

Metabolites of *Polygonum* L. Plants Having Antitumor Properties

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Abstract—The article represents the results of research of antitumor activity of different structural types of plant flavonoids extracted by authors from *Polygonum* L. plants in commercial reserves at the territory of the Republic of Kazakhstan. For the first time ever the results comparative research of antitumor activity of plant flavonoids of different structural groups and their synthetic derivatives have been represented. The results of determination of toxicity of flavonoids in single parenteral infusion conditions have been represented. Experimental substantiation of possible mechanisms of antiproliferative and cytotoxic action of flavonoids has been suggested. The perspectives of usage of plant flavonoids as medications and creation of effective dosage forms of antitumor medicines on their basis have been substantiated.

Keywords—Antitumor activity, cytotoxicity, flavonoids, *Polygonum* L., secondary metabolites.

I. INTRODUCTION

MEDICAL therapy is high-efficient and promising method in the modern oncology. Efficient and timely usage of it along with other methods creates real opportunity of adequate aid to oncologic patients. Over 50 antitumor medicines were introduced in clinical practice. However, due to the fact that antitumor medicines are quite toxic and have undesirable side effects (toxic hepatitis, phlebitis, dermatitis, agranulocytosis, dysbacteriosis etc.), during chemotherapy it is necessary to use traditional medicines, in particular, medicines of plant origin [1], [2].

The main advantages of medical plant and medicines of plant origin is width of their therapeutic effect on a body, absence of side effects and complications even if they are used for a long time, well tolerability by patients. Moreover, medical plants are often the only and (or) economically preferred sources of natural bioactive substances and medicines.

Scientific researches aimed at search for medicines of plant origin for usage in oncology are conducted in two areas of focus: selection of medicines with cytotoxic activity; search for biological response modifiers [2].

One of the most promising classes of plant antitumor substances is natural flavonoids that are also able to suppress free radicals, decrease their concentration in cellular membranes. Owing to antioxidant and membrane protection

function they influence on immunological properties, protect DNA molecules from damaging action of intermediants and reoxidation. Moreover, flavonoids, hydroxycumarines are transformed into quinoid form during oxidation and owing to that they interact with DNA, decrease antioxidant activity of lipids of tumor cells, i.e. decrease their viability. Plants containing phenolglycosides and flavonoids have diuretic and hepatoprotective effect that contributes to deactivation and elimination of toxins and harmful substances accumulated in big amounts in case of cancer diseases. Thus, search for new highly effective and non-toxic antineoplastic agents of plant origin are highly topical and promising [3]-[7].

For the first time ever the article represents comparative analysis of the available and own experimental data on the correlation of structural peculiarities of flavonoids of plant origin of different types and their antineoplastic activity. The perspectives of usage of flavonoids of plant origin as medications and creation of effective dosage forms of antitumor medicines of broad spectrum on their basis have been substantiated.

II. MATERIALS AND METHODS

A. Plant Materials

Plant raw materials (*Polygonum amphibium* L., *Polygonum minus* Huds. Fl. Angl.) were collected in the foothill of Zailiyskiy Alatau (the Republic of Kazakhstan) in blossoming period in July 2013.

B. Extraction and Isolation

To extract flavonoids milled air-dry raw material was exhaustively extracted with 30% proof spirit. Ethanol extract was concentrated in mild conditions (temperature 40-50°C, water-jet pump vacuum) and fractionated with benzene and ethyl acetate.

Concentrated ethyl acetate extract was applied on the column with Sephadex LH-20 eluting components with water and water-spirit mixtures of composition at the ratio from 1:9 to 1:1. Separation of components was controlled using TLC method on Silufol UV-254 platelets. The fractions were combined, concentrated and re-chromatographed on column with silica gel using mixture of chloroform with ethanol with increasing ethanol concentration as eluent [8]. The extracted compounds were additionally purified and analyzed using HPLC on 250x25 mm, LiChrospher 60 RP, 15 µm (Merck), 40 ml/min⁻¹, MeOH : H₂O (60:40) [9].

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C. Antitumor Examinations

Cytotoxic action of the extracted flavonoid-based products, their antitumor activity was determined in Kazakh Scientific-Research Institute of Oncology and Radiology of the Ministry of Health of the Republic of Kazakhstan using standard methods [10].

Study of *Polygonum* L. flavonoids specific antitumor activity has been carried out with 60 mongrel white mice, 40 mice of C₃HA line, 40 mice of C₅₇BI line and 40 hybrid mice BDP₁ to whom 17 strains of transplanted tumors of different histogenesis and 10 substrains of these tumors resistant to antitumor preparations had been transplanted: Pliss lymphosarcoma, sarcoma-45, Herene carcinoma, Woker carcinosarcoma, alveolar mucous cancer of liver PC-1, Zaidel ascite hepatoma, sarcoma-180, ascite and solid Erlich tumors, lymphocytic leukemia P-388, lymphoid leukemia L-1210, lungs Lewis carcinoma, adenocarcinoma Ca-755, melanoma B-16, carcinoma HK, Harding-Passi melanoma, hemacytoblastos La, mouse hepatoma 22a.

Flavonoids were tried against various lymphogenous-hematogenous-metastasizing transplanted tumors of white mongrel rats and mice: Pliss lymphosarcoma, Woker carcinosarcoma, Pliss carcinoma, Woker carcinosarcoma, Herene carcinoma, Erlich tumor and lungs Lewis carcinoma being transplanted to animals by various methods.

Following tumors were used as medicinally resistant: Woker carcinoma resistant to sarcolysine, sarcoma-45 – to rubomycine, Pliss lymphosarcoma – to rubomycine, 5-fluorouracil and sarcolysine, three substrains of rat ovary ascite tumor – to 6-mercaptopurine, thiophosphamide and sarcolysine as well.

Treatment of animals was started when measurable tumor ganglions appeared and lasted for 10 days, in case of mice with ascite tumors – for 8 days. All the trials were reproduced again to reveal results recurrence. Histological investigations were carried out by classic methods [11].

Cutted pieces of tumor tissue were fixed in neutral formalin, Karnau and Buen liquid and poured over paraffin. Sections were colored by hematoxyline-eosine, collagen fibers – according to van-Gizone, impregnation of argiophilic fibers – by the Foot method [12].

Histochemical methods used: revealing of DNA-granules according to Felgene therewith preparations without preliminary hydrolysis worked as reaction control; RNA revealing – by the Brasche method therewith sections were treated by ribonuclease to control the reaction; neutral lipids revealing – by coloring with sudan III [13].

D. Statistical Analysis

For mathematic processing of the results methods of setting average values and their average errors were used. The results were statistically processed using 'Statistica 6.0' software package. The reliable values were those at achieved significance point $p < 0.05$.

III. RESULTS AND DISCUSSION

An impressive body of information exists on the antitumor action of plant flavonoids. *In vitro* work has concentrated on the direct and indirect actions of flavonoids on tumor cells, and has found a variety of anticancer effects such as cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and of tumor invasive behavior. Furthermore, some studies have reported the impairment of *in vivo* angiogenesis by dietary flavonoids. Experimental animal studies indicate that certain dietary flavonoids possess antitumor activity. The hydroxylation pattern of the B ring of the flavones and flavonols, such as luteolin and quercetin, seems to critically influence their activities, especially the inhibition of protein kinase activity and antiproliferation. The different mechanisms underlying the potential anticancer action of plant flavonoids await further elucidation. Certain dietary flavonols and flavones targeting cell surface signal transduction enzymes, such as protein tyrosine and focal adhesion kinases, and the processes of angiogenesis appear to be promising candidates as anticancer agents. Further *in vivo* studies of these bioactive constituents are deemed necessary in order to develop flavonoid-based anticancer strategies.

As noted before, flavonoids as promising antitumor substances are of great interest of researchers. Compared to medicines usually used in therapy to neoplasms, flavonoids with antitumor activity are non-toxic and are able to prevent secondary tumors in some types of liposarcoma. If to rank tested flavonoid as per their antitumor activity in decreasing order, the compounds will be represented in the following order: leucoanthocyanines (leave sarcolysine behind) > chalcones > flavonoids > (with aglycones acting on solid tumors, and glycosides - on ascitic tumor) > catechins.

In researches conducted by us on cell culture, radioprotective and antitumor action of flavonoids was demonstrated. Toxicity of morin, (+)-catechine, apigenin, scoparone, myricetin, rutin, quercetin, kaempferol to ascitic tumor of rats, Erlich carcinoma, Pliss lymphosarcoma, Woker and Heren carcinoma, sarcoma-45 has been demonstrated. At that, it has been noted that cytotoxicity to tumor cells increases with the increase in molecular weight of flavonoid compounds and the amount of phenol OH-groups. The ability of some flavonoids to inhibit proliferation and induce apoptosis of different tumor cell cultures has been established. Quercetin flavonoid contained in the majority of higher plants initiated apoptosis in leukemic human cells (HL-60, K562), adenocarcinoma of colon cells (HT29, LS-180) and prostate cancer (PC-3 DU-155). In melanoma cell culture of mice (B16), however did not have cytotoxic effect. Genistein inhibited proliferation of human tumor cells MCF-7 and induced apoptosis of HL-60 leukemic cells. Chalcones furetin and butein initiated apoptosis of melanoma cell of mice (B16) and human leukemia (HL-60), at that butein inhibited proliferation of human adenocarcinoma cells and HeLa. Out of 21 researched flavonoids extracted by us from *Polygonum* L. plants, flavone, 6-hydroxyflavon and apigenin were the most effective in inhibition of growth of humane carcinoma cells

(ZR-75-1). Cytotoxicity of most flavonoids was higher to tumor cells than to cells obtained from normal tissues. Silymarin and silybin inhibited DNA growth and synthesis in different cell lines of human tumor.

During research of mechanisms of antiproliferative and cytotoxic action of flavonoids, it was established that fisetin induced apoptosis of melanoma cell of a mouse (B16) by means of inhibition of transmembrane glucose transport. Under the effect of baicalin in cells *Jurkat* transmembrane potential of mitochondria was decreased and caspase-3 was activated. Buteine decreased synthesis of anti-apoptogenic proteins Bcl-2 and Bcl-X, but exceeded expression of *Bax* protein and enhanced activity of caspase-3, as well as suppressed tyrosine kinase. Quercetin suppressed expression of heat shock proteins in human's colon adenocarcinoma cells and prostate cancer and inhibited ($IC_{50} = 10.5 \mu M$) transcriptional activity of cyclooxygenase gene 2 in tumor cells (DLD-1) of human's colon. Antiproliferative action of quercetin can be mediated by its ability to be associated with tubulin molecules, as a result of which its conformation properties change and cell mitosis is disturbed. Silymarin inhibited tumor promoter of tumor necrosis factor in epithelial cells. To suppress the growth of human's tumor cells the maximum number of OH-groups in the structure of B-ring of flavonoids as well as unsaturated C2-C3 link was important, glycosidation of ring A enhanced the effect. However, the presence of OH-groups is not obligatory, as high antiproliferative activity to melanoma cells was demonstrated tangeretin, which does not have free OH-groups in its structure, it has only methylated groups. Aglycone flavonoids suppressed proliferation more effectively than glycosidated forms. Research of cytotoxic effect of flavonoids and their derivatives to tumor cells of salivary gland (HSG) and squamous-cell carcinoma of human's oral cavity (HSC-2) demonstrated that compounds containing hydrophobic prenyl or geranyl groups, demonstrated ultimate activity [7]. Some indicators of cytotoxicity are shown in Table I.

TABLE I
CYTOTOXICITY OF PLANT FLAVONOID TO HUMAN'S ORAL CAVITY CARCINOMA (HSC-2), TUMOR CELLS OF SALIVARY GLANDS (HSG) AND NORMAL FIBROBLASTS OF HUMAN'S GUMS (HGF)

<i>Polygonum</i> L. flavonoids	IC_{50} , mM			Tumor specificity
	HSC-2	HSG	HGF	
Apigenin	1.39	1.85	>1.85	>1.3
Chrysin	0.25	0.61	1.36	5.5
Norartocarpetin	0.16	>1.75	1.71	10.9
Kaempferol	>1.87	>1.87	>1.87	> 1
Morin	0.44	0.71	0.65	1.5
Coumataxinin	1.19	0.40	1.15	1.0
Quercetin 3,4'-dimethyl ether	0.69	0.28	0.62	0.9
Morusin	<0.02	0.05	0.03	<1.5
Gankaonin P	0.04	0.11	0.12	3.2
Brusofflavonol E	0.02	0.04	0.04	1.6
Brusofflavonol C	<0.02	0.02	<0.02	<1

Along with that, it was proved that toxicity of flavonoids to mouse's leukemia cells is determined with two parameters: ability to form radicals and hydrophilic nature.

On the model of cancer-induced mammary tumor on mice 5,7,3',4'-tetrahydroxy-3-methoxyflavon and quercetin demonstrated high antitumor activity. Research of anticarcinogenic activity of flavonoid on the model of hepatocarcinogenesis on rats induced by aflatoxin B1 demonstrated that the compounds which do not have OH-groups (flavone, flavanone, tangeretin) had protective effect both at initiation and at prolongation stage; quercetin did not demonstrate antitumor properties in this model. Rutin and quercetin prolonged the time of mice's live after inoculation with KN/Ly cell of ascitic tumor. Silymarin had high anticarcinogenic effect to induced photocarcinogenesis of mice's skin [8].

Antitumor effect of flavonoids can be connected with their anti-proliferative action: thus, some flavonoids (fisetin, apigenin, luteolin) significantly inhibit cell proliferation; quercetin and apigenin suppress melanoma growth on mice.

Comparative pathomorphological research of tumors - both control ones and treated with leukocompounds and flavonols demonstrated that tumors were undergone to significant morphological changes: dystrophic, deformed and deteriorated cells, cells with nuclei in pycnosis and lysis appear, sclerosis, and tumor tissue remains in the form of small islets among necrotic and necrobiotic masses.

Comparative research was carried out relating to antitumor activity of plant flavonoids and their synthetic derivatives. It has been demonstrated that introduction of nitrogroup and halogens into the structure not only increases antitumor activity but also has affect specificity of their effect on tumors.

Tables II and III show experimental findings showing interrelation of antioxidant and antitumor activity of different groups of flavonoids extracted by us from *Polygonum* L. plants:

TABLE II
ANTITUMOR ACTIVITY OF FLAVONOIDS OF *POLYGONUM* L.

<i>Polygonum</i> L. flavonoids	Antitumor action		
	PLs	S-180	ECa
Leukoanthocyanins complex	84.3	78.2	38.0
Leukoanthocyanins and catechins complex	93.8	72.0	50.6
Catechins and polycatechins complex	9.0	78.5	-
Anthocyanins complex	95.5	94.5	88.5
Cyanidin 3,5-diglycoside	58.8	43.7	39.6
Quercetin	50.8	65.1	51.3
Rutin	37.8	44.2	37.7
Myricetin	58.2	72.7	73.5
Morin	47.7	38.6	32.5
Proanthocyanins complex	51.0	42.5	68.9
2-Hydroxychalcone	11.9	12.9	17.0
4,2',5'-Trihydroxychalcone	51.3	71.5	31.1

Note: PLs - Pliss lymphosarcoma, S-180 - sarcoma-180, ECa - Erkhlich carcinoma.

TABLE III
ANTIOXIDANT ACTIVITY OF FLAVONOIDS OF *Polygonum L.*

<i>Polygonum L.</i> flavonoids	pH	pK	Antioxidant action	
			conc., %	DI, Ep
Leukoantho-cyanins complex	5.40	6.20	0.1	2.86
Leukoanthocyanin and catechins complex	4.85	6.00	0.1	2.86
Catechins and polycatechins complex	4.15	5.25	0.1	2.34
Anthocyanins complex	2.35	4.95	0.1	2.34
Cyanidin 3,5-diglycoside	5.90	7.90	0.1	1.65
Quercetin	3.10	6.80	0.1	2.55
Rutin	5.50	pK ₁ =5.65 pK ₂ =10.4	0.1	1.26
Myricetin	4.05	5.85	0.1	2.14
Morin	5.60	5.90	0.1	1.05
Proanthocyanins complex	3.30	5.30	0.1	3.67
2-Hydroxy-chalcone	5.15	pK ₁ =5.80 pK ₂ =10.7	0.1	1.57
4,2',5'-Tri-hydroxychalcone	2.95	pK ₁ =4.95 pK ₂ =9.35	0.1	1.00
			0.05	2.79
			0.05	2.50
			0.1	1.82
			0.05	1.10
			0.1	2.63
			0.05	2.40

Comparison of antitumor activity of flavonoid complexes of *Polygonum L.* plants with synthetic substances for chemotherapy used in medicine is shown in Table IV.

TABLE IV
COMPARISON OF ANTITUMOR ACTIVITY OF *Polygonum L.* FLAVONOID WITH CHEMOTHERAPY MEDICINALS

<i>Polygonum L.</i> complexes and medicinals	Single dose mg/kg	Tumor growth inhibition, %		
		PLs	S-45	WCs
Polyflavans	45	87.6	76.2	63.9
Leukoantocyanins	45	83.1	38.1	61.6
Catechins	45	91.4	67.7	58.5
Anthocyanins	45	88.9	80.8	69.1
Flavanols	45	92.6	86.9	79.7
Chalcones	45	61.7	68.3	74.9
Sarcosine	3	48.8	97.6	98.4
Thiophosphamide	5	61.4	88.4	96.2
Dopan	2	57.3	80.2	95.8
6-Mercaptopurine	2	21.5	12.6	2.9
<i>Polygonum L.</i> complexes and medicinals	HC	Tumor growth inhibition, %		
		PC-1	S-180	ESt
Polyflavans	63.5	71.3	58.9	83.4
Leukoantocyanins	58.2	59.0	51.2	37.7
Catechins	41.6	59.8	41.1	51.6
Anthocyanins	61.7	55.4	73.2	84.9
Flavanols	68.8	76.1	78.4	79.5
Chalcones	22.7	33.8	61.3	24.3
Sarcosine	62.6	83.6	38.9	32.2
Thiophosphamide	3.7	56.7	25.4	19.1
Dopan	56.3	91.8	51.7	43.8
6-Mercaptopurine	18.8	2.4	32.6	39.1

Note: PLs - Pliss lymphosarcoma, C-45 - sarcoma-45, WCs - Woker carcinosarcoma, HC - Herene carcinoma, PC-1 - alveolar mucous cancer of liver, S-180 - sarcoma-180, ESt - Erlich solid tumor.

IV. CONCLUSION

Researches of flavonoids in conditions of one-time and prolonged oral supplementation give evidence of their low toxicity. Even if rutin, quercetin, quercitrin is fed with a food (1% to dietary intake) to rats for a long time (within 1-2 years) neither weight growth, nor loss of appetite nor changed in blood and internals was observed. Substitution of hydroxyl group with halogen atoms, introduction of methyl and acetyl substitutes as well as pyran ring opening (chalcone formation) increases toxicity of flavonoids.

The important area in research of flavonoids is also the creation of composite medicines with medications of different groups and preparation of semisynthetic derivatives. Such researches can open new perspectives of usage of flavonoids as therapeutic agents and creation of effective dosage forms on their basis.

ACKNOWLEDGMENT

Thanks to project: 2028/GF financial support by The Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

The authors are very much indebted to the Kazakh Scientific-Research Institute of Oncology and Radiology for having provided the equipment where the work has been developed.

REFERENCES

- [1] *State Pharmacopoeia of the Republic of Kazakhstan*. – Almaty: Silk Way, 2008.
- [2] E. B. Vladimirkaya, *Biological bases of antitumor therapy*, Moscow, 2001.
- [3] D. A. Muravyeva, I. A. Samylina, G. P. Yakovlev, *Pharmacognosy*, Moscow: Medicine, 2007.
- [4] V. Petkov, *Modern phytotherapy*, Sofia: Medicine and physical culture, 1998.
- [5] M. Sun, J. Han, J. Duan, *Phytotherapy Res.*, vol. 21, no. 3, pp. 269–277, 2007.
- [6] L. H. Shi, Y. H. Qiu, *J. Tumor*, vol. 7, pp. 95–97, 2001.
- [7] G. F. Gonzales, L. G. Valerio, *Anticancer Agents Med. Chem.*, vol.6, no. 5, pp. 429-444, 2010.
- [8] R. A. Muzychikina, D. Yu. Korulkin, *Bioactive constituents of plants. Extraction, separation and analysis*, Almaty: Atamura, 2006.
- [9] H. Chen, Y. Zuo, Y. Deng, *J. Chromatogr. A*, vol. 913, pp. 387-395, 2001.
- [10] R. T. Skil, *Antitumor chemotherapy*, Moscow: Geotar-media, 2011.
- [11] V. E. Steele, R. A. Lubet, R. C. Moon, *Cancer Chemoprevention*, vol. 2, pp. 568-571, 2005.
- [12] A. A. AlFattah Amara, *Am. J. Cancer Sci.*, vol. 2, no. 1, pp. 1-27, 2013.
- [13] S. K. Gupta, *Drug Screening Methods*, Kent: Anshan Publishers, 2009.