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The selection of the amount of anthracene derivatives from raw materials of plant origin and the study of their influence on the rat periodontal

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Abstract

The article presents a new method of ethanol extraction of anthracene derivatives from the herb St. John's wort, the total content of which was evaluated using a calibration graph. The drug improved mineral metabolism, showed anti-inflammatory effects in the bone tissue of the periodontal rats.

Key words: laboratory technology, anthracene derivatives, Hypericum perforatum grass, mineral metabolism, ethanol.

Anthracene derivatives - a group of natural compounds of polyphenolic nature, which are based on the anthracene core of varying degrees of oxidation. They are widely distributed in nature, found in higher plants: the legume family (senna), lily (aloe), and St. John's wort (St. John's wort).

The above-ground part of Hypericum contains various classes of biologically active substances: flavonoids, phenol carboxylic acids, tannins, anthraquinones, as well as anthracene derivatives (hypericin, pseudohypericin).

Hypericum herb (Hypericum perforatum L.) has long been known for its antibacterial, antiviral, cytotoxic, antidepressant, antitumor properties, but traditionally it is used to treat inflammation [1]. Hypericin is one of the most powerful natural photodynamic agents. It is able to generate a superoxide anion radical and a large amount of singlet oxygen [2]. The pro-oxidant photodynamic properties of hypericin are used in photodynamic therapy of cancer, since when activated by light, it very effectively causes apoptosis and / or necrosis of cancer cells [2]. Light-activated pseudohypericin reduces prostaglandin E2 (PG E2) production caused by lipopolysaccharides [3].

The purpose of the work is to isolate and quantify anthracene derivatives of the herb St. John's wort, as well as to study its effect on the periodontal bone of experimental animals.

Materials and methods

Experiments on the study of the periodontoprotective properties of the preparation of anthracene derivatives with the working name AP, obtained under laboratory conditions, were carried out on 11 white female rats 18 months old. age Group 1 (5 individuals) - intact, in group 2, 6 rats were given per os a drug at 0.1 ml / 100 g of body weight of rats per 55 days. At the end of the experiment, the rats were scored by total bleeding from heart vessels under anesthesia (sodium thiopental 40 mg / kg). All animal experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals. The object of biochemical studies was the supernatant of the homogenates of the bone of the alveolar process (50 mg / ml), where the content of malondialdehyde (MDA) was determined [4]; standardized methods: total protein content, alkaline phosphatase activity, calcium and phosphorus content, using commercial reagent kits (manufactured by DAC SpectroMed, Moldova).

The results of the experiments were processed with the determination of the criteria for the reliability of student differences.

Research results

Obtaining drugs based on the modification of the method [5]. The essence of the method consists in extracting the amount of anthracene derivatives from the initial air-dry raw material with 70% ethyl alcohol. According to the method, the optimal conditions for their production are the extraction with ethanol of a certain concentration and the duration of its conduct from dry plant materials.

The optimal conditions for obtaining the drug is a double extraction with 70% ethanol in the course of 90 minutes.

The modification of the method [5] consisted in separating the fraction of lipophilic substances of non-flavonoid nature with a polar solvent, namely hexane [6]. To this end, hexane was added to the preparation obtained in the first stage and thoroughly mixed, after which the mixture was allowed to settle. After spontaneous separation of the phases, the alcohol phase was separated.

Then ethyl alcohol was distilled off under moderate vacuum conditions using a water-jet pump at a temperature of 70 ± 5 ºC, as in dental practice it is preferable to use aqueous solutions. At the final stage, the obtained extract was concentrated in a drying cabinet at a temperature of 45-50 ºC.

The drug AP, obtained from the herb Hypericum perforatum by the above method, was characterized by the presence of anthracene derivatives, in particular hypericin [5].

Anthracene derivatives are characterized by a peak with an absorption maximum at a wavelength of 590 nm. The study of the absorption spectrum of the AP preparation obtained by us showed the presence of a corresponding peak at a given wavelength (Fig. 1).

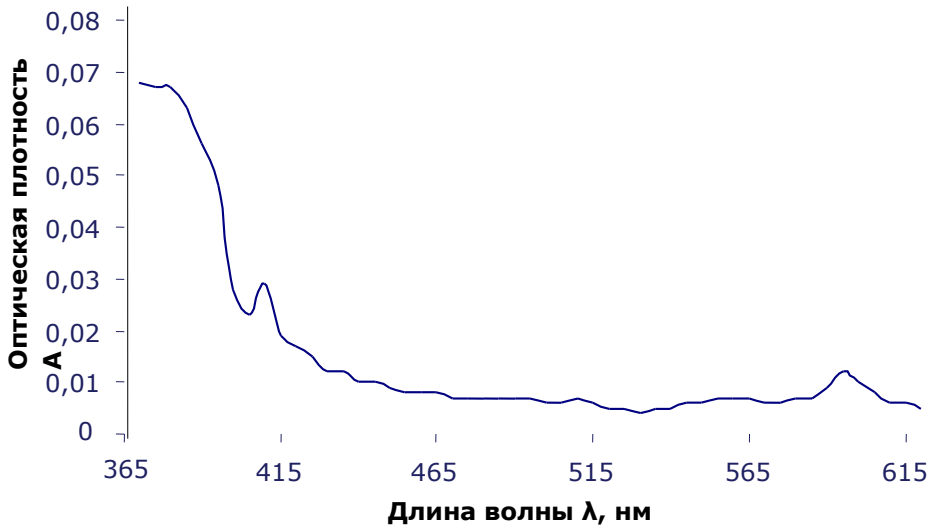

Fig. 1 - Absorption spectrum of the alcohol solution of the drug AP

Previously, to prove the presence of anthracene derivatives in the resulting preparation, a qualitative reaction was carried out, the essence of which was to change the color of the test solution, into which hypericin reversibly enters due to redox processes. So, after adding a few drops of an alkali solution to the preparation, a change in the initial red color to a yellow-green one was observed. The subsequent addition of a certain amount of

hydrochloric acid solution led to a shift in equilibrium towards the formation of the original form of hypericin, and, as a result, to the restoration of the original color of the solution.

Quantitative determination of anthracene derivatives in the resulting preparation was carried out using direct spectrophotometry at a wavelength of 590 nm. Ethanol served as a reference solution, and 1% hypericin solution served as a standard comparison solution (Fig. 2).

The amount of anthracene derivatives in terms of dry raw materials was calculated by the formula:

$$X = \frac{A}{718 \times m} * k,$$

where A is the optical density of the test solution; 718 - specific absorption rate of 1% hypericin solution; m is the mass of the feedstock (g); k is the dilution factor.

Thus, according to the results of the studies, the total content of anthracene derivatives (AP) from the aerial part of Hypericum perforatum L. was determined, which amounted to 0.25 mg / ml of the extract.

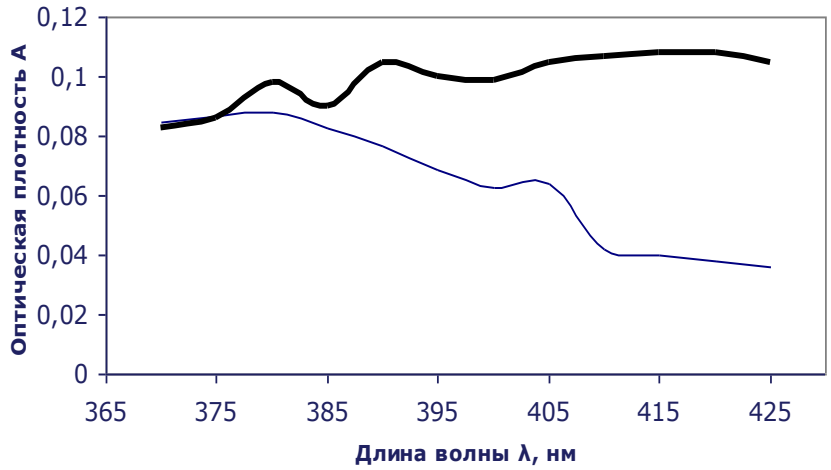

Fig.2. Absorption spectra of the alcohol solution of the drug AP

Later, the effect of anthracene derivative (AP) on the condition of periodontal bone tissue of rats was studied (table).

Studies have shown that under the influence of the drug in the bones of the alveolar process, the level of MDA decreased by 25% ($p = 0.001$) (Table), which indicates the antioxidant properties of the drug, manifested in the periodontal bone tissue. On the other

hand, a decrease in the content of MDA indirectly indicates a decrease in inflammatory events in this object of study (see table).

Table

The effect of anthracene derivatives on biochemical parameters in the bone tissue of periodontal rats (M ± m; p)

Group Animals	Content				Activity
	protein (mg/g)	MDA (nmol/g)	Ca (mmol/g)	P (mmol / g)	AP (mmol / s·g)
Intact	0,97±0,049	2,79±0,12	0,016±0,0012	0,016±0,0010	0,18±0,015
Anthracene	1,16±0,031 p=0,02	2,08±0,15 p=0,001	0,048±0,00026 p<0,001	0,015±0,0021	0,20±0,010

Note. In tab. confidence index p is calculated relative to the intact group.

The preparation of anthracene derivatives from the herb Hypericum perforatum improved mineral metabolism in periodontal bone. During the 55 days of the experiment, the drug increased the alkaline phosphatase activity by 11% (p> 0.05); the calcium content at the same time increased by 3 times (p <0.001); the phosphorus content remained almost unchanged (Table), i.e. the results of the experiment indicate a positive effect of the drug AP on the bone tissue of periodontal rats.

Conclusion

Thus, in the laboratory, a preparation of anthracene derivatives from plant materials, the herb St. John's wort, was obtained. In the course of the research, the anti-inflammatory effects of the preparation obtained were established. The improvement of mineral metabolism in the bone of the alveolar process is apparently associated with a decrease in the production of PGE2 under its influence along the cyclooxygenase pathway during enzymatic membrane oxidation of polyene acids.

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