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*Kaypnazarov Turdibay Nzamatdinovich,
Junior Researcher. S. Yu. Yunusov
Institute of the Chemistry of Plant Substances AS Uzbekistan,
Tashkent, Uzbekistan
E-mail: kturdibay1@mail.ru*

*Ramazonov Nurmurod Sheralievich,
Doktor of Chemical Sciences. S. Yu. Yunusov Institute
of the Chemistry of Plant Substances AS Uzbekistan,
Tashkent, Uzbekistan*

*Egamova Feruza Rustamovna,
Junior Researcher. S. Yu. Yunusov
Institute of the Chemistry of Plant Substances AS Uzbekistan,
Tashkent, Uzbekistan*

*Khushbaktova Zaynab Abduraxmanovna,
Doktor of Medical Sciences. S. Yu. Yunusov
Institute of the Chemistry of Plant Substances AS Uzbekistan,
Tashkent, Uzbekistan*

*Sirov Vladimir Nikolaevich,
Doktor of Medical Sciences. S. Yu. Yunusov
Institute of the Chemistry of Plant Substances AS Uzbekistan,
Tashkent, Uzbekistan*

ISOLATION AND STUDY OF EFFECT OF CYCLOARTAN GLYCOSIDES ON METABOLIC PROCESSES IN CARDIAC MUSCLE OF EXPERIMENTAL ANIMALS

Abstract. The paper describes the preparation of *Astragalus janischewskyi* extract and defines cycloartane glycosides. Experimentally in male rats (180–200 g), 10 daily oral administration of this extract (10 mg/kg) and riboxine (50 mg/kg) has been found to promote the activation of metabolic processes in cardiac muscle. In terms of the effect on carbohydrate-energy and lipid exchanges, the tested extract *Astragalus janischewskyi* is not inferior to riboxine, in terms of antioxidant action it is reliably superior to the effect of the reference preparation used.

Keywords: *Astragalus*, cycloartane glycosides, qualitative and quantitative analysis, metabolic processes.

Introduction: Genus *Astragalus*, cem. Leguminosae, has about 2.500 species and in this regard has no equal among flowering plants. This genus is also

one of the largest in the flora of Central Asia. In the territory of the Republic of Uzbekistan, 254 species of this plant grow [1].

Many plants of the genus *Astragalus* containing cycloartane triterpenoids (cycloartane glycosides) have long been widely used in folk and waitinal medical practice as sufficiently effective cardiotropic agents [2; 3; 4]. This report analyzes the effects of *Astragalus janischewskyi* Popov extract on the state of certain metabolic processes in the heart muscle that ultimately determine its functional activity. Experiments were carried out in comparison with the known preparation riboxine, which has a positive effect on myocardial metabolism and is applied due to this in myocardiodystrophy, ischemic heart disease and in some other forms of cardiac pathology [5].

Experimental chemical part. The dried ground surface portion (1 kg) of the *Astragalus janischewskyi* Popov plant was used. Leguminosae [6] harvested in June 2019 in Surkhandarya region. Extraction was carried out with methyl alcohol (4 L) at room temperature 25 °C for 24 hours under periodic shaking (5 times). Extract was concentrated under reduced pressure and temperature 40–50 °C to consistency of thick resinous mass. Distilled water (300 ml) was added to the initial resinous mass obtained after the primary (methanol) extraction (38 g), and a coloured homogeneous solution was obtained with vigorous stirring. The aqueous solution was then extracted successively with chloroform, ethyl acetate and n-butyl alcohol. Extraction uparit dry. 21.5 g of butanol recovery were obtained.

The presence of cycloartane triterpenoids was carried out by thin layer chromatography on “Silufol” plates in solvent systems: chloroform-methanol-water (4: 1: 0.1; 70: 23: 3; 60: 35: 5). Chromatography of alcohol extracts was carried out with samples of previously known cycloartanes. After chromatography, it was sprayed with a 20% alcohol solution of phosphoronolphramic acid, then heated in an oven for 8–10 minutes at 100 °C. The adsorption zones of cycloartane substances and witnesses were colored brown.

The sum of the extractive substances was then diluted (100 ml) with n-butyl alcohol. The concentrated alcohol solution on the Schott funnel was eluted

through an alumina layer (neutral, Brockman type II). The butanol solution obtained after multiple elution through the adsorbent layer was concentrated to dryness under reduced pressure at a temperature not higher than 70 °C. A crystalline mass was obtained in an amount of 7.75 g (36% based on the amount (21.5 g) of the starting resinous mass).

Column chromatography was performed on KSK silica gel using the chloroform-methanol-water solvent system (9:1:0.01; 6:1:0.05; 70:12:0.1; 70:23:3). As a result, four glycosidic substances were obtained. These compounds were the previously described cyclosiversiosides E, F, G and astragolose VII.

Quantitative analysis of astragolose VII was also carried out by HETLC using external standards.

To prepare the samples, 1 mg of the substance or test mixture was dissolved in 1 ml of methanol. Applied on a chromatographic plate on 3:1 solution by means of Linomat 5. The latitude of the tracks is 3 mm, the distance between them is 7 mm.

Sigma-Aldrich silica gel on TLCAL foils (Germany) chromatography plates were used for analysis.

Solvent systems: chloroform: methanol: water – 70: 15: 0.1 were used as eluant. Elution was carried out in a glass chamber (distance 7 cm). The plates were air-dried after elution for 15 minutes and developed with 20% alcohol solution of phosphoronolphramic acid, then heated in an oven for 8–10 minutes at 100 °C. The adsorption zones of cycloartane substances and astragolose VII were colored brown.

Chromatography was performed at room temperature on a CAMMAG TLC SCANNER3 instrument. UV detection at wavelengths 400 nm.

The content of astragolose VII (x) in the sample was calculated by the formula:

$$x = \frac{C_{std} \times S_{sub} \times M_{sub}}{S_{std} \times C_{sub}}$$

Where:

C_{std} – is the concentration of the standard solution (mg/ml);

S_{sub} – is the area of the substance peak in the substance (3655.6);

M_{sub} – is the mass of the crystalline substance (7.75 g).

S_{std} – is the peak area of the substance in standard solution (20749);

C_{sub} – is the concentration of the substance solution (mg/ml);

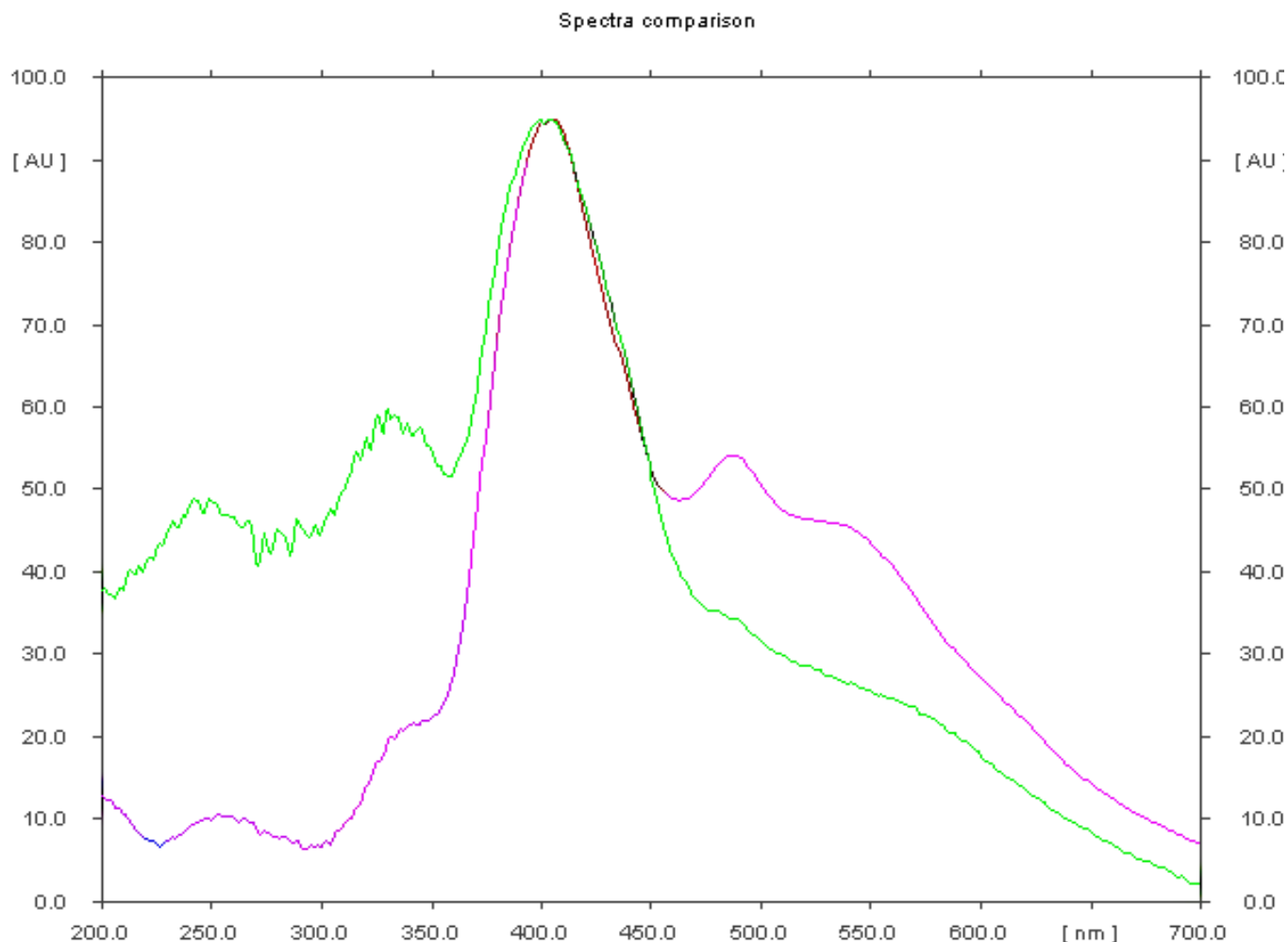


Figure 1. HETL chromatogram astragaloside VII and the sum of cycloartane glycosides

Cyclosiversioside E (1) [7]. 80 mg of compound of composition $C_{40}H_{66}O_{13}$ was isolated, mp 252–254 °C (from methanol).

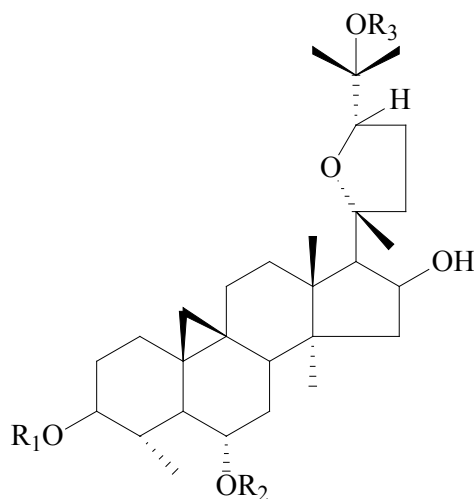
NMR spectrum ^1H (400 MHz, C_5D_5N , δ , ppm, J/Hz, 0-TMS): 0.42 and 0.48 (2H-19, $d, {}^2J=4.95$ of Hz), 0.97, 1.16, 1.18, 1.29, 1.45, 1.66 and 1.80 ($d, 7 \times CH_3$), 3.32 (1H, $dd, {}^3J = 11.74$ and 4.44 Hz, H-3), 3.75 (1H, $td, {}^3J = 8.95$ and 5.29, H-6), 2.58 (1H, $d, {}^3J=7.8$ of Hz, H-22), 3.91 (1H, $dd, {}^3J=8.24$ and 7.34 Hz, H-24), 4.69 (1H, $d, {}^3J=7.45$ of Hz, Xylp H-1'), 4.76 (1H, $d, {}^3J=7.28$ of Hz, H-1'' Xylp).

Cyclosiversioside F (2) [8]. 50 mg of compound of composition $C_{41}H_{68}O_{14}$ was isolated, mp 260–261 °C (from methanol).

NMR spectrum ^1H (400 MHz, C_5D_5N , δ , ppm, J/Hz, 0-TMS): 0.42 and 0.48 (2H-19, $d, {}^2J=4.51$ of Hz), 0.97, 1.16, 1.18, 1.25, 1.45, 1.80 and 1.99 ($d, 7 \times CH_3$), 3.32 (1H, $dd, {}^3J=11.79$ and 4.28 Hz, H-3), 3.75 (1H, $td, {}^3J=8.88$ and 5.30, H-6), 2.56 (1H, $d, {}^3J=9.04$ of Hz, H-22), 3.92 (1H, $dd, {}^3J=8.26$ and 7.01 Hz, H-24), 4.69 (1H, $d, {}^3J=7.45$ of Hz, Xylp H-1'), 4.76 (1H, $d, {}^3J=7.30$ of Hz, H-1'' Glcp).

Cyclosiversioside G (3) [9]. 45 mg of the compound of composition $C_{46}H_{76}O_{17}$ was isolated, mp 226–228 °C (from methanol).

Range of nuclear magnetic resonance ^1H (400 MHz, $CDCl_3$, CD_3OD , δ , m of, J/Hz, 0-TMC): 0.18 and 0.48 (2H-19, dd, $^2J = 4.54$ Hz), 0.95. 1.07. 1.14. 1.17. 1.18. 1.20 and 1.21 (d, $7 \times CH_3$), 3.08 (1H, dd, $^3J = 9.65$ and 3.56 Hz, H-3), 3.46 (1H, td, $^3J = 8.70$ and 3.53 , H-6), 2.30 (1H, d, $^3J = 7.85$ Hz, H-22), 3.77 (1H, dd, $^3J = 5.17$ and 4.98 H-24), 4.59 (1H, d, $^3J = 7.20$ Hz, H-1' Xylp), 4.61 (1H, d, $^3J = 7.56$ Hz, H-1'' Xylp), 4.31 (1H, d, $^3J = 6.74$ Hz, H'-1' Rhap).



Astragoloside VII (4) [10]. 230 mg of compound of composition $C_{47}H_{78}O_{19}$ was isolated, mp 291–292 °C (from methanol).

NMR spectrum ^1H (400 MHz, C_5D_5N , δ , ppm, J/Hz, 0-TMS): 0.18 and 0.56 (2H-19, dd, $^2J = 4$ Hz), 0.90. 1.24. 1.34. 1.37. 1.38. 1.60 and 2.01 (s, $7 \times CH_3$), 3.50 (1H, dd, $^3J = 11.79$ and 4.44 Hz, H-3), 3.76 (1H, td, $^3J = 8.95$ and 5.30 , H-6), 2.77 (1H, k, $^2J = ^3J_1 = ^3J_2 = 11.04$ Hz, H-22), 3.50 (1H, dd, $^3J = 8.26$ and 7.31 H-24), 4.89 (1H, d, $^3J = 7.75$ Hz, H-1' Xylp), 4.86 (1H, d, $^3J = 8.10$ Hz, H-1'' Glcp), 5.06 (1H, d, $^3J = 8.05$ Hz, H-1''' Glcp).

$R_1 = R_2 = Xylp$, $R_3 = OH$

$R_1 = Xylp$, $R_2 = Glcp$, $R_3 = OH$

$R_1 = Rhap(-2-Xylp)$, $R_2 = Xylp$, $R_3 = OH$

$R_1 = Xylp$, $R_2 = R_3 = Glcp$

Experimental biological part. Biological experiments were performed on male rats weighing 180–200 g. The extract of *Astragalus janischewskyi* containing the sum of said cycloartan glycosides (EA) was administered orally at a dose of 10 mg/kg for 10 days. At the end of this period, animals were killed by instant decapitation under mild essential anesthesia. The heart was extracted and used for biochemical studies. Glycogen was determined by Lo S. et.al (1970), lactic and pyruvic acids (MK and PVC) were determined by the methods of Gutman I, Wahlefeld A. W. and Friedeman F., Haugen G. E. (1974; 1943). The redox potential of the milk-pyruvic acid system (M.C./PVK OBP) was calculated by Raiskin M. E. et al. (1970), adenine nucleotides were determined by paper descending

chromatography by Wencstern T. V. and Baev A. A. (1957). Content of cholesterol, triglycerides, phospholipids and not esterified fatty acids defined as it is described in [7], low-new dialdehyde (MDA) according to Steel I. D. and Garishvili T. G. (1977), catalases on Korolyuk M. A., etc. (1988), superoxide dismutases (SOD) according to Dubinina E. E., etc. (1982) and catalases on Korolyuk M. A., etc. (1983).

Statistical processing of the obtained data was carried out by the method of variation statistics with assessment of reliability of differences between control and experimental groups using Student's t-criterion.

Results and their discussion. Studies have shown that ten days of EA administration has a

positive effect on carbohydrate metabolism in myocardium. Under its influence glycogen content increased by 27.7%, PVK content increased by 23.6%, MK content, on the contrary, decreased by 15.8%. As a result, the MK/PVK ORP increased by 5.1 mV. Increase of glycogen level under the action of EA and increase of redox potential of milk-pyruvic acid system in myocardium indicates the prevalence of aerobiosis processes in this case (Table 1). These data have already indicated an increase in the energy input of cardiomyocytes.

However, it was further confirmed that the ATP content of the myocardium was increased by 25% upon multiple administration of EA to animals, the ADF content was not significantly changed, and the AMP level was decreased by 15.4%. Therefore, the ratio of ATP/ADP to ATP/AMP increased by 21.4 and 47.7%. The sum of adenine nucleotides in the myocardium increased by 14.8%. The energy charge of the system increased by 5.3%. The detected changes in the ratio of adenine nucleotides indicate the prevalence of synthesis processes over macroergues recycling processes (Table 1).

Table 1. – Effect of *Astragalus janischewskyi* plant extract on the metabolite content of energy metabolism in the heart muscle of rats ($M \pm m$, $n = 6$)

Experiments medium	Intact animals	Extract of <i>Astragalus</i>	Riboxin
Glycogen, mg%	282 ± 12.2	$360 \pm 16.4^*$	$330 \pm 14.6^*$
Pyruvic acid (PA), mg%	1.48 ± 0.06	1.83 ± 0.08	1.76 ± 0.05
Lactic acid (LA), mg%	52.6 ± 2.4	$44.3 \pm 2.2^*$	$45.6 \pm 1.8^*$
Relatively recovery potential of the system LA/PA, millivolt	-251.6	-246.5	-247.4
Adenosine triphosphoric acid (ATPh), micromole/gramme	2.32 ± 0.06	$2.90 \pm 0.12^*$	$2.76 \pm 0.10^*$
Adenosine diphosphoric acid (ADPh), micromole/gramme	0.68 ± 0.03	$0.70 \pm 0.03^*$	0.70 ± 0.04
Adenosine monophosphoric acid (AMPh), micromole/gramme	0.52 ± 0.02	$0.44 \pm 0.02^*$	$0.46 \pm 0.01^*$
ATPh/ADPh	3.41 ± 0.20	$4.14 \pm 0.22^*$	$3.94 \pm 0.18^*$
ATPh/AMPh	4.46 ± 0.24	$6.59 \pm 0.32^*$	$6.00 \pm 0.28^*$
Sum of adenine nucleotides	3.52 ± 0.08	$4.04 \pm 0.12^*$	$3.92 \pm 0.102^*$
Energy charge	0.76 ± 0.007	$0.80 \pm 0.01^*$	$0.79 \pm 0.005^*$

Note: Reliability with respect to the corresponding indicators of intact animals (reliability level is accepted at $p < 0.05$)

Table 1 also shows that EA, by its effect on the carbohydrate-energy metabolism of the heart muscle, acted similarly to the comparison preparation riboxine, although in some cases it exhibited a more distinct effect. Thus, when riboxine was administered to animals, glycogen and PVK content in the heart increased by only 17.0 and 18.9%, MK decreased by 13.3%. The MC/PVK ORP increased by 4.2 mV. The sum of adenine nucleotides increased

by 11.4% and the energy charge of the system increased by 3.9%.

The effects of EA and riboxine on the lipid content of the myocardium were also similar. Under their influence, cholesterol content decreased by 17.1–16.8 ($p < 0.05$), triglycerides by 15.9–14.0 ($p < 0.05$), and NEGA by 20.5–27.0% ($p < 0.05$). Phospholipid content increased by 21.7–19.8% ($p < 0.05$). At the same time, the effectiveness of

EA and riboxine varied significantly when considering their effects on the activity of the body's antioxidant protection enzymes and lipid peroxidation processes. It can be seen from Table 2 that the administration of EA increases the activity of the key enzymes of the antioxidant system.

Catalase activity in the heart muscle under its influence increased by 27.3% and SOD activity by 17.7% relative to intact animals.

Riboxine contributed to an increase of only 9.1 and 9.8% in the activity of these enzymes in the myocardium.

Table 2. – Effect of *Astragalus janischewskyi* plant extract on activity of body antioxidant protection enzymes and lipid peroxidation processes ($M \pm m$, $n = 6$)

Experiment medium	Katalaza mkat/min/g protein	Superoxide dismutase conventional units/min/mg/ protein	Raspberry dialdehyde nmol/mg protein
Intact animals	13.2 ± 0.52	0.620 ± 0.008	0.280 ± 0.022
Extract of <i>Astragalus janischewskyi</i>	$16.8 \pm 0.64^{*1}$	$0.730 \pm 0.009^{*1}$	$0.150 \pm 0.010^{*1}$
Riboxin	14.4 ± 0.58	$0.680 \pm 0.006^*$	$0.190 \pm 0.012^*$

Note: * – Reliability with respect to the corresponding indicators of intact animals; 1 is the level of validity between groups of animals treated with EA and riboxine ($p < 0.05$)

The level of one of the end products of lipid peroxidation – MDA in the heart of animals treated with EA decreased by 46.4 and those treated with riboxin – 32.1%. The difference in activity of antioxidant protection enzymes in the heart muscle of rats treated with EA and riboxine was in all cases significant.

Thus, in the experiments carried out, it was found that the tested extract *Astragalus janischewskyi* on activating effect on carbohydrate-energy and lipid metabolism of heart muscle in healthy animals is not inferior to the effect of riboxin, but in its antioxidant effect it is reliably superior to the reference preparation used in practice.

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