ONCOLOGY

THE EFFECT OF EXTRACTS OF HIPPOPHAE RHAMNOIDES UPON THE DEVELOPMENT OF TUMOR TRANSPLANTS IN ANIMALS

E. Ch. Pukhalskaia

From the Laboratory of Experimental Chemotherapy (Chief – Corresponding Member Acad. Med. Sci. USSR L. F. Larionov) of the Institute of Experimental Pathology and Therapy of Cancer (Director – Corresponding Member Acad. Med. Sci. USSR N. N. Blokhin) Acad. Med. Sci. USSR, Moscow

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A previous communication [1] has already reported the fact that an alcohol extract of the bark of Hippophae rhamnoides (sea buckthorn) inhibits the growth of an Ehrlich tumor introduced subcutaneously. The present study presents the results of a further investigation of the antitumor properties of this extract.

EXPERIMENTAL METHODS

The extract was prepared by M. F. Petrova in the laboratory of the chemistry of native extracts (Chief – G. P. Menshikov). The specimens of the plant had been collected in the summers of 1955 and 1956 by the Central Asiatic expedition accompanied by P. S. Massagetov. The method for obtaining the alcoholic extracts has been described previously [1]. After the removal of the alcohol, the extract was treated with gelatin or talc for the purpose of separating from it the woody, bark substances.

The doses were calculated in mg of the dried extract per kg weight of the animals.

The treatment of the animals with transplanted tumors was begun at various time intervals after the inoculation, the actual time depending on the initial growth of the particular tumor strain. The injections were given daily. The treatments were continued from 10 to 20 days, this factor also being varied in dependence on the individual characteristics of the growing tumors.

In order to arrive at an opinion as to the effectiveness of the preparation against the growing tumors, the average weights of the tumors in the control group would be compared with the average weight found in the experimental. This difference was then expressed as per cent of the average in relation to the weight of the controls and served as the basic indicator of the inhibiting effect of the preparation upon the tumor growths. In addition, we measured the growth of the tumors in the experimental and control animals by taking the comparative diameters of the tumor swellings every 5 days.

EXPERIMENTAL RESULTS

Experiments on mice and rats [1] had already shown that the extract was much more toxic when gelatin had been used to remove the tannates than when talc had been employed for that purpose; there was but little difference in toxicity between the gelatin treated substance and the native material still containing the tannates so that subcutaneous or intra-abdominal injections caused marked irritation and could be used only in small doses. In the majority of the experiments we used the extract which had been treated with the talc, as this preparation did not have a local irritating action.

The lethal dose (LD_{100}) of the extract purified with the talc, by adding talc, when injected intraabdominally

into rats, was 1 g of the dry substance per 1 kg weight of the animals; deaths beginning 3-4 hours after the injection. The average lethal dose (LD_{100}) was 750 mg/kg, while the maximum bearable dose was 600 mg/kg.

In the rats which died (LD_{100}) there was noted cyanosis of the extremities, ears and nose. At autopsy there was generalized venous hyperemia of the abdominal organs as well as hyperemia and hemorrhages into the lungs in addition to thymic edema.

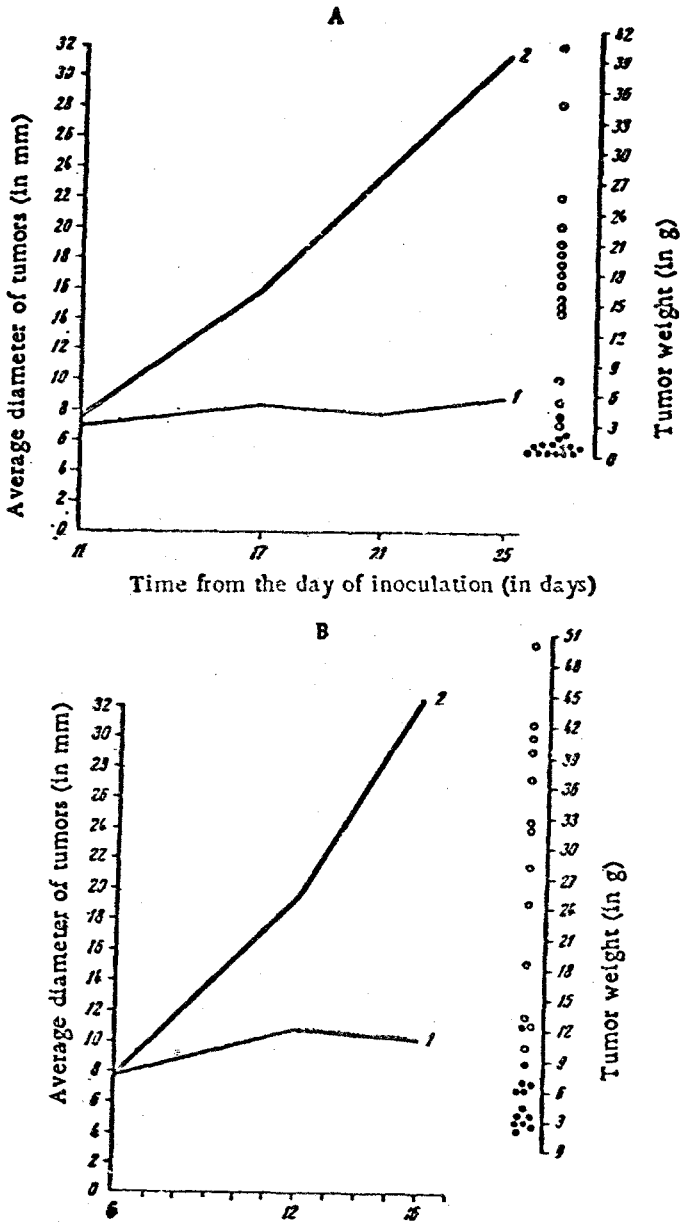
In subsequent experiments we established that repeated injections into the rats of 400 mg/kg doses neither caused death nor produced marked alterations in the organs. However, after each injection of the extract there could be observed inhibition and weakening of the motor activity of the animals followed by ataxia and simultaneous increase in the respiratory rate while the respiratory movements became more shallow. With a dose of 600 mg/kg the respiratory repression continued for several hours.

In rabbits we also observed the effects of extract injections upon the blood picture. For 9 days 3 rabbits received daily subcutaneous injections of 150 mg/kg of the extract cleared with the talc; daily leucocyte counts were made and an erythrocyte count was made initially and at the end of the course. The extract failed to produce any quantitative changes.

We studied the action of the alcohol extract of the bark of Hippophae rhamnoides upon 10 different transmittable rat and mouse tumors: small doses of the native extract, small doses of the gelatin-cleared extract and, finally, various doses of the extract cleared with the talc (see Table).

The Effects Produced by Extracts of the Sea Buckthorn Bark Upon Tumor Growth

Extract	Dose (in mg/kg)	Method of introduction	Tumor	No. of animals		Days after inoculation: treatment with extract begun	Length of treatment (days)	No. of days until death		Inhibition of tumor growth in exptl. animals, (% of controls)
				experimental	control			experimental	control	
Native	150	Subcutaneous	Ehrlich mouse tumor	15	15	5	10	0	2	51
	150	»	Mouse hepatoma	20	20	6	14	1	1	50
Cleared with gelatin	100	Subcutaneous	Ehrlich mouse tumor	20	20	6	10	3	1	42
	150	»	Rat carcinoma of Guerin	14	14	12	11	0	2	54
	150	»	Rat sarcoma 45	15	15	1	14	1	2	29
Cleared with talc	200	Subcutaneous	Ehrlich mouse tumor	20	20	5	12	0	0	61
	400	Intra-abdominally	"	15	15	5	11	4	6	69
	200	Subcutaneous	Mouse mammary cancer	15	15	11	18	0	1	64
	400	»	Mouse cancer of the forestomach	17	17	5	18	2	0	19
	400	Intra-abdominally	Mouse melanoma	21	21	20	21	5	3	60
	150	Subcutaneous	Crocker mouse sarcoma	25	25	6	10	1	2	44
	400	»	M-1 rat sarcoma	16	16	6	11	3	3	82
	400	Intra-abdominally	Rat sarcoma 45	15	15	7	15	0	0	93
	200	Subcutaneous	Walker rat carcinosarcoma	15	15	4	10	1	0	70
	400	»	Rat carcinoma of Guerin	15	15	11	14	1	0	96



Effect of sea buckthorn extract upon the growth of Guerin carcinoma (A) and M-1 sarcoma (B) (experiments No. 15 and No. 12).
1) Experimental; 2) control. Circles indicate distribution of tumors by weight at the end of the experiment. • – experimental, o – control.

As can be seen from the Table, all three extracts retarded the growth of a subcutaneously inoculated Ehrlich tumor to about the same extent, i.e., 50-60%.

The native extract was tested on mice inoculated with mouse hepatoma C_3HA (strain obtained by V. I. Gel'shtein with the aid of orthoamidoazotoluol) and it was noted that it produced tumor inhibition.

The extract, cleared with gelatin had a feeble antitumor effect upon rat sarcoma 45 and a more pronounced effect upon Guerin carcinoma whose original strain had been obtained from a spontaneous rat uterine carcinoma.

The extract cleared with talc and used in doses of 400 mg/kg inhibited markedly the growth of Guerin carcinoma, sarcoma 45 and sarcoma M-1 (see Figure) but complete resolution of the tumors did not occur: in some nodes weighing several score mg the microscope would show individual, undamaged blastomatic cells.

A marked effect was observed with experiments on the Walker carcinosarcoma 256 (strain obtained from a spontaneous carcinoma of rat mammary gland), in experiments with mammary carcinoma (RMZh) in hybrid C_{57} x A (strain obtained by L. A. Bolonin from spontaneous mouse mammary carcinoma), in experiments with melanoma of Harding–Passy on mongrel mice, however, the tumors did not dissolve completely. The extract was particularly resisted by strain PRZh (gastric cancer)in mice breed CC_{57} (strain obtained by Dobryninin by the action of 9 : 10 dimethyl-1,2-benzanthracene).

The results of these experiments has been to demonstrate that the bark of Hippophae rhamnoides has a principle which can be extracted with alcohol and which will inhibit markedly the growth of 8 strains of tumors out of the 10 tested; in other words, this principle has a broad antitumor spectrum of activity. However, the toxicity of the extract is such that it cannot be offered, as yet, for clinical testing; further study of the essential active substances must be continued.

SUMMARY

The antitumor activity of alcohol extracts of the bark of Hippophae rhamnoides as well as the bark of the tree itself was tested upon 10 different forms of transplantable tumors found in mice and rats.

Tumors were markedly inhibited in 8 of the 10 types of tumor tested even if complete resorption failed to be observed.

The preparation is still very crude and toxic. More studies of the active ingredients are needed before the material will be suitable for clinical trial.

LITERATURE CITED

[1] E. Ch. Pukhalskaia, M. F. Petrova and P. S. Massagetov, Biull. Eksptl. Biol. i Med. XLIV, No. 6, 57-60, 1957. *

* Original Russian pagination. See C. B. Translation.