PLANT EXTRACTS TESTED FOR CYTOTOXIC AND ANTITUMORIGENIC ACTION

E. Ch. Pukhalskaya, M. F. Petrova and P. S. Massagetov

From the Laboratory of Experimental Chemotherapy (Head - Prof. L. F. Larionov, Corresponding Member of the USSR Academy of Medical Sciences), the Laboratory of the Chemistry of Natural Substances (Head - Prof. G. P. Menshikov), the Institute for the Experimental Pathology and Therapy of Cancer (Director - N. N. Blokhin, Corresponding Member of the USSR Acad. of Med. Sci.) and from the Botanical Laboratory (Head - P. S. Massagetov) of the All-Union Chemical and Pharmaceutical Institute for Research

(Presented by Prof. A. D. Timofeevsky, Active Member of the USSR Academy of Medical Sciences

Received January 19, 1957.)

Research by massive experimentation is a method which can be used in the search for antitumorigenic preparations from plants due to the diversity of chemical compounds, the biological action of which is yet unknown, existing in plants. In a series of works [2, 3, 4], the order in which different representatives of the higher plants should be tested was determined by the definite pharmocopeian group with which they were connected.

Although there were no definite reasons for the choice of the material to be tested, in some cases, we were directed by various considerations concerning the type of biological action possessed by substances known to be or proposed to be contained in the given plant (for example, the presence of growth-inhibiting substances or of alkaloids with unknown biological action, etc).

EXPERIMENTAL METHODS

Only alcohol extracts were tested as, in this way, almost the whole mass of protein substances were excluded from the study. The alcohol extracts were prepared by the standard method: 50 g of dried plant was ground up and extracted with ethyl alcohol. The alcohol was completely evaporated in a vacuum (15 mm of residual pressure). The residue was dissolved in 100 ml of distilled water, filtered to remove undissolved matter and neutralized to pH 7.

Since most of the compounds with antitumorigenic properties also possessed cytotoxic properties, we first selected extracts according to their cytotoxic activity in vitro on tumorous cells of Ehrlich's ascites. We chose the method developed by V. A. Talyzina[1], already used in the laboratory of Prof. M. M. Maevsky for preliminary culling of antibiotics, to examine the cytotoxic effect in vitro. The test extract (neutralized to pH=7), in two dilutions, was added to fresh Ehrlich's ascites in a ratio of 1:1. The resulting mixture was kept in a refrigerator for 4 hours at a temperature of 4°, and then injected subcutaneously into 10 mice in a dose of 0.3 ml (each dilution was injected into 5 mice).

In this way, the entire process of the extract's action on the tumor cells proceeded in vitro, and only the result of this action was tested by transplanting the material processed with the test extract into the animal.

The control was the development of tumors on the opposite sides of the same mice caused by the simultaneous transplantation of an ascites diluted 1 : 1 with a physiological solution and also kept for 4 hours in the refrigerator.

The extract was considered to have cytotoxic properties if tumors either did not develop where the ascites processed with the test extract had been injected or developed a few days later than in the control.

Ninety alcohol extracts from 78 plants were examined, representing 39 different families. In some cases, extracts from different parts of the plant were tested separately.

Sixty-five of the plant extracts showed no cytotoxic effect in the experiments in vitro. The length of this article does not permit their enumeration.

When a cytotoxic effect was observed, the experiment was repeated once or twice.

Fifteen of the 90 extracts studied had, in some degree, cytotoxic properties. Our next task was to determine from in vivo experiments which of these extracts possessed an antitumorigenic action. However, during the in vitro experiments, we discovered that the cytotoxic effect of some of these extracts was evidently due to the fact that they contained a considerable amount of tannins, which, according to a previous test, do not have antitumorigenic properties and hinder the in vivo testing of an extract by causing extensive necroses to form at the injection place of the extract.

Therefore, the extracts in which a large tannin content had been determined were purified of the tannins by using skin powder before being tested again in vitro before the in vivo experiments.

To study the antitumorigenic properties of the selected extracts in vivo, we conducted the usual therapeutic experiments on mice with Ehrlich's tumor, implanted subcutaneously. Each extract was tested on 40 animals (20 in the experiment and 20 in the control). Treatment was begun on the 5th-6th day after implantation, when the tumors were of a measurable size (average diameter, 5-6 mm), and continued for 12 days. The extracts were injected subcutaneously in doses established earlier in experiments conducted on mice to determine the toxicity of the individual extracts.

At the end of the treatment period, the animals were killed, and the difference between the average weight of the tumors in the control and in the experiment was determined. This difference, expressed in percent of the average weight of the tumors in the control, served as the principal index of the antitumorigenic action of the extract.

Thirteen of the fifteen extracts which had been selected for their cytotoxic action were examined for antitumorigenic properties.

EXPERIMENTAL RESULTS

Positive results from both the in vitro and in vivo experiments coincided in 3 cases, i.e., the cytotoxic properties of the extract were coincidental with antitumorigenic properties (see table) these cases were the extracts from the above-ground portions of Anagallis and Datisca cannabina and from the fruit of the honey locust (Gleditsia triacanthos). However, the cytotoxic and antitumorigenic effects of the Anagallis extract were weakly expressed; treatment with this extract only inhibited growth in Ehrlich's tumor 30%, and the extract was highly toxic.

The extract from the fruits of the Kirghiz honey locust had a stronger effect. There were many tannins contained in the native extract, which were removed before therapeutical use of the extract, but the second in vitro test showed that the extract retained its cytotoxic properties after the removal of the tannins. This extract inhibited the growth of Ehrlich's tumor 63%. One must mention, however, that the extract was rather toxic. By the end of the course of treatment, the experimental mice had lost weight considerably, and when they were dissected, a series of dystrophic changes were found in the liver and spleen. The Datisca cannabina extract (Gissar) had approximately the same effect.

Examination of the extract made from the rind of the Tyan-Shan Hippophae rhamnoides first gave us the impression that the cytotoxic effect of this extract was coincidental with its antitumorigenic properties. This impression proved to be false. The therapeutic experiment was first set up with the native (containing tannins) extract, greatly diluted to prevent necroses. The result obtained was an antitumorigenic effect, inhibiting the growth of Ehrlich's tumor 51%. When the extract was tested in vitro again, after the tannins had been removed, we found that its cytotoxic properties had been removed with the tannins. However, the antitumorigenic effect of treatment with the purified extract increased to 60.6% (as the concentration of the solution increased).

Results from In Vitro and In Vivo Tests of Alcohol Extracts

Family: genus and species of plant	Part of plant extracted	Result of test	
		in vitro	in vivo
Compositae			
Acroptilon picris	Above-ground portion	+	-
Artemisia Tournefortiana	The same	+	-
Saussurea sp.	" "	+	-
Centaurea balsamita	" "	+	Not studied
Oleaster			
Hippophaë rhamnoides:	" "		
Native extract after precipitation of tannins		+	+
			+
Rosaceae			
Padus racemosa:	" "		
Native extract after precipitation of tannins		+	Not studied
		-	-
Nymphaeaceae			
Nuphar luteum	Roots	+	-
Leguminosae			
Gleditsia triacanthos:	Fruit		
Native extract after precipitation of tannins		+	Not studied
		+	+
Equisetaceae			
Equisetum hellocharis	Above-ground portion	+	-
Primulaceae			
Anagallis sp.	The same	+	±
Umbelliferae			
Angelica brevicaulis	" "	+	-
Cucurbitaceae			
Cucumis melo	" "	+	-
Zygophyllaceae			
Peganum harmala	" "	+	-
Aceraceae			
Acer negundo	" "	±	Not studied
Datiscaceae			
Datisca cannabina	" "	+	+

Note: Symbols for in vitro experiments designate: + presence of cytotoxic effect (tumors did not dvelop); ± weak effect (all tumors in the experiment at least twice as small as in control on the 8th day after implantation); - no effect. For in vivo experiments: + clear positive effect (tumor growth inhibited more than 50% as compared with the control); ± weak positive effect (tumor growth inhibited 30-50%); -negative effect (tumor growth inhibited less than 30%).

Therefore, in the first in vitro experiments, the cytotoxic effect was caused by the presence of the tannins, but the antitumorigenic effect is connected with the presence of other substances which have not yet been investigated.

Nine of the extracts which manifested a cytotoxic effect in vitro did not show antitumorigenic activity in the therapeutic experiments. One must mention, however, that in some cases this may have been due to the rapid combination of the active substances directly on the place of injection into the body and to the insufficient concentration of these substances in a given extract. Therefore, some of the experiments which were done with less than the maximum therapeutic dosage bear repeating with more concentrated extracts.

We shall further investigate the three plants which had marked antitumorigenic properties in order to isolate the source of antitumorigenic activity and determine its chemical nature.

SUMMARY

90 extracts of 39 different plants were investigated, regarding their cytotoxic effect on the cells of Ehrlich's ascites. This effect was found to be present in 15. Out of this number, 13 were tested in experimental treatment of Ehrlich's tumor. The extracts of Hippophae rhamnoides, Datisca cannabina and Gleditsia triacanthos had pronounced retarding effect on the growth of this tumor.

LITERATURE CITED

[1] Talyzina, V. A., Antibiotiki, 1956, Vol. 1, No. 5, pp. 35-39.

[2] Belkin M., Fitzgerald D., J. Nat. Cancer Inst. 1952, 13, p. 139.

[3] Belkin M., Fitzgerald D, Felix M., Ibidem, 1952, N 3, p. 741.

[4] Belkin M., Fitzgerald D., Ibidem, 1953, 11, 13, N 4, p. 889.