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THE TOTAL FLAVONOIDS FROM *Thermopsis alterniflora*, *Th. dolichocarpa*, *Vexibia alopecuroides*, AND *Rhaponticum carthamoides* AND THEIR HYPOLIPIDEMIC ACTIVITY

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The total flavonoids have been isolated from the epigeal part of Thermopsis alterniflora *and* Th. dolichocarpa, *and the roots and rhizomes of* Vexibia alopecuroides, *and* Rhaponticum carthamoides *the compositions of the flavonoids present in each of the total extracts have been determined. It was found that the total flavonoid extracts investigated exhibited different degrees of hypolipidemic activity in relation to normal animals and animals with experimental hyperlipidemias. The total flavonoids from* Th. alterniflora *possessed the most pronounced hyperlipidemic action.*

In recent years flavonoids have attracted great attention from researchers as potential effective drugs of low toxicity. The are being used successfully in practical medicine as capillary-strengthening, cholagogic, hypoazotemic, and hepatoprotective agents [1, 2]. More profound experimental-clinical investigations of a series of individual and combined flavonoid preparations as antioxidants, radioprotectors, and antiatheromatous agents are being pursued [2—7].

In the present paper we consider the isolation of the total flavonoids from *Thermopsis alterniflora* Rgl. et Schmalh., *Thermopsis dolichocarpa* V. Nikitin, *Vexibia alopecuroides* (L.) Yakovl., and *Rhaponticum carthamoides* (Willd.) Iljin and give the results of a study of their hypolipidemic activities in relation to normal animals and animals with experimental pathologies accompanied by phenomena of hypercholesterolemia and hypertriglyceridemia.

The epigeal part of *Th. alterniflora* was gathered in the village of Khumsan, Tashkentskaya oblast (Uzbekistan), in the flowering period. We obtained the total flavonoids from a concentrated alcoholic extract, and from them, by column chromatography, we isolated six individual flavonoids: formononetin (20.4% of the total), chrysoeriol (21.4%), apigenin (3.2%, luteolin (22.3%) thermopsoside (2.1%), and cynaroside (4.8%) [8]. The epigeal part of *Th. dolichocarpa* was gathered in the Hissar region (Tadzhikistan) in the fruit-bearing period. After the elimination of lipophilic and resinous substances, a concentrated alcoholic extract yielded the total flavonoids, and from them, by column chromatography, we isolated five individual flavonoids: orobol (14.8% of the total) luteolin (26.3%), genistein (11.2%), cynaroside (9.4%), and genistin (7.6%) [9].

Rhizomes with roots of *Rh. carthamoides* were gathered in Altaiskii krae (Russia) in the flowering period. With the aid of thin-layer chromatography we isolated nine individual flavonoids: quercetin, quercetagetin, luteolin, kaempferol, isorhamnetin, chrysanthemin, cyanin, hesperetin, and hesperetin dirhamnoglycoside.

The method of obtaining the total flavonoids from the roots of *V. alopecuroides* has been published in [10]. In view of the fact that the individual flavonoid compounds and their combined totals exhibited a positive influence on a disturbed lipid metabolism, we studied the action of the total flavonoids obtained on the level of cholesterol and triglycerides in the blood serum. The administration of the substances under investigation in a dose of 50 mg/kg to intact animals for 10 days led to falls in the levels of cholesterol and triglycerides. The greatest falls in the level of cholesterol (by 30%) and of triglycerides (by 33.4%) were observed on the administration of the total flavonoids from *Th. alterniflora* (Table 1).

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TABLE 1. Influence of Flavonoid Preparations on the Level of Lipids (mmole/liter) in the Blood Sera of Intact Rats and of Animals with Experimental Hyperlipidemias ($M \pm m$; $n = 8—10$)

Experimental conditions, indices	Norm		Endogenous HL		Ethanol HL		Triton HL		Adrenaline HL	
	cholesterol	triglycerides	cholesterol	triglycerides	cholesterol	triglycerides	cholesterol	triglycerides	cholesterol	UEFAs
Intact animals	1.63± ± 0.067	0.683± ± 0.056	-	-	-	-	-	-	-	486± ±31
Control (hyperlipidemia)	-	-	2.27± ±0.2	2.38± ±0.15	2.13± ±0.12	1.55± ±0.096	8.24± ±0.28	5.74± ±0.19	1.74± ±0.059	629± 40.9
Total flavonoids of Th. Alterniflora	1.14± ± 0.11	0.455± ±0.023	1.72±* ±0.14	1.78±* ±0.13	1.82± ±0.18	0.993±* ±0.046	5.02±* ±0.87	4.28±* ±0.48	1.21±* ±0.039	442±* ±35
Total flavonoids of Th. Dolichocarpa	1.31± ± 0.16	0.495± ±0.070	1.82± ±0.16	1.87±* ±0.09	1.93± ±0.29	1.14±* ±0.083	5.85±* ±0.17	4.78±* ±0.28	1.35±* ±0.011	504±* ±36.3
Total flavonoids of V. alopecuroides	1.25± ± 0.062	0.526± ±0.036	1.83± ±0.11	1.86±* ±0.12	2.01± ±0.073	0.993±* ±0.1	5.1±* ±0.48	5.0±* ±0.17	1.41±* 0.051	480±* 23.5
Total flavonoids of Rh. Carthamoides	1.32± ± 0.06	0.554± ±0.029	1.91± ±0.17	1.94±* ±0.13	1.96± ±0.11	1.02±* ±0.13	5.32±* ±0.59	4.91±* ±0.18	1.48±* ±0.047	521±* ±28.6

*Significant change in relation to the control, $P < 0.05$. HL — hyperlipidemia.

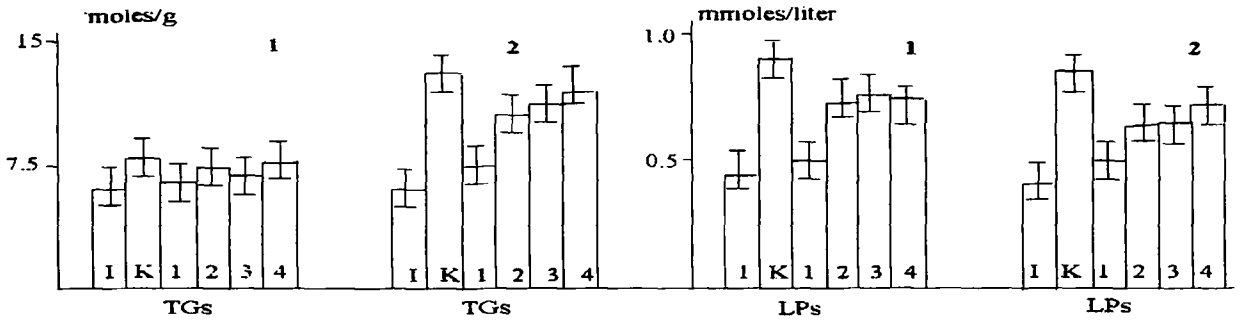

Fig. 1. Influence of flavonoid preparations on the level of triglycerides (TGs) in the liver, and of pre-β- and β-lipoproteins (LPs) in the blood serum, under the conditions of endogenous (*I*) and ethanolic (*II*) hyperlipidemia (HL): I — intact animals; K — control (hyperlipidemia); *1*) HL + total flavonoids of *Th. alterniflora*; 2) HL + total flavonoids of *Th. dolichocarpa*; *3*) HL + total flavonoids of *V. alopecuroides*; *4*) HL + total flavonoids of *Rh. carthamoides*.

The flavonoid preparations investigated prevented the appearance of endogenous hyperlipidemia. The levels of cholesterol and triglycerides in the blood serum of rats that had received the total flavonoids from *Th. alterniflora* were lower than in the untreated animals by 24—25%. At the same time, the level of apo-β-containing lipoproteins (pre-β- and β-lipoproteins) in the blood serum was lower by 34.3% and the levels of liver triglycerides lower by 12%. The effect of the other preparations studied was less pronounced (see Table 1 and Fig. 1)

The administration of the preparations to animals with ethanolic hyperlipidemia also promoted the normalization of an ethanol-disrupted lipid metabolism. Falls were observed in the levels of cholesterol and triglycerides (Table 1) and of apo-β-lipoproteins in the blood serum and of triglycerides in the liver (see Fig. 1). In comparison with the control group, the level of the lipids studied in the blood serum of the experimental animals was lower by 6—36% and that of triglycerides in the liver lower by 5—19.4%.

The therapeutic and prophylactic administration of flavonoid preparations to rats with Triton-induced hyperlipidemia prevented the development of a pronounced disturbance of the lipid metabolism. While in the control group the level of cholesterol rose more than fivefold and that of the triglycerides ninefold, on administration of the preparations under investigation the levels of cholesterol and triglycerides in the blood serum were significantly lower than in the control (see Table 1).

An analogous effect was noted on the administration of these preparations to animals with adrenaline hyperlipidemia. In the blood serum of animals treated with the total flavonoids from *Th. alterniflora* the levels of cholesterol and of unesterified fatty acids (UEFAs) were lower than in the intact animals (Table 1).

The results obtained show that the flavonoid preparations investigated are capable, to various degrees, of lowering the levels of cholesterol, triglycerides, and apo-β-lipoproteins in the blood serum and of triglycerides in the liver. In the mechanism of the hypolipidemic action of these preparations there is, apparently, a suppression of the processes of lipolysis and of the synthesis of triglycerides and of very low-density lipoprotein in the liver, since, in the experiments performed, the degree of expression of endogenous, ethanolic, Triton, and adrenaline hyperlipidemias, the development of which is due to these processes, decreased substantially. The most pronounced fall in the indices of the lipid metabolism that were investigated, both in intact animals and in those with experimental pathologies was observed on the administration of the total flavonoids from *Th. alterniflora*, which is obviously due to the ratio and composition of the individual flavonoids.

EXPERIMENTAL

General Observations. For column chromatography we used silica gel 100/250 μm (Chemapol, Czech Republic) and polyamide. The preparative chromatography of the flavonoids was conducted on Silufol UV-254 plates using the solvent system of [8]. The flavonoids were detected by spraying the plates with a 1% solution of vanillin in concentrated sulfuric acid. Individual flavonoids were identified with the aid of mass, PMR, and ^{13}C NMR spectra, taken on the instruments specified in [9 and 10]. The circular dichroism curve was measured on a Jasco-20 spectropolarimeter.

Isolation of the Total Flavonoids of *Th. alterniflora*. The air-dry comminuted epigeal part (14 kg) was extracted with water three times to eliminate carbohydrates, salts, alkaloids, organic acids, and other hydrophilic substances, and was then extracted exhaustively with 80% ethanol at room temperature. The extract was evaporated to 6 liters and was shaken with chloroform (5 × 2 liters). The aqueous solution was left to stand. After 20—24 the precipitate that had deposited from the chloroform-treated aqueous solution was filtered off, washed with 0.5 liter of chloroform, and dried at 95—100°C. This gave 330.0 g (2.35% on the air-dry weight of the plant) of total flavonoids. Individual flavonoids were isolated from 33.0 g of the total by chromatography on a column of silica gel in a chloroform—ethanol gradient.

Isolation of the Total Flavonoids of *Th. dolichocarpa*. The air-dry comminuted epigeal part of the plant (1.0 kg) was extracted three times with water to eliminate carbohydrates, alkaloids, and other hydrophilic substances and was then extracted exhaustively (8 times) with 80% ethanol at room temperature. The extract was evaporated to a volume of 2 liters and, to free the aqueous alcoholic extract from chlorophyll and resinous substances, it was washed with chloroform (5 × 600 ml). After a day, the purified aqueous extract deposited a precipitate, which was separated off, washed with 0.2 liter of chloroform. and dried at 95—100°C. This gave 26.0 g (2.6% of the air-dry weight of the plant) of total flavonoids. Individual flavonoids were isolated from this mixture by chromatography on a column of silica gel in a chloroform—ethanol gradient system.

Isolation of the Total Flavonoids of *Rh. carthamoides*. Air-dry comminuted rhizomes with roots (35 kg) were exhaustively extracted with 80% ethanol at room temperature. The extract was evaporated to 20 liters and was treated with chloroform (5 × 10 liters). The residual aqueous solution was slowly passed through a column of polyamide sorbent (2000 g), and the flavonoids were desorbed with ethanol. The ethanolic eluate was evaporated in vacuum, and the residue was dried, to give 175 g (0.5% of the weight of raw material) of total flavonoids.

Experimental hyperlipidemia was induced in rats by a day's starvation with no restriction of drinking water [11], by the oral administration of ethanol [6], or by the intraperitoneal injection of Triton WR-1339 in a dose of 225 mg/kg [12] or of adrenaline in a dose of 1.5 mg/kg [6].

We determined the amounts of total cholesterol [13], of UEFAs [14], of apo-β-lipoproteins [15], and of triglycerides in the blood serum and also the levels of triglycerides in the liver [16]. Statistical treatment was conducted by the method of M. L. Belen'kii (1963).

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