Antioxidant activity of some herbal extracts genus Chernogolovka (Prunella L.): in vitro and in vivo researchA.A. Shamilov[1], D.I. Pozdnyakov[1], V.N. Bubenchikova[2], M.V. Chernikov[1], E.R. Garcia[1]

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Antioxidant activity of extracts from species of genus Prunella L .: in vitro and in vivo investigation

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SUMMARY

The article presents the results of an experimental study devoted to in vitro and in vivo assessment of the antioxidant activity of aqueous and aqueous-alcoholic extracts obtained from the herb of three representatives of the genus Prunella L .: Prunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L. As a result, it was It was found that the highest antioxidant potential is possessed by the dry residues of water-alcohol extracts (extractant: ethyl alcohol 70%), which, when introduced into the model medium, most significantly inhibited the formation of superoxide, hydroxyl and DPPH radicals, and also improved the pro / antioxidant balance in animals without a pathological background, which was expressed in an increase in the activity of endogenous antioxidant enzymes: superoxide dismutase, catalase, glutathione peroxidase, and a decrease in the serum concentration of TBA-active products.

Key words: antioxidants, antioxidant activity, herb, plant extracts,blackhead species, Prunella L.

RESUME

In article there are results of antioxidant activity of water and water-alcohol extracts from aerial parts Prunella L. species: Prunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L by in vitro and in vivo tests. The highest antioxidant activity was established to air-dried residues of water-alcohol extracts (solvent - 70% ethanol) which inhibited generation of superoxide, hydroxyl- and DPPH-radicals and improved pro-oxidant antioxidant balance to animals without pathological forms. It was expressed in the increasing activity of endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and the deceasing of TBARS.

Keywords: antioxidants, antioxidant activity, aerial part, plant extracts, Prunella L.

INTRODUCTION

The intensification of free radical oxidation processes can underlie the aging of the body, the intensification of the action of unfavorable environmental factors, as well as the pathogenesis of a number of diseases, for example, oncopathology [1], atherosclerosis [2], ischemic stroke [3], arrhythmias [4], etc. Correction of oxidative stress is of undoubted scientific and practical interest and can be used as an independent link in pharmacotherapy. In the present, it has been established that the use of agents with antioxidant activity promotes accelerated convalescence in atherosclerosis, coronary heart disease (IHD), diabetes mellitus [5]. The antioxidants include substances of both natural [6] and synthetic [7] origin. However, despite the high therapeutic efficacy of synthetic drugs,

According to various estimates in the genus Prunella L. includes about 15 species, of which three are found in the flora of Russia: part of the ordinary (Prunella vulgaris L.), a representative of Europe (from Karelia to the Upper Dnieper and Upper Volga regions), the Caucasus, Western and Northern Siberia and Central Asia; h. split (Prunella laciniata L.) and large-flowered (Prunella grandiflora L.) are found in the flora of Eastern Europe and the Caucasus. [ten]. According to the literature, three types

Prunella: Prunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L. are characterized by the presence of various groups of biologically active substances, including phenol carboxylic acids and their derivatives, flavonoids, including anthocyanins, tannins, fatty acids, sesquiterpenoids, coumarins, carbohydrates, essential oils, iridoids and saponins [11, 12, 13] ... Triterpenoid speciesPrunella L. are represented by ursolic and oleanolic acids. Among phenol carboxylic acids, caffeic, 4-caffeoylquinic, neochlorogenic, chlorogenic, and rosemary were identified; among flavonoids, quercetin, quercetin 3-glucoside, kaempferol, rutin were identified [11, 14]. Thus, the rich chemical compositionPrunella grandiflora L., Prunella laciniata L., Prunella vulgaris L. suggests a potentially high antioxidant activity of extracts obtained from raw materials of these representatives of the genusPrunella L.

Objective of the study: To evaluate the antioxidant activity of extracts obtained from the herbPrunella grandiflora L., Prunella laciniata L .; Prunella vulgaris L. in in vitro and in vivo testing.

MATERIALS AND METHODS

The extracts studied in this work were obtained by extraction of the herb Prunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L. (sample 15.0 grams) with purified water and ethyl alcohol of various concentrations (95%, 70%, 40%), which was carried out in a boiling water bath with a reflux condenser for 2 hours at a raw material: extractant ratio of 1:50. The extracts cooled to room temperature were filtered through a yellow ribbon filter paper and concentrated under reduced pressure on a Qyre-2A rotary evaporator (Qiyu Industrial (Shanghai) Co., Ltd.) at 40 ± 2 ° C until a thickened mass was obtained. After they were dried in a thermostat (TS-1/20 SPU, Russia) at a temperature of 40 ± 2 ° C, dry residues were obtained.

Investigation of the antioxidant properties of extracts from species Prunella L. performed in 2 stages. The first stage was the study of the antioxidant activity of the dry residue obtained by extraction with purified water and ethyl alcohol of various concentrations from grassPrunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L., in in vitro testing by evaluatingradical-inhibiting properties.

The antiradical properties of the investigated dry residues were studied using model media for the generation of DPPH, superoxide, and hydroxyl radicals. DPPH-inhibiting properties were assessed according to the method proposed byMensor LL et al. (2001), at518 nm. Superoxide radical scavenger properties were studied using the model describedWinterbourn CC et al. (1975) based on the photo-reversal reactionriboflavin at 560 nm. Hydroxyl radical binding activity was investigated by the method Elizabeth & Rao (1990) Spectrophotometric Detection of Colored Degradation Products Complex 2-deoxyribose with thiobarbituric acid during the Fenton reaction at 532 nm.

The obtained dry residues, dissolved in ethyl alcohol 70%, were introduced into the incubation medium in the following concentration range: 1000 μg / ml, 500 μg / ml, 250 μg / ml, 125 μg / ml and 62.5 μg / ml. When conductingIn vitro studies, quercetin (Hunan Warrant Pharmaceuticals, China) was used as a positive control in a similar concentration range. Based on the receivedof results by probit analysis was used to calculate the half-inhibition coefficient (IC_{50}). All tests were performed in a triplet version (according to [15]).

At the second stage of the study, the antioxidant properties of the obtained dry residues from the herb species of the genus Prunella L in in vivo testing. This block of experimental work was performed on 140 male Wistar rats kept in standard vivarium conditions in compliance with the requirements of international standards of experimental ethics (Directive 2010/63 / EU of the European Parliament and of the council on the protection of animals used for scientific purposes, September 22, 2010).

The studied dry residues were administered to animals at a dose of 100 mg / kg (per os in the form of an aqueous suspension) onfor 10 days, after which blood was taken from the rats (from the abdominal part of the aorta), which was centrifuged at 1000 g for 10 minutes to obtain serum. In the blood serum of animals, the changes in the activity of endogenous antioxidant defense enzymes were assessed: superoxide dismutase (SOD), glutathione peroxidase (HP), and catalase (CAT), as well as the concentration of TBA-active products (TBA-AP) in terms of malondialdehyde. At this stage, quercetin was used as a reference compound at a dose of 100 mg / kg, administered according to a scheme similar to the studied dry residues. The number of animals in each experimental group was 10 (according to [16]).

SOD activity was assessed in a xanthine-xanthine oxidase test based on the dismutation reaction of the superoxide radical formed during the xanthine oxidation and 2- (4-

iodophenyl) -3- (4-nitrophenol) -5-phenyltetrazolium chloride at 505 nm. HP activity was determined by the method Pierce & Tappel (1978), where at At 340 nm, a decrease in NADPH was recorded during the conjugated glutathione reductase reaction. The activity of CAT was studied in the course of setting the reaction of hydrogen peroxide with ammonium molybdate in the presence of the analyzed sample at 410 nm. The concentration of TBK-AP was determined in the condensation reaction with 2-thiobarbituric acid at 532 nm (according to [17]).

The results obtained were statistically processed. Data were expressed as M ± SEM. Comparison of groups of means was carried out by the ANOVA method with post-processing Newman-Keisle at $p < 0.05$. The software package for statistical analysis "STATISTICA 6.0" (StatSoft, USA) was used in the work.

CHARACTERISTICS OF THE OBJECTS OF STUDY

Chernogolovka (lat. Prunella L .; English - Self Heal, it. - Brunella or Brakne) is a genus of perennial herbaceous plants from the Lamiaceae family with erect or ascending stems, with serrated, entire or pinnately lobed, pinnately incised or pinnately divided leaves. The most common are three types: common blackheadPrunella vulgaris L., large-flowered blackhead Prunella grandiflora L., blackhead cut Prunella laciniata L. and (Fig. 1–3) [10, 18].

Rice. 1. Common chernogolovka (Prunella vulgaris L.) (source: https://obs.infoflora.ch/assets/db_doc/taxa_images / 2012/06/20/20120620003524-d110b3ad.jpg)

Rice. 2. Chernogolovka large flowering (Prunella grandiflora L.) (a source: https://obs.infoflora.ch/assets/db_doc/taxa_images / 2012/06/20 / 20120620003523-40ecb181.jpg)

Rice. 3. Chernogolovka split(Prunella laciniata L.) (source: https://obs.infoflora.ch/assets/db_doc/taxa_images / 2012/06/20 / 20120620003521-6faa2410.jpg)

For more than a hundred years, the common blackhead has been used in traditional and official medicine in China [10, 19].

Extracts from common, large-flowered and cut have a number of medicinal properties: hemostatic, wound healing, anti-inflammatory, expectorant, antimicrobial, antipyretic, tonic and anti-complementary. These three types can be used with the following list of diseases: for cancer of the thyroid gland, mediastinum, lymphogranulomatosis, lymphoma, respiratory diseases and infections, hemoptysis, empitigo, psoriasis, scrofula, seborrhea, exudative diathesis, laryngitis, nephritis, hemorrhoids, diarrhea (, skin, lungs), diphtheria, dysentery, hypertension, arthritis, rheumatic fever, lymphadenitis, hyperthyroidism and thyrotoxicosis, gastralgia, epilepsy, scurvy, leukorrhea, mastitis, metritis, colpitis, bruises, dislocations [20].

The flowering aerial part of the common part is used to prepare a restorative drink [21], in cooking it is served in the form of a salad for fatty foods, and is used as a component of some dishes [22]. Common chernogolovka is a fodder plant for cattle [23].

RESULTS OF THE STUDY

Data obtained during in vitro assessment of the antioxidant activity of the studied dryresidues are presented in table. 1.

Table 1

The results of studying the antiradical activity of dry residues, grass-derived representatives of the genus Prunella L.

Dry residues	IC_{50}, μg / ml		
	Superoxide radical	Hydroxyl radical	DPPH radical
PVW	264.2 ± 1.2 *	513.4 ± 1.9 *	368.8 ± 2.4
PV95	216.5 ± 2.7 *	463.3 ± 1.6 *	323.1 ± 1.4 *
PV70	40.8 ± 1.7 #	228.8 ± 2.3 #	185.3 ± 1.6 #
PV40	155.3 ± 2.6 *	324.7 ± 2.0 *	217 ± 1.4 *
PG95	294.5 ± 1.4 *	590.4 ± 1.7 *	497.3 ± 2.1 *
PG70	55.7 ± 1.3 #	286.3 ± 1.1 #	173.9 ± 2.5 #
PG40	144 ± 1.5 *	327.2 ± 2.0 *	285.1 ± 1.5 *
PGW	277.9 ± 2.3 *	492 ± 3.0 *	327.3 ± 1.7 *
PL95	321.3 ± 2.5 *	433.6 ± 2.6 *	371.3 ± 1.5 *
PL70	49.7 ± 1.1 #	224.9 ± 1.3 #	211.1 ± 1.6 #
PL40	184.9 ± 1.5 *	348.8 ± 2.0 *	240 ± 1.8 *
PLW	310.1 ± 2.8 *	583.6 ± 2.7 *	387.2 ± 1.7 *
Quercetin	20.3 ± 2.1	109.2 ± 2.7	119.3 ± 2.2

Note: PVW is the dry residue of water extraction from Prunella vulgaris L .; PV95 - dry residue of alcohol extraction (extractant: ethyl alcohol 95%) fromPrunella vulgaris L .; PV70 - dry residue of aqueous-alcoholic extraction (extractant: ethyl alcohol 70%) fromPrunella vulgaris L .; PV40 - dry residue of aqueous-alcoholic extraction (extractant: ethyl alcohol 40%) fromPrunella vulgaris L .; PGW - dry residue of water extraction fromPrunella grandiflora L .; PG95 - dry residue of alcohol extraction (extractant: ethyl alcohol 95%) fromPrunella grandiflora L .; PG70 - dry residue of water-alcohol extraction (extractant: ethyl alcohol 70%) fromPrunella grandiflora L .; PG40 - dry residue of aqueous-alcoholic extraction (extractant: ethyl alcohol 40%) fromPrunella grandiflora L .; PLW - dry residue of water extraction fromPrunella laciniata L .; PL95 - dry residue of alcohol extraction (extractant: ethyl alcohol 95%) fromPrunella laciniata L .; PL70 - dry residue of water-alcohol extraction (extractant: ethyl alcohol 70%) fromPrunella laciniata L .; PL40 - dry residue of aqueous-alcoholic extraction (extractant: ethyl alcohol 40%) fromPrunella laciniata L .; # - statistically significant relative to quercetin ($p < 0.05$); * - statistically significant relative to quercetin ($p < 0.001$).

As you can see from the table. 1, IC values closest to quercetin$_{50}$ were obtained in the study of dry residues of aqueous-alcoholic extracts (extractant: ethyl alcohol 70%) obtained from herbsPrunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L. So, when studying the superoxide radical inhibitory activity, it was found that the IC_{50} for dry residues from Prunella grandiflora L., Prunella laciniata L., Prunella vulgaris L., obtained by extraction with ethyl alcohol 70%, were 2.7 ($p < 0.05$); 2.5 ($p < 0.05$) and 2.0 ($p < 0.05$) times, respectively, are higher than that of quercetin. At the same time, the evaluation of the DPPH radical scavenger properties made it possible to establish that IC_{50} for PV70, PG70 and PL70 extracts was at 1.55 ($p < 0.05$); 1.46 ($p < 0.05$) and 1.77 ($p < 0.05$) times higher than that of quercetin. It should be noted that IC_{50} in the study of the hydroxyl radical of the inhibitory activity of dry residues under the codes PV70, PG70 and PL70 exceeded the similar value of quercetin by 2.1 ($p < 0.05$); 2.62 ($p < 0.05$) and 2.06 ($p < 0.05$) times, respectively.

results in vivo evaluation of antioxidant properties of dry residues obtained from Prunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L. are presented in table. 2.

In the course of this block of experimental work, it was found that in rats that were injected with quercetin, the activity of endogenous antioxidant defense enzymes SOD, HP and SAT in blood serum increased by 36.9% ($p < 0.05$); 28.1% ($p < 0.05$) and 18.9% ($p < 0.05$) in relation to the group of intact animals. Also, in animals, on the background of quercetin administration, a decrease in the serum concentration of TBA-AP by 24.1% ($p < 0.05$) was observed relative to the same indicator of the IL group.

table 2

Influence of dry residues obtained from herbs of some members of the genus Prunella L.,

on the change in pro / antioxidant balance in animals without concomitant pathological background

Group	SOD, U / L	GP, U / L	CAT, μat / L	TBK-AP, μmol / l
IZH	300.5 ± 7.1	602.6 ± 5.9	0.863 ± 0.015	5.4 ± 0.5
PV95, 100 mg / kg	317.2 ± 29.7 #	599.6 ± 14.6 #	0.941 ± 0.015 #	5.9 ± 0.7 #
PV70, 100 mg / kg	396.1 ± 23.5 *	645.2 ± 45.9 *	0.999 ± 0.024 *	4.5 ± 0.1 *
PV40, 100 mg / kg	302.7 ± 89.5 #	603.0 ± 51.7 #	0.720 ± 0.045 #	5.8 ± 0.1 #
PVW, 100 mg / kg	293.6 ± 14.6 #	615.6 ± 21.6	0.955 ± 0.017 #	5 ± 0.2 #
PG95, 100 mg / kg	312.3 ± 54.7 #	588.6 ± 23.7 #	0.774 ± 0.047 #	5.74 ± 3.0 #
PG70, 100 mg / kg	393.5 ± 22.0 *	601.7 ± 25.9 *	1.210 ± 0.013 *	4.04 ± 0.2 *
PG40, 100 mg / kg	307.3 ± 20.4 #	586.3 ± 85.6 #	0.825 ± 0.085 #	5.92 ± 0.3 #
PGW, 100 mg / kg	315.9 ± 15.9 #	593.9 ± 41.2 #	0.738 ± 0.011 #	5.2 ± 0.6 #
PL95, 100 mg / kg	305.87 ± 33.7 #	584.8 ± 20.6 #	0.895 ± 0.014 #	5.84 ± 1.0 #
PL70, 100 mg / kg	365.2 ± 41.4 *	652.3 ± 85.6 *	1.234 ± 0.052 *	4.2 ± 0.1 *
PL40, 100 mg / kg	306.5 ± 74.7 #	590.8 ± 42.0 #	0.821 ± 0.017 #	5.23 ± 0.1 #
PLW, 100 mg / kg	303.9 ± 47.3 #	582.9 ± 98.7 #	0.799 ± 0.041 #	5.2 ± 0.6 #
Quercetin, 100 mg / kg	401.3 ± 15.9 *	671.9 ± 24.3 *	1.020 ± 0.162 *	4,100 ± 0.7 *

Note: IL - a group of intact animals; * - statistically significant relative to intact animals (p <0.05); # - statistically significant relative to the group of rats treated with quercetin (p <0.05); the designations of the dry residues, which were received by the groups of animals, are similar to the designations of the table. 1.

In animals that received a suspension of dry residue PV70, the activity of SOD, GP and CAT exceeded the analogous indicators of intact animals by 31.8% (p <0.05); 23.7% (p <0.05) and 15.8% (p <0.05), respectively, with a decrease in the concentration of TBA-AP by 16.7% (p <0.05). Against the background of course administration to rats of a suspension of the dry residue obtained by extraction with ethyl alcohol 70% grass Prunella grandiflora L., an increase in the activity (relative to intact animals) of SOD by 31% (p <0.05), HP - by 24.9% (p <0.05) and SAT - by 40.2% (p <0, 05).

In rats that were injected with a suspension of dry residue PG70, there was a decrease in the content of TBA-AP in blood serum relative to IL by 25.2% (p <0.05). In the group of animals that received a suspension of dry residue PL70 for 10 days, the activity of SOD, GP and SAT was 21.5% (p <0.05); 19.2% (p <0.05) and 43% (p <0.05), respectively, are higher than that of IL. At the same time, the concentration of prooxidants (TBA-AP) in the blood serum of rats injected with a suspension of dry residue PL70 decreased by 22.2% (p <0.05) in relation to intact animals.

No statistically significant differences in the pro / antioxidant state were established between the groups of animals that received the studied suspensions of dry residues PV70, PG70, and PL70, and the group of rats that received quercetin in an equivalent dose. At the same time, the use of dry residues obtained by extraction with purified water and ethyl alcohol 40% and 95% from grassPrunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L., did not have a significant effect on the change in the pro / antioxidant status in rats (Table 2).

DISCUSSION

Oxygen free radicals (Oxygen radicals) are important signaling molecules, usually formed during electron transport reactions along the mitochondrial respiratory chain. Along with the signaling function, IBS can have a damaging effect on the ultrastructure of the cell, which is expressed in the intensification of lipid peroxidation [24]. The pathological properties of IBS acquire only when high concentrations are reached, which is observed when the optimal functioning of the electron transport system is disturbed, or the antioxidant defense is insufficiently active. It is also important that IBS are formed as a result of exposure to radiation, environmental pollutants and as by-products of drug metabolism.

It is possible to reduce the toxic effect of IBS on the body by using exogenous antioxidants - molecules that have antioxidant properties and the ability

to form stable, non-reactive free radicals. Antioxidants can have direct radical-binding ("scavenger") activity and, in addition, can indirectly (by increasing the catalytic properties of endogenous antioxidant enzymes) inactivate CPK. As a rule, to achieve a high antioxidant potential, a combination of both direct and indirect antioxidant properties is necessary [25].

This study showed that the dry residues (extractant: ethyl alcohol 70%) obtained from herbs of different types of blackheads - Prunella grandiflora L., Prunella laciniata L. and Prunella vulgaris L., possess antiradical properties, expressed in the inhibition of the formation of superoxide, DPPH- and hydroxide-radicals in the model mixture. At the same time, the introduction of the studied dry residues in the form of a suspension to animals without an accompanying pathological background promoted an increase in the activity of endogenous antioxidant defense enzymes: SOD, HP, and CAT, as well as a decrease in the concentration of prooxidants - TBK-AP. Solids of alcohol extraction (extractant: ethyl alcohol 70%) during settingin vivo studies on the severity of pharmacologicalthe activities were comparable to the individually isolated compound, quercetin in an equivalent dose.

CONCLUSION

Based on the results obtained, we can assume the relevance of further study of the dry residues of aqueous-alcoholic extracts (extractant: ethyl alcohol 70%) from the herb of a number of representatives of the genus Chernogolovka: Prunella grandiflora L., blackhead cut Prunella laciniata L. and common blackhead Prunella vulgaris L. for increasing the spectrum of pharmacological activity.

Also represents the undoubted scientific and practical interest grade possibilities therapeutic or prophylactic use of the investigated aqueous-alcoholic extracts in various pathological processes in which the development of oxidative stress plays a decisive role, for example, in atherosclerotic vascular lesions, ischemic stroke, discirculatory encephalopathy, ischemic heart disease.

Considering that agents with antioxidant activity, as a rule, are used in a course, which in some cases can be a rather long time interval, it seems expedient to study the general toxic properties of aqueous-alcoholic extracts obtained from the herb of large-flowered blackhead Prunella grandiflora L., blackhead cutPrunella laciniata L. and common blackhead Prunella vulgaris L. by extraction with 70% ethyl alcohol, in order to avoid a significant negative effect on the functional activity and structural integrity of organs and organ systems.

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