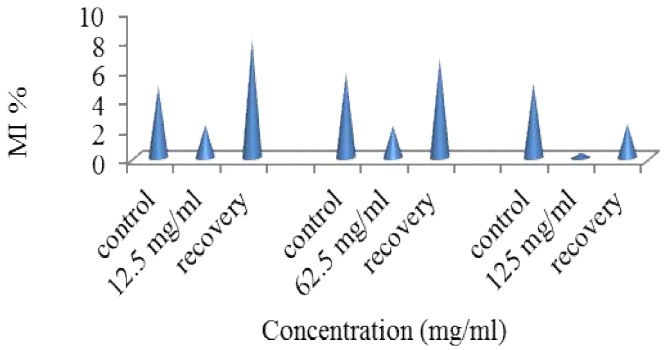

Figure1. Mitotic indexes of *A. cepa* root-tip cells treated for 24 hours with different concentrations of *A. kurdica* aqueous extract, recovered for 24 hours in water, and their controls

Table 2 shows the chromosome aberrations in *A. cepa* root tip cells treated with different concentrations of *A. kurdica* flower extract in the experiments. Other significant observations made for the various concentrations and treatments are presented in the form of micrographs (Figure 2). *A. kurdica* flowers extract induced chromosome and cytological alterations both in 62.5 and 125 mg/ ml treatment. Analysis of chromosome aberrations indicated that the extract causes bridges in anaphase, sticky Chromosomes (metaphases), micro nucleocytes, multi polar anaphases, ghost cells and cells with damaged nucleus. In addition, the observation of these aberrations was dose dependent. In *Allium* test, a strong toxic effect of *A. kurdica* flowers extract was observed, supported by great occurrence of sticky metaphases, leading to cellular death (mitotic index decrease).

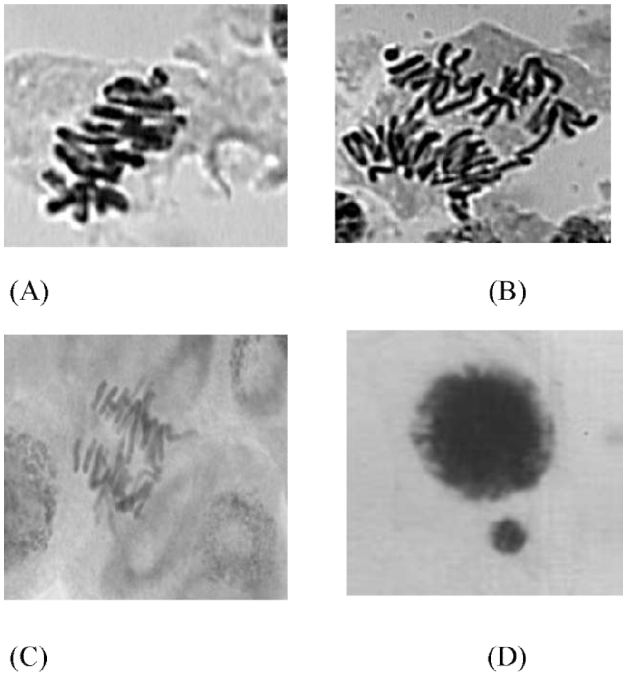

Figure 2. Effect of *A. kurdica* flower extracts on mitosis of Allium cepa root (a) Sticky chromosome (metaphases); (b) Multi polar anaphase; (c) Anaphase bridge; (d) Micro Nucleocytes

Aberration Concentration	Anaphase bridges	Micro nucleocyte	Multi polar anaphases
12.5 mg/ml	-	-	-
62.5 mg/ml	-	Yes	Yes
125 mg/ml	Yes	Yes	-

Table 2. Chromosome aberrations of the *A. cepa* root tip cells treated with different concentrations of *A. kurdica* flower extracts

	Phase indexes				
Concentration (mg/ ml)	PI	Me1	AI	TI	MI (%)
Control	129	100	67	75	6.1
0	65	97	71	59	4.75
Control	96	79	75	45	4.91
12.5	40	41	26	27	2.23
Control	87	81	68	112	5.8
62.5	79	37	6	9	2.18
Control	59	101	75	70	5.083
125	12	-	4	3	0.31

Table 3. Phase and mitotic indexes in *Allium cepa* root- tip cells treated with different concentrations of *A. kurdica* extract and their respective controls.

Discussion

The potential cytotoxic and genotoxic effects of aqueous extracts of *Althea kurdica* on *Allium cepa* were evaluated. Data on the effects of the extract on root growth of *Allium cepa* showed that there was concentration dependent decrease in normal root morphology. There is a linear relationship between macroscopic and microscopic parameters for all the extracts. In *Allium cepa*, whenever there is root growth and morphology characterization inhibition, there is always reduction in the number of dividing cells. Inhibition of root growth in *Allium cepa* might be due to the presence of some heavy metals in the extract. In the other word, when the aqueous extract were tested on *A. cepa* root- tip cells to evaluate their action on the kinetics of the cell cycle, a decrease in the mitotic indexes was observed, which was more marked at 62.5 and 125 mg/ml concentrations (Table 3). At these concentrations the extracts

proved to be extremely cytotoxic and inhibited *A. cepa* root growth. The cytotoxic effects observed gradually disappeared when the bulbs were allowed to recover in water.

The reduction in the number of dividing cell at tested concentrations suggests that the extract of this plant have mitodepressive effect on the cell division of *Allium cepa*. The action on cell division probably resulted from a reversible toxic action of the extracts. Reasons of reduction of mitotic activity might be due to blockade of G2 phases of cellular cycle, inhibition of DNA/protein synthesis [27]. Mitodepressive effects of some plant extracts resulting from their interaction with DNA nucleotides thus inhibiting DNA synthesis and subsequent mitotic inhibition have been reported by [28, 29, 30]. In addition in the other study we have reported that *A. kurdica* extract have antiproliferative on human leukemic cells and lymphocyte cells [31] indicating that such extract may have therapeutic potential in the treatment of cancers. Our results regarding the cytotoxic activity of *A. kurdica* support our previous work of that has attributed these effects to the presence of phenol derivatives (quercitrin, a glicosidic flavonoid, tanines, saponines, and resins). Taken together our results indicate that not only is the onion root-tip test useful in assessing infusions prepared from *A. kurdica* extract but that such infusions have cytotoxic and anti- activities and may have potential for the therapeutic inhibition of the cell cycle in eukaryotic organisms. A chromosome anomaly, abnormality or aberration reflects on a typical number of chromosomes or a structural abnormality in one or more chromosomes. Our results showed induction of chromosome or chromatide type of aberration in the treated cells (Table 2). Frequencies of total chromosome aberrations increased significantly upon exposure to *A. kurdica* flower extracts which indicate clastogenic activity. In the other studies, it was reported that alkaloids be responsible for chromosome aberration. In this study, metaphase and anaphase disorders in the cell of *Allium cepa* by this extract were observed and might have been due to the presence of alkaloids in the tested extract. This is because extract of this plant studied has been reported to contain various alkaloids, tannins, quercitrin, glicosidic flavonoid, tanines, saponines, resins and essential oils that may lead to aberration in disturbance of chromosomes in cells at the next stage of cell division [21].

Finally, we conclude that when applied in high doses, *A. kurdica* leaf extract shows cytotoxic and genotoxic activity. In addition, the results herein suggest that the tested extract possess inhibitory, mitodepressive and turbagenic effects on root growth, cell division and chromosomes behavior of *Allium cepa*. Moreover, there is a need for a closer look at the genotoxicological effects of the tested extract in animal test systems for human welfare as *A. kurdica* has been consumed to cure varieties of diseases.

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