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Changes in elemental composition of *Lewis* carcinoma tumors in mice due to the supply of water–ethanolic extracts of *Artemisia dracunculus* and *Pastinaca sativa*

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Abstract. Macro– and trace elements play an important role in biological processes, connected with malignant tumors emergence and development. Despite numerous data on elemental composition of tumour tissues up to date, the effect of plant preparations on elemental composition of tumors has been poorly investigated. In order to evaluate the elemental composition of mice Lewis carcinoma tumors under *Artemisia dracunculus* (tarragon) and *Pastinaca sativa* ('black parsnip') ethanolic extracts ingestion and to characterize mineral content in the above herbs, ICP–MS– method was used. The results demonstrated significant decrease of Cd, Pb, Hg, Al, Mn, Sr and Se concentrations in Lewis carcinoma tumors of mice under tarragon and 'black parsnip' ethanolic extracts loading compared to control group of mice without supply of herbs extracts. 'Black parsnip' and tarragon extracts also increased the concentrations of Ca, Si and V in tumors. Contrary to tarragon extract, which increased I content in tumors, 'Black parsnip" significantly decreased the accumulation of I. Taking into account the anti–carcinogenic and anti–metastatic effects of 'black parsnip' and the pro–carcinogenic effect of *A. dracunculus*, their utilization in preventive medicine is supposed to arise interesting prospects.
Keyword: *Lewis carcinoma; tarragon; 'black parsnip'; plant extracts; elemental composition; iodine.*

Introduction

Macro– and trace elements play an important role in biological processes, connected with malignant tumors emergence and development.

They participate in energy provision of cells, their growth and development, hormone production regulation, maintenance and function of the immune system being cofactors of several enzymes [SINHA, 2014].

Numerous investigations of tumor element composition allow to reveal several patterns, in particular high levels of Ca, Fe, Zn in breast tumors [SILVA *et al.*, 2012], priority Se accumulation in tumor tissues [GHERASE, FLEMING, 2020], increased concentrations of Cd, Fe, Mg, Mn, Pb and Zn in patients with malignant glioma [ARSLAN *et al.*, 2011]. Elemental composition of tumors, biological fluids (serum, urine, bronchoalveolar lavage) and hair elemental composition of patients with lung cancer have been previously described [CALLEJÓN–LEBLIC *et al.*, 2019].

Epidemiological investigations have indicated a relationship between several microelements' consumption (Se, Ni, As, Zn) and risks of malignant neoplasms development [SILVERA, ROHAN, 2007], and in this respect carcinogenicity of trace elements has been described [MULWARE, 2013].

Despite numerous studies devoted to tumor elemental composition and the role of trace elements in tumor development, literature reports are still lacking about changes in tumor elemental composition consequent to the supply of different preparations with anti–carcinogenic effect, including herbs.

Plants provide interesting opportunities in revealing new anti–carcinogens with low toxicity, as anti–carcinogenic properties of many herbs may be largely connected with both high antioxidant properties and peculiar elemental composition [WANG *et al.*, 2012].

Many plants biologically active compounds and products with high antioxidant properties demonstrate their ability in antitumor potential, preventing free radicals formation, changing carcinogen transformation, and affecting DNA reparation, pro–oncogenes expression, proliferation, apoptosis, antitumor immunity system. They may also bound toxic and carcinogenic compounds, inducing their elimination from the body [DERYAGINA *et al.*, 2019; BELITSKY *et al.*, 2016]. Recent studies revealed the anti–metastatic effect of parsnip, produced in conditions of prolonged root storage under elevated temperature and humidity (the so–called 'black parsnip') [DERYAGINA *et al.*, 2019; GOLUBKINA *et al.*, 2020].

In our previous experimental studies on male BDF mice with subcutaneous transplantable Lewis carcinoma, it was shown that a water–ethanolic (3.2%) extract of 'black parsnip' inhibited tumor growth up to 56% during 17 days, and also demonstrated its anti–metastatic effect, decreasing the number of animals with metastasis by up to 60% and the total lung metastasis by twice compared to control. Water–ethanolic extract of tarragon did not significantly affect the growth of transplanted Lewis carcinoma, but increased lung metastasis number.

Despite the fact that a relative number of animals with metastasis was non–significantly decreased in mice under tarragon 1.2% and 2.0% water–ethanolic extract, the total number of lung metastasis was 1.55 and 1.65 times higher respectively than that of control animals [DERYAGINA *et al.*, 2019].

The aim of the present study was to comparatively evaluate the elemental composition of transplanted Lewis carcinoma tumors in mice supplemented with water–ethanolic extract of 'black parsnip' and tarragon.

Material and methods

Preparation of herbs

Leaves of tarragon (cultivar Izumrud) from Nikita Botanic Gardens (Crimea) selection were dried at root temperature to constant weight and homogenized.

'Blach parsnip' was obtained by parsnip root storage at 70°C and 95% humidity in a climatic chamber [GOLUBKINA et al., 2019]. In order to produce herb extracts an appropriate amount of 'black parsnip' and tarragon was extracted by 3.2% ethanol at 37°C during 4 hours. The resulting mixtures were filtered through a filter paper and used for soldering mice.

Experiment

The experiment was carried out on 50 BDF mice (males) obtained from the Stolbovaya Farm of Federal Medical and Biological Agency (http://www.scbmt.ru). Two–month old mice weighing 19–21 g were used. The protocols planned for the experiments were approved by the Animal Care and Use Committee of the N.N. Blokhin NRMCO and corresponded to the guidelines for the welfare and use of animals in cancer research adopted by The United Kingdom Coordinating Committee on Cancer Research [WORKMAN *et al.*, 2010]. The mice were grouped in 7–8 units per cage, with free access to drinking water and a pelleted basal diet.

Lewis carcinoma strain was obtained from N.N. Blokhin NRMCO collection, and the third and the fourth *in vivo* tumor passages were used in the work. Tumor cells were subcutaneously injected in the right axillary region exposed ($5x10^6$ cells per mouse).

The mice were randomly split into five groups, each containing 9–11 animals:

1) control, mice with transplanted Lewis carcinoma consuming 3.2% ethanol solution;
2) mice supplied with 1.2% water–ethanol extract of tarragon;
3) mice receiving 2.0% water–ethanol extract of tarragon leaves;
4) mice applied with 3.2% water–ethanol extract of 'black parsnip';
5) mice supplied with 5.6% water–ethanol extract of 'black parsnip' [DERYAGINA *et al.*, 2019].

At the end of the experiment the mice were euthanized, tumor tissues were isolated, weighed and dried at 70° C to constant weight, homogenized and subjected to the elemental analysis.

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Elemental composition

The Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Si, Sn, Sr, V, and Zn contents of dried herbs and mice tumors were assessed using an ICP–MS on a quadruple mass spectrometer Nexion 300D (Perkin Elmer Inc., Shelton, CT 06484, USA) equipped with the 7–port FAST valve and ESI SC DX4 autosampler (Elemental Scientific Inc., Omaha, NE 68122, USA) in the Biotic Medicine Center (Moscow, Russia). Rhodium 103 Rh was used as an internal standard to eliminate instability during measurements. Quantitation was performed using an external standard (Merck IV, multi–element standard solution), potassium iodide for iodine calibration, and Perkin–Elmer standard solutions for P, Si, and V. All the standard curves were obtained at five different concentrations. For quality checking purposes, internal controls and reference materials were tested together with the samples on a daily basis.

Microwave digestion of samples was achieved with sub–boiled HNO_3 diluted 1:150 with distilled deionized water (Fluka No. 02, 650 Sigma–Aldrich, Co., Saint Louis, MO, USA) in the Berghof SW–4 DAP–40 microwave system (Berghof Products + Instruments Gmb H, 72, 800 Eningen, Germany).

The instrument conditions and acquisition parameters were as follows: plasma power and argon flow, 1500 and 18 L min^{-1}, respectively; aux argon flow, 1.6 L min^{-1}; nebulizer argon flow, 0.98 L min^{-1}; sample introduction system, ESI ST PFA concentric nebulizer and ESI PFA cyclonic spray chamber (Elemental Scientific Inc., Omaha, NE 68122, USA); sampler and slimmer cone material, platinum; injector, ESI Quartz 2.0 mm I.D.; sample flow, 637 L min^{-1}; internal standard flow, 84 L min^{-1}; dwell time and acquisition mode, 10–100 ms and peak hopping for all analytes; sweeps per reading, 1; reading per replicate, 10; replicate number, 3; DRC mode, 0.55 L min^{-1} ammonia (294993–Aldrich Sigma–Aldrich, Co., St. Louis, MO 63103, USA) for Ca, K, Na, Fe, Cr, V, optimized individually for RPa and RPq; STD mode, for the rest of analytes at RPa = 0 and RPq = 0.25.

Statistical Analysis

Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level, using IBM SPSS Statistics software version 21 (USA). Shapiro–Wilk test allowed us to check that the data related to all the variables examined showed a normal distribution, referring to 0.05 probability level.

Results and discussion

Antioxidants content and elemental composition of herbs preparations

Data presented in Figure 1 demonstrate the antioxidant status peculiarities of herb preparations used: the higher antioxidant activity of tarragon compared to 'black parsnip' is apparently connected with the relatively high essential oil content (0.62%).

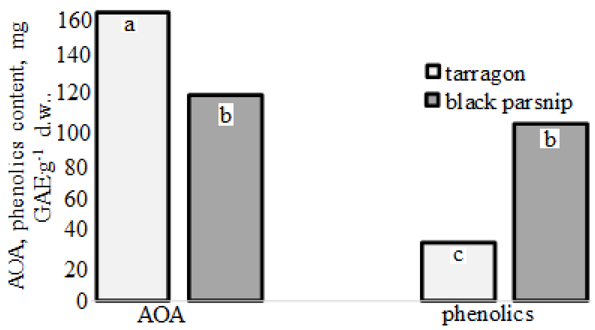

Figure 1. AOA and polyphenol content in tarragon and 'black parsnip' (values with the same letters do not differ statistically according to Duncan test at p ≤0.05).

At the same time, the significantly higher levels of polyphenols can be highlighted in parsnip roots, which almost totally determine the antioxidant activity of the preparation.

Table 1.

Macroelement content in tarragon and 'black parsnip' (g·kg^{-1} d.w.)

Element	Tarragon	Parsnip	Significance
Ca	13.59±1.36	2.10±0.25	*
K	20.90±2.09	20.84±2.38	n.s.
Mg	1.39±0.14	1.67±0.19	n.s.
Na	0.269±0.027	0.057 ±0.09	*
P	4.71±0.47	3.97±0.32	n.s.

Taking into account the well–known anti–carcinogenic properties of polyphenols [WANG et al., 2012], the latter fact confirms to a large extent the previously revealed anti–carcinogenic effect of 'black parsnip' [DERYAGINA et al., 2019].

The elemental composition data of 'black parsnip' and tarragon indicated similar content of K, Mn and P among macro elements, and Fe, Cr, V among microelements and heavy metals.

Table 2

Microelement content in tarragon and 'black parsnip' (mg·kg^{-1} d.w.)

Element	Tarragon	'Black parsnip'	Significance
B	69.42±6.94	20.24±4.91	*
Co	0.09±0.013	0.057±0.018	*
Cu	16.74±1.67	3.49±0.76	*
Fe	63.5±6.35	57.38±10.36	n.s.
I	0.36±0.043	0.195±0.070	*
Li	0.24±0.028	0.05±0.01	*
Mn	47.13±4.71	9.68±1.94	*
Se	23.5±1.2	0.060±0.001	*
Si	7.53±0.75	28.60±9.06	*
Zn	51.12±5.11	11.99±0.92	*

n.s., not statistically significant; * statistically significant at p≤0.05.

At the same time significantly higher concentrations of Ca, Na, B, Co, Cu, I, Li, Mn, Se and Zn were recorded in tarragon plants, contrary to Si and Pb, whose content was 4 and 7 times higher in 'black parsnip' than in tarragon respectively (Tables 1–3).

Table 3

Content in Al, heavy metals and arsenic in tarragon and 'black parsnip' (mg·kg^{-1} d.w.)

Element	Tarragon	Parsnip	Significance
Heavy metals and As			
As	0.090±0.014	0.018±0.001	*
Cd	0.090±0.014	0.130±0.030	*
Cr	0.160±0.019	0.160±0.050	n.s.
Ni	2.10±0.21	1.33±0.41	*
Sr	48.78±4.88	19.4±1.55	*
Pb	0.100±0.015	0.170±0.020	*
V	0.09±0.01	0.08±0.03	n.s.
Light element			
Al	40.61±4.06	32.53±3.05	*

n.s., not statistically significant; * statistically significant at p≤0.05.

Nevertheless, the lower water–ethanolic extraction efficiency in tarragon (a precipitation took place during extraction), contrary to 'black parsnip' full solubility in 3.2% water–ethanol solution, indicated much lower bioavailability of macro– and trace elements in tarragon leaves.

Changes in elemental composition of tumor tissue under water–ethanolic extract of tarragon and 'black parsnip' supply

Tables 4 and 5 present the results of macro and microelements determination in Lewis carcinoma tumors of mice supplied with herbal preparations.

Table 4.

Element content in tumor not affected by tarragon and 'black parsnip' water–ethanolic extracts (mean values in mg·kg^{-1} d.w. ± standard deviations)

Element	Control	'Black Parsnip' extract 3.2%	'Black Parsnip' extract 5.6 %	Tarragon extract 1.2 %	Tarragon extract 2.0 %
Macroelements					
K	12912±1291	11220±1122	11051±1105	10646±1065	12536±1254
Mg	832±83	727±73	752±75	701±70	822±82
Na	10881±1088	10981±1098	11247±1125	12367±1237	11528±1153
P	15120±1512	13146±1315	13378±1338	12986±1299	14537±1454
Microelements					
B	0.46±0.055	0.38±0.045	0.41±0.049	0.38±0.046	0.39±0.046
Co	0.07±0.011	0.07±0.01	0.08±0.012	0.08±0.012	0.09±0.014
Fe	277±28	236±24	252±25	265±27	263±26
Li	0.04±0.005	0.03±0.005	0.03±0.004	0.03±0.004	0.05±0.008
Mo	0.23±0.027	0.22±0.027	0.27±0.032	0.23±0.028	0.27±0.032
Zn	86.3±8.63	77.86±7.79	78.35±7.83	85.26±8.53	94.71±9.47
Heavy metals and arsenic					
As	0.03±0.004	0.03±0.004	0.03±0.004	0.03±0.004	0.03±0.005
Cu	6.97±0.70	6.40±0.64	7.07±0.71	7.11±0.71	7.58±0.76
Ni	0.100±0.015	0.080±0.012	0.110±0.013	0.110±0.014	0.100±0.012

Two groups of elements have been identified as: 1) not affected (Table 4) and 2) greatly affected by herbal supplementations (Table 5).

Table 5

Element content of Lewis carcinoma tumors affected by tarragon and 'black parsnip' water–ethanolic extract supply (mean values in mg·kg^{-1} d.w. ± standard deviations)

Element	Control	3.2% extracts of 'black parsnip'	5.6% extracts of 'black parsnip'	1.2% extract of tarragon	2.0% Extracts of tarragon
Macro–elements					
Ca	1099±110^{b}	1425±143	1541±154^{a}	1560±156^{a}	1537±154^{a}
Micro–elements					
I	0.63±0.05^{c}	0.31±0.04^{d}	0.25±0.03^{d}	0.88±0.10^{b}	1.11±0.11^{a}
Mn	0.53±0.064^{a}	0.32±0.039^{b}	0.30±0.036^{b}	0.34±0.041^{b}	0.27±0.032^{b}
Se	0.28±0.021^{a}	0.17±0.02^{b}	0.20±0.02^{b}	0.18±0.02^{b}	0.24±0.02^{a}
Si	13.4±1.3^{b}	29.6±3.0^{a}	29.4±2.9^{a}	30.4±3.0^{a}	28.9±2.9^{a}
I/Se	1.89	1.85	1.26	5.00	4.64
Heavy metals and Al					
Al	10.55±1.05$_{a}$	6.39±0.64^{b}	3.28±0.33^{c}	3.65±0.36^{c}	3.52±0.35^{c}
Cd	3.59±0.36^{a}	0.38±0.05^{c}	0.07±0.01^{d}	0.31±0.04^{c}	1.09±0.11^{b}
Cr	0.18±0.02^{b}	0.19±0.02^{b}	0.26±0.03^{a}	0.26±0.03^{a}	0.22±0.03^{ab}
Hg	0.010±0.002^{a}	0.006±0.0013^{b}	0.010±0.002^{a}	0.005±0.0011^{b}	0.010±0.002^{a}
Pb	4.82±0.48^{a}	0.50±0.05^{c}	0.22±0.03^{d}	0.26±0.03^{d}	0.84±0.10^{b}
Sr	3.14±0.31^{a}	2.25±0.22^{b}	1.48±0.15^{d}	1.93±0.19^{c}	1.36±0.14^{d}
V	0.11±0.01^{d}	0.14±0.02^{cd}	0.18±0.03^{bc}	0.22±0.03^{ab}	0.26±0.03^{a}
Ca/Sr	350	633	1041	808	1130

Along each line, values followed by different letters are significantly different according to Duncan test at $P \leq 0.05$.

Element concentration in tumor not affected by tarragon and 'black parsnip' supply

The group of tumor elements not affected by the plant extracts supplied includes the macroelements K, Mg, Na, P, the heavy metals Ni, Cu and As, and the micro– elements B, Co, Fe, Mo and Zn (Table 4). It can be inferred that these elements either do not participate directly in growth and development of tumors in mice or their concentrations in water–ethanolic solutions were too low to cause any significant consequences.

As far as macroelements are concerned, it has been reported that only brain tumors show high concentrations of K, Mg, P and Ca [CANELAS *et al.*, 1969].

Investigations of Willebrand et al [WILLEBRAND *et al.*, 2019] demonstrated a significant reduction of mice tumor development due to high sodium consumption level.

However, neither of the two aforementioned findings took place in the case of Lewis carcinoma tumors, which may be connected with the different location and histological type of tumors.

Tumor concentration of elements significantly affected by tarragon and 'black parsnip' supply

Significant changes in the concentrations of other elements were recorded in tumor (Table 5). Notably, a significant increase of Ca, Si, Cr and V levels was detected in the cases of tarragon and 'black parsnip' extracts supply (Table 3). Cancer cells are known to possess the ability of Ca signal systems modification, changing expression and function of cationic channels, pumps and transporters, affecting proliferation, angiogenesis, invasion and apoptosis [SANTONI *et al.*, 2020].

Clinical investigations indicate increased Ca levels in tumors of bladder, kidney and lung tumors [MOGÁRRIO, 2016]. Increase in tumor Ca content as a result of tarragon and 'black parsnip' supply (by 39.8–41.8 % and 29.7–40.2 % respectively) may be potentially connected with the necrotic processes detected in large tumors, as it is known that necrosis in tumors, especially at the last stages of the process, increases calcification process [ZIMMERMANN, 2017]. On the other hand, some epidemiological studies have associated calcium intake with decreased colon adenoma and cancer [LANGNER, RZESK, 2012; PARK *et al.*, 2007].

The mechanism underlying this protective effect is mainly due to binding and inactivating the pro–cancerogenic activity of bile acids on colon epithelial cells. In addition, calcium was found to inhibit epithelial cell proliferation or induce their differentiation through altering cell signaling pathways [FEDIRKO *et al.*, 2009; LAMPRECHt *et al.*, 2002; MILNER *et al.*, 2001; HOLT *et al.*, 2001].

Previous publications indicate Si accumulation in tumor tissues [NAWI et al., 2019]. The results obtained from the culture of neuroblastoma cells SH–SY5Y, depending on the concentrations of organic silicon, indicate that Si is able to both prevent damage to neurons and induce neurotoxicity at higher concentrations, leading to the death of tumor cells using necrotic processes and apoptosis [CARCIMARTÍN *et al.*, 2015]. The detected increase of Si content in Lewis carcinoma tumor in the present work on the average of 2,2 times compared to control, may be connected with high concentration of Si in parsnip (Table 1).

The high solubility of 'black parsnip' in 3.6% water–ethanol entails that Si is present in the herb preparation in a bioavailable form, contrary to tarragon, which is poorly dissolved. But this assumption seems to be a moot point, as similar Si content increase in Lewis carcinoma tumor was recorded upon tarragon extract supply. Increase in chromium and vanadium concentration in Lewis carcinoma tumors was more pronounced in case of tarragon than 'black parsnip' extracts supply, especially at high doses reaching 1,2 and 2.4 times respectively (Table 5).

Literature data indicate that positive [EVANGELOU, 2002; VOROBIEVA *et al.*, 2013] or negative [MILNER *et al.*, 2001] vanadium effect on tumor growth and development depends on concentration, element valence and histotype of tumor cells. The main mechanisms of anti–carcinogenic effect of

V are connected with: participation in xenobiotics detoxification; ability to produce reactive oxygen species, inhibit tyrosine phosphatases and activate tyrosine phosphorylases, leading to apoptosis or suppressor gene activation; ability to initiate cell cycle arrest and modulate the expression of cell adhesion molecules [HANAUSKE *et al.*, 1987]. The detected vanadium concentrations in Lewis tumors of the present work are close to those able to cause a cytotoxic effect on proliferating cells, stimulating their death [CRUZ et al., 1995; ALIABAD *et al.*, 2018].

Chromium is another carcinogen capable to accumulate in tumor tissue, and the most efficient protection against carcinogenicity of this element is the protection through polyphenols [WANG *et al.*, 2017]. Out of the two herb preparations used in the present work the highest concentrations of phenolics were detected in parsnip, known to decrease the metastasis of Lewis carcinoma.

Interestingly, contrary to Cr and V, the accumulation levels of other heavy metals (Cd and Pb) and Al in Lewis carcinoma tumor dramatically decreased in mice supplied with herbs preparations (Table 5).

In particular, the accumulation levels of Al, Pb, Cd in Lewis carcinoma tumors decreased by 1.65–3.0, 9.6–22 and 9–51 times respectively in an experiment with 'black parsnip'supply and the intensity of this phenomenon was positively correlated with the dose applied Tarragon. supply remarkably inhibited Al, Cd and Pb accumulation (2.9–3.0; 3.3–11.6 and 5.7–18.5 times respectively), though the inhibition was negatively correlated with the dose used, as a higher concentration of tarragon extract was less effective in Pb and Cd concentrations decrease.

Carcinogenicity of Al, Cd, Pb, Hg has been well studied and depends to a large extent on the development of oxidative stress [KIM *et al.*, 2012; CARVER, GALLICCHIO, 2017], and also on epigenetic changes, inhibition of DNA reparation processes and effect on apoptosis [XIAO *et al.*, 2016]. On the other hand, the high antioxidant activity of parsnip and tarragon due to high concentrations of polyphenols, may induce the inactivation of reactive oxygen species formation, triggered by the above metals, that may lead to slowing down the growth of genetic instability in tumor cells. In this respect, the detected decrease in Pb, Cd, Hg and Al accumulation upon to tarragon and 'black parsnip' application is negatively correlated with the high flavonoid's concentration in the above herbs; in particular, the flavonoid chelating ability to Cd has been previously described [SILVERA and ROHAN 2007]. Summarizing the results relevant to Cd, Pb, Hg, and Al, it can be assumed that a pronounced decrease in their concentrations in tumors due to supply of "black" parsnip and tarragon extracts is possibly caused by element chelation by organic molecules of the tested materials with subsequent excretion from the body [SEARS, 2013].

As far as the not radioactive Sr is concerned, this element does not possess carcinogenic effect and its most important biological role is the participation in bone tissue formation. In the present study, the increases in both Sr accumulation in tumor tissue by 1.4–2.1 times, compared to the control, and in Ca content are of special interest due to the close relationship between the two elements. Indeed, Table 3 data indicate a 2–3 times increase in tumors Ca/Sr ratio under herb extracts supply, both for 'black parsnip' and tarragon, and in both cases the effect was dose dependent. At present, there is no information about Ca/Sr ratio values in tumors, that makes the present results the first reports relevant to this phenomenon.

Among the other elements examined, a special attention has been paid to I and Se whose metabolisms are closely connected with each other [ARTHUR, 1999]. 'Black parsnip' and tarragon effects on I accumulation are multidirectional: tarragon extract increased the tumor iodine concentration by 2–2.6 times; contrary, 'black parsnip' decreased the tumor iodine level by 1.4–1.7 times. Up to date iodine accumulation in tumors has been investigated predominately in the case of radioiodine therapy of thyroid carcinoma, while no data are still available for the iodine accumulation in tumors of other cancer types without radioactive supplementation. Our previous investigations demonstrated a strong antimetastatic effect of 'Black parsnip', contrary to tarragon extract which stimulated metastases formation [DERYAGINA et al., 2019]. Furthermore, the present results demonstrate a positive correlation between tumor I/Se ratio and number of metastases (Figure 2).

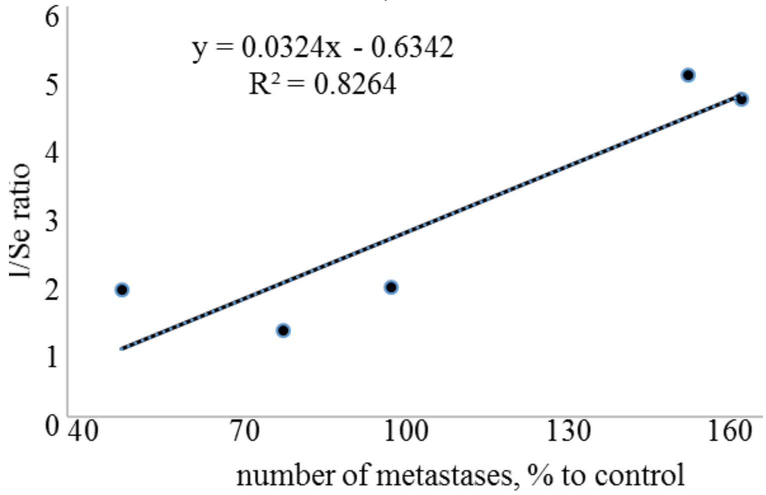

Figure 2. Relationship between the number of metastases and tumor I/Se ratio (data of metastases number are taken from the work [DERYAGINA et al., 2019].

Selenium may actively affect both the thyroid hormones homeostasis and iodine bioavailability, as it is a part of the active center of tri–iodothyronine deiodinases [CANN et al., 2000].

With regard to Se, the effects of tarragon and 'black parsnip' extracts were similar: parsnip decreased Se content in tumor tissue by 1.4–1.7 times and tarragon extract by 1.2–1.6 times. It is significant that higher doses of both parsnip and tarragon had a less pronounced effect on selenium accumulation.

The selenium hyperaccumulation in tarragon, Izumrud variety, recorded in previous research [PLUGATAR et al., 2018], did not affect Se accumulation in Lewis tumor that may be connected with the peculiarities of Se metabolism in tumor tissues and low solubility of tarragon selenium compounds. The results indicate that tarragon extract increases I/Se ration in Lewis carcinoma tumors by tree times, whereas the effect of parsnip on this parameter is less pronounced. Up to date, studies carried out on the elemental composition of tumors have examined the relationship between iodine and selenium exclusively for the thyroid gland [FIORE et al., 2019]. Most of investigations provided fragmental data on iodine accumulation in tumors, whereas the joint homeostasis of these elements in tumors is to be still investigated. Notably, high selenium levels in tumors may cause toxic and pro–oxidative effect on tumor and stromal cells, and also stimulate the activity of Se–dependent glutathione peroxidases [ARTHUR, 1999]. Comparison of the present results on metastatic Lewis carcinoma tumors with the appropriate data on Se accumulation in non-metastatic Ehrich carcinoma in mice [XIAO, et al., 2016] indicate higher Se accumulation in the latter case (the differences reached 1.3 times).

In this connection it is interesting to note that according to Gao et al [GAO et al., 2016], the data iodine level in malignant thyroid tumor was twice lower than in benign neoplasm.

The detected iodine concentration decrease in Lewis carcinoma due to 'black parsnip' supply may be connected with the known iodine accumulation inhibition caused by flavonoids, described for thyroid gland [KOHRLE, 2000].

Manganese is another element with significant concentration decrease up to 1.6–2 times in Lewis carcinoma tumors due to herbs' extracts supply (Table 3). The role of Mn in carcinogenesis remains unknown though its ability to cause mutation was described [GERBER *et al.*, 2002]. The mechanism of Mn^{2+} cytostatic effect may be connected with predominant disturbances of processes, participating in replication and reparation of DNA [ZAKHARCHEVA *et al.*, 2017].

A relationship between tumor Mn content and tumor radioresistance was detected: tumors with low Mn content (testicular cancer) were more sensitive to radiation therapy than those with high Mn content [DOBLE, MIKLOS, 2018]. The authors explained this phenomenon by highlighting the Mn^{2+} ability to delete not enzymatically active forms of oxygen through complexes formed between Mn^{2+} and peptides, amino acids, nucleosides and organic acids.

Conclusions

The present work has been innovatively targeted to evaluate the elemental composition of tumor tissue under supply of plant preparations with different anti–carcinogenic potential. The lack of literature reports in this research field has caused multiple difficulties in interpreting the results. Nevertheless, the several significant changes in elemental composition of tumors under herb extracts supply unveiled in the present investigation have arisen new chances for studying more in depth the specific peculiarities of carcinogenesis and finding out new ways of tumor growth regulation.

Taking into account the antitumor and anti–metastatic effects of 'black parsnip' and its pronounced ability to decrease the heavy metals content in tumors, this herb species may be considered as an interesting perspective for a more comprehensive study of its prophylactic utilization in conditions of increased exposition of human beings to heavy metals.

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References

1. Aliabad, H.B.; Falahati–pour, S.K.; Ahmadirad, H.; Mohamadi, M.; Hajizadeh, M.R.; Mahmoodi, M. Vanadium complex: an appropriate candidate for killing hepatocellular carcinoma cancerous cells –*Biometalls*, **2018**. 31, 981–990. doi: 10.1007/s10534–018–0139–x.
2. Arslan, M.; Demir, H.; Arslan, H.; Gokalp, A.S.; Demir, C. Trace Elements, Heavy Metals and Other Biochemical Parameters in Malignant Glioma Patients. *Asian Pacific Journal of Cancer Prevention*, **2011**. 12, 447–451.
3. Arthur, J.R. Functional indicators of iodine and selenium status. *Proceedings of the. Nutrition Society,* **1999**. 8, 507–512.
4. Belitsky, G.A.; Kirsanov, K.I.; Lesovaya, H.A.; Jakubovskaya, M.G. Natural inhibitors of carcinogenesis. Molecular carcinogenesis. Moscow: ABC–press, **2016**. 158–177 (in Russian).
5. Callejón–Leblic, B.; Arias–Borrego, A.; Pereira–Vega, A.; Gómez–Ariza, J.L.; García–Barrera, T. The Metallome of Lung Cancer and its Potential Use as Biomarker Review. *International Journal of Molecular Sciences*, **2019**. 20, 778, https://doi.org/ 10.3390 /ijms20030778
6. Canelas, H.M.; De Jorge, F.B.; Pereira, W.C.; Sallum, J. Biochemistry of cerebral tumours: sodium, potassium, calcium, phosphorus, magnesium, copper and sulphur contents of astrocytomata, medulloblastomata and glioblastomata multiforme. *Journal of Neurochemistry,* **1969.** 15, 1455–1461. doi: 10.1111/j.1471–4159.1968.tb05927.x.
7. Cann, S.A.; van Netten, J.P.; van Netten, C. Hypothesis: iodine, selenium and the development of breast cancer. *Cancer Causes Control*, **2000**. 11, 121–127.
8. Carcimartín, A.; Merino, J.J.; Santos–López, J.A.; López–Oliva, M.E.; González, M.P.; Sánchez–Muniz, F.J.; Benedi, J. Silicon as neuroprotector or neurotoxic in the human neuroblastoma SH–SY5Y cell line. *Chemosphere*, **2015**. 135, 217–224. doi: 10.1016/j.chemosphere. 2015.04.060.
9. Carver, A.; Gallicchio, V.S. Heavy metals and cancer. doi: 10.5772/ intechopen.70348. **2017**.
10. Cruz, T.F.; Morgan, A.; Min, W. In vitro and in vivo antineoplastic effects of orthovanadate. *Molecular and Cellular Biochemistyr*. **1995**. 153, 161–166. doi: 10.007/ bf01075933
11. Deryagina, V.P.; Reutov, V.P. Modulation of nitrogen reactive forms production via ingredients of plants products in

carcinogenesis inhibition. *Advances in Molecular Oncology,* **2011**. 6, 18–36 (in Russian).
12. Deryagina, V.P.; Rizhova, N.I.; Kirsanov, K.I.; Savluchinskaya, L.A.; Golubkina, N.A. Effect of water–ethanolic extracts of tarragon (*Artemisia dracunculus* L.) and 'black parsnip' (*Pastinaca sativa* L.) on growth and metastasis of subcutaneous transplantable Lewis carcinoma of mice, *Questions of Biological, Medical and Pharmaceutical Chemistry,* **2019.** 22, 47–54. https://doi.org/10.29296/25877313–2019–10–00. (in Russian).
13. Doble, P.A.; Miklos, G.L.G. Distributions of manganese in diverse human cancers provide insights into tumour radioresistance. *Metallomics*, **2018**. 9, doi: 10.1039/c8mt 00110C.
14. Evangelou, A. Vanadium in cancer treatment. *Critical Reviews on Oncology/Hematology*, **2002**. 42, 249–265. doi: 10.1016/S1040–8428(01)00221–9.
15. Fedirko, V.; Bostick, R.M.; Flanders, W.D.; Long, Q.; Sidelnikov, E.; Shaukat, A.; Daniel, C.R.; Rutherford, R.E.; Woodard, J.J. Effects of vitamin D and calcium on proliferation d differentiation in normal colon mucosa: a randomized clinical trial. *Cancer Epidemiology, Biomarkers and Prevemtion,* **2009**. 18, 2933–2941.
16. Fiore, M.; Conti, G.O.; Caltabiano, R.; Buffone, A.; Zuccarello, P.; Cormaci, L.; Cannizzaro, M.A.; Ferrante, M. Role of Emerging Environmental Risk Factors in Thyroid Cancer: A Brief Review. *International Journal of Environmental Research and Public Health,* **2019**. 16, 1185, doi:10.3390 /ijerph16071185.
17. Gao, S.–Y.; Zhang, X.–Y.; Wei, W.; Li, X.–T.; Li, Y.–L.; Xu, M.; Sun, Y.–S.; Zhang, X,–P. Identification of benign and malignant thyroid nodules by in vivo iodine concentration measurement using single–source dual energy CT. A retrospective diagnostic accuracy study. *Medicine (Baltimore)*, **2016.** 95, e4816. doi: 10.1097/MD.0000000000004816
18. Gerber, G.B.; Léonard, A.; Hantson, P. Carcinogenicity, mutagenicity and teratogenicity of manganese compounds. *Critical Reviews on Oncology/Hematology,* **2002**. 42, 25–34.
19. Gherase, M.R., Fleming, D.E.B.; Probing Trace Elements in Human Tissues with Synchrotron Radiation, Cristalls 2020; 10: 12; doi:10.3390/cryst10010012
20. Silva, M.P.; Soave, D.F., Ribeiro–Silva, A., Poletti, M.E. Trace elements as tumor biomarkers and prognostic factors in breast cancer: a study through energy dispersive X–ray fluorescence. *BMC Research Notes*, **2012.** 5, 194, doi:10.1186/1756–0500–5–194.
21. Golubkina, N.; Zayachkovsky, V.; Stepanov, V.; Deryagina, V.; Rizhova, N.; Kirsanoiv, K.; Caruso, G. High Temperature and Humidity Effect on Biochemical Characteristics of Organically–Grown Parsnip Roots Compared to Garlic Bulbs. Plant Foods Hum. Nutr. **2020**; 75(2): 292–297.doi.org/10.1007/s11130–019–00775–1
22. Hanauske, U.; Hanauske, A.R.; Marshal, M.H.; Muggia, V.A.; Von Hoff, D.D. Biphasic effects of vanadium salts on in vitro tumor colony growth. *International Journal of Cellular Cloning,* **1987**. 5, 170–178. doi: 10.1002/stem.5530050209
23. Holt, P.R.; Wolper, C.; Moss, S.F.; Yang, K.; Lipkin M. Comparison of calcium supplementation or low–fat dairy foods on epithelial cell pro–liferation and differentiation. Nutr. Cancer **2001**; 41: 150–155
24. Kim, H.S.; Kim, Y.J.; Seo, Y.R. An Overview of Carcinogenic Heavy Metal: Molecular Toxicity Mechanism and Prevention. Anticancer Agents. *Journal of Medicinal Chemistry*, 2**012.** 12, 1281–1305.
25. Kohrle, J. Flavonoids as risk factor for goiter and hypothyroidism. In: The thyroid and environment. Peter F, Wiersinga W, Hostalek U. (eds). Schattauer, Stuttgart; Proceedings of Merck European Thyroid Symposium, Budapest, 2000. June 22–25, **2000**, pp. 41–63.
26. Lamprecht, S.A.; Lipkin, M. Cellular mechanisms of calcium and vitamin D in the inhibition of colorectal carcinogenesis. *The Annals of the New York Academy of Sciencies*, **2002.** 952, 73–87. https://doi.org/10.1111/j.1749–6632.2001.tb02729.x.
27. Langner, E.; Rzesk, W. Dietary derived compounds in cancer chemoprevention. *Wspolczesna Onkology*, **2012.** 16, 394–400. doi: 10.5114/wo.2012.31767.
28. Li, X.; Jiang, X.; Sun, J.; Zhu, C.; Li, X.; Tian, L.; Liu, L.; Bai, W. Cytoprotective effects of dietary flavonoids against cadmium–induced toxicity. *The Annals of the New York Academy of Sciencies*, **2017**. 1398, 5–19. doi: 10.1111/nyas.13344.
29. Milner, J.A.; McDonald, S.S.; Anderson, D.E.; Greenwald, P. Molecular targets for nutrients involved with cancer prevention. *Nutrition and Cancer*, **2001**. 41, 1–16.
30. Mogárrio, A.M.F. PhD Thesis. Study of trace elements concentration in cancerous and healthy bladder, colon and lung tissues. Diss. Faculdade de Ciências e Technologia Universidade Nova de Lisboa, **2016**.
31. Mulware, S.J. Trace elements and carcinogenicity: a subject in review, *Biotechnology,* **2013**. 3, 85–96. doi: 10.1007/s13205–012–0072–6
32. Nawi, A.M.; Chin, S.–F.; Shan, S.A.; Jamal, R. Tissue and trace elements concentration

among colorectal patients: a systematic review of case–control studies. *Iranian Journal of Public Health,* **2019**. 48, 632–643.
33. Park, S.Y.; Murphy, S.P.; Wilkens, L.R.; Nomura, A.M.; Henderson, B.E.; Kolonel, L.N. Calcium and vitamin D intake and risk of colorectal cancer: the Multiethnic Cohort Study. *Americal Journal of Epidemiology*, **2007**. 165, 784–793
34. Plugatar, J.V.; Kantseva, U.I.; Logvinenko, L.A.; Golubkina, N.A.; Molchanova, A.V.; Kovalsky, J.G. Food additive from tarragon Izumgud variety. Paternt RU 2689711, 14.08.**2018**.
35. Santoni, G.; Morelli, M.B.; Marinelli, O.; Nabissi, M.; Santoni, M.; Amantini, C. Calcium signaling and the regulation of chemosensitivity in cancer cells: Role of the transient receptor potential channels. *Advances in Experimental Medicine and Biology*, **2020.** 1131, 505–517. doi: 10.007/978–3–030–12457–1_20.
36. Sears, M.E. Chelation: Harnessing and enhancing heavy metal detoxification. A Review. *Scientific World Journal*, **2013.** 2013, 219840. doi. 10.1155/2013/219840
37. Silvera, S.A.N.; Rohan, T.E. Trace elements and cancer risk: a review of the epidemiologic evidence, *Cancer Causes and Control,* **2007**. 18, 7–27, doi:10.1007/s10552 –006–0057–z.
38. Sinha, S.K. Trace elements deficiency and cancer. *Journal of Pharmacy and Bioallied Science*, **2014**. 9, 21–32.
39. Vorobieva, N.M.; Fedorova, H.V.; Baranova, N.I. Vanadium: biological role, toxicology and pharmaceutical utilization. *Biosphere*, **2013**. 5, 77–79 (in Russian).
40. Wang, H.; Khor, T.O.; Shu, L.; Su, Z.; Fuentes, F.; Lee, J.–H.; Kong, A.–N.T. Plants Against Cancer: A Review on Natural Phytochemicals in Preventing and Treating Cancers and Their Druggability, *Anti–cancer Agents in Medicinal Chemistry*, **2012.** 12, 1281–1305.
41. Wang, Y.; Su, H.; Gu, Y.; Song, X.; Zhao, J. Carcinogenicity of chromium and chemoprevention: a brief update. *Onco Targets and Therapy*, **2017**. 10, 4065–4079. doi: 10.2147/OTT.S 139262.
42. Willebrand, R.; Hamad, I.; Van Zeebroeck, L.; Kiss, M.; Bruderek, K.; Geuzens, A.; Swinnen, D.; Côrte–Real, B.F.; Markó, L.; Lebegge, E.; Laoui, D.; Kemna, J.; Kammertoens, T.; Brandau, S.; Van Ginderachter, J.A.; Kleinewietfeld, M. High Salt Inhibits Tumor Growth by Enhancing Anti–tumor Immunity. *Frontiers in Immunology*, **2019**. 10, 1141. https://doi.org/ 10.3389/fimmu.201901141.
43. Workman, P.; Aboagye, E.O.; Balkwill, F.; Balmain, A.; Bruder, G.; Chaplin, D.J. Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer*, **2010.** 102, 1555–1577.
44. Xiao, C.L.; Liu, Y.; Tu, W.; Xia, Y.J.; Tian, K.M.; Zhou, X. Research progress of mechanisms underlying cadmium–induced carcinogenesis. *Zhonghua Yu Fang Yi Xue Za Zhi.,* **2016.** 50, 380–384. doi: 10.3760/cma.J.issn.0253–9624.2016.04.021.
45. Zakharcheva, K.A.; Gening, L.V.; Kazachenko, K.Y.; Tarantul, V.Z. Cells resistant to toxic concentrations of manganese have increased ability to repair DNA. *Biochemistry* (Moscow), **2017.** 82, 38–45, doi: 10.1134/S0006297917010047
46. Zimmermann, A. Metastatic Liver Disease: Secondary Alterations of Hepatic Metastases. In: Tumors and Tumor–Like Lesions of the Hepatobiliary Tract, Springer, Cham., **2017.** doi https://doi.org /10.1007/978–3–319–26956–6_109.

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