

*Pharmacochemistry*

## Tribusponin and Atherosponin - Remedies of Vegetable Origin

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**ABSTRACT.** Tribusponin, which is used for treatment and prevention of atherosclerosis, is a nonhormonal anabolic agent developed on the basis of steroid glycosides of the *Tribulus terrestris* L. - a plain annual plant. Tribusponin is suitable for treatment of cerebral atherosclerosis, atherosclerosis in combination with hypertension, angina pectoris against the background of coronary atherosclerosis, and mesenterial atherosclerosis complicated with atonia of intestine and/or biliary tracts, male infertility, for using in a rehabilitation period after serious illness and surgical interventions, and for increasing the athletes' exercise tolerance. Dosage form Atherosponin (0.25 g capsules), containing a mixture of the dried aqueous-alcoholic extract and the fine-dispersed powder of the plant in the ratio of 1:1.5, was developed from the aerial parts of *Tribulus terrestris* L. The preparation retains the whole complex of substances of the plant in the natural form. The therapeutic effects and indications of the food additive Atherosponin are similar to those of Tribusponin. © 2013 Bull. Georg. Natl. Acad. Sci.

**Key words:** *Tribulus terrestris* L., Tribusponin, Atherosponin.

*Tribulus terrestris* L. - ground burnut, Fam. Zygophyllaceae is a widespread plain annual plant with creeping radiating stems. The ground burnut has been used in oriental folk medicine for treatment of various diseases from time immemorial [1].

At the end of the 1960, at the Institute of Pharmacochemistry of the Georgian Academy of Sciences, the presence of steroid glycosides - diosgenin derivatives in the *Tribulus terrestris* (Tt) was revealed for the first time [2]. Afterwards diosgenin, gitogenin, ruscogenin, tigogenin, hecogenin, yamogenin and their glycosides were isolated from the Tt growing in

different natural regions of the world [1].

On the basis of steroid glycosides of the Tt, we produced preparation Tribusponin for treatment and prevention of atherosclerosis and as a nonhormonal anabolic agent [3]. Pharmacological tests [4] and clinical trials, performed at four clinics of the First and the Second Moscow Medical Institutes, at the Institute of Clinical and Experimental Therapy of Georgia, at hospital therapy clinics of Kalinin, Izhevsk and Tbilisi Medical Institutes, and at the Central Institute of Sports Medical and Biological Problems, showed the following: Tribusponin promotes a decrease in the

blood cholesterol content and an increase in the lecithin/cholesterol factor, reduces the index of phospholipids and lipoproteids, decreases lipopexia in the aorta, myocardium and liver, controls blood tension, and exhibits vasodilating and anticoagulant properties.

Tribusponin belongs to preparations that act via the cranial division. It possesses the ganglion blocking action and is capable of eliminating the effect of irritation of sympathetic (adrenergic) nerves. The preparation improves the evacuation and digestion functions of the gastrointestinal tract, stimulates the small intestine tonus and contraction, intensifies cholesteris and possesses a diuretic action.

Tribusponin exhibits nonspecific anabolic activity, stimulates physical skeletal myopachynsis, enhances the organism tone and hence the capacity for work, and stimulates the generative function [3, 4].

Commercial production and wide application of Tribusponin was permitted by the order of the Ministry of Public Health of the USSR (No. 1359, 1981). It was registered at the Ministry of Public Health of the Russian Federation in 1998, 2002, 2005 and 2007, and at the Ministry of Public Health of Georgia in 2007 (DA NR-003323). Since 2008 Tribusponin by the name "Colkhisponin" has been registered as a biologically active food additive in the USA. Tribusponin is produced in the form of 0.25 g tablets containing 0.1 g active substance with 0.015-0.017 g furostanolic glycosides.

The Tribusponin substance was produced at the Chinkent Chemical pharmaceutical factory, while the final dosage form – at the Tbilisi Chemical pharmaceutical factory and then at the experimental-production base of I. Kutateladze Institute of Pharmacochimistry of the Academy of Sciences of Georgia. Several million packages of Tribusponin were annually supplied to the network of pharmacies of the USSR and later - of the NIS.

The remedy is recommended for treatment of cerebral atherosclerosis, atherosclerosis in combination with hypertension, angina pectoris against the back-

ground of coronary atherosclerosis, and mesenterial atherosclerosis complicated with atonia of intestine and/or biliary tracts, at the conditions accompanied by blood hypercoagulation. Tribusponin is used as a rehabilitation remedy after severe diseases, surgery, etc. [3, 4].

The analysis of the chemical composition of the tribusponin substance was performed by high-performance liquid chromatography in combination with mass- spectrometry (HPLC/MS/MS) by using system Agilent Series 1110 with mass-spectrometer Thermo Finnigan Deca Ion-Trap (CA, the USA). The mobile phase was the following: water + 0.1% trifluoroacetic acid (TFAA) and acetonitrile + 0.1% TFAA. The separation was performed by gradient elution in the reversed-phase column Waters Symmetry C18 (2.1x150 mm, 5  $\mu$ m). As the result, 24 furo- and spirostanol glycosides, the derivatives of diosgenin, tigogenin, gitogenin and hecogenin, including 5 tri-, 10 tetra-, 7 penta- and 2 hexaglycosides, 8 sulfated glycosides and, probably, ecdisteroids were found in the tribusponin substance. The presence of phenolic compounds, namely gallic acid and 3 flavonoid glycosides of quercetin and isoramnetin, was revealed.

Tribusponin differs from other Tt preparations in a rich and rare composition, which likely determines its efficient therapeutic action.

The tribusponin substance has found application in allelopathy as well. The effect of Tribusponin on the growth and development of crops was studied at specialized institutions of the country. It was established that presowing treatment of seeds of grain-leguminous plants or spraying of vegetable seedlings with low-concentration (0.002-0.005%) Tribusponin water solutions increased the crop capacity by 25-60% and provided the yield of high-quality ecologically pure products [6].

Intensive chemical and pharmacological investigations of the Tt began in the 1990s. Its versatile therapeutic efficacy in respect of almost all functions of the human body was established, various medici-

nal preparations, including food additives, based on the GB appeared.

We set ourselves the task of producing a natural dosage form from the Tt with preservation of the content of biologically active substances of the plant in natural form.

The research on the selection of optimal conditions of production of the aqueous-alcoholic extract and fine-dispersed powder of the plant was carried out. As a result, the following procedure of extraction was proposed: air-dry granular raw material was extracted with 75% ethanol in the ratio of 1:7 at room temperature for 1 hour with permanent stirring. For the second and the third extractions, 5-fold amount of ethanol was used, the third extraction being performed at the extractant temperature. The united extract was distilled from ethanol, and the water phase was purified with chloroform in the ratio of 1.0:0.5 three times, condensed and dried in vacuum. In this way, 12-14% pale brown powder-like extract containing 30-35% steroid glycosides was obtained.

The powder with the dispersion of 0.25 mm turned out to be the most suitable. In model tests, in buffer solutions of sodium phosphate simulating the stomach and bowels media with pH1.5 and pH8.5, respectively, a good degree (60-65%) of transition of the sum of steroid saponins into the liquid was observed [7]. This fact allowed us to consider the granular raw material suitable for production of the preparation. By mixing the dried aqueous-alcoholic extract and the fine-dispersed powder of the Tt in the ratio of 1:1.5, we obtained the mass for the final capsule-shaped dosage form, representing the nonhygroscopic bitter greenish-brown powder with a characteristic smell containing 12-14% furostanol glycosides. The chemical analysis of the powder showed that it retained the content of substances of the initial plant in the natural state. One 0.25 g capsule contains 0.025-0.035 g furostanol glycosides. This preparation called Atherosponin with the action similar to Tribusponin is recommended as a biologically active food additive.

The quality certificate issued by Global Test, Ltd. certified the complete compliance of the analytical sample of Atherosponin with the specifications stated in the normative document executed as required by the State Pharmacopoeia, the microbiological purity complying with the European Pharmacopoeia Ph. Eur. 43.5.1.4., Category 4.

It is well known that the steroid glycosides of Tt stimulate the secretion of gonadotropic hormones (LH and FSH) from the hypophysis, which in its turn enhances the testosterone secretion. Under the influence of testosterone, protein synthesis, and muscular oxygen and carbohydrate availability improve, which increases the provision of muscles with energy. Under the influence of Tt glycosides, cholesterol metabolism in the liver and blood coagulability improve, which in turn promotes a decrease in the arterial tension. In the case of male infertility, as a result of the increase in the testosterone content, the Tt glycosides improve spermatogenesis and increase the spermatozoon mobility. These facts determine the therapeutic action of Tt preparations.

As was mentioned above, Atherosponin as a biologically active food additive has the same application indications as Tribusponin.

It is essential to resume the production of Tribusponin and to establish the production of Atherosponin for providing the population with affordable high-performance preparations of vegetable origin.

*Tribulus terrestris* L. - ground burnut is a biologically progressive species being in the process of intensive dissemination. I. Shreter studied the Tt resources in the European part of the former Soviet Union, Central Asia and Transcaucasia [8]. Their wide spread was established. At some studied field massifs, total Tt resources made up several thousand tons air-dried raw materials per year, and on the territory of Georgia – several hundreds tons per year. The expected crops are even more. The high seed production of the Tt and the large amounts of mericarps in the soil in different habitats guarantee natural reproduction of

the Tt population after annual harvesting of medicinal raw materials in autumn [8].

In addition to that, at I. Kutateladze Institute of Pharmacochemistry of the Georgian Academy of Sciences, A. Shtromberg and A. Jorbenadze studied the biological specific features and the ways of reproduction and cultivation of the Tt. As a result, the Tt

was cultured. Under dense sowing, the habit of the Tt changes: its creeping stems become standing, which increases the crop capacity and allows mowing the plant. Under local conditions, it is possible to harvest twice a year. The crop capacity makes up 6-7 tons of air-dried commercial production per hectare a year [9].

## ფარმაკოქიმია

# ტრიბუსპონინი და ათეროსპონინი - მცენარეული წარმოშობის სამკურნალო საშუალებები

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ფართოდ გავრცელებული ერთწლიანი მცენარის *Tribulus terrestris* L. - კუროსთავის სტერილიზებული გლიკოზიდების საფუძველზე შემუშავებულია პრეპარატი “Tribusponin” – “ტრიბუსპონინი” – ათეროსკლეროზის მკურნალობისა და პროფილაქტიკისათვის განკუთვნილი არაპორმონალური ანაბოლიტური საშუალება. ტრიბუსპონინის გამოყენების ჩვენებებია: თავის ტვინის სისხლძარღვთა ათეროსკლეროზი, სისხლში ქოლესტერინის დონის მომატება, ათეროსკლეროზი ჰიპერტონული დაავადებით, სტენოკარდია კორონალური სისხლძარღვების ათეროსკლეროზის ფონზე, სისხლის ჰიპერკოაგულაცია, მეზენტერიული სისხლძარღვების ათეროსკლეროზი მუცლის ღრუს ორგანოთა და ნაღვლმდენი გზების ატონიით, შეკრულობა; რეაბილიტაცია მძიმე დაავადების და ქირურგიული ჩარევის შემდეგ, სპორტსმენთა გამძლეობის მომატება, მამაკაცთა უნაყოფობა. ტრიბუსპონინი გამოდის 0.1 გ ტაბლეტებში N50.

კუროსთავის მიწისზედა ნაწილებიდან მომზადებულია ნატურალური სამკურნალო ფორმა 0.25 გ კაფსულების სახით, რომელიც შეიცავს სპირტ-წყლიანი მშრალი ექსტრაქტისა და მცენარის წვრილდისპერსული ფხვნილის ნარევის შეფარდებით 1:1.5. მასში შენარჩუნებულია მცენარეში არსებული ნივთიერებები ბუნებრივი სახით და ტრიბუსპონინის იდენტური თერაპევტული მოქმედებით ხასიათდება. პრეპარატს “Atherosponin” – “ათეროსპონინი” ეწოდა. ათეროსპონინი რეკომენდებულია, როგორც ბიოლოგიურად აქტიური კვების დანამატი და ტრიბუსპონინის ანალოგიურად გამოიყენება.

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