

INHIBITING EXPRESSION OF ATHEROGENIC MICRORNA-24 AND
MICRORNA-125 AND INDUCING EXPRESSION OF ANTI-ATHEROGENIC
MICRORNA-126, MICRORNA-222 AND MICRORNA-LET7B

The invention relates to the field of clinical medicine, and more particularly to the possibility of influencing the expression profile of specific microRNAs in human blood plasma by ingestion of angelica extract, allowing the future use of angelica extract as an agent for protecting against the effect of specific epigenetic atherogenic factors in human blood plasma.

Known from prior art is the following method for using angelica extract, entitled "Agent for preventing the skin from aging or improving its age-related condition" (US20040223942A1, 3/6/2003, Japan). In this method, the angelica extract is used in a composition of components that influence the functional state of skin fibroblasts by increasing the expression of Rho kinase or myosin light chain kinase. The main difference of this method is the absence of any effect of using angelica extract on the epigenetic atherogenic and antiatherogenic factors, in particular on the expression level in the blood plasma of atherogenic microRNA-24, -125 and antiatherogenic microRNA-126, -122, -let7b.

The technical result of the claimed method is the possibility of influencing the expression profile of specific microRNAs (microRNA-24, -125, -126, -222, -let7b) in the blood plasma by inhibiting the expression of atherogenic microRNAs (microRNA-24, -125) and by inducing the expression of antiatherogenic microRNAs (microRNA-126, -222, -let7b) by ingestion of angelica extract.

The aforementioned technical result is achieved in that the method involves successively carrying out the following steps: 1) obtaining the angelica extract; 2) ingesting the

obtained angelica extract in a daily amount of 5 drops per 10 kg of body weight for a period of 4 weeks.

The angelica extract is obtained by drying the roots of the angelica plant for one week at $24\pm 1^{\circ}\text{C}$, then grinding the dried roots into a powder, then adding 100 g of ground angelica to 100 ml of distilled water, mixing it, after which the obtained mixture is boiled in a water bath for 1-3 hours, until the volume of the liquid has been reduced by 10-fold, centrifuged at 15000 RPM for 30 minutes at $24\pm 1^{\circ}\text{C}$, and then filtering the obtained supernatant on a Whatman filter 1, after which the solution is concentrated with a Concentrator plus rotary evaporator (Eppendorf) and a WP6122050 pump (Millipore) under the following conditions: 1400 RPM, temperature 30°C , duration 16 hours, vacuum 10 mbar.

5 ml of the obtained aqueous angelica extract are removed, and diluted to 1:5 in a physiological solution (0.9% aqueous sodium chloride solution), and 5 drops per 10 kg of body patient body weight are recommended for consumption *per os*.

Example "Inhibiting the expression of atherogenic microRNA-24, -125 and inducing the expression of antiatherogenic microRNA-126, -222, -let7b based upon data from a study of microRNA in the blood plasma during the daily administration of the angelica extract over 4 weeks at a dose of 5 drops per 10 kg of body weight".

A 55-year old man weighing 82 kg and a 43-year old woman weighing 75 kg ingested an aqueous angelica extract prepared in the manner described above in a daily dose of 5 drops per 10 kg of body weight for 4 weeks. The expression level of atherogenic and antiatherogenic microRNA in the blood plasma was determined on day 1 and on day 28 of administering the aqueous angelica extract.

In order to evaluate the expression level of atherogenic and antiatherogenic microRNAs, microRNAs were isolated from peripheral venous blood (5 ml, collected in a vacuum tube with anticoagulant) with the RNeasy Mini Kit (Qiagen, Germany), after which the reverse transcription reaction was performed with the miScript II RT Kit (Qiagen, Germany), and the PCR analysis was performed in real time with primers for microRNA-24, -125, -126, -222, let-7b, 16. The expression level is indicated in relation to the expression level of microRNA-16 (miRNeasy Mini Kit, miScript II RT Kit, Qiagen, Germany). The microRNA values are indicated as the ratio of the expression level of the examined microRNA, depicted as the number of copies of the examined microRNA/mcl, to the expression level of the microRNA-16, also depicted as the number of copies of the microRNA-16/mcl. This is a common approach to reflect the dynamics of the microRNA expression level, since the expression level of the microRNA-16 in relation to the expression level of other microRNAs is stable, and can serve as a reference relative to which the expression level of other microRNAs can be estimated and evaluated, and the levels of microRNA expression between different objects (cellular lines, culture, living organisms) can be estimated.

The table lists the values obtained for the expression level of atherogenic and antiatherogenic microRNAs against the backdrop of ingesting the aqueous angelica extract.

Table "Expression level of atherogenic microRNA-24, -125 and antiatherogenic microRNA-126, -222, -let7b on days 1 and 28 during the *per os* administration of angelica extract".

Expression level of microRNA (examined microRNAs/microRNA16)	Control (Day 1 of ingesting the angelica extract)	Experiment (Day 28 of ingesting the preparation)
Change in the expression level of the atherogenic microRNA		

	Men	Women	Men	Women
microRNA-24	12.1	5.4	10.4	7.2
microRNA-125	7.5	7.6	5.8	6.0
Change in the expression level of the antiatherogenic microRNA				
	Men	Women	Men	Women
microRNA-126	4.5	5.1	6.0	6.6
microRNA-222	3.8	5.8	6.7	7.1
Let-7b	2.2	7.9	4.9	10.3

The expression level of atherogenic microRNAs in the blood plasma dropped after 4 weeks of ingesting the angelica extract in a dose of 5 drops per 10 kg of body weight: The microRNA-24 dropped from 12.1 to 5.4 in men, and from 10.4 to 7.2 in women; the microRNA-125 from 7.5 to 5.8 in men, and from 7.6 to 6.0 in women. The expression level of antiatherogenic microRNAs in the blood plasma rose after four weeks of ingesting the angelica extract: The microRNA-126 from 4.5 to 5.1 in men, and from 5.1 to 6.6 in women; the microRNA-222 from 3.8 to 6.7 in men, and from 5.8 to 7.1 in women, and the let-7b from 2.2 to 4.9 in men, and from 7.9 to 10.3 in women.

CLAIMS

1) A method for inhibiting the expression of atherogenic microRNA-24, microRNA-125, and for inducing the expression of antiatherogenic microRNA 126, microRNA-222, microRNA-let7b in human blood plasma, in which the preparation is obtained by drying the roots of the angelica plant for one week at $24\pm 1^{\circ}\text{C}$, the dried roots are then ground into a powder, after which 100 g of ground angelica is added to 100 ml of distilled water, mixed, after which the obtained mixture is boiled in a water bath for 1 to 3 hours, until the volume of the liquid has been reduced by 10-fold, centrifuged at 15000 RPM for 30 minutes at $24\pm 1^{\circ}\text{C}$, after which the obtained supernatant is filtered on a Whatman filter 1, and the solution is further concentrated with a Concentrator plus rotary evaporator (Eppendorf) and a WP6122050 pump (Millipore) under the following conditions: 1400 RPM, temperature 30°C , for 16 hours with a 10 mbar vacuum, after which 5 ml of the obtained aqueous extract are dissolved in a 0.9% aqueous sodium chloride solution at a ratio of 1:5, after which the obtained preparation is administered to the patient *per os* in a quantity of 5 drops per 10 kg of their body weight once a day for 4 weeks.