

Original Article

Allelopathy and Anti-mitotic Effects of *Cuscuta campestris* and *Cuscuta monogyna* Extracts on Plant Cell Division

Azra Ataei Azimi* and Babak Delnavaz Hashemloian

Department of Biology, Islamic Azad University, Saveh Branch, Saveh, Iran.

Article History: Received: 25 March 2017 /Accepted in revised form: 16 July 2017

© 2013 Iranian Society of Medicinal Plants. All rights reserved

Abstract

Some natural compounds of plants including phenols and alkaloids induce mitosis depressive that are blocked cell division. Field dodder (*Cuscuta campestris* Yunck.) and eastern dodder (*Cuscuta monogyna* Vahl.) are the most widespread obligate parasitic species. In the present study, end of summer 2015, field dodder and eastern dodder are collected from *Alhagi maurorum* Medik. host and Vine (*Vitis sylvestris* C.C.Gmel.) host respectively from Markazi province of Iran and identified by department of botany of Islamic Azad University of Saveh. The aerial parts of dodders were used for aqueous extract. Dodders alkaloid and poly phenol were visualized by TLC and Dragendorff (for alkaloid) and ferric salt (for poly phenol) reagents. Total phenols and alkaloid were measured by using the calibration and spectrophotometry methods. Root length and roots tip meristem model of onion bulbs and barley seedlings were utilized to allelopathy and antimitotic effects of dodders aqueous extracts. Standard cytotoxic 8-Hydroxyquinoline served as controls. The bulbs and barley seedlings were treated at various concentrations: 0.05, 0.1 and 0.2 mg ml⁻¹ (dw) of and aqueous extract of dodders and 8-Hydroxyquinoline for 6 and 12 h. The inhibitory effect of dodders extract were evaluated on the growth and mitotic activity (Mitotic index) of barley seedlings and onion root meristems and the effect was compared with standard 8-Hydroxyquinoline. The One Way ANOVA test was used for statistical analysis.

The poly phenols of field dodder was condensed tannins (include flavonoids) but for eastern dodder hydrolysable tannins. Like 8-Hydroxyquinoline, both field and eastern dodders extracts significantly inhibited the growth of roots and mitotic activity in incubation time and dose-dependent manner. However, *C. campestris* extract was more allelopathy and antimitotic potent in this regard and produced root decay and mitosis arrest. The extract of dodders had inhibitory and mitosis depressive effect on root tip meristem cells. In the present study we found, the dodders aqueous extracts can be used to produce desirable effects as it pertains to chromosome condensation and spread, and though the roles of these chemicals in spindle fiber inhibition have been elucidated, as 8-Hydroxyquinoline.

Keywords: Field dodder, Eastern dodder, Meristem, 8-Hydroxyquinoline, Onion, Barely

Introduction

A wide variety of anti-mitotic substances exhibit cytotoxic effect by interfering with cell-cycle kinetics. Some natural compounds of plants including phenols and alkaloids induce mitosis depressive that is blocked cell division and cancer [1]. These materials are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the

cell cycle or by blocking the formation of the mitotic spindle in M-phase [2]. Some of these microtubule inhibiting substances include colchicine, 8-hydroxyquinoline and ice cold water. A number of these substances have been used to enhance the spread of mitotic chromosomes during squashing. 8-hydroxyquinoline is believed to block mitosis by disrupting the mitotic spindle tubules at metaphase [3, 4]. These chemicals have been observed to produce desirable effects as it pertains

*Corresponding author: Department of Biology, Islamic Azad University, Saveh Branch, Saveh, Iran.
Email Address: attaei@iau-saveh.ac.ir

to chromosome condensation and spread, and though the roles of these chemicals in spindle fiber inhibition have been elucidated by many authors [5]. Natural products produced by plants and their synthetic derivatives are expected to play an important role in the development of innovative agents to inhibit cell mitosis and cancer by blocking the formation of the mitotic spindle in M-phase. There is a growing interest in the pharmacological evaluation of various plants used in the world traditional system of medicine [6].

Field dodder (*Cuscuta campestris*) and Eastern dodder (*C. monogyna*) are the most widespread obligatory parasitic species in the genus *Cuscuta* (dodder) [7]. Extracts of dodders include various flavonoid compounds, a range of polysaccharides, a number of different alkaloids and various other chemicals [8, 9]. Seeds extracts of *C. reflexa* have antimitotic and anticancer activities [10] and one study suggest that *C. chinensis* water extract induced skin papillomas and carcinomas in mice [11]. *Nigella sativa* L. seeds extracts were toxic on root number and length and reduced the mitotic index of meristem cells of onion roots [12]. Cuscutic resinoside A from the seeds of *C. chinensis* is a stimulator of breast cancer cell proliferation [13]. Flavonoids extracts of *C. chinensis* can protect PC12 cells against oxidative stress. The mechanism of it may be the ability of scavenging ROS and increasing the activity of antioxidant enzyme [14]. Barley [15] and onion [16] are outstanding tests organisms because of its sensitivity to xenobiotic as well as their suitable cells and chromosome features.

The present study was carried out to evaluate the phenol and alkaloid content, cytotoxic and antimitotic potential of *C. campestris* and *C. monogyna* aqueous extracts by standard assay method using barley (*Hordeum vulgare* L.) seedlings and onion(*Allium cepa* L.) roots meristem model [17]. The effects were compared with 8-Hydroxyquinoline, a standard cytotoxic drug.

Material and Methods

Collection of Dodders

Field dodder (*C. campestris* Yunck.) and eastern dodder (*C. monogyna* Vahl.) are collected from Alhagi (*Alhagi maurorum* Dest.) host and Vine (*Vitis sylvestris* Gmelin) host respectively from Markazi province of Iran and identified by Department of Botany of Islamic Azad University

of Iran. The aerial parts of dodders were taken out from their hosts, cleaned and dried in oven at 60°C for 34 hours and then powdered.

Dodders Aqueous Extracts Preparation

50 g of dodders powders were extracted by 100 ml distilled water in 80°C warm bath for 2 h. The extracts were filtered through a filter paper and dried by rotary evaporator at 80°C .

Dodders Extract TLC and Phenol and Alkaloid Presentation

TLC was used according to modified methods of MORRIS *et al.* as described by Baerhem-Suendsen and Verpoorte (1983) [18]. 50µl aqueous extract (0.1 g.ml⁻¹) was applied onto thin layer chromatography (TLC) plates. TLC solvent systems routinely used was ethanol: distilled water (4:1) and TLC plates(20×20 cm) were formed by coating it to a thickness of 0.2 mm of silica gel G60 (Merck). Phenols and alkaloids were visualized using TLC and the color reaction by ferric chloride (6.7g in 100ml distilled water) and Dragendorff reagent (0.4 g of bismuth subnitrate in 10 ml of concentrated hydrochloric acid mixed with 5.0 g of potassium iodide in 50 ml of distilled water and the solution volume was reached to 100 mL by distilled water) spray reagents respectively. The Dragendorff reagent [18], widely used in the visualizing of alkaloid (orange) and ferric salts used in the visualizing of poly phenols. The hydrolysable tannins (gallitannins and ellagitannins) give blue-black precipitates and condensed tannins brownish-green ones [19].

Total Phenols

Total phenols were determined by using the Folin Ciocalteu reagent [20]. The calibrations solutions were prepared using 0, 25, 50, 75 and 100 µg.ml⁻¹ solutions of gallic acid (Merck) in distilled water. 0.5 ml of each aqueous of plant extract (0.1 g.ml⁻¹) or quercetin (the phenolic compound commonly used as the standard) was mixed with the 2.5 ml of Folin Ciocalteu reagent (the reagent diluted tenfold with distilled water) and 2 ml of aqueous Na₂CO₃ (1 M). The mixtures were allowed to stand for 15 min and total phenols were determined by colorimetric at 765 nm by a double beam UV/visible spectrophotometer (Shimadzu, Japan). The total phenol value was expressed in terms of gallic acid equivalent (mg.g⁻¹ of dry weight), which is a commonly used reference value.

Total Flavonoids

Aluminum chloride colorimetric was used for flavonoids determination [21]. The calibration solutions were prepared by using gallic acid solutions at concentrations from 0 to 10 $\mu\text{g.ml}^{-1}$ in distilled water. 0.5 ml of quercetin and each plant extracts (0.1 g.ml^{-1}) was mixed separately with 2 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 M potassium acetate and 2.3 ml of distilled water. After keeping the mixture at room temperature for 30 min, the absorbance of the reaction maximum was measured at 415 nm.

Total Alkaloid

10 mg of dry aqueous extracts were placed in a 10 ml flask and dissolved in 10 ml ethanol. Ephedrine alkaloid (Caspian Tamine Drugs Company of Iran) was used for calibrations solutions. Six calibrations solutions (0, 20, 40, 60, 80 and 100 $\mu\text{g ml}^{-1}$) were prepared with ethanol. Total alkaloids were measured at 254 nm, according to modified methods of Renaudin (1984) [22].

Onion (*Allium cepa* L.) and Barley (*Hordeum vulgare* L.) Tests

To cytotoxic and antimitotic effects of dodders aqueous extracts, we used root of onion bulbs and barley seedlings [23]. The treatment (incubation) of roots was carried out in two applications under the same conditions.

Application 1

Onion bulbs ($50 \pm 10 \text{ g}$) were grown in 100 ml pots in distilled water in light at 25 °C for 48 h (until the roots have grown to approximately 0.5- 1 cm). The bulbs were prefixed to the distilled water (control) and to 8-Hydroxyquinoline and aqueous extract of dodders (*C. campestris* and *C. monogyna*) at various concentrations: 0.05, 0.1 and 0.2 mg ml^{-1} (dw) for 6 and 12 h. The difference of length of roots was determined after 6 and 12 h after treatment by 8-Hydroxyquinoline and aqueous extract of dodders, prior to fixation with ethanol-acetic acid (3:1, v/v). Roots of bulbs 6 and 12 h grown in water prior to fixation, were used as control.

Application 2

The barley seeds were germinated in Petri dishes at 25°C and dark, for 4 days after seeds water imbibition (until the roots have grown to approximately 0.5-1 cm). The seedlings were

incubated with 0.05, 0.1 and 0.2 mg ml^{-1} (dw) of dodders extracts and 8-Hydroxyquinoline for 6 and 12 h. The difference length of roots was determined after 6 and 12 h after treatment by 8-Hydroxyquinoline and aqueous extract of dodders, prior to fixation with ethanol-acetic acid (3:1, v/v). Barley seedlings 6 and 12 h grown in water prior to fixation were used as control.

Microscopic Examination and Determination of Mitotic Index

After the completion of treatment, the roots of each onions bulbs and barley seedlings were cut and fixed in ethanol-acetic acid (3:1, v/v). After the fixation, the roots were hydrolyzed in 5 ml 1N HCl at 25°C for three minutes and washed in distilled water. They were subsequently processed for cytological study by the conventional 2% aceto-carmine squash technique (for 10 minutes). After removing the root caps from well-stained root tips, 1 mm of the mitotic zones were immersed in a drop of aceto-carmine on a clean slide and squashed under a cover glass and examined microscopically. For each root tip the number of mitotic and total meristem cells was counted in 5-8 fields using Olympus microscope BX-41 [24]. In all 400-500 cells were counted and cells manifesting different stages of mitosis i.e., interphase and prophase (P), metaphase (M), anaphase (A) and telophase (T) were recorded. The mitotic index was calculated using the following formula:

$$\text{Mitotic index} = (\text{P} + \text{M} + \text{A} + \text{T}) / \text{Total cells}$$

The inhibitory effect of Field and eastern dodder extracts was evaluated on the growth(Macroscopic effect) and mitotic activity(Microscopic effects) of barley seedlings and onion root meristems and the effect was compared with standard 8-Hydroxyquinoline.

Statistical Analysis of Data

All experiments were done with 3 repeat, at least. The SPSS 11.5 statistical package program was used for statistical analysis. Differences among treatments were compared by analysis of variance of the One Way ANOVA test (significance at the $p < 0.05$ and 0.01 level) and Duncan test to compare means.

Results

The aqueous extracts of two dodders were compared by TLC analysis. Result of TLC showed the phenols of two dodders are poly phenols. The poly phenols of field dodder were condensed tannins because it was visualized brownish-green by ferric salts reagent but hydrolysable tannins were poly phenols of eastern dodder because visualized blue-black (Fig. 1 left). Dragendorff reagent visualized of aqueous extracts TLC of two dodders showed the both dodders have alkaloid but their pattern of TLC are very different one another (Fig. 1 right).

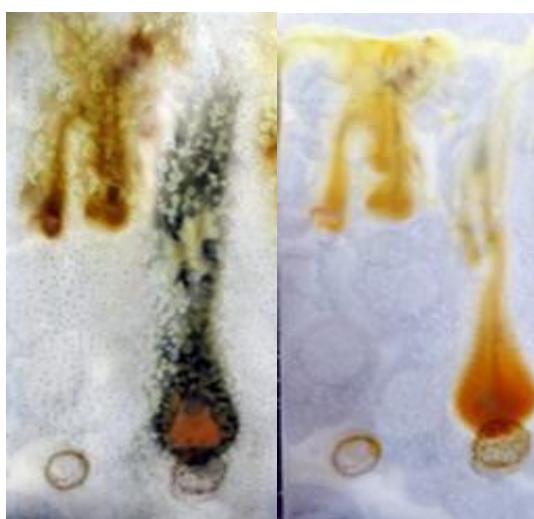


Fig. 1 TLC patterns of aqueous extracts of *C. campestris* and *C. monogyna*:

Left: condensed tannins of *C. campestris* (brownish-green left) and hydrolyzable tannins of *C. monogyna* (blue-black right) were visualized by ferric salts reagent.
Right: alkaloids of *C. campestris* (orange left) and *C. monogyna* (orange right) were visualized by Dragendorff reagent.

Total Phenol and Flavonoid

Total Phenol and flavonoid of *C. campestris* and *C. monogyna* were 18.51 and 15.98, and 22.61 and 7.68 mg.g⁻¹ respectively (Table 1). Total Phenol of *C. monogyna* was higher than *C. campestris* but its total flavonoid was very lower.

Total Alkaloid

Table 1 Phenol, flavonoid and alkaloid content of *Cuscuta campestris* Yunck. and *Cuscuta monogyna* Vahl.

| Plants | Phenol (mg.g ⁻¹) | Flavonoid (mg.g ⁻¹) | Alkaloid (mg.g ⁻¹) |
|----------------------|------------------------------|---------------------------------|--------------------------------|
| <i>C. campestris</i> | 18.51 b | 15.98 a | 7.65 b |
| <i>C. monogyna</i> | 22.61 a | 7.68 b | 9.55 a |

Total alkaloid of *C. campestris* and *C. monogyna* were 7.65 and 9.55mg.g⁻¹ respectively (Table 1). Total alkaloid of *C. monogyna* was higher than *C. campestris*.

Allelopathy Effects

The root length for control and each extracts is given in Table 2. A progressive increase in root length of barley seedlings and onion bulbs were observed in control group. The root length of barley in control group at 0, 6 and 12 h were 0.39, 0.43 and 0.48cm and for onion were 0.48, 0.50 and 0.84cm respectively. On the other hand, the root growth of barley in control group at 6 and 12 h were 0.14 and 0.48cm and for onion were 0.50 and 0.84cm respectively. Incubation of barley seedlings and roots of onion bulbs in different concentrations of cytotoxic agents produced a growth retarding effect that was associated with a decrease in the root length.

The different concentration of dodders extracts and 8-Hydroxyquinoline (0, 0.05, 0.1 and 0.2 mg.ml⁻¹) induced root decay and decreased the root length significantly at 6 h and 12 h were compared by analysis of variance of the One Way ANOVA test (significance at the p<0.05 and 0.01 level) and Duncan test to compare means. The root growth of barley and onion were reduced by increasing of concentrations and time treatments. The root growth of barley seedlings at 0.1 and 0.2 mg ml⁻¹ extracts of dodders were at 0.05- 0.08 cm at 6h and 12h respectively, without significantly difference but for 8-Hydroxyquinoline, with significantly difference were 0 cm (Table 2).

The root growth of onion at 0.2 mgml⁻¹ of extracts of dodders, and 0.1 and 0.2 mgml⁻¹ of 8-Hydroxyquinoline were 0- 0.05 cm at 6h and 12h, without significantly difference. All agents arrested the root growth but the force of 8-Hydroxyquinoline was better than dodders extracts. Field dodder extract was more effective on root length when compared with the eastern dodder extract.

Table 2 Root length (cm) of control (non-treated) onion bulbs and Barley seedling, bulbs and Barley seedling treated with dodders (*Cuscuta campestris* Yunck. and *Cuscuta monogyna* Vahl.) extract and 8-Hydroxyquinoline continuously for 6 and 12h.

| Plant | Con. mgml ⁻¹ | Root (cm) | <i>C. campestris</i> Root(cm) | | <i>C. monogyna</i> Root (cm) | | Hydroxyquinolin Root (cm) | |
|--------------------|----------------------------|--------------|----------------------------------|--------|---------------------------------|---------|------------------------------|--------|
| | | | 0h. | 6h. | 12h. | 6h. | 12h. | 6h. |
| Barley seedling | 0 | 0.39 | 0.43 a | 0.48 a | 0.43 a | 0.48 a | 0.43 a | 0.48 a |
| | 0.05 | 0.39 | 0.11 bc | 0.16 b | 0.12 bc | 0.14 b | 0.14 b | 0.18 b |
| | 0.1 | 0.39 | 0.05 c | 0.07 c | 0.07 c | 0.08 c | 0.01 d | 0.02 d |
| | 0.2 | 0.39 | 0.06 c | 0.07 c | 0.06 c | 0.05 c | 0.00 d | 0.00 d |
| Onion bulb | 0 | 0.48 | 0.50 b | 0.84 a | 0.50 b | 0.84 a | 0.50 b | 0.84 a |
| | 0.05 | 0.48 | 0.23 c | 0.27 c | 0.2 c | 0.3 c | 0.18 cd | 0.2 c |
| | 0.1 | 0.48 | 0.1d | 0.13 d | 0.12 d | 0.17 cd | 0.02 e | 0.05 e |
| | 0.2 | 0.48 | 0.05 e | 0.03 e | 0.01 e | 0.04 e | 0.01 e | 0.00 e |

Values for particular concentration and time treatments followed by different small letter are significantly different at $P < 0.05$ and 0.01

Table 3 Mitotic index in roots of barley (*Hordeum vulgare*) seedlings and onion bulbs(*Allium cepa*) meristem cells following incubation with different concentrations(Con mg l⁻¹) of extracts of two dodders(*Cuscuta campestris* Yunck. and *Cuscuta monogyna* Vahl.) and 8-Hydroxyquinoline at 6 h and 12 h.

| T | Plant | Con mgml ⁻¹ | 6h. | | | | | | 12h | | | | | |
|--------------------|--------------------|---------------------------|-----|----|----|----|----|----|-----|----|----|----|----|----|
| | | | mi | pr | me | an | te | cy | mi | pr | me | an | te | cy |
| <i>C.comp...</i> | Barley seedling | 0 | 30 | 11 | 9 | 8 | 2 | 0 | 25 | 7 | 8 | 3 | 6 | 1 |
| | | 0.05 | 22 | 8 | 8 | 3 | 2 | 1 | 18 | 2 | 10 | 1 | 3 | 2 |
| | | 0.1 | 17 | 5 | 10 | 1 | 3 | 2 | 15 | 2 | 13 | 0 | 0 | 0 |
| | | 0.2 | 8 | 2 | 6 | 0 | 0 | 0 | 6 | 0 | 6 | 0 | 0 | 0 |
| <i>C. monogyna</i> | Onion bulb | 0 | 27 | 8 | 5 | 6 | 5 | 3 | 24 | 2 | 6 | 4 | 6 | 6 |
| | | 0.05 | 18 | 5 | 7 | 3 | 2 | 1 | 14 | 3 | 7 | 2 | 1 | 1 |
| | | 0.1 | 14 | 3 | 7 | 2 | 1 | 1 | 13 | 2 | 8 | 1 | 2 | 0 |
| | | 0.2 | 8 | 3 | 5 | 0 | 0 | 0 | 7 | 0 | 7 | 0 | 0 | 0 |
| <i>Hydro...</i> | Barley seedling | 0 | 30 | 11 | 9 | 8 | 2 | 0 | 25 | 7 | 8 | 3 | 6 | 1 |
| | | 0.05 | 18 | 6 | 10 | 1 | 1 | 0 | 14 | 2 | 9 | 1 | 2 | 1 |
| | | 0.1 | 15 | 4 | 9 | 1 | 1 | 0 | 12 | 0 | 10 | 0 | 0 | 2 |
| | | 0.2 | 12 | 0 | 10 | 0 | 1 | 1 | 8 | 0 | 8 | 0 | 0 | 0 |
| | Onion bulb | 0 | 27 | 8 | 5 | 6 | 5 | 3 | 24 | 2 | 6 | 4 | 6 | 6 |
| | | 0.05 | 20 | 8 | 8 | 2 | 1 | 1 | 18 | 3 | 10 | 1 | 1 | 3 |
| | | 0.1 | 17 | 6 | 9 | 1 | 1 | 0 | 14 | 1 | 11 | 0 | 0 | 2 |
| | | 0.2 | 8 | 2 | 6 | 0 | 0 | 0 | 6 | 0 | 6 | 0 | 0 | 0 |

(Mitotic index (mi)%), interphase and prophase (pr), metaphase (me), anaphase (an), telophase (te) and cytokinesis(cy))%

Cytotoxic Effects

The mitotic cells (Mitotic index or number of dividing cells) were counted in the root meristems in above groups at 0, 6 and 12 h of incubation with each agents. In Table 3, the mitotic indexes (MI)

are given for control and for each extract. The MI ranged of root meristems of barely seedlings 30 and 25 and for onion 27 and 24% in the control group over a period of 6 and 12 h respectively.

All agents produced a significant decrease in MI that was dose and time dependent. It is evident that

all agents reduced the mitotic index significantly (to 0-8%). The reduction in number of dividing cells in the root meristem shows the antimitotic effects of the substances that found in dodders extracts. 8-Hydroxyquinoline was more effective on mitotic index when compared with the Field and eastern dodders extracts. In respect of this results, Field and eastern dodders extracts contains antimitotic constituents that can stop the mitosis in anywhere of the cell cycle. Furthermore these constituents probably affect the cytoskeleton or tubulin polymerization or degradation.

The MI values for each group are given in Table 3. The MI rates were decreased with increasing concentrations of dodders extracts and 8-Hydroxyquinoline compared with the control (water). Decrease of MI rates explains cytotoxicity of dodder aqueous extracts and 8-Hydroxyquinoline in plant test system. Similarly the reduction of MI was also found in all concentrations of dodder aqueous extracts and 8-Hydroxyquinoline treated groups, especially 0.2 mg ml⁻¹. The reduction in MI showed that substances in dodders aqueous extracts have high cytotoxic effects.

The stage of mitosis at which the block occurred was determined by counting relative numbers of cells at each stage of mitosis after staining. As shown in Table 3, the ratio of the number of cells of roots meristem of barley seedlings and onion in anaphase to those in metaphase decreased to zero over the concentration range and incubation time increasing that induced mitotic block, indicating chromosome condensation and a block specifically in metaphase (Fig. 2, 3). At concentrations of 0.1 and 0.2 mg ml⁻¹ dodders extracts, 0.05 and 0.1 mg ml⁻¹ 8-Hydroxyquinoline, cells in anaphase were absent. Thus, the block occurred specifically at the transition from metaphase to anaphase. The potency of the three compounds with respect to both inhibition of cell proliferation and mitotic block was 8-Hydroxyquinoline > *C. campestris* > *C. monogyna*.

Discussion

Plants are known to synthesis allelochemical [25]. Allelochemical compounds belong to different categories of secondary metabolites such as phenols, tannins, flavonoids and alkaloids [26].



Fig. 2 The chromosome condensation and block effects specifically in metaphase of Field dodder (*Cuscuta campestris* Yunck.) and eastern dodder (*Cuscuta monogyna* Vahl.) extracts in meristematic cells mitosis of roots of *Allium cepa*: A) 8-Hydroxyquinoline 0.05 mg ml⁻¹, B) *C. campestris* extract 0.1 mg ml⁻¹, C) *C. monogyna* extract 0.1 mg ml⁻¹, G, H) *C. campestris* extract 0.2 mg ml⁻¹, K) *C. monogyna* extract 0.2 mg ml⁻¹.



Fig. 3 The chromosome condensation and block effects specifically in metaphase of Field dodder (*C. campestris*) and eastern dodder (*C. monogyna*) extracts in meristematic cells mitosis of roots of *Hordeum vulgare* seedlings: A) 8-Hydroxyquinoline 0.05 mg ml⁻¹, B) *C. monogyna* extract 0.1 mg ml⁻¹, C) *C. monogyna* extract 0.2 mg ml⁻¹.

The poly phenols of *C. campestris* and *C. monogyna* were condensed and hydrolyzable tannins respectively. The condensed tannins or proanthocyanidins are polyflavonoids, consisting of chains of flavan-3-ol units. The hydrolyzable tannins are usually subdivided into gallotannins and ellagitannins [27]. *C. campestris* and *C. monogyna* extracts were including poly phenols and alkaloid. Extracts of dodders include various flavonoid compounds, a range of polysaccharides, a number of different alkaloids and various other chemicals [8, 9]. Plant growth is dependent to cell division in root or stem tips. It is also known that proper cytoskeleton orientation that accompanies cell divisions is crucial for correct proceeding of mitosis [28]. Cell divisions are in turn prerequisite for root growth as newly formed cells are the only factors of the organ elongation via increasing their dimensions (mainly in line with long axis of the root [29, 30]. Rudrappa *et al.* (2007) found that water extract (20%, v/v) from noxious weed (*Phragmites australis* Cav.) caused disruption in root cell microtubule network in *Arabidopsis thaliana* [31]. In the present study, we found anti-mitotic properties of two dodders (*C. campestris* and *C. monogyna*) extracts. Data on the effects of the dodders extracts on roots growth of barley seedlings and onion bulbs showed that there were incubation time and concentration-dependent decrease. This results shows that the extracts from these dodders have inhibitory effects on root growth of barley seedlings and onion bulb. In conformity with animal and human cell cytotoxicity [32, 33]. It was found that dodders extracts have cytotoxic properties also in plant test systems. The extracts of dodders have inhibitory and mito depressive effects on root tip meristem cells. Mito depressive effects of some plant extracts, being the ability to block the synthesis of DNA and nucleoproteins had earlier been reported [34, 35]. They may not even allow the initiation of their biosynthesis and such action occurring in the interphase nucleus apart from influencing the ultimate structure of the chromosome during cell division could also cause reduction of number of other stages [36]. Extracts of dodders include various flavonoid compounds and different alkaloids [8, 9]. Seeds extracts of *C. reflexa* have antimitotic and anticancer activities [10] and one study suggest that *C. chinensis* water extract induced skin papillomas and carcinomas in mice [11]. *N. sativa* L. seeds extracts were toxic on root

number and length and reduced the mitotic index of meristem cells of onion roots [12]. Flavonoids extracts of *C. chinensis* can protect PC12 cells against oxidative stress. The mechanism of it may be the ability of scavenging ROS and increasing the activity of antioxidant enzyme [14]. There is a linear relationship between macroscopic and microscopic parameters for all treatments. Dodders extracts in 0.1 and 0.2 mg ml⁻¹ concentrations reduced the mitotic index, significantly (cytotoxic effects). The cytotoxic effects of dodders aqueous extracts was comparable to that of 8-Hydroxyquinoline and all agents inhibited root growth and mitosis to a significant extent.

Conclusions

In the present study we found, the cytotoxic effects of those are comparable to that of 8-Hydroxyquinoline and all agents inhibited root growth and mitosis to a significant extent (as anti-cancer drugs). Our results showed aqueous extracts of these two dodders (*C. campestris* and *C. monogyna*) are rich sources of poly phenols and alkaloids that some these compound having anti-mitosis potential and usable for cytology studies and anti-cancer drug production. Dodders aqueous extracts can be used to produce desirable effects as it pertains to cytology, chromosome condensation and spread, and though the roles of these chemicals in spindle fiber inhibition have been elucidated, as 8-Hydroxyquinoline and anti-cancer drugs.

Acknowledgments

We are grateful to the biological department at the Islamic Azad University of Saveh for providing financial and laboratorial support.

References

1. De Araujo Junior RF, De Souza TP, Pires JG, Soares LA, De Araujo AA, Petrovick PR. A dry extract of *Phyllanthus niruri* protects normal cells and induces apoptosis in human liver carcinoma cells. *Exp Bio Med.* 2012;237:1281-1288.
2. Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr. Med. Chem Anti-Canc Agents.* 2002;2:1-17.
3. Osalou AR, Rouyandezagh SD, Alizadeh, B, Er C, Sevimay CS. A comparison of ice cold water pretreatment and - bromonaphthalene cytogenetic

- method for identification of *Papaver* species. Science World J. 2013;8:88-96.
4. Ekong NJ, Akpan GA, Udo IJ. Comparative effects of colchicine, 8-hydroxyquinoline and paradichlorobenzene on arm ratio of mitotic chromosomes of *Allium cepa* L. Int. J. Med: Plants and Alternative Med. 2014;2:21-26.
 5. Burley J. Karyotype of *Sitka Spruce*, *Picea sitchensis*. Silvae Genetica. 1964;14:127-137.
 6. Xua HS, Wuc YW, Xud SF, Xsuna HX, Chend FY, Yao L. Antitumor and immune modulatory activity of polysaccharides from the roots of *Actinidia eriantha*. J Ethnopharmacol. 2009;125:310-317.
 7. Kanwal D, ABID R, QAISER M. The seed atlas of Pakistan-iii. Cuscutaceae. Pakistan J. Bot. 2010;42:703-709.
 8. Shoji Y, Haruya D, Toshihiro N. An alkaloid and two lignans from *Cuscuta chinensis*. Phytochem. 1994;37:1755-1757.
 9. Ye M, Yan YN, Qiao L, Ni XM. Studies on chemical constituents of *Cuscuta chinensis*. China J Chinese Materia Medica. 2002;27:115-117.
 10. Udavant PB, Satyanarayana SV, Upasani CD. Preliminary screening of *Cuscuta reflexa* stems for anti-inflammatory and cytotoxic activity. Asian Pacific J Tropical Biomed. 2012;51:303-307.
 11. Nisa M, Akbar S, Tariq M, Hussain Z. Effect of *Cuscuta chinensis* water extract on 7, 12-dimethylbenz [a] anthracene-induced skin papillomas and carcinomas in mice. J Ethnopharma. 1986;18:21-31.
 12. Ozmen A, Gamze B, Tugba A. Antimitotic and antibacterial effects of the *Nigella sativa* L. seed. Caryologia. 2007;60:270-272.
 13. Umehara K, Nemoto K, Ohkubo T, Miyase T, Degawa M, Noguchi H. Isolation of a new 15-membered macrocyclic glycolipid lactone, *Cuscutic resinoside* a from the seeds of *Cuscuta chinensis*: a stimulator of breast cancer cell proliferation. Planta Medica. 2004;70:299-304.
 14. Zhen G, Jiang B, Bao Y, Li D, An L. The protect effect of flavonoids from *Cuscuta chinensis* in PC12 cells from damage induced by H2O2. Zhong Yao Cai. 2006;29:1051-5.
 15. Georgieva M, Kruppa K, Molnár-Láng M, Liu L, Manova V, Stoilov L. Cytogenetic effects in barley root apical meristem after exposure of dry seeds to lithium ion beams nikolova I. Genetics Plant Physiol. 2015;5:3-9.
 16. Leme DC, Marin-Morales MA. Chromosome aberrations and micronucleus frequencies in *Allium cepa* cells exposed to petroleum polluted water. Mutation Res. 2008;650:80-86.
 17. Sharma CB. Plant meristems as monitors of genetic toxicity of environmental chemicals. Current Sci. 1983;52:1000-1002.
 18. Baerhemi-Suendsen A, Verpoorte R. Chromatography of alkaloids, part A. TLC, Elsevier, Amsterdam. 1983;253-277.
 19. Bamoniri A, Behpour M, Khayat Kashani M. Quantification of total phenolics and tannins of pomegranate extraction for standardization to ellagic acid. J Optoelectronics Biomed Mater. 2010;2:25 – 31.
 20. Gutfinger T. Phenols in olive oils. J American Oil Chem Soci. 1981;58:966–968.
 21. Chang C, Yang M, Wen H, Chem J. Estimation of total flavonoid contents in plants by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178–182.
 22. Renaudin JP. Reversed phase High performance liquid chromatographic. J Chromato. 1984;291:165-174.
 23. Fiskejso G. A 2-3 Day plant test for toxicity assessment by measuring the mean root growth of onions (*A. cepa* L.). Environ. Toxic. Water Qual. 1993;8:461-470.
 24. Badria FA, Houssein WE, Zaghloul MG, Halim AF. Antimitotic activity of gossypol and gossypolone. Pharmace Biol. 2001;39:120-126.
 25. Inderjit I and Weinert J. Plant allelochemical interference. 2001; Perspective in plant ecology. 2001;4:4-12.
 26. Inderjit I and Dakshini KMM. On Laboratory Bioassays in allelopathy. The Botanical Review. 1995;1:29-43.
 27. Haslam E. Condensed tannins- structure, and Chapter 4: The hydrolyzable tannins. In: Chemistry of vegetable tannins. Ed. E. Haslam. Academic Press, London. 1966;275-390.
 28. Wade RH. On and around microtubules: an overview. Molle. Biotech. 2009;43:177-191.
 29. Ding L, Qi L, Jing H, Li J, Wang W, Wang T. Phytotoxic effects of leukamenin E (an ent-kaurene diterpenoid) on root growth and root hair development in *Lactuca sativa* L. seedlings. J Chem Ecol 2008;34:1492-1500.
 30. Wasteneys GO, Ambrose JC. Spatial organization of plant cortical microtubules: close encounters of the 2D kind. Trends Cell Bio. 2009;19:62-71.
 31. Rudrappa T, Bonsall J, Gallagher JL, Seliskar DM, Bais HP. Root-secreted allelochemical in the noxious weed *Phragmites australis* deploys a reactive oxygen species response and microtubule assembly disruption to execute rhizotoxicity. J Chem Ecol. 2007;33:1898-1918.
 32. Swamy SMK, Tan BKH. Cytotoxic and immune potentiating effects of ethanolic extract of *Nigella sativa* seeds. J Etnopharma. 2000;70:1-7.
 33. Thabrew MI, Mitry RR, Morsy MA, Hughes RD. Cytotoxic effects of a decoction of *Nigella sativa*, *Hemidesmus indicus* and *Smilax glabra* on human hepatoma HepG2 cells. Life Sci. 2005;77:1319-1330.
 34. Mercurykutly VC, Stephen J. Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test. Cytologia. 1980;45:769-777.
 35. Schulze E, Kirscher S. Microtubule dynamics in interphase. Microtubule dynamics in interphase. Cellular J Cell Bio. 1996;102:1020-1021.
 36. Akinboro A, Bakare AA. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* L. J Ethno pharm. 2007;112:470-475.