

Comparative Investigation of the Diuretic and Anti-Depressant Activity of Liquid Extracts of *Crataegus Sanguinea*

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Abstract.

The different types of raw material of hawthorn blood-red (*Crataegus sanguinea* Pall., *Rosaceae* family) are promising sources of medicinal products. The comparative study of the diuretic and antidepressant activities of liquid extracts based on the leaves, flowers, shoots and fruits of hawthorn blood-red there was carried out. In the course of the experiments, the diuretic activity of the liquid extract of hawthorn leaves of blood red was revealed, as well as the antidepressant activity of liquid extracts of the flowers, shoots and fruits of hawthorn blood-red.

Key words: flavonoids, hyperoside, rutin, diuretic activity, saluretic activity, creatininuretic activity, antidepressant activity, liquid extracts, hawthorn blood-red, *Crataegus sanguinea* Pall.

INTRODUCTION

Hawthorn blood-red (*Crataegus sanguinea* Pall., the *Rosaceae* family) is widespread in the territory of the Russian Federation [1]. Currently only medicines based on hawthorn flowers and fruits are used as cardiogenic agents in the medical practice of the Russian Federation [2, 4]. Previously, in preclinical studies using white outbred rats the presence of a diuretic and antidepressant effect for a soft extract from fruits of hawthorn blood-red was established [3]. However, the literature data indicate that hawthorn medicines have a complex effect [2]. Leaves and shoots of hawthorn blood-red are one of the promising types of medical plant raw materials [7]. It should be noted that the leaves of hawthorn together with flowers (shoots) are used in foreign medicine [10], moreover flavonoids (hyperoside, rutin, rhamnoside vitexin, etc.) are considered active ingredients. In prior years the presence of diuretic activity was established for rutin, and antidepressant activity was established for hyperoside [2, 9]. The purpose of this research is a comparative study of the diuretic action and antidepressant activity of liquid extracts based on hawthorn blood-red flowers, shoots, leaves and fruits.

MATERIALS AND METHODS

We obtained liquid extracts based on dried leaves, flowers, shoots and fruits of hawthorn blood-red in laboratory conditions. The raw materials were harvested in the Samara Region in 2016. The all liquid extracts were obtained in the ratio «raw materials-extragent» 1: 1. Ethanol 70% was the extragent in all cases. The content of total flavonoids in terms of hyperoside was evaluated in all medicines. The analysis of the content of total flavonoids was carried out based on the methods developed by us earlier [4]. The results are shown in Table 1. The all medicines obtained were investigated by us for the presence of diuretic and antidepressant activity.

Studies were carried out on white outbred rats of both sexes weighing 200-220 g. Animals were kept in a vivarium on a normal diet with free access to water. Each group was consisted of ten animals. The investigated medicines were administered intragastrical through a probe at a dose of 100 µl/kg [5]. The ethanol 70% was used as a control.

The diuretic activity of hawthorn liquid extract at a dose of 100 mg / kg was determined in chronic experiments [5, 6]. The furosemide in dose of 1 mg / kg was used as a comparative drug for 4-hour experiment because it is fast and short-acting diuretic

agent. The hydrochlorothiazide in high therapeutic doses of 20 mg / kg was a comparative drug for 24-hour experiments because it is slow and long-acting diuretic agent. On the experiment day the animals of the study group were administered the study medicines intragastrical on a background of 3% of the water load, the control for comparative drugs was an identical volume of water. The animals were placed in the exchange cells. The 4 h and 24 h portions of urine were assembled. The renal excretion of water was determined, concentration of sodium and potassium was detected by flame photometry using flaming fluid analyzer PAZH-1, concentration of creatinine was registered by colorimetric method using photocolormeter KFK-3.

The study of antidepressant activity was carried out using the "Desperation" test [8]. In this test individual time of active attempts of animals to get out of the water for five minutes were fixed. The amitriptyline at a dose of 5 mg / kg was used as a synthetic reference drug with antidepressant activity. In this case the water in a volume of 0.5% of the animal body weight was used as a control. All medicines were administered once against a background of a similar water load. The experiment was performed 1 hour after the medicine administration.

The data were processed statistically by the Mann-Whitney test with the Bonferroni adjustment.

Table 1. The content of total flavonoids in the medicines of hawthorn blood-red

No.	Medicine	The content of total flavonoids calculated on hyperoside
1.	Liquid extract from flowers of hawthorn blood-red	0.238±0.012%
2.	Liquid extract from leaves of hawthorn blood-red	0.314±0.016%
3.	Liquid extract from fruits of hawthorn blood-red	0.254±0.013%
4.	Liquid extract from shoots of hawthorn blood-red	0.050±0.003%

RESULTS AND DISCUSSION

During the experiments it was found that after 4 hours only a liquid extract from hawthorn blood-red leaves at a dose of 100 µl / kg contributed to a significant increase in diuresis (by 20%), natriuresis (by 38%) and kaliuresis (by 77%) in the animal experimental group relative to indicators of water-alcohol control,

which indicates a decrease in tubular reabsorption (Table 2). Liquid extracts from flowers, shoots and fruits of hawthorn blood-red did not lead to significant changes of tested parameters of the excretory kidneys function in comparison with water-alcohol control.

The comparative drug furosemide with single intragastric administration at a threshold dose of 1 mg / kg

promoted a significant increase in diuresis (by 23%) and natriuresis (by 31%) for 4 hours of the experiment in the animals experimental group relative to the water control mainly due to reduced tubular reabsorption (Table 3).

Table 2. The effect of hawthorn blood-red liquid extracts at a dose of 100 µl / kg on the rat's excretory function of the kidneys after 4 hours of the experiment

Medicine	Duresis,ml	Natriuresis, µm	Kaliuresis,µm	Renal excretion of creatinine,mg
Control (Ethanol 70%)	1.48±0.13	216.15±30.58	114.77±13.17	2.35±0.22
Liquid extract from flowers of hawthorn blood-red	1.10±0.08	226.21±20.95	143.03±13.81	2.68±0.28
Liquid extract from leaves of hawthorn blood-red	1.77±0.11*	297.94±26.37*	168.74±10.58*	2.80±0.19
Liquid extract from fruits of hawthorn blood-red	1.41±0.06	255.45±21.36	150.31±13.16*	2.47±0.21
Liquid extract from shoots of hawthorn blood-red	1.24±0.09	229.40±21.01	108.09±6.06	2.59±0.21

* - the statistical assurance of the differences in the parameters of the experimental group from the parameters of the animal control group receiving 70% ethanol, p <0.05.

Table 3. The effect of furosemide intragastric administration at a threshold dose of 1 mg / kg on the rat's excretory function of the kidneys after 4 hours of the experiment

Medicine	Duresis,ml	Natriuresis, µm	Kaliuresis,µm	Renal excretion of creatinine,mg
Control(Water)	1.98±0.11	403.68±32.08	104.10±10.72	2.73±0.29
Furosemide	2.44±0.13*	529.24±43.46 *	123.76±6.83	3.14±0.25

* - the statistical assurance of the differences in the parameters of the experimental group from the parameters of the animal control group receiving water, p<0.05.

Table 4. The effect of hawthorn blood-red liquid extracts at a dose of 100 µl / kg on the rat's excretory function of the kidneys after 24 hours of the experiment

Medicine	Duresis, ml	Natriuresis, µm	Kaliuresis,µm	Renal excretion of creatinine,mg
Control (Ethanol 70%)	1.70±0.14	303.15±26.88	140.03±16.55	3.29±0.38
Liquid extract from flowers of hawthorn blood-red	1.41±0.13	316.37±35.27	139.03±16.74	3.23±0.31
Liquid extract from leaves of hawthorn blood-red	2.19±0.11*	392.55±29.20*	167.98±18.34	4.49±0.32*
Liquid extract from fruits of hawthorn blood-red	1.65±0.14	309.74±36.82	156.40±18.67	3.86±0.42
Liquid extract from shoots of hawthorn blood-red	1.67±0.14	364.89±33.45	146.86±20.97	3.37±0.30

* - the statistical assurance of the differences in the parameters of the experimental group from the parameters of the animal control group receiving 70% ethanol, p <0.05.

Table 5. The effect of hypothiazide intragastric administration at an average therapeutic dose of 20 mg / kg on the rat's excretory function of the kidneys after 24 hours of the experiment

Medicine	Duresis,ml	Natriuresis, µm	Kaliuresis,µm	Renal excretion of creatinine,mg
Control (Water)	2.73±0.17	462.88±52.16	155.86±20.70	5.27±0.55
Hypothiazide	3.83±0.22 *	711.31±90.84 *	241.60±19.26 *	6.85±0.59

* - the statistical assurance of the differences in the parameters of the experimental group from the parameters of the animal control group receiving water, p<0.05.

Table 6. Antidepressant activity of hawthorn blood-red liquid extracts

Medicine	The time of active movement, s	The time of active movement, %
Control (Water)	91.14±8.62	100%
Amitriptyline	140.00±10.02 *	154%
Control (Ethanol 70%)	87.14±3.11	100%
Liquid extract from flowers of hawthorn blood-red	174.86±5.98 Δ	201%
Liquid extract from shoots of hawthorn blood-red	146.14±10.88 Δ	168%
Liquid extract from leaves of hawthorn blood-red	100.86±5.94	116%
Liquid extract from fruits of hawthorn blood-red	113.86±7.59 Δ	131%

* - the statistical assurance of the differences in the parameters of the experimental group from the parameters of the animal control group receiving water, p<0.05;

Δ - the statistical assurance of the differences in the parameters of the experimental group from the parameters of the animal control group receiving 70% ethanol, p<0.05.

Consequently, a liquid extract of hawthorn blood-red leaves at a dose of 100 µl / kg with a single intragastric administration for 4 hours of experiment promoted an increase in diuresis and natriuresis similarly to the action of furosemide at a threshold dose of 1 mg/kg.

At the same time, within 24 hours of the experiment, the liquid extract of the hawthorn blood-red leaves in the animals experimental group resulted in an increase in renal excretion of

water (by 29%), sodium (by 30%) and creatinine (by 36%) relative to the water-alcohol control which indicates a decrease in tubular reabsorption and an increase in glomerular filtration (Table 4). Liquid extracts of flowers, shoots and fruits of hawthorn blood-red did not cause significant changes of tested parameters of the excretory kidneys function in animals of experimental groups.

Whereas the comparative drug hypothiazide with single intragastric administration at a dose of 20 mg / kg substantially contributed to a significant increase in diuresis (by 40%), natriuresis (by 54%) and kaliuresis (by 55%) relative to water control in 24 hours of the experiment (Table 5).

As can be seen from the above the liquid extract of hawthorn blood-red leaves with single intragastric administration at a dose of 100 µl/kg for 24 hours of the experiment promoted a moderate increase of diuresis and natriuresis, somewhat inferior in strength to hypothiazide at an average therapeutic dose of 20 mg/kg, but showed creatininurative effect, which is uncharacteristic for hypothiazide.

In the study of antidepressant activity, the comparison drug amitriptyline at a dose of 5 mg / kg increased the motor activity of the rats by 54% relative to the water control (Table 6). At the same time, a liquid extract of hawthorn blood-red leaves in a dose of 100 µl / kg did not affect the motor activity of animals in comparison with water-alcohol control. The liquid extract of hawthorn blood-red shoots in a similar dose of 100 µl/kg increased the motor activity of animals by 68% in comparison with water-alcohol control, acting similarly to amitriptyline in the above dose. A liquid extract of hawthorn blood-red flowers in a dose of 100 µl/kg significantly increased the motor activity of animals by 101% compared to water-alcohol control, this effect is comparable to the effect of amitriptyline at a dose of 5 mg/kg. A liquid extract of hawthorn blood-red fruits at a dose of 100 µl/kg increased the motor activity of animals by 31% compared to water-alcohol control, somewhat less than amitriptyline at a threshold dose of 5 mg/kg.

CONCLUSIONS

Because of the study of the diuretic and antidepressant action of phytopharmaceutical products, a number of features of their pharmacological action were revealed. Thus, the liquid extract of hawthorn blood-red leaves at a dose of 100 µl/kg had a rapid progress of diuretic and saluretic action (tubular effect), a prolonged effect, showing an additional creatininurative effect (glomerular effect) by the end of 24 hours of the experiment. Other studied drugs had no effect on the excretory kidneys function, probably due to the lower content of flavonoid rutin, for which a dose-dependent diuretic effect was proven in earlier experiments [9].

Liquid extracts of hawthorn blood-red flowers, shoots and fruits at a dose of 100 µl/kg exhibited an antidepressant effect

comparable to the effect of amitriptyline at a threshold dose of 5 mg/kg. Antidepressant activity of phytopharmaceutical products increases in the following series: liquid extract of hawthorn blood red fruit - liquid extract of hawthorn blood-red shoots - liquid extract of hawthorn blood-red flowers. It should be noted that the liquid extract of hawthorn blood-red leaves, despite the high content of the amount of flavonoids, did not show antidepressant activity. It can be assumed that this drug effect is associated with a high content of flavonoid hyperoside in flowers and shoots of hawthorn blood-red, for which antidepressant activity was previously found [1].

As can be seen from the above the medicines of hawthorn blood-red have complex nephrotropic and neurotropic action and can be used in the treatment of cardiovascular pathology.

REFERENCES

1. *Basyrova I.R., Libis R.* The prevalence of the main risk factors for cardiovascular diseases and their combinations among residents of Orenburg // *Aspirantskiy vestnik Povolzh'ya*. 2017. No. 1-2. P. 48-53.
2. *Kurkin V.A.* Pharmacognosy. Samara, 2016. 1279 p.
3. *Kurkin V.A., Kurkina A.V., Zaitceva E.N., Dubishchev A.V., Pravidtseva O.E., Morozova T.V.* Diuretic and antidepressant activity of liquid extract from the fruits of the hawthorn blood-red // *Bulletin of Siberian medicine*. 2015. Vol. 14, No. 3. P. 18-22.
4. *Kurkina A.V.* Flavonoids of pharmacopoeial plants. Samara, 2012. 290 p.
5. *Zaitceva E.N., Zaitcev, A.V.* *The device for the introduction of water load to laboratory animals*: Patent 115651, Russian Federation; publ. 10.10.12, Bull. No. 13.
6. *Zaitceva E.N.* A method of producing diuresis in laboratory animals: Patent 2494703, Russian Federation; publ. 10.10.13, Bull. No. 28.
7. *Trofimova S.V., Khasanova S.R., Kudashkina N.V.* Study of the cardioprotective properties of the leaves of hawthorn blood-red and species «Cardiophyt» // *Medical Bulletin of Bashkortostan*. 2011. Vol. 6, No. 2. P. 299-302.
8. *Khabriev R.U.* Guidance on experimental (preclinical) study of new pharmacological substances. Moscow, 2005. 832 p.
9. *Kurkin V.A., Zaitceva E.N., Kurkina V.V., Dubishchev A.V., Pravidtseva O.E.* *Comparative study of the diuretic activity of water-based and alcohol extracts of medicinal plants containing flavonoids* // *Bulletin of Experimental Biology and Medicine*. – 2015. Vol. 159, No. 3. P. 348-352.
10. *European Pharmacopoeia*. 6-th Ed. Rockville: United States Pharmacopoeial Convention, Inc., 2008.