Antimutagenic Properties of Medicinal Angelica (*Angelica archangelica L.*) Studied with the Micronucleus Test

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The present-day environment is being increasingly polluted with chemical mutagens. This, together with a high background level of radiation, leads to a rise of the mutation pressure, accompanied by an increased frequency of spontaneous abortions, developmental defects, and hereditary disorders. All this adds urgency to the search for antimutagenic preparations protecting the human hereditary apparatus. A large number of antimutagens have now been studied, including various chemical compounds as well as bioactive substances of natural origin. Since pure chemical antimutagenic compounds often have side effects, natural antimutagens, specifically of plant origin, attract special interest.

This work was devoted to the study of the antimutagenic effect of medicinal angelica with respect to the mutagen thiophosphamide (thioTEP).

MATERIALS AND METHODS

Experiments were carried out on outbred white mice weighing 25-30 g. Roots and rootstocks of medicinal angelica, gathered in September, 1991 in the area of Nagaevo village (Bashkiria), served as raw material for preparing aqueous and alcohol extracts. Dried roots were ground in a homogenizer and passed through a sieve (pore diameter 1 mm). The extraction was conducted according to the eleventh edition of the State Pharmacopeia of the USSR (1989). Fifty milliliters of distilled water or methanol were added to 1 g homogenate, and the mixture was weighed and held at 20°C for 1 hour. The mixture-containing retort was then connected with a reflux condenser and heated in a gently boiling water bath for 2 hours. The extract was cooled at 20°C for 10-15 min, and the weight was adjusted to the initial one with the appropriate solvent. The extract was passed through dry paper filter, and the sediment was pressed out. The extracts were prepared ex tempore. Aqueous extract was introduced on the basis of the air-dried mass. The filtrate of alcohol extract was vacuum evaporated in a rotary evaporator at 60°C, with the subsequent determination of the humidity. The dry preparation was dissolved in water prior to administration. The animals received aqueous and alcohol extracts via a gastric probe in a dose of 50, 100, 500, and 1000 mg per kg body weight in a volume of 25 ml per kg, 2 hours before and/or simultaneously with thioTEP. The mutagen solution was prepared from the medicinal preparation thioTEP, which was dissolved in distilled water ex tempore and injected intraperitoneally in a dose of 2 mg per kg and in a volume of 10 ml per kg.

Bone marrow preparations and blood smears were made 24 hours after mutagen administration [8]. The preparations were air-dried, fixed in absolute methanol for 3 min, and stained after May-Grunwald-Giemsa. The frequency of micronuclei was

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TABLE 1. Frequency of Micronuclei–Containing Cells in Mice Treated with thioTEP and Extracts of Medicinal Angelica

Treatment	Dose, mg per kg		Percent of micronuclei-containing cells	
	extract	thioTEP	bone marrow	blood
Aqueous extract	50	–	0.20±0.05*	0.18±0.05*
	100	–	0.22±0.09*	0.20±0.06*
	500	–	0.18±0.07*	0.19±0.08*
	1000	–	0.17±0.08*	0.17±0.03*
Aqueous extract simultaneously with thioTEP	50	2	0.78±0.07*	0.75±0.07*
	100	2	0.69±0.12*	0.64±0.06*
	500	2	0.65±0.11*	0.66±0.11*
	1000	2	0.73±0.10*	0.70±0.10*
Aqueous extract 2 hours before thioTEP	50	2	0.54±0.12*	0.50±0.10*
	100	2	0.43±0.13*	0.48±0.09*
	500	2	0.45±0.13*	0.48±0.07*
	1000	2	0.39±0.04*	0.40±0.13*
Alcohol extract (humidity 8.9%)	50	–	0.28±0.07*	0.27±0.05*
	100	–	0.30±0.10*	0.31±0.07*
	500	–	0.33±0.11*	0.30±0.08*
	1000	–	0.27±0.08*	0.24±0.04*
Alcohol extract simultaneously with thioTEP	50	2	0.82±0.11*	0.80±0.09*
	100	2	0.80±0.10*	0.78±0.14*
	500	2	0.75±0.13*	0.75±0.11*
	1000	2	0.72±0.09*	0.73±0.13*
Alcohol extract 2 hours before thioTEP	50	2	0.61±0.08*	0.59±0.08
	100	2	0.57±0.12*	0.56±0.05*
	500	2	0.59±0.05*	0.55±0.10*
	1000	2	0.46±0.04*	0.45±0.13*
Negative control	–	2	1.70±0.11	1.55±0.11
Positive control	–	–	0.20±0.06*	0.17±0.05*

Note: an asterisk indicates reliable difference from the negative control ($p<0.05$).

determined by examining 1000 polychromatophilic bone marrow erythrocytes and 2000 normochromic peripheral blood erythrocytes. The statistical assessment of the results was performed routinely [2]. The significance of the differences between the mean values was determined using Student's t test.

RESULTS

The study of the antimutagenic activity of medicinal angelica was carried out in two stages; first we studied the activity of aqueous extract, and then of an alcohol extract. The results are presented in Table 1. The experiments included two control groups. Mice of the positive control group received intragastrically distilled water in a volume of 25 ml per kg, while animals of the negative control group were injected intraperitoneally with thioTEP in a dose of 2 mg per kg.

Both the aqueous and alcohol extracts of angelica failed to induce micronuclei. The range of extract concentrations tested was quite broad, but the variations of micronuclei number between the maximum and minimal doses were negligible. Administration of aqueous extract simultaneously with thioTEP reduced the number of micronuclei by 55-62%, whereas in the case of alcohol extract the reduction was about 52-58%. The inhibition of the mutagenic effect of thioTEP was more pronounced when the extracts were introduced 2 hours prior to thioTEP. In this case aqueous extract inhibited the appearance of micronuclei by 70-77%, and alcohol extract by 65-73%. In both the positive control and test group the number of micronuclei was lower ($p<0.01$) than in the negative control group. These results lead to the conclusion that angelica extracts possess antimutagenic activity. Antimutagenic activity of angelica was also shown in the Ames test [7].

A series of coumarins has been obtained from the roots of angelica grown in Bashkiria: angelicin, archangelicin, xanthotoxin, umbelliferon, scopletin, bergapten, and isoimperatorin [3]. The total quantity of coumarins in the roots of plants grown in the collection area was 1.98%. It can be assumed that the antimutagenic effect of angelica is mediated by the coumarins present in it. It has been shown [6] that coumarins given in a dose of 65-130 mg per kg per day for a week reduce benz(a)pyrene genotoxicity in male mice, as follows from a micronuclei assay.

Benz(a)pyrene is a mutagen requiring metabolic activation for the manifestation of its effect. As coumarins inhibit its mutagenic effect, one could suppose that the antimutagenic effect of coumarins is related to their interference with the processes of metabolic activation of mutagens. However, thioTEP used in our experiments does not require metabolic activation for the realization of its effect. ThioTEP possesses prooxidant properties [1]: it is therefore plausible to assume that the antimutagenic effect of coumarins is connected with their capacity to bind free radicals. The disclosed antioxidant properties of coumarins [5] may serve as indirect confirmation of this assumption.

It follows from our results that aqueous and alcohol extract of medicinal angelica possess antimutagenic activity, as manifested by their ability to reduce the frequency of thioTEP-induced micronuclei in mouse bone marrow and peripheral blood. Most probably, the antimutagenic effect of the extracts is due to their antioxidant properties. Thus, medicinal angelica contains antimutagens that, according to the classification offered by G. G. Poroshenko and S. K. Abilev [4], belong to the free radical-binding antimutagens.

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The Role of the Sympatheticoadrenal Structures in Hematopoiesis Regulation under Cytostatic Myelodepression

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As has been demonstrated previously, the neuroendocrine system markedly affects the hemopoietic response under extreme conditions which do not cause myelodepression [2,3,8,9]. At the same time, the role neurohormonal signals play in the proliferation and differentiation of the hemopoietic cells during various hemodepressive states remains unclear. It is known from the fragmentary data that glucocorticoids are able to reduce the toxic effects of antitumor drugs on hemopoiesis [1], and a mild halothane anesthesia significantly decreases the sensitivity of CFUs to cytostatics [7].

The aim of the present investigation was to establish the role of the sympathetic nervous system in

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