BIOLOGICA NYSSANA
8 (2) • December 2017: 151-158
DOI: 10.5281/zenodo.1135968

Original Article

Received: 14 September 2017
Revised: 21 November 2017
Accepted: 22 December 2017

Evaluation of cytotoxicity of 'anti-diabetic' herbal preparation and five medicinal plants: an *Allium cepa* assay

Višnja Madić*, Jelena Jovanović, Aleksandar Stojilković, Marina Jušković, Perica Vasiljević

University of Niš, Faculty of Science and Mathematics, Department of Biology and Ecology, Višegradska 33, Niš, Serbia

** E-mail: visnja.madic@gmail.com*

***Abstract*:**

Madić, V., Jovanović, J., Stojilković, A., Jušković, M., Vasiljević, P.: ***Evaluation of cytotoxicity of 'anti-diabetic' herbal preparation and five medicinal plants: an Allium cepa assay. Biologica Nyssana, 8 (2), December, 2017: 151-158.***

Traditional medicine is often used as the treatment for diabetes mellitus, but little is known about potential toxicity of medicinal plants used for this purposes. The cytotoxic effect of aqueous extracts of a traditional 'anti-diabetic' herbal preparation, as well as its constituents: *Rubus fruticosus*, *Vaccinium myrtillus*, *Potentilla erecta*, *Geum urbanum* and *Phaseolus vulgaris* was evaluated using the *Allium cepa* assay. Onion bulbs were exposed to 400 μg/ml, 800 μg/ml and 1200 μg/ml concentrations of each extract. There was concentration dependent inhibition of root growth by the extracts when compared with the control. All the tested extracts had mitodepressive effect on cell division. 'Anti-diabetic' herbal mixture (1200 μg/ml) and *V. myrtillus* (400 μg/ml) caused surprisingly high prophase accumulation. These events manifested the cytotoxic effect of this herbal preparation as well as all the tested medicinal plants at some of the tested concentrations.

Key words: Diabetes, 'anti-diabetic' herbal preparation, cytotoxicity

***Apstrakt*:**

Madić, V., Jovanović, J., Stojilković, A., Jušković, M., Vasiljević, P.: ***Ispitivanje citotoksičnosti "antidijabetičkog biljnog preparata" i pet medicinskih biljaka: Allium cepa test. Biologica Nyssana, 8 (2), Decembar, 2017: 151-158.***

Tradicionalna medicina se često koristi u terapiji dijabetes melitusa, ali malo toga se zna o potencijalnoj toksičnosti medicinskih biljaka korišćenih u ove svrhe. *Allium cepa* testom ispitali smo citotoksični efekat vodenih ekstrakata tradicionalnog "antidijabetičkog" biljnog preparata, kao i njegovih sastojaka: *Rubus fruticosus*, *Vaccinium myrtillus*, *Potentilla erecta*, *Geum urbanum* i *Phaseolus vulgaris*. Lukovice su bile izložene trima koncentracijama svakog od ovih ekstrakata, i to: 400 μg/ml, 800 μg/ml and 1200 μg/ml. Svi testirani ekstrakti pokazali su mitodepresivni efekat na ćelijsku deobu. "Antidijabetički" biljni preparat (1200 μg/ml) i *V. myrtillus* (400 μg/ml) su, uz to, izazvali iznenađujuće veliku akumulaciju ćelija u profazi. Ovi događaji su manifestacija citotoksičnog efekta ovog biljnog preparata i ovih medicinskih biljaka u nekim od ispitivanih koncentracija.

Ključne reči: Dijabetes,"antidijabetički" biljni preparat, citotoksičnost

Introduction

The use of medicinal plants has always been part of the human tradition. According to the World Health Organisation, 80% of the world population relies on the traditional medicine (Farnsworth et al., 1985). In recent years there has been an increased popularity of traditional medicine globally, mostly due to the high costs of nursing care and international commercial medicines.

However, some constituents of medicinal plants have been found to be potentially toxic, mutagenic, carcinogenic and teratogenic (Ping et al., 2012). Also, potentional contraindications between herbal preparations and modern pharmaceutical products are not sufficiently examined, both for short-term and long-term use. For all this, the purpose of this study is to evaluate the toxicological effects of some of the herbal preparations used in traditional medicine.

Traditional medicine is used as the treatment for a variety of diseases, including diabetes mellitus, commonly referred to as diabetes. In 2015, there were 415 milion adults and half a million children with diabetes globally, most in low- to middle-income countries, predicted to rise to over 642 milion in the next 23 years. Every 6 s someone dies from diabetes (International Diabetes Federation (IDF), 2015). UK Diabetologist Edwin Gale's quoted, 'What is the commonest cause of death in a child with diabetes? The answer – from a global perspective – is a lack of access to insulin' (Gale & Wagner, 2006).

Although herbal substituents can not replace insulin, traditional treatments can lead to the development of new hypoglycemic drugs, especially for T2D.

There is a variety of herbal mixtures intended for maintaining normal glycemic values, and one of the most commonly used in Europe, primarily in the Balkan countries, is made of blackberry (*Rubus fruticosus* L., *Rosaceae*) leaves (RFL), blueberry (*Vaccinium myrtillus* L., *Ericaceae*) leaves (VML), tormentil (*Potentilla erecta* Uspenski ex Ledeb., *Rosaceae*) roots (PER), St. Benedict's herb (*Geum urbanum* L., *Rosaceae*) aerial parts (GUA), and kidney bean (*Phaseolus vulgaris* L., *Fabaceae*) pods (PVP).

There is a long history of using these medicinal plants in traditional therapy for diabetes. Their hypoglycemic effect was examined by a number of studies (Swanston-Flatt et al. 1990; Eddouks et al., 2002; Tomczyk & Latté, 2009; Kyznietsova et al., 2015; Koupý et al., 2015; Paun et al., 2015; Johnson & De Mejia, 2016; Sidorova et al., 2017), but a little is known about their potential toxicity.

For the most of the substances on this planet there are doses which are toxic and which are non-toxic on the cell level. For this reason, the aim of this study was to investigate cytotoxic effect of aqueous extracts of the entire 'anti-diabetic' herbal preparation as well as its individual ingredients and predict their EC50 on the root meristem cells of *Allium cepa* L., fam. *Amaryllidaceae*.

Allium cepa test is often used in assessing cytological and cellular effects of medicinal plant's extracts (Akinboro & Bakare, 2007; Çelik & Aslantürk, 2010; Ping et al., 2012). It is a short-term test with many advantages, such as low cost and good chromosome conditions for the study of disturbance of cell division or chromosome aberrations (Fiskesjo, 1985). Some researchers show certain restriction in regards to using plant test systems for evaluating cytotoxicity of medicinal plants. However, *A. cepa* test, as a plant test systems *in vivo*, is validated by several researches, which jointly performed animal testing *in vitro* and the results obtained were similar (Camparoto et al., 2002; Teixeira et al., 2003), providing valuable information for human health. Moreover, Rank & Nielsen (1994) showed a correlation of 82% between the *A. cepa* test and the carcinogenicity test in rodents and concluded that the same was even more sensitive than the Ames test.

Material and methods

Preparation of extracts

The medicinal plants utilized in this study, i.e. blackberry (*R. fruticosus*) leaves (RFL), blueberry (*V. myrtillus*) leaves (VML), tormentil (*P. erecta*) roots (PER), St. Benedict's herb (*G. urbanum*) aerial parts (GUA), and kidney bean (*P. vulgaris*) pods (PVP), were purchased immediately before evaluation in the certified herbal pharmacy in Niš, Serbia. Originally, these medicinal plants were collected on the mountains of South-Eastern Serbia. *R. fruticosus*, *V. myrtillus*, *G. urbanum*, *P. vulgaris*, were collected on the Rtanj Mt., near Soko Banja, while *P. erecta* was collected on the Stara planina Mt. Pirot. *Rubus fruticosus*, *V. myrtillus*, *G. urbanum* and *P. erecta* were found in the wild, while *P. vulgaris* was organically grown, therefore, the possible impact of pesticide use on results of the *A. cepa* test was excluded.

Dried plant material was finely grinded, and 0.48 g of each tested material was separately boiled in 800 ml of distilled water until half of the liquid evaporated. The extract was then filtered and used as a stock solution. Stock solutions and dilutions were made immediately before use. Three concentrations of each extract, viz: 400 μg/ml, 800 μg/ml and 1200 μg/ml were considered.

Allium cepa assay

Small bulbs (1 – 2 cm in diameter) of the common onion, *A. cepa* (2 n = 16) were purchased at the agricultural pharmacy in Niš, Serbia. The base of each of the bulbs was suspended on distilled water for 24 h. After that period, the bulbs with satisfactory root lengths were exposed to the tested materials, 5 bulbs per every concentration, for 48 h. Test extracts were changed daily. The negative control was 5 bulbs suspended on distilled water for 72 h. The positive control was 5 bulbs suspended 24 h on distilled water, and afterwards, 48 h on 500 µg/ml toluene diluted in 2% DMSO. The experiment was performed at 25 ± 1 ºC in the dark.

At the end of the exposure period, in order to prevent drying of the material, root length of the whole root bundle was measured outside of the test tube by the ruler, as shown on **Fig. 1.** This method gives one value for each bulb, and permits the experiment to be continued (F i s k e s j o , 1985).

Fig. 1. Measuring the root length of the whole root bundle. Root length of the whole root bundle was measured outside of the test tube by the ruler. This method gives one value for each bulb, and permits the experiment to be continued.

Root tips were cut by scalpel, fixed in methanol / glacial acid (3:1, v/v) for 24 h at 4 ºC. The next day they were placed in 70% ethanol alcohol and refrigerated until used.

Slides were prepared immediately before microscopic analysis. The root tips were hydrolyzed in 1 N HCl at 60 ºC for 13 minutes and washed three times in distilled water. After that, they were stained with 1% acetocarmine at room temperature for 15 minutes or, alternatively, on a Bunsen burner for 1 - 2 minutes and washed three times in distilled water. The root tips were afterwards transferred onto the cold slides, squashed by cover slips and heat fixed.

The following parameters were used for determination of cytotoxicity: treated root growth inhibition (TRG), mitotic index (MI) and phase index (PI).

For the treated root growth inhibition, from the length of bundle for each concentration (LTR) the percentage of root growth inhibition in relation to the negative control (LNC), i.e.

$$TRG = \frac{LTR\ (cm)}{LNC\ (cm)} x100\%$$

and the EC_{50} (the effective concentration where root growth amounts to 50% of the controls) for each extract was determined (F i s k e s j o , 1985).

For the MI and PI analysis, for every concentration, as well as for the controls, 5 microscopic slides were made, and 500 cells per slide, i.e. 2500 cells per concentration were observed by Leica microscope with 400 X (**Fig. 2.**) and 1000 X magnification (**Fig. 3.**), and analyzed by the same microscope with 400 X magnification. MI was calculated as the total number of cells in prophase (P), metaphase (M), anaphase (A), and telophase (T) in relation to the number of observed cells (T e d e s c o & L a u g h i n g h o u s e IV, 2012).

$$MI = \frac{P + M + A + T}{500} x100\%$$

The proportion of mitotic phases was determined as a percent ratio between cells in specific phase and the dividing cells (F i s k e s j o , 1985).

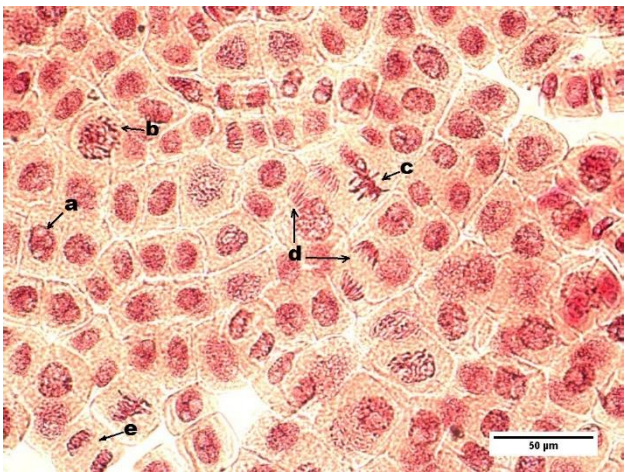

Fig. 2. Mitosis of *A. cepa root* meristems. Magnification 400 X. Leica light microscope. Normal stages of mitotic division. (a) interphase (b) prophase (c) metaphase (d) anaphase (e) telophase.

Statistical analysis

Statistical analysis was carried out using Microsoft Excel 2010 and Systat 13 with five replicates for each group. Data were expressed as mean ± standard deviation (SD) calculated by one way analysis of variance (ANOVA). Differences between controls and the individual dosage group of each extract were analyzed by means of the paired t-test, with 95% confidence limits where significance was accepted at $p<0.05$.

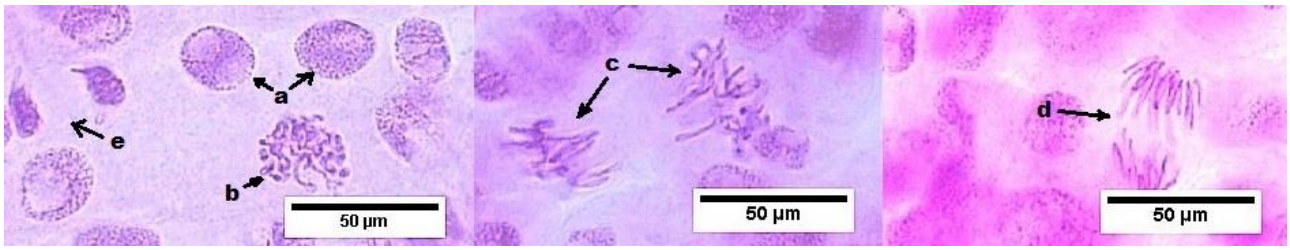

Fig. 3. Stages of mitotic division in cells of *A. cepa* treated with aqueous extracts of 'anti-diabetic' herbal mixture, *R. fruticosus, V. myrtillus, P. erecta, G. urbanum* and *P. vulgaris*. Magnification 1000 X. Leica light microscope. Normal stages of mitotic division. (a) interphase (b) prophase (c) metaphase (d) anaphase (e) telophase.

Results ad discussion

The effects of the aqueous extracts of 'anti-diabetic' herbal mixture, *R. fruticosus, V. myrtillus, P. erecta, G. urbanum* and *P. vulgaris* on root growth of *A. cepa* are shown on **Tab. 1.** Good root growth was achieved in the negative control, while the positive control inhibited root growth.

At tested concentrations, root growth was the highest at the 400 µg/ml concentration of all the extracts while it was the lowest at 1200 µg/ml, with the exception of *P. vulgaris* extract, where the highest root growth was in exposure to 800 µg/ml concentration of extract.

Inhibition of root growth implying toxicity was concentration dependent and statistically significant ($p<0.05$) at all tested concentrations of *P. erecta* and *G. urbanum* extracts, while extracts of 'anti-diabetic' herbal mixture, *R. fruticosus* and *V. myrtillus* triggered concentration dependent and statistically significant ($p<0.05$) inhibition of root growth at higher concentrations (800 µg/ml and 1200 µg/ml). The EC_{50} for the extracts of 'anti-diabetic' herbal mixture, *R. fruticosus, V. myrtillus, P. erecta, G. urbanum* and *P. vulgaris* were 1400 µg/ml, 800 µg/ml, 990 µg/ml, 690 µg/ml, 550 µg/ml, and 1590 µg/ml, respectively.

The effects of tested extracts on cell division of *A. cepa* root meristem cells are presented in **Tab. 2**. Mitotic index in the negative and the positive control were 20.28 ± 2.17 and 3.88 ± 0.92, respectively.

With increasing concentration of the all the tested extracts there was concentration dependent decrease in the mitotic index with the exception of *P. erecta* extract, where 400 µg/ml concentration did not cause statistically significant decrease of MI compared to the positive control. Of all the lowest concentration of tested extracts (400 µg/ml), the

Table 1. The effects of aqueous extracts of 'anti-diabetic' herbal mixture, *R. fruticosus, V. myrtillus, P. erecta, G. urbanum* and *P. vulgaris* on root growth of *A. cepa.* RG (%) of the control, treated root growth expressed as % of the control. MIX, 'anti-diabetic' herbal mixture. RFL, leaves of R. *fruticosus*. VML, leaves of *V. myrtillus*. PER, roots of *P. erecta*. GUA, aerial parts of *G. urbanum*. PVP, pods of *P. vulgaris*. NC, negative control. PC, positive control.

* Significant compared to its negative control at 0.05 level.

º Significant compared to its positive control at 0.05 level.

	MIX		RFL		VML		PER		GUA		PVP	
Treatment	Mean root length (cm) ±SD	TRG (%) of control	Mean root length (cm)±SD	TRG (%) of control	Mean root length (cm) ±SD	TRG (%) of control	Mean root length (cm) ±SD	TRG (%) of control	Mean root length (cm) ±SD	TRG (%) of control	Mean root length (cm) ±SD	TRG (%) of control
NC	3.04±0.59	100	3.04±0.59	100	3.04±0.59	100	3.04±0.59	100	3.04±0.59	100	3.04±0.59	100
400 µg/ml	2.82±0.46	92.8 º	2.7±0.48	88.8 º	2.44±0.59	80.1 º	2.3±0.21	75.7 *	1.56±0.45	51.3 *	2.9±0.22	95.4 º
800 µg/ml	2.1±0.24	69.1 *	1.52±0.53	50 *	1.7±0.47	55.1 *	1.3±0.41	42.8 *	1.48±0.53	48.7 *	3.12±0.25	102.6 º
1200 µg/ml	1.82±0.49	59.8 *	0.84±0.17	27.6 *	1.5±0.47	49.3 *	1.04±0.11	34.2 *	1±0.14	32.9 *	2.16±0.18	71 *
PC	1.38±0.7	45	1.38±0.7	45	1.38±0.7	45	1.38±0.7	45	1.38±0.7	45	1.38±0.7	45
EC50	1400 µg/ml		800 µg/ml		990 µg/ml		690 µg/ml		550 µg/ml		1590 µg/ml	

Table 2. Cytological effects of aqueous extracts of 'anti-diabetic' herbal mixture, *R. fruticosus*, *V. myrtillus*, *P. erecta*, *G. urbanum* and *P. vulgaris* on meristematic cells of *A.cepa.* MI, mitotic index. MIX, 'anti-diabetic' herbal mixture. RBL, leaves of *R. fruticosus*. VML, leaves of *V. myrtillus*. PER, roots of *P. erecta*. GUA, aerial parts of *G. urbanum*. PVP, pods of *P. vulgaris*. NC, negative control. PC, positive control.

* Significant compared to its negative control at 0.05 level.

º Significant compared to its positive control at 0.05 level.

	MIX	RFL	VML	PER	GUA	PVP
Treatment	**Mitotic index (%) ± SD**	**Mitotic index (%) ± SD**	**Mitotic index (%) ± SD**	**Mitotic index (%) ± SD**	**Mitotic index (%) ± SD**	**Mitotic index (%) ± SD**
NC	20.28±2.17	20.28±2.17	20.28±2.17	20.28±2.17	20.28±2.17	20.28±2.17
400 µg/ml	13.96±0.64 * º	16.12±1.36 * º	10.96±0.78 * º	16.76±2.67 º	16.92±2.61 * º	16.16±1.38 * º
800 µg/ml	9.8±0.55 * º	12.2±0.6 * º	10.04±1.91 * º	14.68±0.84 * º	12.76±0.48 * º	9.68±0.48 * º
1200 µg/ml	6.4±0.93 * º	9.56±1.08 * º	7.72±1.4 * º	11.84±0.73 * º	9.12±1.47 * º	7.96±0.68 * º
PC	3.88±0.92	3.88±0.92	3.88±0.92	3.88±0.92	3.88±0.92 * º	3.88±0.92 * º

highest decrease of mitotic index was influenced by *V. myrtillus* extract (10.96 %).

Allium cepa root meristem cells of the control groups displayed the existence of all phases of mitosis. The different concentrations of the tested extracts caused different changes in the proportion of mitotic phase's distribution in comparison to the positive and negative controls, as shown on **Tab. 3**. Compared to the positive and negative control, influence of 'anti-diabetic' herbal mixture, *R. fruticosus*, *V. myrtillus* extracts on phase index was statistically significant concentration depended.

Prophase index was, as expected, the most frequent one in all of the tested groups. Extracts of 'anti-diabetic' herbal mixture (400 µg/ml and 800 µg/ml concentrations) and *R. fruticosus* (800 µg/ml and 1200 µg/ml concentrations) decreased the prophase index of *A. cepa* root meristem cells, while 400 µg/ml and 800 µg/ml extract of *V. myrtillus* and 1200 µg/ml of 'anti-diabetic herbal mixture' increased the prophase index. The extracts of *P. erecta*, *G. urbanum* and *P. vulgaris*, with the exception of 400 µg/ml concentrated *P. erecta* which increased prophase index, had no influence on it.

Extract of 'anti-diabetic' herbal mixture did not change metaphase index with statistical significance, while higher concentrations of *R. fruticosus* (1200 µg/ml) and *V. myrtillus* (800 µg/ml) increased it. Extracts of *P. erecta*, *G. urbanum* and *P. vulgaris* produced no change in metaphase index.

None of the extracts had any influence on anaphase index.

As expected, telophase index was the least frequent one in all the tested groups. Extracts of *R. fruticosus* (400 µg/ml and 1200 µg/ml), *V. myrtillus* (all the tested concentrations), *G. urbanum* (800 µg/ml) and *P. vulgaris* (1200 µg/ml) decreased this index.

Higher plants such as *A. cepa* are accepted as admirable genetic models to evaluate genotoxic effects. Results of the current study reflected the utility of root tips of cells of *A. cepa* for monitoring the cytotoxic effects of medicinal plant extracts.

Data on the effects of the extracts on root growth of *A. cepa* showed that there was concentration dependent decrease in root growth. Based on the EC50 the order of induction of root growth inhibition was PVP < MIX < VML < RFL < PER < GUA, as shown on **Tab. 1.**

As it is already said, little is known about their potential toxicity. Our results are consistent with what has been done so far, i.e. a blackberry extract has cytotoxic capabilities as it can induce apoptosis in human leukemia HL-60 cells (Sun et al., 2002); *P. erecta* rhizome extracts is considered safe with respect to acute toxicity when applied to humans (Shushunov et al, 2009), and *G. urbanum* is considered non-toxic as well (Paun et al., 2015).

There is a linear relationship between macroscopic and microscopic parameters for all the extracts. In *A. cepa*, whenever there is root growth inhibition, there is always reduction in the number of dividing cells (Fiskesjo, 1985).

Mitotic index was characterized by the total number of dividing cells in the cell cycle. Mitotic index is used as an indicator of cell proliferation biomarkers which measures the proportion of cells in the mitotic phase of the cell cycle. Therefore, the decrease in the mitotic index of *A. cepa* meristem cells could be interpreted as cellular death. Low mitotic index may be reflecting a direct cytotoxic effect of the tested extract. (Sasidharan et al., 2012.)

The cells of *A. cepa* root tips after treatment with extracts of all the tested extracts showed decrease in mitotic index with increasing concentration. There were significant differences (p

Table 3. Phase index of *A. cepa* root meristem cells treated with different concentrations of aqueous extracts of 'anti-diabetic' herbal mixture, *R. fruticosus*, *V. myrtillus*, *P. erecta*, *G. urbanum* and *P. vulgaris.* MIX, 'anti-diabetic' herbal mixture. RFL, leaves of *R. fruticosus*. VML, leaves of *V. myrtillus*. PER, roots of *P. erecta*. GUA, aerial parts of *G. urbanum*. PVP, pods of *P. vulgaris*. NC, negative control. PC, positive control.

* Significant compared to its negative control at 0.05 level.

º Significant compared to its positive control at 0.05 level.

Tested extract	Phase index (%) ± SD	Treatment				
		NC	400 µg/ml	800 µg/ml	1200 µg/ml	PC
MIX	Prophase	52.02±4.17	44.47±5.05 * º	48.91±3.84 º	62.46±6.41 * º	84.0±16.04
	Metaphase	20.61±4.39	22.06±2 º	22.47±2.3 º	14.93±2.63 º	4.29±4.72
	Anaphase	14.07±6.41	16.47±5.6	16.76±5.23	13.49±3.8	5.34±5.29
	Telophase	13.36±2.88	16.99±6.34	11.87±6.48	7.86±4.78	6.29±11.23
RFL	Prophase	52.02±4.17	55±3.59 * º	48.55±3.59 º	49.36±3.17 * º	84.0±16.04
	Metaphase	20.61±4.39	24.1±1.56 º	24.85±2.27 º	30.18±2.25 * º	4.29±4.72
	Anaphase	14.07±6.41	13.41±4.03	22.19±6.42	17.17±3.47 º	5.34±5.29
	Telophase	13.36±2.88	7.4±3.474 *	7.42±3.61	3.28±1.69 *	6.29±11.23
VML	Prophase	52.02±4.17	77.18±3.22* º	59.57±1.95 * º	49.66±7.05 º	84.0±16.04
	Metaphase	20.61±4.39	9.44±1.99 * º	30.84±3.98 * º	17.34±11.53	4.29±4.72
	Anaphase	14.07±6.41	7.15±2.8	8.44±2.27	23.8±10.91 º	5.34±5.29
	Telophase	13.36±2.88	6.23±1.75 *	1.14±1.07 *	9.19±2.9 *	6.29±11.23
PER	Prophase	52.02±4.17	58.25±2.55* º	51.42±7.98 º	48.62±10.12 º	84.0±16.04
	Metaphase	20.61±4.39	18.26±3.12 º	19.56±3.47 º	26.11±14.82 º	4.29±4.72
	Anaphase	14.07±6.41	13.14±2.21 º	15.13±3.04 º	12.15±4.89 º	5.34±5.29
	Telophase	13.36±2.88	10.34±3.49	13.9±5.18	13.13±5.88	6.29±11.23
GUA	Prophase	52.02±4.17	53.28±5.81 º	56.17±6.35 º	50.24±4.65 º	84.0±16.04
	Metaphase	20.61±4.39	19.31±3.19 º	18.14±5.1 º	22.49±4.1 º	4.29±4.72
	Anaphase	14.07±6.41	15.07±5.89 º	17.88±3.13 º	17.45±3.31 º	5.34±5.29
	Telophase	13.36±2.88	12.23±4.58	8.78±2.97 *	9.82±4.35	6.29±11.23
PVP	Prophase	52.02±4.17	58.12±4.96 º	55.46±3.92 º	65.94±10.26	84.0±16.04
	Metaphase	20.61±4.39	23.23±1.69 º	21.09±2.61 º	16.29±5.38 º	4.29±4.72
	Anaphase	14.07±6.41	9.95±2.87	13.62±2.16 º	10.79±4.29	5.34±5.29
	Telophase	13.36±2.88	8.7±3.78	9.82±4.88	6.97±1.67 *	6.29±11.23

< 0.05) between treated groups and a control group in mitotic index, as shown on **Tab. 2**.

Observed mitodepressive effect suggests that tested extracts had some effects on cell division of *A. cepa*. This may be due to abnormal conditions of the cells induced by the treatments. The reduction of the mitotic index might be explained as being due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis (Briand & Kapoor, 1989).

Of all the tested aqueous extracts, only 'anti-diabetic' herbal preparation, *R. fruticosus*, *V. myrtillus* showed an influence on the mitotic phases, and 'anti-diabetic' herbal preparation (1200 µg/ml) and *V. myrtillus* (400 µg/ml) caused surprisingly high prophase accumulation. This highly prophase accumulation could be correlated to the blockage of the dividing cells at Chfr point which prevents prophase-metaphase transition as proved by Scolnick and Halazonetis (2000) who explained that Chfr protein delays chromosome condensation and nuclear envelope breakdown in response to drug such as taxol and nocodazole that disrupt microtubule structure. Although chromosome abnormalities were not analyzed in this paper, it is observed that 'anti-diabetic' herbal mixture, *R. fruticosus*, *V. myrtillus* and the positive control caused spindle disturbance, especially c-mitosis, as the most common type of abnormalities, which indicates that these extracts not only caused disruption in the spindle structure, but also caused inhibition of spindle formation and prevented its polymerization (Salmon et al., 1984).

Conclusion

The diabetes problem is a global epidemic, mostly in low- to middle income countries. Costs of international commercial medicines are high, which caused an increased popularity of traditional medicine. However, some constituents of medicinal plants have been found to be potentially toxic (Ping et al., 2012). According to our literature review, a little is known about potential toxicity of *R. fruticosus*, *V. myrtillus*, *P. erecta*, *G. urbanum* and *P. vulgaris*, medicinal plants used as a traditional remedy for diabetes.

The results presented in this paper are therefore important since they suggest the cytotoxic effect of these medicinal plants in some concentrations. Exposure to the high concentrations of all the tested extracts, especially to 'anti-diabetic' herbal mixture, *R. fruticosus* and *V. myrtillus*, initiates a cascade of deleterious changes within *A. cepa* root cells, visible as the inhibition of root growth and the reduction of the mitotic index. Moreover, 'anti-diabetic' herbal preparation (1200 µg/ml) and *V. myrtillus* (400 µg/ml) caused surprisingly high prophase accumulation as well as spindle disturbance, especially c-mitosis, indicating that these extracts at these concentrations have a toxic effect.

Further cytogenetic studies dealing with clastogenicity and genotoxicity of these extracts with more comprehensive genotoxicity assessment in animal model may reveal further interesting results for their usage as a traditional medicine.

References

Akinboro, A., Bakare, A.A. 2007: Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *Journal of Ethnopharmacology*, 112 (3): 470-475.

Briand, C.H., Kapoor, B.M. 1989: The cytogenetic effects of sodium salicylate on the root meristem cells of *Allium sativum* L. *Cytologia*, 54: 203–209.

Camparoto, M.L., Teixeira, R.O., Mantovani, M.S., Vicentini, V.E.P. 2002: Effects of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth infusions on onion root-tip and rat bone-marrow cells. *Genetics and Molecular Biology*, 25: 85-89.

Çelik, T.A., Aslantürk, Ö.S. 2010: Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with *Allium* test. *Journal of biomedicine and biotechnology*: 1-8.

Eddouks, M., Maghrani, M., Lemhadri, A., Ouahidi, M.L. 2002: Ethnopharmacological survey of medicinal plants used for the treatment of *diabetes mellitus*, hypertension and cardiac diseases in the South-East region of Morocco (Tafilalet). *Journal of Ethnopharmacology*, 82 (2-3): 97-103.

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z. 1985: Medicinal plants in therapy. *Bulletin of the World Health Organization*, 63 (6): 965–981.

Fiskesjo, G. 1985: The *Allium* test as a standard in environmental monitoring. *Hereditas*, 102: 99-112.

Gabriela, P., Neagu, E., Albu, C., Radu, G.R. 2015: Inhibitory potential of some Romanian medicinal plants against enzymes linked to neurodegenerative diseases and their antioxidant activity. *Pharmacognosy Magazine*, 11 (1): S110-116

Gale, E.A.M., Wagner, T.H. 2006: Dying of Diabetes. *BMJ Publishing Group Limited and RPS Publishing*, Lancet, London. 368 (9548): 1626-1628.

International Diabetes Federation (IDF). 2015: IDF Diabetes Atlas 7th Edition. International Diabetes Federation. 47-65

Johnson, M.H., De Mejia, E.G. 2016: Phenolic compounds from fermented berry beverages modulated gene and protein expression to increase insulin secretion from pancreatic cells *in vitro*. *Journal of Agricultural and Food Chemistry*, 65 (30): 6211-6221.

Koupý, D., Kotolová, H., Kučerová, J. 2015: Effectiveness of phytotherapy in supportive treatment of type 2 *diabetes mellitus* billberry (*Vaccinium myrtillus*). *Ceska a Slovenska Farmacie*, 64 (1-2): 3-6.

Kyznietsova, M. Y., Halenova, T. I., Savchuk, O. M., Vereschaka, V. V., Ostapchenko, L. I . 2015: Carbohydrate metabolism in type 1 diabetic rats under the conditions of the kidney bean extract application. *Fiziolohichnyi Zhurnal*, 61 (6): 96–103.

Paun, G., Neagu, E., Albu, C., Radu, G. L., 2015: Inhibitory potential of some Romanian medicinal plants against enzymes linked to neurodegenerative diseases and their antioxidant activity. *Pharmacognosy Magazine*, 11 (1): 110–116.

Ping, K.Y., Darah, I., Yusuf, U.K., Yeng, C., Sasidharan, S. 2012: Genotoxicity of *Euphorbia hirta*: an *Allium cepa* assay. *Molecules*, 17 (7): 7782-7791.

Rank, J., Nielsen, M.H. 1994: Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutation Research*, 312 (1): 17- 24.

Salmon, E.D., McKeel, M., Hays, T. 1984: Rapid rate of tubulin dissociation from microtubules in the mitotic spindle *in vivo* measured by blocking polymerization with colchicine. The Journal of *Cell Biology*, 99 (3): 1066–1075.

Sasidharan, S., Ping, Darah, I., Yusuf, U.K., Yeng, Y., 2012: Genotoxicity of *Euphorbia hirta*: An *Allium cepa* assay. *Molecules*, 17: 7782-7791.

Scolnick, D.M., Halazonetis, T.D. 2000: Chfr defines a mitotic stress checkpoint that delays entry into metaphase. *Nature*, 406 (6794): 430–435.

Sidorova, Y., Shipelin, V., Mazo, V., Zorin, S., Petrov, N., Kochetkova, A. 2017: Hypoglycemic and hypolipidemic effect of *Vaccinium myrtillus* L. leaf and *Phaseolus vulgaris* L. seed coat extracts in diabetic rats. *Nutrition*, 41: 107–112.

Shushunov, S., Balashov, L., Kravtsova, A., Krasnogorsky, I., Latté, K.P., Vasiliev, A. 2009: Determination of acute toxicity of the aqueous extract of *Potentilla erecta* (Tormentil) rhizomes in rats and mice. *Journal of Medicinal Food*, 12 (5): 1173-1176

Sun, J., Chu, Y.F., Wu, X., Liu, R.H. 2002: Antioxidant and antiproliferation activities of fruits. *Journal of Agricultural and Food Chemistry*, 50: 7449–7454.

Swanston-Flatt, S.K., Day, C., Bailey, C.J., Flatt, P. R. 1990: Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetologia*, 33 (8): 462–464.

Tedesco, S.B., Laughinghouse IV, H. D. 2012: Bioindicator of genotoxicity: the *Allium cepa* test. In: Strivastava, J.K.: *Environmental Contamination*, 137-156. InTech.

Teixeira, R.O., Camparoto, M.L., Mantovani, M.S., Vicentini, V.E.P. 2003: Assesment of two medicinal plants *Psidium guajava* L. and *Achillea millefolium* L., in *in vitro* and *in vivo* assays. *Genetics and Molecular Biology*, 26 (4): 551-555.

Tomczyk, M., Latté, K. P. 2009: *Potentilla* - a review of its phytochemical and pharmacological profile. *Journal of Ethnopharmacology*, 122 (2): 184–204.