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EFFECT OF *HYPERICUM ALPESTRE* AND *RUMEXOB TUSIFOLIUS L.* EXTRACTS ON THE ALTERATIONS OF PROLINE QUANTITY IN VARIOUS RAT ORGANS

H.M. KARAPETYAN, E.KH. BARSEGHYAN

Department of Biochemistry, Microbiology and Biotechnology,
Yerevan State University,
hkarapetyan@ysu.am

The effect of extracts of *Hypericum alpestre* and *Rumex obtusifolius L* herbals on the amount of free proline and the activity of proline biosynthesis enzymes in various organs of the rat was studied. It was shown that the activity of proline biosynthesis enzymes under the influence of *R. obtusifolius L.* extracts decreased in the liver by 74.7%, in the brain by 51.7%, and no changes in enzyme activity were observed in the kidneys. Compared with the control group, proline biosynthesis in the liver of rats under the action of extracts of *H. alpestre* was completely inhibited, in the brain – by 29%. Inhibition of the activity of proline biosynthesis enzymes is explained by the presence of severalbiologically active compounds with anti-inflammatory, antiproliferative, and anti-aging properties in the extracts of herbals.

Proline – proline biosynthesis enzymes – herbal extract – H. alpestre – R. obtusifolius L.

Ուսումնասիրվել է սրոհունդ ալպիական (*Hypericum alpestre*) և ավելուկ լայնատերև (*Rumex obtusifoliusL.*) դեղաբույսերի լուծամզվածքների ազդեցությունը ազատ պրոլինի քանակության և պրոլինի կենսասինթեզի ֆերմենտների ակտիվությունների վրա առնետի տարբեր օրգաններում: Ցույց է տրվել, որ ավելուկ լայնատերևի լուծամզվածքի ազդեցությամբ պրոլինի կենսասինթեզի ֆերմենտների ակտիվությունը լյարդում ընկճվել է 74.70 %-ով, ուղեղում՝ 51.77 %-ով, իսկ երիկամներում գրեթե փոփոխություն չի դիտվել: Ալպիական սրոհունդի լուծամզվածք ստացած կենդանիների լյարդում ստուգիչ խմբի համեմատությամբ պրոլինի կենսասինթեզը լիովին ընկճվել է, իսկ ուղեղում արգելակվել է 29.02%-ով: Պրոլինի կենսասինթեզի ֆերմենտների ակտիվության ընկճումը բացատրվում է դեղաբույսերի լուծամզվածքներում պարունակվող հակաբորբոքային, հակապրոլիֆերատիվ և հակատարիքային հատկություններով օժտված մի շարք կենսաբանորեն ակտիվ միացությունների առկայությամբ:

Պրոլին – պրոլինի կենսասինթեզի ֆերմենտներ – դեղաբույսերի լուծամզվածքներ – Hypericum alpestre – Rumex obtusifolius L.

Изучено влияние экстрактов *Hypericum alpestre* и *Rumex obtusifolius L.* лекарственных растений на количество свободного пролина и активность ферментов биосинтеза пролина в различных органах крысы. Показано, что активность ферментов биосинтеза пролина под влиянием экстрактов *R. Obtusifolius L.* снижалась в печени на 74,7 %, в мозге на 51,7 %, в почках изменений активности ферментов не наблюдалось. По сравнению с контрольной группой биосинтез пролина в печени крыс под действием экстрактов *H. alpestre* был полностью угнетен, в мозге – на 29 %.

Ингибирование активности ферментов биосинтеза пролина объясняется наличием в составе экстрактов лекарственных растений ряда биологически активных соединений с противовоспалительными антипролиферативными и антивозрастными свойствами.

Пролин – ферменты биосинтеза пролина – экстракты растений –
H. alpestre – R. obtusifolius L.

Like other amino acids, L-proline serves as the basis for proteins and, due to its special structure, makes them rigid. Proline plays a highly beneficial role in cells exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule, and a signaling molecule [8, 15, 16].

Proline is synthesized by two pathways: glutamate pathway and ornithine pathway. The glutamate pathway accounts for major proline accumulation during osmotic stress. The proline is synthesized from glutamic acid via intermediate Δ'-pyrroline-5-carboxylate (P5C). The reaction is catalyzed by Δ'-pyrroline-5-carboxylate synthetase (P5CS) and Δ'-pyrroline-5-carboxylate reductase (P5CR) [15, 17]. In an alternative pathway, proline can be synthesized from ornithine, which is transaminated to P5C by ornithine-δ-aminotransferase. Accumulation of proline has been suggested to contribute to stress tolerance in many ways. As proline acts as the molecular chaperone it is able to maintain the protein integrity and enhance the activities of different enzymes [8]. Numerous studies have reported proline as an antioxidant suggesting its role as ROS scavenger and singlet oxygen quencher [4, 8].

Transcriptomics, metabolomics, and proteomics studies have demonstrated the role of proline-5-carboxylate reductase-mediated proline synthesis in cancer development. It has been proven that an excess amount of proline affects the clinical course of cancer [5, 9]. It is known, that mammary tumor tissues are full of collagens, providing a large reservoir of free L-proline [2]. Prolyl-specific peptidases are induced in cancer cells and can release L-Pro-rich peptides and free L-proline in their microenvironment by degrading ECM collagens [12]. For instance, free L-proline is accumulated in esophageal carcinoma tissue, where it reaches significantly higher levels than in neighboring normal tissues [14]. Free L-proline is transported inside cancer cells, where it can be used for anabolic and catabolic purposes. Inhibition of the enzyme pyrroline-5-carboxylate reductase reduces the growth of tumor cells and increases the cytotoxicity of chemotherapeutic drugs, and the regulation of proline biosynthesis is considered a potential therapeutic target for tumor treatment. In this sense, promising studies can create a basis for the use of natural compounds that regulate the biosynthesis of proline [9]. Such biologically active compounds may be contained in the herbal plant's plant's *Hypericum alpestre* and *Rumex obtusifolius* growing in the territory of Armenia. Herbal plants have fewer side effects compared to other drugs and contribute to the stimulation of mechanisms that prevent and correct the development of various pathological conditions, including the regulation of apoptosis, a decrease in the rate of tumor cell proliferation, etc. [11].

Given the above, the aim of the research was to study the effect of plant extracts of *H. alpestre* and *R.obtusifolius L.* on the change in the amount of free proline and the activity of proline biosynthesis enzymes in various organs of the rat.

***Materials and methods*.** 90-120 g laboratory albino rats served as the research object. Rats were kept at constant environmental and nutritional conditions with room humidity (50-55%) at 12 h light/12 h dark cycle and were fed a standard pellet diet and with water ad libitum (Animal care house, Yerevan State University YSU)). All experimental procedures were approved by the National Center of Bioethics (Armenia) and were in accordance with procedures outlined in the "Guide for Care and Use of Laboratory Animals" (NIH publication 80-23).

Plant materials. The methanol extracts of *Hypericum alpestre* subsp. polygonifolium (Rupr.) Avet. &Takht. (aerial part) (ERCB 13206: numbers of Voucher specimens, which were deposited to the Herbarium of YSU) and *Rumex obtusifolius L*. (seed) (ERCB 13208) were used during the study. The plant materials were harvested from Tavush region of Armenia (900-1600 m above sea level). Identification of plant materials was done at the Department of Botany and Mycology, YSU (Armenia) [5]. Plant crude extracts were prepared by maceration technique using methanol (98%) at 10:1 solvent-to-sample ratio (v/w). The stock solutions of the samples have been prepared by dissolving crude dried extracts in pure dimethyl sulfoxide (DMSO) (Sigma-Aldrich).

One group of animals was injected subcutaneously with plant extracts at the following doses: 2.4 mg/kg/day for 8 weeks: 12 injections every 4 days. After the rats were decapitated, their organs (liver, kidney, brain) were separated in cold conditions.

To estimate the activity of enzymes of proline biosynthesis [1, 17], the 5% homogenate has been prepared. The homogenization was carried out in potassium –sodium–phosphate buffer (pH 7.4). The incubation mixture, which contained 50 mM ornithine, 50 mM α-ketoglutarate, 100 mM potassium–sodium–phosphate buffer (pH 7.4), 1 mM pyridoxal-5-phosphate, 0.5 ml homogenate has been prepared. The incubation was carried out at 37^0C for an hour. During this period the environment ornithine under transaminase action was transformed into proline-5-carbon acid, then was incubated again for 15 min and proline-5-carbon acid under pyroline-5-carboxylase action was transformed into proline. The reaction was stopped by three chlorine acetic acids. The patterns were centrifuged at 8000 g for 10 min. Enzyme activity was determined according to the formed proline quantity. Proline quantity was estimated by chemical method [3]. 1 ml of ninhydrin reagent (3 g ninhydrin dissolved in 180 ml of glacial acetic acid and 20 ml of formalin) was added to 1 ml of a pattern. The mixture was heated at 100ºC for 1 min or at 75ºC for 4 min in a water bath, frozen and the optical density of red colored product has been measured by a photoelectric calorimeter at 490 nm wavelengths. Proline was used as a standard.

The obtained results were subjected to statistical analysis by the "Biostat" program. The veracity was estimated according to the Student t-standard.

***Results and Discussion*.** The effect of extracts of *H. alpestre* and *R. obtusifolius L*. plants on the content of free proline and the activity of proline biosynthesis enzymes (ornithine transaminase and proline-5-carboxylate reductase) in various organs of rats (liver, kidneys, brain) was studied. Animals fed a standard diet without herbal extracts served as the control group. The results of the study are presented in figures 1 and 2. (fig.1 and fig.2).

As can be seen from the data obtained, the largest amount of free proline in the studied organs was found in the liver, the amount of which was 1.5 times more than in the kidneys, and 2.6 times more than in the brain tissue (fig. 1). The low content of proline in the intact brain is apparently due to the fact that proline-rich peptides with antibacterial activity are synthesized here, as a result of which proline is most likely not in the free state in the brain and is included in the composition of the mentioned proteins [16]. Results presented in figure 1 show, that the amount of free proline in the organs of the rat changes under the influence of *R. obtusifolius L.* extracts, in particular, the amount of proline in the liver decreases by 19.3%, in the kidneys by 15%, and in the brain by 18.7% according to compared with the control group. Regarding the effect of *H. alpestre*

extracts, no significant changes were found at this stage of the study compared to the control group (fig.1). The total activity of ornithine transaminase and proline-5-carboxylate reductase enzymes in the corresponding organs was also studied both in the control group and under the influence of herbals. As shown by the results obtained in the control group, the activity of proline biosynthesis enzymes in organs decreases in the series liver>kidney>brain, which coincides with the amounts of free proline found in the corresponding organs (fig. 2).

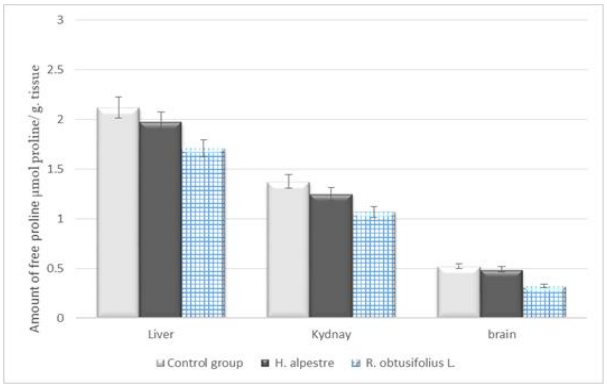

Fig 1. The effect of extracts of *H. alpestre* and *R. obtusifolius L.* on the amount of free proline in different organs of rats (µmol/g tissue, n=3, p<0.01)

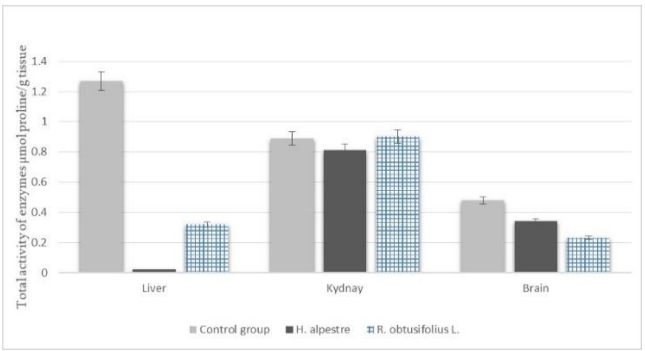

Fig. 2. The effect of extracts of *H. alpestre* and *R. obtusifolius L*. on the total activity of proline biosynthesis enzymes (ornithine transaminase and proline-5-carboxylate reductase) in different organs of rats (µmol proline /g tissue n=3, p<0.01)

Compared with the control group, the total activity of proline biosynthesis enzymes in the liver of rats that received *R. obtusifolius L*. extract was reduced by 74.70%, in the brain - by 51.77%, and no changes were found in the kidneys. Compared with the control group, proline biosynthesis was completely inhibited in the liver of animals receiving the *H. alpestre* extract, as for the brain, biosynthesis was inhibited by 29% (fig 2). The suppression of the activity of proline biosynthesis enzymes can most likely be explained by the presence in the extracts of herbals of a number of compounds: polyphenols, flavonoids, and terpenes, which have anti-inflammatory, antiproliferative, and anti-aging properties [10].

It has also been shown that plant extracts have high antioxidant properties due to the presence of phenolic compounds that are involved in redox reactions and neutralization of reactive oxygen species, preventing the development of various pathological conditions [6, 11].

The use of plant extracts probably contributed to both a decrease in the number of free radicals and an increase in the antioxidant defense of cells, which in turn caused a decrease in the biosynthesis of proline, which performs an additional protective role in the cell. The use of plant extracts probably contributed to both a decrease in the number of free radicals and an increase in the antioxidant defense of cells, which in turn caused a decrease in the biosynthesis of proline, which performs an additional protective role in the cell.

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