Effect of blueberry paste on experimental malignant tumor growth

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Recently there has been observed an increasing number of works dealing with anti-tumor properties of such plant compounds as polyphenols of tea, soya, red grapes, etc. Green tea flavonoids, resveratrol of red grape, soybean isoflavons have been found to have essential anti-carcinogenic and anti-tumor effects, and certain mechanisms of these effects have been defined. Specifically our experiments have shown that green tea extract and several soybean foods can essentially retard the growth of grafted experimental tumors [1 – 4]; the molecular mechanisms of this effect have been studied [5, 6].

Blueberry (*Vaccinium myrtillus L.*) is among the plants having a lot of biologically-active substances. Blueberry contains carbohydrates (glucose, sucrose, fructose, pectins), organic acids (citric, lactic, malic, succinic, oxalic), vitamins (A, B, C, PP), flavonoids (giperin, quercetin, astragalin), anthocyanins (delphinidin, malvidin, idain, mirtillin), phenols, minerals, macro- and microelements (iron, manganese, selenium, cobalt, copper, gold, silver, zink), a lot of tannin and rutin. Due to this blueberry has antioxidant, antiseptic, astringent, antiinflammatory, diuretic, cholagogic, spasmolytic, hypoglycemic characteristics. Blueberry is widely used in folk medicine. It's therapeutic properties were known thousands years ago in China, Egipt, Greece, Rome.

Blueberry fruits and leaves are used to treate diarrea, bleeding, anaemia, leukemia, gastritis, hepatitis, hypostases, hypertensia, atherosclerosis, rheumatism, arthritis, podagra, psoriasis, insular diabetes, quinsy, bronchitis, cystitis, urethritis, gastrointestinal colic, ophtalmic diseases, etc.

There has also been information on anti-tumor characteristics of blueberry and its bioflavonoids. Thus, pterostylben /blueberry bioflavonoids/ in the diets of

the experimental animals, inhibits the {beta}-catenin/p65 downstream signaling pathway and colon carcinogenesis in rats [7]. Similar data have been obtained on mice. *Mutanen M. et al; Cooke D. et al.* have shown that freeze-dried blueberry and blueberry anthocyanin mixture diminish the frequency of intestinal adenoma formation in the Apc(Min) mice, a genetic model of human familial adenomatous polyposis [8, 9]. *According to the authors*, these results confirm the efficiency of blueberry and its components for prevention of intestinal cancer which is one of the most widespread cancer diseases. Thomasset S. et al. have performed pilot studies as to the effect of mirtocyan, an anthocyanin-rich standardized blueberry extract, on tumor cells proliferation (Ki-67 protein expression served as a criterion) in colorectal cancer patients. The 7-days-long mirtocyan consumption before surgical operation led to diminished tumor cells proliferation [10].

Some other investigators have ascertained that blueberry extract inhibits growth of leukemia cells. Thus, the blueberry extract inhibited the growth of HL60 human leukemia cells *in vitro* through the induction of apoptosis [11].

Ashutosh K. et al .have revealed that ursolic acid (one of the blueberry components) inhibited both constitutive and interleukin-6–inducible STAT3 activation in a dose- and time-dependent manner in multiple myeloma cells. Ursolic acid down-regulates the expression of STAT3-regulated gene products such as cyclin D1, Bcl-2, Bcl-xL, survivin, Mcl-1, and VEGF. Ursolic acid also inhibits proliferation and induced apoptosis and the accumulation of cells in G1-G0 phase of cell cycle. As they suggest, these results testify to the fact that ursolic acid is a novel blocker of STAT3 activation that may have a potential in prevention and treatment of multiple myeloma and other cancers [12]. *Shishir Shishodia et al.* [13] suggest that one of the ursolic acid anti-carcinogenic and anti-tumor effects may be inhibition of activation of NF-κB transcription factor which plays an important role in carcinogenesis and tumor growth, and decreased expression of NF-κB-dependent genes that take part in proliferation. In their experiments, ursolic acid inhibited NF-κB (p50/p65) activation and its binding to DNA.

It has been shown that anthocyanin-rich mixtures extracted from bilberry and grapes inhibit the kinase activity of receptor tyrosine kinases in human vulva carcinoma or porcine aortic endothelial cells [14].

The aim of our work has been to study the effect of a bluelbery paste *(a new domestic product made by an original new domestic technology and equipment developed in SIPE "Institute "TEKMASH")* on the growth of grafted experimental tumor strains with different animal species (rats and mice) and of different organ and tissue origin.

Materials and methods

Product pre-screening. With the view of determining the blueberry paste advantages in specialized diets for cancer prevention, we have studied, using our original methods [15, 16], blueberry paste effect on cancerolysis activity of blood serum of healthy animals (mice and rats).

Tumor strains and animals. The following grafted experimental tumor strains of two animal species (rats and mice) and of different organ and tissue origin have been used in our investigations, namely:

- *Rat Guerin's carcinoma,* derived from a spontaneous uterus carcinoma but its growth is not estrogen-dependent and that's why these tumors can be grafted to both male and female non-inbred rats; in our experiments, these tumors were grafted subcutaneously with 0.5 ml of 20% suspension of small tumor tissue bits per an animal; non-inbred female rats of near 170 g body mass were used;
- *Lewis lung carcinoma* (synonims – LLC, 3LL) of C57Bl/6 mice; males of near 17 g body mass were used; these tumors were grafted by injection of 5×10^5 tumor cells in 0.2 ml of isotonic NaCl solution into a leg musccle; suspension of tumor cells was obtained without protease treatment, by tumor tissue punching shear throuhh thin metal mesh and further filtering it through a nylon mesh;

- *Mouse Ehrlich carcinoma*, has been derived from a mouse mammary carcino but, being re-grafted for some decades,it has lost almost all histologic differentiating signs including H2-antigens and, because of this, can be equally well grafted to male or female mice of different strains or non-inbred and can grow in ascites form if grafted intraperitoneally; in our experiments, non-inbred male mice of about 33 g body mass were used; tumors were grafted with 3×10^5 tumor cells injected intraperitoneally;
- *L1210 lymphatic leukemia of DBA2 mice* (may be grafted to both male or female DBA2 mice or to F1 (C57Bl/6 × DBA2) crossbreed mice, known as BDF1; in our experiments, female mice of about 22 g body mass were used; tumors were grafted with 2×10^5 tumor cells injected intraperitoneally).

Animals feeding. We prepared the food mixing standard crushed compound feed and blueberry paste in the ratio of 1.7 g of paste to 80 g of standard compound feed. This food mix was fed to the animals in the experimental group therapeutically from the moment of tumors grafting up to the moment of the animal slaughter or death. The animals in the control groups consumed standard compound food only.

Effect criteria and statistics. The complete oncological experiments with the solid tumors (Guerin's carcinoma and Lewis carcinoma) were evaluated by the development of average tumor size, by the average tumor mass at the moment of the animals slaughter and by the average lifetime (ALT) of animals after tumor grafting (the latter concerns the groups of animals that have not been slaughetred). In similar experiments with ascites tumors (Ehrlich carcinoma and L1210 lymphatic leukemia) – average number of tumor cells per an animal was considered instead of average tumor size and mass. The number of tumor cells was calculated after the cells had been washed from the animal's abdominal cavity with isotonic NaCl solution. The Student's t-criterion was used for statistical evaluations.

Results and discussion

Pre-screening experiments

Effect of bilberry paste on cancerolysis activity of healthy animals blood serum. The results of bilberry paste pre-screening are given in the table 1.

Table 1

Effect of bilberry paste on cancerolysis activity of healthy animals blood serum.

Species, strain and group of animals	*Cancerolysis index, % versus average activity of control serum*
Rats, non-inbred, control (n=5)	Conventional zero ± 5
Rats, non-inbred, experiment (+ bilberry paste) (n=5)	+19 ± 4*
Mice, C57Bl/6, control (n=7)	Conventional zero ± 3
Mice, C57Bl/6, experiment (+ bilberry paste) (n=7)	+55 ± 9*

* – *Difference between control and experimental group is significant (P<0.05).*

According to table 1 if the healthy animals, both rats and mice, were fed with bilberry paste, cancerolysis activity of their serum was significantly elevated versus its control value. With mice the effect was surprisingly high (such great cancerolysis characterisics have been found only tree times during our 13-year period of work with cancerolysis reaction). This fact leads to the doubtless conclusion that blueberry paste is a very promising produc for further oncological investigations. This preliminary conclusion has been confirmed by full-sized oncological experiments.

Oncological experiments

Effect of bilberry paste on growth of Guerin's carcinoma in rats. Blueberry paste added to diet of rats grafted with ***Guerin's carcinoma*** caused significant slowing-down of tumor growth which is confirmed both by growth dynamics (Fig. 1) and final average tumor mass in slaughtered animals (Table 2). Slowing-down index was 49,2 % (Table 2).

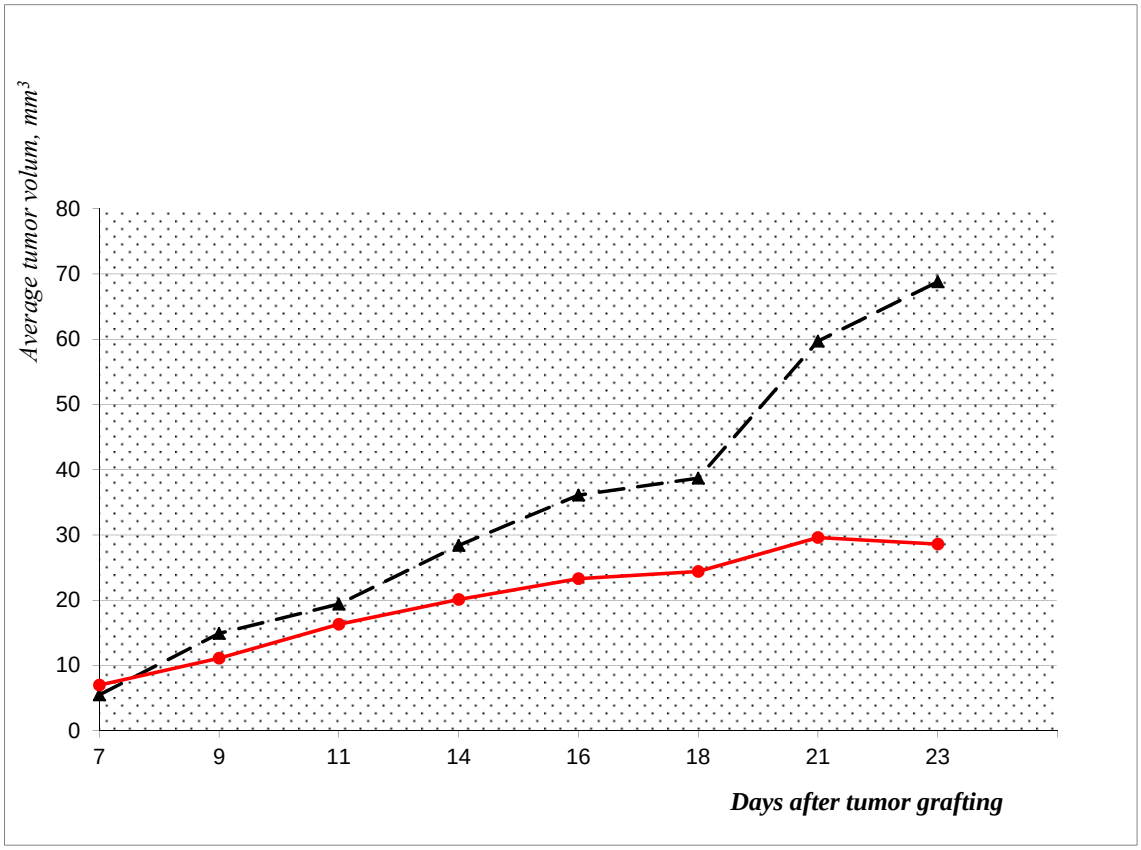

Fig.1. Growth dynamics of Guerin's carcinoma in rats fed with blueberry paste *(black curve – control animals, n = 11; red curve – animals fed with blueberry paste, n = 10).*

Table 2

Effect of blueberry paste on average tumor mass of Guerin's carcinoma in rats

Group of animals	Tumor mass *(g, M±m)*
Control (n = 11)	35.8 ± 6.9
Experiment (n = 10)	18.2 ± 7.1**

*** – 0.05<P<0,1*

Effect of blueberry paste on Lewis lung carcinoma growth of tumor-bearing animals and their survival. If blueberry paste was added to the diet of ***Lewis lung carcinoma*** bearing mice, it caused slowing-down of tumor growth but its rate was slower compared to the effect on Guerins carcinoma (49,5%) while the average lifetime of these animals increased substantially compared to the one on the conventional ration (Table 3).

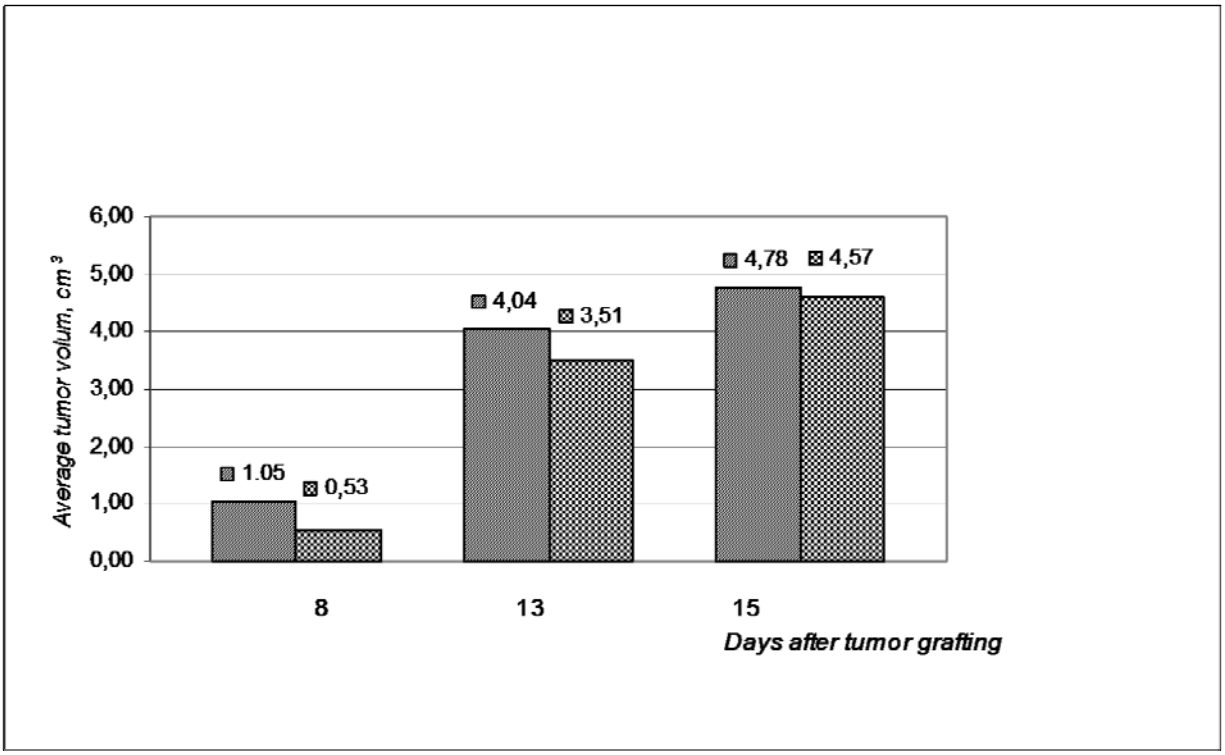

Fig.2. Effect of blueberry paste on growth of Lewis lung carcinoma in mice (*yellow bars – control animals, n = 10; green bars – experimental animals, n = 10). * - difference between control and experimental groups is statistically significant (P<0.05).*

Table 3

Effect of blueberry paste on average lifetime of Lewis carcinoma bearing mice

Group of animals	Average lifetime, *(days, M ± m)*
Control, *n = 13*	19.1 ± 1.0
Experiment, *n = 10*	23,3 ± 1.2*

** - Difference between control and experimental group is significant (P<0,02).*

Effect of bilberry paste on survival of L1210 lymphatic leukemia bearing mice. Increasing effect of bilberry paste on AL of mice with ***L1210 lymphatic leukemia*** was not great but of high statistical significance (Table 4).

Table 4

Effect of bilberry paste on average lifetime of L1210 lymphatic leukemia bearing mice.

Group of animals	Average lifetime, *(days, M ± m)*
Control, *n = 21*	**9,0 ± 0.1**
Experiment, *n = 17*	**9,9 ± 0.1***

** - Difference between control and experimental group is significant (P<0,001).*

Effect of blueberry paste on ascites Ehrlich carcinoma bearing mice. *T*he experiments with ***Ehrlich ascites carcinoma*** bearing mice have shown the decreased average number of tumor cells / by 12,9%/ per an animal and the decrease of ascites (from 4,0 ml to 3,6 ml/. These effects seem to represent an evident trend (0.05<P<0.1).

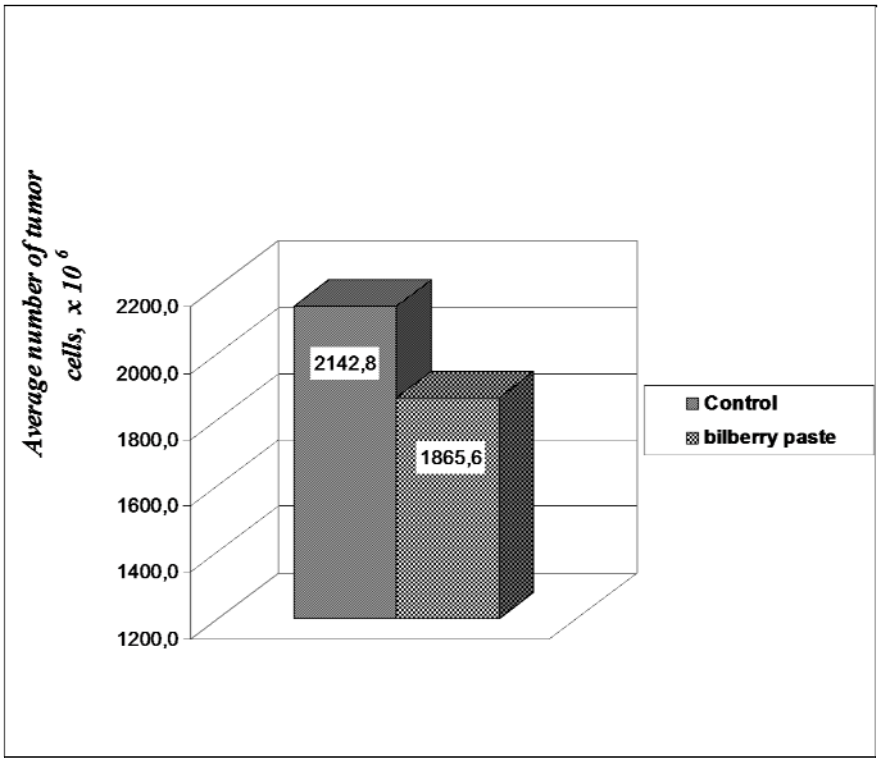

Fig.4. Effect of blueberry paste on growth of ascites Ehrlich carcinoma in mice (*yellow bar – control animals, n = 10; blue bar – experimental animals, n = 10*).

Thus, the data obtained in this study seem promising and require further investigations of blueberry-paste diets for cancer prevention and treating oncological patients.

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