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RESEARCH ARTICLE

Study of Chemical Composition and Diuretic Activity of Cherry Fruit Extract

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ABSTRACT:

Qualitative composition and content of flavonoids and hydroxycinnamic acids in cherry (*Prunus cerasus)* fruits extract have been studied by HPLC, system Shimadzu LC-20 Prominence module system equipped with LC-20AD quaternary pump, CTO-20A column oven, SIL-20A autosampler, SPD-M20A diode-array detector, Luna C 18 (4.6mm×150mm, i.d. 5 mkm) column from Phenomenex®. 5 phenolic compounds were identified as flavonoid glycosides and hydroxycinnamic acids: chlorogenic acid (1.88%), quercitrin (1.69%), and rutin (1.11%) in high amount. It also contains tannins: gallic acid (0.07%), and catechin (0.23%). The total amount of determined phenolic compounds in cherry extract was 4.99%. The ratio of flavonoids to hydroxycinnamic acids was 3:2. Toxicity, diuretic activity, rheological properties of blood and membrane stabilizing activity were determined. Rats were randomly divided into 2 groups: control group (given water) and experimental group (given Cherry extract). Results show diuretic, hypouricosemic, and membrane-stabilizing effects due to improvement of kidney filtration and suppressing water reabsorption, excretion of uric acid and creatinine from the body and reducing the degree of hemolysis of red blood cells and preventing damage to erythrocyte membranes. Therefore, according to our study, cherry fruit extract might potentially be used in treatment of various chronic diseases (e.g. gout).

KEYWORDS: cherry, fruits, extract, hydroxycinnamic acids, flavonoids, diuretic, hypouricosemic, and membrane-stabilizing activity

INTRODUCTION:

Cherry *Prunus cerasus* L. from family *Rosaceae* is one of the well-known plants and used as a fresh fruit, but also in the form of canned food, jam and juice[1]. Medicinally regular uses of cherry and cherry juice tend to have uricosuric, anti-inflammatory effects[2]. Chemical constituents of cherry fruits have been investigated last time quite well. Ripe cherry fruits contain carbohydrates as sugars 8-15%, hemicellulose– 0,1%, fibers 0,5%, pectins up to 11%; organic acids till

2% (malic acid prevail 1,2% and citric, lactic, quinic, acetic, succinic, formic); phenolic compounds 0,11-0,49%; among them coumarins (umbelliferon, herniarin, magalebosid), flavonoids–mainly quercetin glycosides up to 2%. Anthocyanidin composition has also been investigated well and cyanidin, mecocyanidin and others have been found out[6]. Presence of leucoantho cyanidins, catechins as well as hydroxycinnamic acids was determined. Skin of cherry fruits contains more vitamins than pulp. Such vitamins as (mg%) ascorbic acid–15, B_1–0,03-0,2, B_6–0,05-0,08, B_{15}–0,08, PP up to 0,4 as well as carotenoids were determined in cherry fruits[1].

It is known that flavonoids possess diuretic activities[2]. The flavonoid acacetin exhibited significant diuretic effect was studied by Lipschitz test methods using Wistar rats as animal model[11]. Perhaps, flavonoids and hydroxycinnamic acids take part in anti-inflammatory activity and they were able to inhibit the arachidonate conversion through the lipoxygenase pathway, as it was seen on Zymosan model[3].

Therefore, obtaining of extract from cherry, study of its flavonoids and hydroxycinnamic acids composition by HPLC, as well as diuretic activity may help to understand the mechanism of action and play role for further standardization of the extract.

The aim of present work:
Was determination of qualitative composition and content of phenolic compounds of cherry fruits extract, its toxicity, diuretic activity, influence on rheological properties of blood and membrane stabilizing activity.

MATERIAL AND METHODS:

The object of the study was extract obtained from *Prunus cerasus* fresh fruits 'Vladimirskaya" variety harvested in July, in Kharkiv region in 2017.

0.5 kg of fresh cherry fruits were splited, stones were removed and fruits were comminuted to puree. 400 g cherry puree was mixed with 800 ml 50% ethyl alcohol in the flask and the mixture was centrifuged. 600 ml of liquid part was decanted into flask. 300 ml of obtained extract was used for chemical analysis, another 300 ml of the extract was used for pharmacological research and alcohol was eliminated in it with the extract concentration till 100 ml.

For preliminary identification of biologically active substances qualitative reactions, paper chromatography (PC) and thin layer chromatography (TLC) were used.

Flavonoids and hydroxycinnamic acids were studied by one-and two-dimensional TLC and PC with valid samples of compounds in the solvent systems: glacial acetic acid-water-ethyl acetate (20:20:60), n-butanol-acetic acid-water (4: 1: 2), and 15% acetic acid. Obtained chromatograms were treated by solution of amino ethyl ester of diphenyl boric acid in methanol, macrogol solution and ammonia solution with azo coupling reagent. After that, the plates were viewed in UV-and daylight before and after treating by reagents.

The profile of flavonoids and hydroxycinnamic acids was determined using an HPLC system Shimadzu LC-20 Prominence module system equipped with LC-20AD quaternary pump, CTO-20A column oven, SIL-20A autosampler, SPD-M20A diode-array detector and LC-20 chemstation for data analysis were used[4, 5, 7]. Chromatographic separations were carried out using Luna C 18 (4.6mm×150mm, i.d. 5 mkm) column from Phenomenex®. All measurements were carried out in areas which meet the requirements of the measurement methods and requirements of the operational documents for the measuring equipment used during the measurements (climatic conditions, lighting, sound and vibration isolation, power supply parameters , availability of water supply and sewage, equipment with grounding, etc.), stable temperature (18-25°C) and relative humidity (70±5%) [4, 5].

Ultrapure water was obtained from Milli-Q® apparatus, trifluoroacetic acid and acetonitrile HPLC grade were purchased from Sigma-Aldrich (USA).

All dilutions were performed in standard volumetric flasks. Solvents and solutions were filtered through 0.45 mkm nylon filters before use[4].

Volume of the test sample introduced–5 µl. The temperature of the column oven was set at 35°C. The mobile phase flow rate was 1 ml/min. Detecting wavelength 330 nm (for hydroxycinnamic acids, flavonoid glycosides), 370 nm (for flavonoid aglycones), 280 nm (for tannins). Mobile phase: eluent A was: 0.1 trifluoroacetic acid in water; eluent B was trifluoroacetic acid in acetonitrile[5]. Program of gradient elution can be seen in table 1.

Table 1. Program of gradient elution

Chromatography time (min.)	Eluent A, %	Eluent B, %
0–5	95	5
5–35	95→75	5→25
35–40	75	25
40–60	75→50	25→50
60–65	50→20	50→80
65–70	20	80
70–85	95	5

The components were identified by retention time and by the correspondence of UV spectra to standard substances.

Calculations were made by the formula, %

$$\text{Assay},\% = \frac{A_{pr} \times m_{st} \times V_{pr} \times P \times 100}{A_{st} \times V_{st} \times m_{pr} \times 100}$$

Where,
A_{pr}*:* Area of peak of the substance on the chromatogram of solution under investigation,
A_{st}*:* Area of peak of the substance on the chromatogram of standard solution,
m_{st}*:* Mass of standard sample of substance in standard solution (mg),
m_{pr}*:* Mass of cherry fruit extract (mg),
V_{pr}*:* Dilution of solution under investigation (ml),
V_{st}*:* Dilution of standard solution (ml),
P: Activity of the standard (%).

Reproducibility of results was confirmed by carrying out the research for three times.

The Cherry extract's safety was studied with investigation of acute toxicity of laboratory animals (male and female white rats) after single intragastric administration for determination of the LD_{50} dose in accordance with the guidelines of Ukrainian State Expert Center of Ministry of Health[12-14]. The research was performed in accordance with National guidelines, "The General Ethical Principles of Animal Experimentation" (Ukraine, 2001), which is consistent with the provisions of "The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [12-14]. The limiting measure in determination of the LD_{50} was the maximum dose of fourth-grade toxicity (low-toxic substances)–5000 mg/kg. If the dose administered to animals does not cause their death, the introduction of high-dose is impractical[14]. So, 24 experimental animals were randomized into 2 groups: 1-st group of Intact Control (IC)–6 male and 6 female rats which were given solvent (water) and 2-nd group–6 male and 6 female rats which were given Cherry extract in dose 5000 mg/kg. The animals were observed for 14 days.

A unified methodical scheme was used to determine the dynamics of diuresis, which makes it possible to estimate the state of the filtration capacity of the kidneys and tubular reabsorption of water, to study the effect of Cherry extract on the state of the urinary system and to determine the presence of diuretic and hypouricosemic activity [15-17].

Diuretic activity was studied in terms of daily spontaneous diuresis and forced diuresis (with a liquid load in a volume of 3% of animal's body weight, which was done 1 hour after the use of Cherry extract) in rats. Spontaneous diuresis was studied on the first day of the study after a single dose and 20th-day of intragastric administration of Cherry extract in dose of 5 ml/kg.

After 2nd and 21st days from the start of the experiment, immediately after the study of spontaneous diuresis, forced diuresis was studied after the 2nd and 21st intragastric administration of Cherry juice in dose of 5 ml/kg. In the same mode, control group rats received an equivalent amount of solvent (drinking water). In the study of spontaneous diuresis for collection of urine, the rats were placed in metabolic cages for 24 hours and in the study of forced diuresis-for 3 hours[15,16]. At the end of the experiment, animals were subjected to euthanasia according to ethical requirements when working with laboratory animals and collected blood for serum production. The concentration of creatinine and uric acid in urine and serum was determined with the biochemical set of reagents from "Filisit" production (Ukraine) [18,19].

Excretion of creatinine and uric acid was calculated according to the formula[20]. According to the given method[21], the velocity of glomerular filtration and relative reabsorption of fluid were calculated.

The influence of Cherry extract on the rheological properties of blood has been investigated. Outbred white rats were used for the experiment. After randomization, IC group animals had intragastric administration of water whereas the experimental group animals had intragastric administration of Cherry extract in dose of 5 ml/kg during 3 weeks. At the end of the experiment, after the incision of the tip of the rat tail, the time of coagulation of the first drop of blood was determined [18]. The degree of effect on blood coagulation was determined by the ability of Cherry extract to reduce the time of blood coagulation in rats compared to IC and expressed as a percentage (%).

Biologically active substances (BAS) of cherry as flavonoids, hydroxycinnamic acids and other polyphenols, that might play role as antioxidant, anti-inflammatory, membrane stabilizing and cytoprotective agents, can restore biomembranes and functional activity of cells, tissues, organs, systems and the organism as a whole. Therefore, it would make sense to study the influence of cherry extract on the functional state of cell membranes under erythrocyte hemolysis in rats and the ability to exhibit membrane-stabilizing activity.

The study of the influence of cherry extract on cell membranes was performed under conditions of spontaneous hemolysis of rat erythrocytes by method of F.C. Jager [22]. The method [22] was based on the

photoelectrocolorimetric determination of ectoglobular hemoglobin, which appeared in the media as a result of spontaneous lysis of erythrocyte membranes caused by lipid peroxidation with air oxygen.

For the experiment, 12 outbred white rats were divided into 2 groups. After randomization, animals received intragastrically Cherry extract in dose of 5 ml/kg for three weeks. IC group rats during this period received an equivalent amount of solvent (water) intragastrically. Further, the degree of hemolysis of erythrocytes was determined by the method[22]. The membrane-stabilizing effect of Cherry extract was determined by the ability to prevent damage to erythrocyte membranes and was estimated by the change in the number of hemolysed erythrocytes in animals from experimental groups compared to IC group rats and expressed as %.

RESULTS:

After our preliminary study of BAS of cherry extract, the presence of hydroxycinnamic acids and flavonoids, including chlorogenic acid, cyanidin-3-O-glucoside and rutin were established by TLC and PC with authentic samples in the extract.

As a result of the study of the extract by HPLC, the phenolic compounds were determined as derivatives of tannins, hydroxycinnamic acids and flavonoids. Graphical result of determination of phenolic compounds can be seen in figure 1 and UV-spectrums of standard samples (SS) of basic phenolic substances–in figure 2.

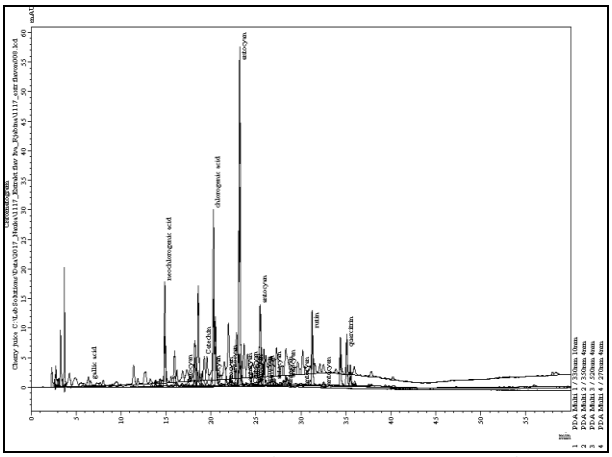
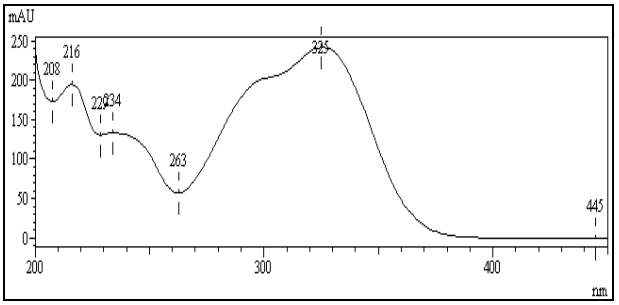


Fig.1: Graphical results of HPLC investigation of phenolic compounds in Cherry fruits liquid extract

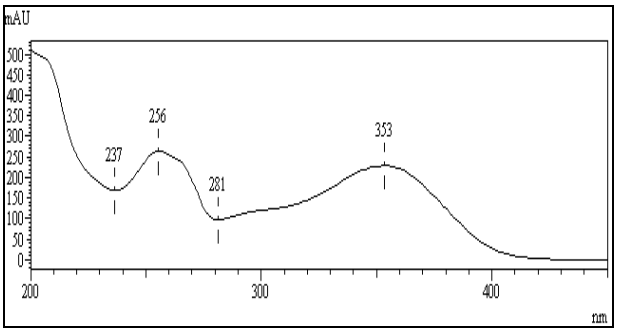
Among Cherry extract compounds 5 were determined authentically: two acids as gallic and chlorogenic, whereas flavonoids were represented as flavan (catechin) and flavonol glycosides (quercitrin, rutin) (Table 2).

Table 2. Phenolic substances of Cherry fruits liquid extract

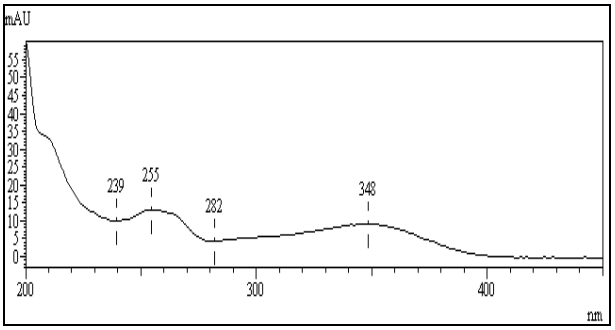
Components	Retention time, min	Area of peak (mAU)	Length of wave (nm)	Content, %
Gallic acid	6.8	8862	280	0,0664
Chlorogenic acid	20.0	278298	330	1,8798
Rutin	31.1	86093	330	1,11295
Quercitrin	35.8	58891	370	1,69213
Catechin	19.6	48634	370	0,23448



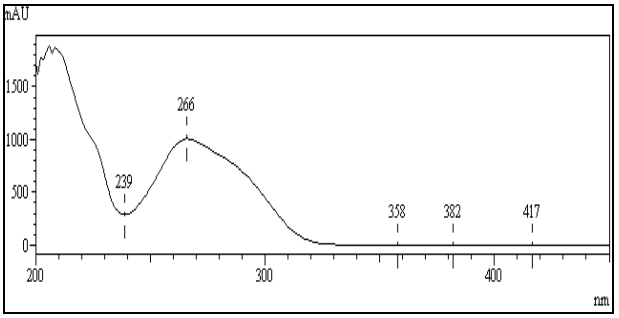
Chlorogenic acid



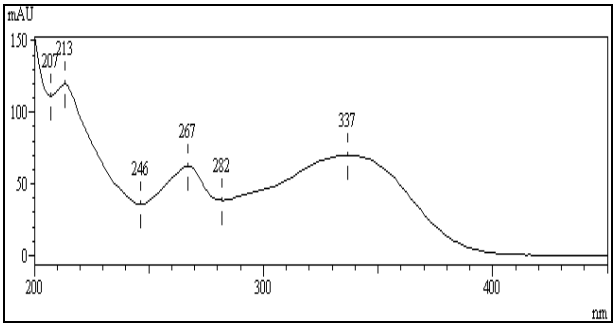
Rutin



Quercitrin



Gallic acid



Catechin

Fig. 2: UV-spectrums of SS of basic phenolic substances

It is known that cherry fruits are part of our everyday diet, but it was obligatory to determine toxicity of Cherry extract according to the toxicological classification of substances by K. K. Sidorov. It was determined that after single intragastric administration of water and Cherry extract all rats were neat, active, responsive to auditory and visual stimuli, processes of urination and defecation were normal, respiratory failure and convulsions were not observed, reflex excitability has been saved. The rats of both experimental groups consumed food and water normally. The death of animals is not registered during the entire period of observation. Thus, single intragastric administration of maximal dose of Cherry juice in 5000 mg/kg in rats did not cause the death of the animals and pathological changes on the functional state of the rats' organism. Therefore, the determination of the LD_{50} dose of the extract was impossible. So, it was established the absence of toxicity of the Cherry extract in dose of 5000 mg/kg and according to toxicological classification of substances by K. K. Sidorov the cherry extract after intragastric administration belong to V toxicity class– practically harmless substances[16].

The results of the study of influence of Cherry extract on indicators of kidney function of rats with spontaneous daily diuresis can be seen in table 3. Influence of Cherry juice on indicators of kidney's function of rats with diuresis with water loading presented in table 4.

Table 3. Influence of Cherry extract on indicators of kidney's function of rats with spontaneous daily diuresis

Indicators	Term	Intact control	Cherry extract, 5 ml/kg
Number of animals in the group, heads	-	6	6
Body weight of animals, g	Initial data	206,67±5,11	213,33±11,01
	3 weeks	219,17±5,39	200,83±10,83
Urine volume (D), ml	Initial data	4,52±0,21	7,23±0,79*
	3 weeks	3,52±0,51	2,93±0,45
Daily diuresis (V), ml/100 g	Initial data	2,20±0,12	3,39±0,33*
	3 weeks	1,62±0,25	1,48±0,22
Amount of creatinine in urine, mg	Initial data	2,47±0,39	6,03±0,43*
	3 weeks	2,77±0,33	8,13±0,81*
Creatinine in urine, mmol/l	Initial data	5,19±0,74	7,59±0,49*
	3 weeks	7,17±0,65	29,81±2,25*/**
Creatinine excretion, mmol/100 g	Initial data	2,64±0,45	2,43±0,39
	3 weeks	4,99±0,78	22,54±3,70*/**
Uric acid in urine, mmol/l	Initial data	2,37±0,16	2,45±0,13
	3 weeks	1,51±0,24	2,97±0,13*
Excretion of uric acid, mmol/100 g	Initial data	1,20±0,13	0,75±0,07*
	3 weeks	1,06±0,25	2,29±0,40*/**

Note: *-rejection rate is likely related to the group of data of intact controls, p<0.05;

****-rejection rate significantly on the initial data, p<0.05.**

Table 4. Influence of Cherry extract on indicators of kidney's function of rats with diuresis with water loading

Indicators	Term	Intact control	Cherry extract, 5 ml/kg
Number of animals in the group, heads	-	6	6
Body weight of animals, g	Initial data	206,67±5,11	213,33±11,01
	3 weeks	219,17±5,39	200,83±10,83
Urine volume (D), ml	Initial data	3,58±0,41	2,93±0,31
	3 weeks	3,20±0,54	0,78±0,13*/**
Daily diuresis (V), ml/100 g	Initial data	1,73±0,18	1,39±0,16
	3 weeks	1,46±0,25	0,39±0,08*/**
Daily diuresis (V), ml/100 g×h	Initial data	0,58±0,06	0,47±0,05*
	3 weeks	0,49±0,08	0,13±0,03*/**
Serum creatinine, mmol/l	Initial data	0,11±0,01	0,08±0,02
	3 weeks	0,14±0,01	0,14±0,01
Creatinine in urine, mmol/l	Initial data	2,79±0,34	2,47±0,48
	3 weeks	3,99±0,57	11,12±1,35*/**
Creatinine excretion, mmol/100 g	Initial data	1,77±0,35	1,75±0,23
	3 weeks	3,41±0,94	35,19±7,94*/**
Velocity of glomerular filtration, ml/100g×h	Initial data	13,91±0,66	14,87±0,94
	3 weeks	13,22±1,86	10,52±2,99
Relative fluid reabsorption, %	Initial data	95,82±0,47	96,84±0,40
	3 weeks	96,29±0,29	96,68±0,35
Serum Uric acid, mmol/l	Initial data	62,00±3,58	87,67±3,36*
	3 weeks	88,51±11,00	94,09±8,37
Uric acid in urine, mmol/l	Initial data	0,73±0,08	0,42±0,04*
	3 weeks	0,55±0,05	0,90±0,19*
Excretion of uric acid, mmol/100g	Initial data	0,44±0,06	0,32±0,04
	3 weeks	0,47±0,12	2,78±0,73*

Note: *-rejection rate is likely related to the group of data of intact controls,.

p<0.05; **-rejection rate significantly on the initial data, p<0.05.

Results of the three-week study of influence of cherry extract on rheological properties of rats blood and the state of rats cell membranes in the model of erythrocytes spontaneous hemolysis by Jager F.C. presented in tables 5 and 6.

Table 5. Three-week influence of Cherry extract on rheological properties of rat blood

Indicators	Intact control	Cherry extract, 5 ml/kg
Number of animals in the group, heads	6	6
Clotting time, sec	69,67±8,04	91,67±9,85*
Hemolytic action,%	-	24,0

Note: *-rejection rate is likely related to the group of data of intact controls, p<0.05

Table 6. Three-week influence of Cherry extract on the state of rats' cell membranes in the model of erythrocytes spontaneous hemolysis by Jager F.C.

Indicators	Intact control	Cherry extract, 5 ml/kg
Number of animals in the group, heads	6	6
Degree of erythrocytes hemolysis, %	10,36±0,74	5,86±0,47*
Membranostabilizing activity, %	-	43,4

Note: *-rejection rate is likely related to the group of data of intact controls, p<0.05

DISCUSSION:

The total amount of determined phenolic compounds in Cherry extract was 4.99%. The extract contained 3.04 % of flavonoids as well as hydroxycinnamic acids–1.88%. The ratio of flavonoids to hydroxycinnamic acids was 3:2.

Cherry may acquire new interest, mainly due to the fact that it can be considered as a "functional food" because of its high content of biologically active substances. As the results showed, it contains chlorogenic acid (1.88%), quercitrin (1.69%), and rutin (1.11%) in high amount. It also contains tannins: gallic acid (0.07%) and catechin (0.23%).

Diuretic and membrane stabilizing activity of cherry extract may be related to the presence phenolic compounds as flavonoid rutin and quercetrin, chlorogenic acid (CGA) and catechin. It is known that rutin possesses antioxidant activity. Traditionally, it is used as an antimicrobial, antifungal, and anti-allergic agent. However, current research has shown its multi-spectrum pharmacological benefits in treatment of various chronic diseases, such as cancer, diabetes, hypertension, and hypercholesterolemia[8, 10, 11]. It is known role of flavonoids and hydroxycinnamic acids in diuretic effects[2, 9, 11]. It was reported that catechins have anti-inflammatory, anti-urolithiasis and anti-oxidative activities[10].

Results of the investigation of spontaneous diuresis demonstrates that Cherry extract after intragastric administration in dose of 5 ml/kg for 21 days (3 weeks) has diuretic effect at the beginning of the experiment– increased urine volume by 60% relatively to the IC group (in 1,6 times) ($p<0.05$) and daily diuresis by 54% (1.5 times) ($p<0.05$) (Table 3). After three weeks of consumption of Cherry extract the volume of diuresis and daily diuresis does not change compared with IC (Table 3). This indicates that the Cherry extract only eliminates excess of water and does not suppress the body's water balance.

It was shown that Cherry extract under conditions of forced diuresis (with water loading) does not change diuretic activity at the beginning of the experiment after single use and suppresses it after prolonged administration (Table 4). Thus, Cherry extract causes a significant reduction of the values of Urine volume on 73% ($p<0.05$) and 76% ($p<0.05$) relatively initial data and IC as well as Daily diuresis on 72% ($p<0.05$) and 73% ($p<0.05$) respectively and this confirms decreasing in the glomerular filtration rate on 30% relative to the initial data and of IC (Table 4). This indicates that in forced diuresis after prolonged use of Cherry extract it improves the filtration ability of the kidneys, inhibits the reabsorption of water and stimulates the elimination of fluid from the body, which is very important in the treatment of gout.

Strong hypouricosemic action of cherry juice was established under conditions of spontaneous diuresis and forced diuresis (with water loading) due to the effect on the value of excretion of creatinine and excretion of uric acid, which are markers of nitrogen balance and purine metabolism respectively (Table 3-4). Thus, under conditions of spontaneous diuresis, Cherry extract after 3 weeks of application with respect to the initial data and data of IC, stimulates creatinine excretion by 9.3 times ($p<0.05$) and 4.5 times ($p<0.05$) respectively, and uric acid excretion by 3 times ($p<0,05$) and 2,2 times ($p<0,05$) respectively (Table 3). In the case of forced diuresis the Cherry extract in 3-week application significantly relative to the initial data and data of intact control stimulates the creatinine excretion by 20 times ($p<0,05$) and 10 times ($p<0,05$) respectively and excretion of uric acid in 8.7 times ($p<0,05$) and 5.9 times ($p<0,05$) respectively (Tab. 4). These results indicate a powerful ability of Cherry extract to excrete uric acid and creatinine (pathogenic products of nitrogen and purine exchanges marker respectively) and allow recommending Cherry extract in gout treatment.

The influence of cherry extract on the rheological properties of blood has been investigated because excessive fluid elimination from the body can lead to increased blood density and the risk of thrombosis. It was established the cherry extract causes increase of blood coagulation time by 24% ($p<0,05$) relatively in comparison with the data of IC (Table 5). This indicates possible weak antiplatelet or anticoagulant properties

perhaps due to some phenolic compounds as coumarins.

It was also established the ability of cherry extract to prevent damage to erythrocyte cells membranes and significantly reduce the degree of erythrocytes hemolysis in 1.8 times ($p<0,05$) compared with intact control (Table 6). The membrane stabilizing activity of the cherry juice is 43% ($p<0,05$) (Table 6) and may determine its cytoprotective, antioxidant and metabolic properties and positive effects on the function of body cells (of the kidneys, blood cells, liver etc.).

Therefore, content of phenolic compounds as flavonoids and hydroxycinnamic acids were determined in obtained extract. The extract showed diuretic, hypouricosemic, membrane stabilizing activity. The results confirmed prospects for further study and development of new drugs, which could be used in treatment of various chronic diseases.

CONCLUSIONS:

Study of chemical composition and content of cherry fruits extract was performed by HPLC. 5 phenolic compounds were identified as hydroxycinnamic acids and flavonoid glycosides.

Thus, the results of pharmacological studies indicate diuretic, hypouricosemic, membrane-stabilizing effect of Cherry extract and its positive effect on the rheological properties of blood, which allows recommend cherry juice for further research in order to create a new drug for the treatment of gout.

Conflict of Interest:

Authors declare no the conflict of interest.

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