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Research Article

EVALUATION OF CYTOTOXIC AND GENOTOXIC POTENTIAL OF AQUEOUS EXTRACTS OF MEDICINAL PLANT *HELICHRYSUM* MILL. USING *ALLIUM CEPA* CHROMOSOME ASSAY

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ABSTRACT. The cytotoxic and genotoxic effects of aqueous extracts of *Helichrysum* was evaluated using the *Allium cepa* assay. The crude extracts of *Helichrysum* flowers were prepared with distilled water. Onion bulbs were exposed to 50 mg/mL, 100 mg/mL, 200 mg/mL concentrations of each of the extracts for macroscopic and microscopic analysis, respectively. There was concentration-dependent and statistically significant ($P < 0.05$) inhibition of root growth by the extracts when compared with the control. The EC50 obtained for decoctions of *Helichrysum*. Aqueous flower extracts decreased MI depending on the treatment time and concentration and caused chromosomal abnormalities at all doses in the root meristems of *A. cepa*. Observed chromosome aberrations such as C-metaphase, sticky metaphase, metaphase with fragments, disturbed metaphase, polyploid metaphase, anaphase bridge, anaphase laggard, disturbed anaphase, telophase bridge and micronucleus reinforces cytotoxic and genotoxic effects of aqueous extracts of this medicinal plant.

Keywords: *Helichrysum, Medicinal plant, Allium assay, cytotoxicity, genotoxicity*

INTRODUCTION

The genus *Helichrysum* belongs to Asteraceae family is native to Europe, Central Asia, and China. *Helichrysum* species called everlasting plants, immortal flower or fadeless flower. It is represented by 24 species in Turkey and 17 species of the genus are endemic [1, 2].

Helichrysum genus has benefits such as diuretic, kidney stone and sand removal, regulation of digestive disorders, uro-genital disorders, strengthening the immune system, has antibiotic and antioxidant effects also has beneficial effect on stomach pain, jaundice, diarrhea, and asthma. They are used for their anti-asthmatic properties, diuretic purposes, against kidney stones and stomach pain [3]. *Helichrysum* species are also used for therapeutic purposes in traditional folk medicine in Turkey for centuries. This herb is widely used as herbal tea. Flowers of everlasting plants are used for diarrhea and intestinal disease (one glass daily for 2-3 days is taken), also flowers and leaves are used as a cure for jaundice [4, 5, 6].

Traditional use of *H. graveolens* controls diabetes mellitus and it is used for diuretic purposes [7]. In Europe infusions of *Helichrysum* flowers are used for regulatory and diuretic effects. In Germany and Italy there are commercial preparations of *Helichrysum* and the capitula of the genus are described in the Polish, Swiss Pharmacopoeia. 10%

infusion of *Helichrysum* flavonoids have regulatory effect on liver, pancreas and stomach.5 The etheric oil, flavones and flavone glycosides, sterins, bitter substances, tannins, dyestuffs, resin, carotene, vitamin C, vitamin P and coumarin found in the *Helichrysum* plant are used in many areas [8]. Ergezer et al. [9] stated that anti-carcinogen, antimutagenic, antimicrobial and antiviral properties of the tannins in the *Helichrysum* genus should be determined with further studies. Although there is a belief among the public that herbal medicines come from nature and have no negative or toxic side effects, a lot of medicinal plants have cytotoxic and genotoxic properties mutagenic, cytotoxic and genotoxic effects of many plants used in traditional medicine are shown in various assay [10, 11, 12, 13].

There are many test systems to evaluate the cytotoxicity and genotoxicity of plants. *A. cepa* bioassay introduced by Levan [14] as an effective test system suitable for toxicity assessment: it is fast, inexpensive, as well as compatible with many animal test systems [15]. Therefore *A. cepa* has been used frequently by many researchers [11, 12, 13, 16, 17, 18].

The aim of this study was to evaluate cytotoxicity and genotoxicity of aqueous extracts obtained from the flowers of medicinal plant *Helichrysum* sp.

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MATERIALS AND METHODS

Methodology

The experiment was conducted at the Cytogenetic laboratory of Hacettepe University Faculty of Science, Department of Biology in January and July, 2021.

Plant Material

Medicinal Helichrysum stoechas (L.). Moench ssp. Barrelieri (Ten) Nyman (everlasting plant) were collected from Bodrum (37.137519-27.31404) Turkey during flowering (July, 2020). The plant material was identified by Hacettepe University herbarium specialist Haşim Altınözlü.

Aqueous Extracts

The extracts of everlasting plant were prepared according to the traditional use in Turkey (decoction). Since the crude extracts of medical plants were traditionally used, dried flowers of the *Helichrysum* sp. were used in this study.

Helichrysum flowers dried at room temperature and ground to a fine powder with mechanical mill. The aqueous extract (decoction) was prepared by boiling 50g powdered capitulum mixed with 1000mL distilled water (5% stock solution) for covered baker for 10 minutes and cooled to room temperature. Subsequently the extract was filtered through a filter paper. The stock solution was diluted with distilled water to prepare three different concentrations of aqueous extracts. Cytotoxicity of aqueous extracts of *Helichrysum*

flowers were evaluated at three different concentrations 50 mg/mL, 100 mg/mL, 200 mg/mL.

Allium Assay:

Allium cepa bulbs (1.5-2 cm in diameter) were obtained from a local market and dried at room temperature for a week. Then brown scales of onion removed carefully leaving the root primordia intact.

Fifty onion bulbs were primarily suspended in jars filled with distilled water for 2 days at room temperature and protected from direct sunlight. The onions showing good root growth were selected and transferred to the jars filled with test solutions.

Three replicates of onion bulbs were suspended in distilled water (negative control), 8mM EDTA (ethylenediaminetetraaceticacid) (positive control) (EDTA CAS no 60004) (Grant,1982, Barbério et al. 2009) and three different concentrations of aqueous extracts (50, 100 and 200 mg/mL) of *Helichrysum*.

After 24 hours to determine the root growth inhibition 10 root samples were taken each of 3 bulbs and measured (in cm). The percentage root growth inhibition in relation to positive and negative control and the EC50 (effective concentration where root growth amounts to 50% of controls) for each extract was determined according to Fiskesjo [16].

After 24 hours and 48 hours of exposure root tips were cut and fixed in acetic alcohol (3:1 ethanol: glacial acetic acid) for a day and then stored in 70% alcohol at 4 Co until used. Root tips were hydrolyzed at 60 C0 in 1 N HCl, stained with Feulgen for an hour. At least 3 root tips were made squash preparations and examined under light microscope (Leika). Photomicrograps were taken by RisingCam (microscope digital camera).

Statistical Analysis

Data on macroscopic and microscopic parameters (root length, mitotic index and chromosomal aberrations) were compared using student t test.

Differences between the control and the individual dosage group of each extract analyzed by means of the Students' t-test of significance at the $P<0,05$ level.

RESULTS AND DISCUSSION

The levels of macroscopic parameter (root growth) after 48 hours are presented in Table 1. In the present study the toxic effect of aqueous extracts of medicinal plant *Helichrysum* was evaluated by analyzing root growth. All tested concentrations of *Helichrysum* aqueous extracts (50mg /mL, 100 mg/mL and 200 mg/mL) caused significant inhibition in the root growth in comparison to negative and positive control. The root length inhibition was higher with increased aqueous concentrations of medicinal herb *Helichrysum*. The highest average root growth rate was determined as 2.70 cm in negative control, 1.25 cm in positive control and 1.30 cm in 200 mg/mL aqueous extract of everlasting plant. Average root lengths were significantly decreased depending on concentrations. Positive control and 200 mg/mL aqueous extract are shown more than 50% root length inhibition (EC 50).

Table 1. *Cytotoxicity of medicinal plant Helichrysum on growing roots of A. cepa after 48h*

Treatment groups	**Concentrations**	**Average root length (cm)±SD**	**Inhibition %**
Negative control	Distilled water	2,70 ± 0,819	-
Positive control (EDTA)	8 mM	1,25* ± 0,654	53,70
Aqueous extracts of Helichrysum	50 mg/mL	1,96* ± 0,766	27,41
	100 mg/mL	1,66* ± 0,441	38,52
	200 mg/mL	1,30* ± 0,485	51,85

*Statistically important p <0,05

Evaluation of Cytotoxicity

Cytotoxicity was investigated by observing cytological parameters such as mitotic index and chromosome aberrations. The effect of different concentrations of *Helichrysum stoechas* aqueous extracts on the MI are shown in Table 2.

Table 2. *Mitotic index values in negative control, positive control and aqueous extract concentrations of Helichrysum*

Time (Hour)	Treatments	Number of Examined cells	Number of Dividing cells	MI ± SD
24	Negative Control	6712	352	5,24 ± 0,91
	Positive Control(EDTA)	9444	284	3,00 ± 0,92
	50mg/mL	7194	333	4,64 ± 0,75
	100mg/mL	6748	328	4,86 ± 0,39
	200mg/mL	7190	381	5,31 ± 0,85**
48	Negative Control	7686	405	5,28 ± 0,56
	Positive Control(EDTA)	12202	342	2,80 ± 0,09*
	50mg/mL	7052	312	4,42 ± 0,66
	100mg/mL	9494	234	2,46 ± 0,55*
	200mg/mL	6570	150	2,28 ± 0,35*&**

*statistically important from NC P<0.05
**statistically important from PC P<0.05

Consequently, 24 hours treatment has no adverse effect compared to negative control. The MI decreased at 48 hours in the positive control and all aqueous extracts compared to negative control. At the same time, a positive correlation was observed between root elongation and decrease of mitotic index. The difference of MI between NC and PC also 100 mg/mL and 200 mg/mL is statistically significant at P <0.05 at 48 hours. Also, with the PC, the difference of MI between 100 mg/mL in 24 hours and 200 mg/mL in 48 hours is statistically significant (P <0.05).

Evaluation of Genotoxicity

Chromosome aberrations (CAs) were observed in all phases of mitosis. The CAs types and percentages are displayed in Table 3. PC and aqueous extracts of *Helichrysum* caused nine type of CAs which are sticky metaphase (Fig.1a), C-metaphase (Fig.1b), metaphase with fragments(Fig.1c), disturbed metaphase (Fig.1d), polyploid metaphase (Fig.1e), anaphase laggard anaphase bridge (Fig.1f), disturbed anaphase (Fig.1g), telophase bridge(Fig.1h) and micronucleus (Fig.1i) (Fig. 1).

Table 3. *Cytogenetic effects of aqueous extracts of Helichrysum*

		%															
Treatment time (h)	Treatment	Interphase	Prohase	Metaphase	Anaphase	Telophase	C metaphase	Sticky metaphase	Metaphase with fragment	Disturbed metaphase	Polyploid	Anaphase bridge	Anaphase laggart	Disturbed anaphase	Telophase bridge	Micronucleus	% aberration
24	NC	94,76	3,1	1,22	0,33	0,38	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01
	PC(EDTA)	96,98	0	0,49	0,00	0,15	0,02	1,62	0,08	0,00	0,00	0,17	0,21	0,17	0,11	0,00	2,38
	50mg/ml	95,44	1,64	1,06	0,17	0,57	0,56	0,19	0,00	0,00	0,00	0,31	0,00	0,00	0,06	0,00	1,12
	100mg/ml	95,14	0,77	1,84	0,41	0,89	0,29	0,21	0,00	0,04	0,00	0,41	0,00	0,00	0,00	0,00	0,95
	200mg/ml	94,69	0,92	1,14	0,64	1,50	0,06	0,36	0,00	0,00	0,00	0,69	0,00	0,00	0,00	0,00	1,11
48	NC	98,99	1,05	2,39	0,22	0,68	0,00	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,03
	PC(EDTA)	95,51	0	0,24	0,00	0,16	0,03	1,55	0,00	1,46	0,00	0,00	0,00	0,55	0,42	0,08	4,09
	50mg/ml	95,42	2,09	0,09	0,11	0,19	1,25	0,14	0,00	0,00	0,34	0,17	0,17	0,00	0,00	0,03	2,10
	100mg/ml	97,32	0,4	1,10	0,23	0,27	0,13	0,23	0,00	0,02	0,00	0,23	0,02	0,00	0,00	0,00	0,63
	200mg/ml	97,71	0,68	0,18	0,15	0,15	0,55	0,33	0,00	0,00	0,00	0,18	0,07	0,00	0,00	0,00	1,13

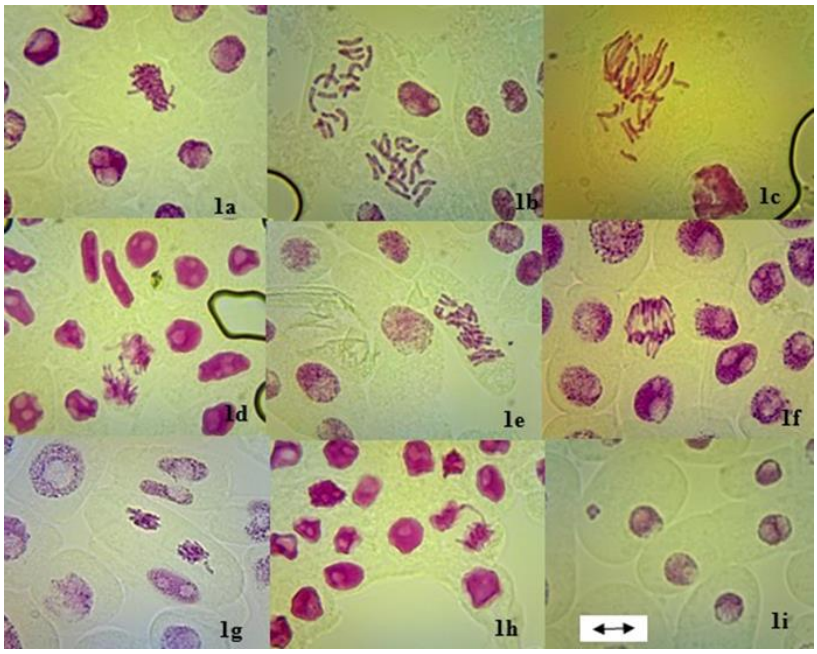


Fig. 1. *Chromosome aberrations induced by aqueous extracts of Helichrysum in the root meristems of A. cepa:* ***1a*** *Sticky metaphase,* ***1b*** *C-metaphase,* ***1c*** *disturbed metaphase with fragments and chromosome breaks,* ***1d*** *disturbed anaphase,* ***1e*** *poliploid metaphase,* ***1f*** *anaphase bridge,* ***1g*** *forward chromosome,* ***1h*** *telophase bridge,* ***1i*** *micronucleus (Bar represents 10 µm)*

CONCLUSION

Although medicinal plants have been used for centuries, there is not enough information about their possible toxic effects on organisms. In the present study, the cytotoxic and genotoxic effects of *H. stoechas* aqueous extracts were evaluated by analyzing Allium assay which are widely used as indicators in toxicity tests. The root length inhibition is accepted as the most important macroscopic parameter and is used to determine EC 50 values [15]. Our results showed that the inhibition of growth was dependent on concentrations of aqueous extracts similar to the other researches findings [21, 22, 23, 24]. Hindrance of root elongation also predicts the decrease in the mitotic index. A similar situation has been observed in many studies with herbal extracts [11, 12, 23].

The lowest MI value was observed in the positive control group at both exposure times with 42% and 46.96% reduced MI respectively as compared to the negative control. MI reduction below 22% in comparison to negative control causes lethal influence on organisms [11, 21].

Sharma [22] referred reduction below 50% is the cytotoxic limit and possess sub lethal influence. In this study the highest sub lethal influence (56.81%) was determined in 200mg/mL concentration in comparison to the negative control at 48 hours. This high decrease in mitotic index can be explained by the toxic effect causing cell death and is in agreement with other studies [23].

The reduction in the mitotic index is an indicator of cytotoxicity, and can be due to the inhibition of DNA synthesis or G2 phase of the cell cycle. Although aqueous solutions of *Helichrysum* have no effect on MI over 24 hours, it can be considered cytotoxic with increasing concentrations at 48 hours.

Stickiness, chromosome bridges and fragments, are irreversible and have toxic effects that can be the cause of cell death [24]. Stickiness may result from chromatid crosslinks [11, 16, 17]. Chromosome breaks showed the clastogenic effect of aqueous extracts of *Helichrysum*. Fragments are the result of chromosome breaks and can be result of anaphase and telophase bridges [11, 17]. Anaphase bridge may have been formed as a result of the breakdown and recombination of chromatids, while it is also likely to be linked to chromosome stickiness [18]. Laggard chromosomes were also observed on this study this type of CAs resulting from spindle fiber inhibition are considered to be irreversible and low toxic [24]. In the present study polyploid cells have been observed and they can be attributed to the breakdown in the spin structure [18]. Micronucleus formation was observed only at 48 hours treatment of PC and 50mg/mL concentration.

Many herbs have been used for medicinal purposes among the public for many years, but there is not much information about their toxic effects on living organisms. Toxicity tests will help to understand these harmful effects. Allium test was used in the analysis of cytotoxic and genotoxic effects of aqueous extracts of everlasting plant used for medicinal purposes.

In this study, *Helichrysum stoechas*, aqueous extracts of the medicinal plant, were evaluated on root tip meristematic cells of *A. cepa* to evaluate cytotoxicity and genotoxicity for its therapeutic purposes. It was concluded that the aqueous extracts of everlasting plant caused genotoxic and cytotoxic effect on the meristematic cells of *A. cepa* when applied in high doses and for a long term. This results propose that failure to use medical herbs at the right dose has a detrimental effect on cells.

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