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Antioxidant effect of ethanolic onion (*Allium cepa*) husk extract in ageing rats

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ABSTRACT

The role of natural antioxidants in preventing of age-relating diseases is evident. The vegetable industry generates a large amount of waste, which is a good source of antioxidants.

The aim of the study was the investigation of the antioxidant effect of long-term consumption of ethanolic yellow onion husk extract in ageing laboratory rodents.

Twenty male Wistar albino rats were divided randomly into two groups (n = 10): a control group and an experimental group that received ethanolic yellow onion husk extract (2 mL/rat diluted with distilled water; activity of 4.44 μmol-equiv. quercetin) for 188 days. Oxygen radical absorbance capacity and ferric reducing antioxidant power assays were used to determine the total antioxidant capacity of the extract, which amounted to 941.4 ± 32.7 μmol equiv. Trolox/g raw material and 167.4 ± 16.4 μmol-equiv. quercetin/g raw material, respectively. Oral intake of the onion husk extract affected the indicators of the antioxidant system of the liver and the brain but not of the blood and plasma, mainly due to elevations in the activity of catalase and superoxide dismutase in the liver by 44.4% and 79.1%, respectively, and in the brain by three-fold and 79.1%, respectively.

The availability, cheapness and high antioxidant potential of onion waste qualifies it a good source of functional ingredients and bioactive substances applicable in the food and pharmaceutical industries.

1. Introduction

Ageing is considered an inevitable biological process (Sadowska-Bartosz and Bartosz, 2014) accompanied by wide range of disorders, including the loss of ability to reproduce and regenerate. Age-related degradation promotes the development of socially significant diseases such as cancer and cardiovascular diseases (Cui et al., 2020), as well as degenerative disorders (Freitas et al., 2017; Go and Jones, 2017; Hayes et al., 2020; Meng et al., 2017). Lifestyle, environment, nutrition and heritable gene mutation also accelerate ageing (Prasad et al., 2017). Currently, there are more than 300 theories of ageing. Nevertheless, correlations of oxidative stress, inflammation, mitochondrial dysfunction, shortening of telomeres and gene mutations with cellular ageing are the most studied (Prasad et al., 2017; Yegorov, 2020). Numerous studies have demonstrated that reactive oxygen species (ROS) are involved in the main processes associated with ageing, such as telomere shortening, autophagy, and stem cell depletion (Vaiserman et al., 2020). These observations formed the free-radical theory of ageing, later termed as the oxidative stress theory of ageing. The theory is based

Abbreviations: ROS, reactive oxygen species; FR, free radical; AOS, antioxidant system; OHE, onion husk ethanolic extract; IICI, integral indicators of chronic intoxication; TAC, total antioxidant capacity; ORAC, oxygen radical absorbance capacity; FRAP, fFerric reducing antioxidant power; GSH, reduced glutathione; CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances; HAT, hydrogen atom transfer; SET, single electron transfer; GC–MS, gas chromatography–mass spectrometry; HPLC-MS, high performance liquid chromatography–mass spectrometry.

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on the hypothesis that the uncontrolled structural damage of lipids, DNA and proteins by ROS lead to damage accumulation, which stimulates ageing (Liguori et al., 2018).

ROS are continuously generated and neutralised during enzymatic and non-enzymatic reactions in organisms and participate in important metabolic processes (Bardaweel et al., 2018). However, excessive generation of free radicals (FRs), in particular ROS, can irreversibly damage cells and biomolecules, with further accumulation of oxidative modified products (Mittler, 2017). Mammals have a complex multi-stage antioxidant system (AOS) for regulation of the number of FRs. An AOS can be enzymatic or non-enzymatic and include endogenous and exogenous antioxidants (Aversa et al., 2016). Exogenous antioxidants play an important role, since they are able to maintain the AOS, whose activity decreases with ageing and the development of pathological disorders (Pohl and Lin, 2018). These substances are absorbed by the organism together with food or consumed as synthetic or natural biologically active additives (Oyewole et al., 2014). Plants are the main source of exogenous natural antioxidants for humans and animals, as they contain a wide variety of antioxidants (Cui et al., 2020), which, in addition, are effective in small concentrations and do not show negative effects.

Every year, a huge number of articles are published devoted to the study of the biological properties of various herbs, spices, fruits and vegetables for their further application in the food industry and in medicine (Aremu et al., 2019; Hariharapura et al., 2014; Malfa et al., 2020). However, in recent years, the interest of scientists and producers has been directed towards the waste of the fruit and vegetable industry, due to the low cost and high content of biologically active substances (Kupaeva and Kotenkova, 2019). It is known that along with the growing demand for food, the amount of waste from the fruit and vegetable industry is also increasing (Bedrníček et al., 2019). Thus, over the past decade, commercial cultivation of onions, which is the second largest in the world, has increased by more than a quarter of the total production, and is currently estimated at 66–85.7 million tons per year (Ren et al., 2020). Onions belong to the genus *Allium* in the family *Alliaceae* and comprise many cultivars (Sidhu et al., 2019), of which three are coloured (red, yellow-brown and white) (Ren et al., 2020). The onion *Allium cepa L.* is the most common plant and ranks second in importance among horticultural crops worldwide, second only to tomatoes (Benítez et al., 2011). The growing demand for onions leads to an increase in waste, which consists of onion skins, two outer fleshy scales and roots, generated during industrial peeling, and undersized, malformed, diseased or damaged bulbs (Benítez et al., 2011). Waste is estimated at thousands of tons (Bedrníček et al., 2019). Onion waste is not used as feed, because of its characteristic aroma, or as organic fertiliser, due to the rapid formation of phytopathogenic agents (Benítez et al., 2011), which makes it an economically attractive source of natural antioxidants.

This study was aimed at investigation of the antioxidant effect of long-term consumption of ethanolic yellow onion husk extract, as a source of natural antioxidants, in ageing laboratory rodents.

2. Material and methods

2.1. Preparation of plant extract

For the preparation of yellow onion (*Allium cepa*) husk ethanolic extract (OHE), onions were obtained from the supermarket, producer OOO 'Agroleto', Krasnodar, Russia. The husks were ground (size less than 5 mm) and soaked in ethanol 70% (60 g/900 mL) for 24 h with gentle shaking at room temperature. The content was filtered through a paper filter and kept in airtight bottles in a refrigerator at 4 °C until use.

2.2. Chemicals

Fluorescein sodium salt (purity ≥ 97%); 2,2′-azobis(2-methyl propionamidine) dihydrochloride (AAPH, purity ≥ 97%); 5,5-dithiobis(2-nitrobenzoic acid) (DTNB, purity ≥ 98%); quercetin (purity ≥ 95%); iron (III) chloride hexahydrate (purity ≥ 99%); acetonitrile (for HPLC, gradient grade, ≥99.9%); hexane (for HPLC, ≥97.0%,GC); gallic acid (purity ≥ 97%); and tetramethylchromane-2-carboxylic acid (Trolox, purity ≥ 97%) were purchased from Sigma-Aldrich (USA). Di-potassium hydrogen phosphate anhydrous (purity ≥ 98%); potassium di-hydrogen phosphate (purity ≥ 98%); sodium acetate anhydrous (purity ≥ 99%); trichloroacetic acid (TCA; purity ≥ 99%); thiobarbituric acid (purity ≥ 98%); 1-butanol (purity ≥ 99.5%); pyrogallic acid (purity ≥ 99%); hydrogen peroxide (H2O2, purity ≥ 33%); sodium carbonate (purity ≥ 99.5%); Folin-Ciocalteu reagent; and hydrochloric acid (HCl; 37%) were purchased from PanReac AppliChem (Germany). Acetic acid (purity ≥ 99.8%); *ortho*-phosphoric acid (purity ≥ 85%) was purchased from Component-Reaktiv (Russia). 2,4,4-Tris(2-pyridyl)-1,3,5-triazine (TPTZ, purity ≥ 99%) was purchased from Acros Organics (China).

2.3. Composition of ethanolic onion husk extracts

2.3.1. Determination of total phenols

Determination total phenols in onion husk extract was performed on a spectrophotometer Cary 50 (Varian, Australia) using Folin–Ciocalteu reagent using to the method of Obanda et al. (1997) with the author's modification. Volumes of 0.25 mL of the sample or standard or 80% ethanol for measuring the control sample were added to the tube. Then, 1.25 mL of Folin–Ciocalteu reagent (diluted with distilled water in a ratio of 1:9) was added, after 3 min incubation 1.0 mL of 7.5% Na_2CO_3 was added to the tube, mixed and incubated for 2 h. Optical density was measured at a wavelength of 765 nm. Total phenols content was determined according to a standard curve using gallic acid in the concentration range of 0–200 mg/L and expressed in μmol-equiv. gallic acid /g plant material.

2.3.2. GC–MS analysis

Determination of ethanolic onion husk extract composition was performed on a gas chromatograph Agilent 7890 (Agilent Technologies, USA) with a mass spectrometric detector Agilent 5975C (Agilent Technologies, USA) and capillary column HP-5MS (30 mm × 0.25 mm × 0.25 μm). Polar (acetonitrile) and a non-polar (hexane) solvents were used for components extraction. Identification of analytes was carried out using software NIST Standard Reference Database 1Av17 (Gaithersburg, USA) by the relative intensity of the main and two confirming ions. The calculation was performed using the internal normalization method.

2.3.3. HPLC-MS analysis

Determination of vitamins content in ethanolic onion husk extract was performed on a Dionex Ultimate 3000 (Thermo Fisher Scientific, USA) with UV detector. Chromatographic column Acclaim Polar Advantage C16, 4.650 mm was used for determination of water-soluble vitamins, LC-PAH 5 μm, 15 × 4.6 mm – for fat-soluble vitamins. The separation was carried out in the gradient elution mode, the volume of the introduced sample was 20 μL. The concentration of water-soluble vitamins was determined using a wavelength of 210 nm, with the exception of B1, C (245 nm) and B2 (254 nm). Fat-soluble vitamin concentrations were determined using 325 nm, 270 nm, and 285 nm wavelengths for vitamins A, D, and E, respectively.

2.4. Animal study design

2.4.1. Animals

A total of 20 male Wistar albino rats, aged 17 months, weighing 458.7 ± 42.7 g average, were purchased from FSUE 'Laboratory animal nursery Rappolovo', v. Rappolovo, Leningrad region, Russia. The rats were kept under standard conditions (temperature 20 ± 3 °C, humidity 48 ± 2%, day/night (from 06.00 to 18.00 h/from 18.00 to 06.00 h), no more than four rats per plastic cage), and water and feed were available ad libitum. A balanced diet of commercial pellets (Laboratorcorm, Russia) was fed. The rats were kept for 7 days before the beginning of the experiments for acclimatisation.

2.4.2. Groups

Animals were divided randomly in two groups: control group (n = 10) and experimental group (n = 10). Rats in control group received a standard feed (Labkorm, Russia) ad libitum during the experiment. Animals in experimental group received a standard feed (Labkorm, Russia) ad libitum during the experiment and OHE diluted with distilled water to an activity of 4.44 μmol-equiv. quercetin (measured by ORAC method), administered orally at 2 mL/rat every day for 187 days. The concentration of ethanol consumed by animals did not exceed 8%. Body weight was measured using electronic scales (Adventurer Pro AV2101, USA) before study for group randomization and at the end of the experiment for integral indicators of chronic intoxication calculation.

2.4.3. Ethics statement

Animal rearing and handling, and the experimental design and procedures, were approved by the bioethics committee of the V. M. Gorbatov Federal Research Centre for Food Systems of RAS, Moscow, Russia (Protocol No. 01/2019 of 09.05.2019).

2.5. Sampling

At the end of the experiment (188 days), rats were fasted for 12 h before being euthanised by carbon dioxide (VetTech UK) according to animal welfare rules.

2.5.1. Blood sampling and plasma preparation

Blood was collected from euthanised rats by cardiac puncture using a small-volume syringe, and then dispensing the collected blood on the inner side of 4-mL test tubes (Vacuette, Austria) with heparin as anticoagulant. Plasma separation was carried out by centrifugation at 2260*g* for 8 min in a CM-6M centrifuge (ELMI, Riga, Latvia). Plasma were separated and stored at −20 °C before measurement of biochemical parameters.

2.5.2. Organ sampling and extracts preparation

The liver, brain, heart, kidney and spleen were removed. The liver and brain were well homogenised in 10 volumes of 50 mM phosphate buffer (pH = 7.0) and centrifuged at 5000*g* for 5 min at 4 °C in a centrifuge 5427R (Eppendorf AG, Germany). The supernatants of each sample were separated and stored at −20 °C before measurement of biochemical parameters.

2.6. Integral indicators of chronic intoxication of rats

Weight of internal organs was measured using electronic scales (Acculab VICON, Canada). Integral indicators of chronic intoxication (IICIs) were calculated using the equation:

$$IICI = (m_1/m_2) \times 100 \quad (1)$$

where m_1: organ weight; m_2: rat weight.

2.7. Determination of total antioxidant capacity (TAC)

2.7.1. Oxygen radical absorbance capacity (ORAC) assay

Determination of TAC was performed by the ORAC method on a Fluoroskan Ascent FL system (TermoLabsystems, Finland) using black 96-well plates (Malta and Liu, 2014) with the author's modification. Volumes of 30 μL of sample or standard and 200 μL of 0.5 μM of sodium fluorescein were added to the wells and the microplates covered with film (SSIbio, USA) and placed into the Fluoroscan Ascent FL for 30 min at 37 °C. Then, 30 μL of 153 μM AAPH was added to each well and fluorescence measured at 37 °C for 60 min at 5-min intervals. Excitation wavelength was 485 nm, emission wavelength was 535 nm. The TAC of each sample was determined four times. TAC was determined according to a standard curve of Trolox in the concentration range 5–75 μM. Depending on their activity, the samples were diluted with 75 mM phosphate buffer (pH = 7.4). TAC was expressed in μmol equiv. Trolox/g raw material.

2.7.2. Ferric reducing antioxidant power (FRAP) assay

Determination of TAC by the FRAP method was performed on a spectrophotometer SF-2000 (OCB «Spectr», Russia) using to the method of Benzie and Strain (Benzie and Strain, 1996) with the author's modification. The fresh FRAP solution was prepared by mixing 300 mM acetate buffer (pH = 3.6), 10 mM TPTZ (prepared in 40 mM HCl) and 20 mM ferric (III) chloride aqueous solution in the ratio of 10:1:1 (v/v/v). Volumes of 1.45 mL of FRAP reagent and 50 μL of the sample or standard or distilled water for measuring the control sample were added to the tube. The reaction mixture was incubated for 30 min at 37 °C in the dark. Optical density was measured at a wavelength of 594 nm. TAC was determined according to a standard curve using quercetin in the concentration range of 1–0.1 μM. Depending on their activity, the extracts were diluted with distilled water. The TAC of the OHE was expressed in μmol-equiv. quercetin/g raw material, plasma in μmol-equiv. quercetin/L. The TAC of the liver and brain extracts were expressed in μmol-equiv. quercetin/g protein.

2.8. Biochemical assays

2.8.1. Protein determination

Protein was measured using the semiautomatic analyser BioChem SA (High Technology Inc., Walpole, MA, USA) with the respective commercial kits (High Technology Inc.) according to the manufacturer's instructions.

2.8.2. Quantitative determination of reduced glutathione (GSH)

Concentration of GSH in blood and organ sample extracts was determined on a spectrophotometer SF-2000 (OCB «Spectr», Russia) assessed by Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid (DTNB) dissolved in methanol) using the method of Ellman (Ellman, 1959) with the author's modification. Volumes of 0.25 mL of sample were transferred to glass tubes and 1.5 mL of distilled water added and thoroughly mixed. Then 0.5 mL of 20% trichloroacetic acid was added, mixed and incubated at 4 °C for 20 min. The tubes were centrifuged at 1660*g* for 15 min in a CM-6M centrifuge (ELMI, Riga, Latvia). A volume of 1 mL of the supernatant was transferred to each of two test tubes and 0.5 mL of 0.3 M phosphate buffer added. Methanol (50 μL) was added to the control sample and 50 μL of Ellman reagent to the experimental sample, mixed, and the optical density measured at 412 nm. The concentration of GSH was calculated using equation (2) for blood plasma and equation (3) for organ extracts:

$$C_{GSH} = (D_{ex} - D_{con}) \cdot r \cdot 10^6/\varepsilon \quad (2)$$

$$C_{GSH} = (D_{ex} - D_{con}) \cdot r \cdot 10^6/\varepsilon/C_{pr} \quad (3)$$

where D_{ex}: optical density of the test sample; D_{con}: optical density of the control sample; ε: molar extinction coefficient (13.1×10^3 M^{-1} cm^{-1}); C_{pr}: protein concentration (g/L). Results were expressed in μmol/L for plasma and in μmol/g of protein for organ extracts.

2.8.3. Catalase activity estimation (CAT)

CAT activity in plasma and organ extracts was determined on a spectrophotometer SF-2000 (OCB «Spectr», Russia) according to method (Beers and Sizer, 1952) with the author's modification. A volume of 720 μL of 50 mm phosphate buffer (pH = 7.0) was mixed with 800 μL of 0.1% hydrogen peroxide. The optical density (D_0) was measured at a wavelength of 240 nm relative to the control sample, using 1-cm cuvettes. Then, 20 μL plasma or brain extract or 5 μL liver extract was added to the test tubes and after 1.5 min of incubation the optical density (D_1) was measured at a wavelength of 240 nm. As a control, 800 μL of phosphate buffer was added instead of hydrogen peroxide. Activity was calculated using the equation:

$$U = (D_0 - D_1) \cdot r \cdot 10^6/\varepsilon/t/C_{pr} \quad (4)$$

where D_0: the optical density before sample addition; D_1: optical density after incubation; ε: coefficient of molar extinction of hydrogen peroxide (39.4 M^{-1} cm^{-1}); t: incubation time (1.5 min); C_{pr}: protein concentration (g/L). The results were expressed as U/g of protein, in which U is amount of H_2O_2 (mmol) neutralised per min.

2.8.4. Superoxide dismutase activity estimation (SOD)

SOD activity in plasma and organ extracts was determined on a spectrophotometer SF-2000 (OCB «Spectr», Russia) according to the method of Marklund and Marklund (Marklund and Marklund, 1974) with the author's modification. To a volume of 1.14 mL of 50 mM phosphate buffer (pH = 8.2), 30 μL of plasma or extract and 30 μL of 10 mM pyrogallol solution were added and mixed. The increase in the optical density of the experimental samples was measured at the beginning (D_0) and after 2 min incubation (D_1) at a wavelength of 340 nm relative to the phosphate buffer, using 1-cm cuvettes. Auto-oxidation of pyrogallol was measured in a control sample in the same reaction mixture, adding 30 μL of 50 mM phosphate buffer instead of the sample. The percentage inhibition of pyrogallol auto-oxidation was calculated using equation (5) and SOD activity was calculated using equation (6):

$$P\% = (\Delta D_{con} - \Delta D_{ex}) \cdot 100\%/\Delta D_{con} \quad (5)$$

$$U = P\%/t/C_{pr}/V_s \quad (6)$$

where ΔD_{con}: difference in optical densities before incubation and after for the control sample; ΔD_{ex}: difference in optical densities before incubation and after the test sample; t: incubation time (2 min); C_{pr}: protein concentration (g/L); V_s: sample volume (mL). The results were expressed in U/g protein, in which U percentage of pyrogallol inhibition per min.

2.8.5. Lipid peroxidation products

The levels of malondialdehyde (MDA), the major lipid peroxidation product, were evaluated as thiobarbituric acid reactive substances (TBARS) (Brazhe et al., 2014) in plasma and organ extracts.

To glass tubes 1.5 mL of 2% (w/v) *ortho*-phosphoric acid, 100 μL of plasma or organ extract or distilled water for the control sample, and 0.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) were added. After incubation at 95 °C for 45 min the samples were cooled to room temperature in an ice bath for 10 min. The reacted malondialdehyde (MDA)-thiobarbituric (TBA) adduct in the mixture was extracted by addition of 2.5 mL n-butanol. After mixing, all samples were centrifuged at 1660*g* for 10 min in a CM-6M centrifuge (ELMI, Riga, Latvia). The supernatant was transferred to 1-cm cuvettes and optical density measured on a spectrophotometer SF-2000 (OCB 'Spectr', Russia) at wavelengths of 535 and 570 nm. Concentration of MDA-TBA complex was calculated using equation (7) for plasma and (8) for organ extracts.

$$C = (D_{535} - D_{570}) \cdot r \cdot 10^6/\varepsilon \quad (7)$$

$$C = (D_{535} - D_{570}) \cdot r \cdot 10^6/\varepsilon/C_{pr} \quad (8)$$

where D_{535}: optical density at 535 nm; D_{570}: optical density at 570 nm; C_{pr}: protein concentration (g/L); ε: molar extinction coefficient of the (Malondialdehyde) MDA-TBA complex (1.56×10^5 M^{-1} cm^{-1}). Results are expressed as μmol MDA formed per L for plasma and μmol MDA formed per g protein for organ extracts.

2.9. Statistical analyses

STATISTICA 17.0 software was used in this study for statistical analysis. The results were calculated as median and 25th–75th percentile. Significant differences were tested by non-parametric statistical Mann–Whitney *U*-tests for independent variables. Differences with P-values of <0.05 were considered statistically significant.

3. Results

3.1. Total antioxidant capacity analysis

The TACs of ethanolic extracts of onion husk were measured by ORAC and FRAP methods. The ORAC fluorescent method was used to evaluate the contribution of antioxidants demonstrating an HAT (hydrogen atom transfer) mechanism of action and describing the ability of antioxidants to neutralise FRs by hydrogen donation. The FRAP photometric method was used to evaluate the contribution of antioxidants demonstrating an SET (single electron transfer) mechanism of action and describing the ability of antioxidants to interact with FRs by transferring a single electron (Siddeeg et al., 2021). Thus, according to the results of ORAC, the TAC of the extract was 941.4 ± 32.7 μmol equiv. Trolox/g raw material. The TAC determined by the FRAP method was significantly lower and amounted 167.4 ± 16.4 μmol-equiv. quercetin/g raw material.

3.2. Composition of ethanolic onion husk extracts

Total phenols content in ethanolic extract of onion husk were measured by Folin–Ciocalteu method and averaged 4.1 ± 0.2 mg-equiv. gallic acid /g plant material.

Component composition of ethanolic extracts of onion husk obtained by GC–MS analysis was detailed by CAS# using National Institute of Standards and Technology (NIST) DataBase, whose library has more than 62,000 compounds, and the National Center for Biotechnology Information (NCBI). The analysis protocol contained the compound, molecular weight, retention time, and percentages of the compound in the test sample. The list of detected substances is presented in Table 1.

The GC–MS method revealed a large number of substances belonging to different chemical classes, such as alkanes, alkenes, chlorine-and bromine-containing substances, long-chain fatty alcohols, and others that are known components of many plants (FooDB). Compounds No 4, 15, 17, 24, 29, are qualified as natural antioxidants (Arora et al., 2017; Namdev and Gupta, 2015). Two phenolic antioxidants (4, 15) that presumably contribute to TAC with the HAT mechanism of action were identified. There were identified a large number of compounds with double bonds (8,

Table 1
Identified components of ethanolic extracts of onion husk.

No	Compound	CAS#	MW*	MF**	RT***	Area****
	Non-polar solvent (hexane)					
1	Benzene, 1,3-bis(1,1-dimethylethyl)-	1014-60-4	190	$C_{14}H_{22}$	4.029	1.13
2	Benzenepropanal, 4-(1,1-dimethylethyl)-	18127-01-0	190	$C_{13}H_{18}O$	4.175	5.26
3	Heptadecane, 8-methyl	13287-23-5	254	$C_{18}H_{38}$	4.569	2.76
4	Phenol, 2,4-bis(1,1-dimethylethyl)	96-76-4	206	$C_{14}H_{22}O$	5.814	4.64
5	Heptadecane	629-78-7	240	$C_{17}H_{36}$	6.380	1.35
6	9-Octadecene, 1,1-dimethoxy-	15677-71-1	312	$C_{20}H_{40}O_2$	7.075	0.08
7	Undecane, 2,9-dimethyl-	17301-26-7	184	$C_{13}H_{28}$	7.179	0.89
8	Cholesta-3,5-diene	747-90-0	369	$C_{27}H_{44}$	14.713	3.28
9	Cholest-5-en-3-ol (3.beta.)-, acetate	604-35-3	429	$C_{29}H_{48}O_2$	14.713	3.28
10	9,19-Cycloergost-24(28)-en-3-ol,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)	10376-42-8	469	$C_{32}H_{52}O_2$	16.197	8.06
11	Silane,(9,19-*cyclo*-9.beta.-lanost-24-en-3.beta.-yloxy)trimethyl-	17608-55-8	499	$C_{33}H_{58}OSi$	18.210	4.28
	Polar solvent (acetonitrile)					
12	Benzene, 1,3-bis(1,1-dimethylethyl)-	1014-60-4	190	$C_{14}H_{22}$	4.034	0.40
13	Benzenepropanal,4-(1,1-dimethylethyl)-	18127-01-0	190	$C_{13}H_{18}O$	4.143	1.63
14	Imidazol-5(2H)-one, 4-amino-2-(2-furyl)-, oxime, 3-oxide	318259-17-5	196	$C_7H_8N_4O_3$	4.896	0.10
15	Phenol, 2,4-bis(1,1-dimethylethyl)	96-76-4	206	$C_{14}H_{22}O$	5.882	1.17
16	Sarcosine, N-(3-methoxybenzoyl)-, dodecyl ester	1000321-50-0	391.5	$C_{23}H_{37}NO_4$	6.157	0.15
17	4-(3-Amino-1H-1,2,4-triazol-5-yliminomethyl)resorcinol	321968-42-7	219	$C_9H_9N_5O_2$	6.618	0.05
18	Pyrrolidine-3-carboxamide, 1-isopropyl-5-oxo-N-(2-thiazolyl)-	351067-38-4	253	$C_{11}H_{15}N_3O_2S$	6.753	0.12
19	Urea, N,N-dibutyl-N'-(3,4-dichlorophenyl)-	15442-05-4	317	$C_{15}H_{22}Cl_2N_2O$	7.002	0.23
20	2-Propenoic acid, 2-methyl-, 2,4,6-tribromophenyl ester	37721-71-4	399	$C_{10}H_7Br_3O_2$	7.568	0.07
21	Tridecanol, 2-ethyl-2-methyl-	1000115-66-1	242	$C_{16}H_{34}O$	8.081	0.35
22	Triacontane, 1-bromo-	4209-22-7	502	$C_{30}H_{61}Br$	9.036	0.10
23	3-Octen-2-ol, 2-methyl-, (Z)-	18521-07-8	142	$C_9H_{18}O$	9.239	0.10
24	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	75581-03-2	342	$C_{25}H_{42}$	12.196	1.39
25	1,19-Eicosadiene	14811-95-1	278.5	$C_{20}H_{38}$	13.623	1.92
26	Cholesta-3,5-diene	747-90-0	369	$C_{27}H_{44}$	14.733	1.77
27	Cholest-5-en-3-ol, 4,4-dimethyl-,(3.beta.)-	1253-88-9	415	$C_{29}H_{50}O$	15.527	2.12
28	Ergost-25-ene-3,5,6,12-tetrol, (3. beta.,5.alpha.,6.beta.,12.beta.)-	56052-97-2	449	$C_{28}H_{48}O_4$	16.804	2.41
29	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-	1259-10-5	469	$C_{32}H_{52}O_2$	18.246	5.57

* Molecular weight (g/mol). ** Molecular formula. *** The retention time (min). **** Peak area, %.

25, 26), as well as polyene No 24. Presumably, the listed compounds may be antioxidants with the SET mechanism of action. However, the activity of polyenes is small, but in the presence of proton donors in higher concentrations, activity can increase. In this case, the compounds could contribute to the TAC through two mechanisms simultaneously.

Concentrations of vitamins in ethanolic extracts of onion husk are presented in Table 2.

Vitamins B2, B9, C и B1 were observed in ethanolic extract of onion husk. Such vitamins as B1 (thiamine), B2 (riboflavin), B3 (nicotinic acid), B6 (pyridoxine), B9 (folic acid) and vitamin H (biotin) are nitrogen-containing heterocyclic compounds that exhibit antioxidant properties according HAT mechanism of action and contribute to the TAC. Essential vitamin C is an integral part of mammals AOS and is the main representative of the antioxidants class of dienols, contributing to the TAC due to SET mechanism of action.

Table 2
Vitamin composition in ethanolic extracts of onion husk.

Vitamin	Content
B1, mg/100 g	0.53 ± 0.05
B2, mg/100 g	0.070 ± 0.007
B3, mg/100 g	n/d*
B5, mg/100 g	n/d*
B6, mg/100 g	n/d*
B9, μg/100 g	4.06 ± 0.41
B12, μg/100 g	n/d*
H, μg/100 g	n/d*
C, mg/100 g	0.27 ± 0.03
A, μg/100 g	n/d*
D2, μg/100 g	n/d*
E, μg/100 g	n/d*

*n/d means non-detected.

3.3. Animal study results

3.3.1. Integral indicators of chronic intoxication analysis

In our study we used ethanolic extract of onion husk administrated to ageing rats for 188 days. Long-term consumption of ethanol could lead to intoxication; therefore, IICIs were evaluated (Table 3):

There were no statistically significant differences between the experimental and control groups in the IICIs of internal organs; therefore, the administration of alcoholic onion husk extract with the selected ethanol concentration (not exceed 8%) to rats for an extended time did not have a toxic effect on the laboratory animals.

3.3.2. Antioxidant status of rat plasma

There were no statistically significant differences in the AOS parameters in the blood and plasma of the rats. However, GSH concentration in blood of experimental group rats showed a slight increase (2.3%; P = 0.316) compared to the control group. Content of MDA-TBA complex in plasma of the experimental group rats demonstrated a negligible decline (1.4%; P = 0.430) compared to control group (Table 4).

There was also increases in SOD and CAT activities in the plasma of the experimental group rats of 18.6% (P = 0.225) and 6.1% (P = 0.753), respectively, relative to the control group, but this increase was not statistically significant due to the wide variability of values within each group reflected in the value of the interquartile ranges.

It was also observed that, despite a noticeable increase in the activity of enzymatic antioxidants, the plasma TAC of experimental group rats determined by the FRAP method, which included protein components, in contrast decreased by 3.3% (P = 0.753) compared to the control group.

Table 3
Integral indicators of chronic intoxication of internal organs of rats.

Internal organ		Control group	Experimental group	P–value
Liver	Median ± SD	3.25 ± 0.42	3.08 ± 0.29	0.600
	P 25/75	2.89/3.67	2.86/3.41	
Brain	Median ± SD	0.40 ± 0.07	0.40 ± 0.04	0.674
	P 25/75	0.35/0.47	0.39/0.45	
Heart	Median ± SD	0.37 ± 0.08	0.38 ± 0.03	0.958
	P 25/75	0.36/0.41	0.36/0.40	
Kidney	Median ± SD	0.32 ± 0.03	0.31 ± 0.02	0.637
	P 25/75	0.29/0.34	0.30/0.32	
Spleen	Median ± SD	0.33 ± 0.13	0.27 ± 0.04	0.875
	P 25/75	0.20/0.44	0.26/0.31	

*Significant difference as compared to control group (based on two-tailed Mann–Whitney *U* test).

Table 4
Parameters of rat AOS in blood and plasma.

Variables		Control group	Experimental group	P–value
CAT, U/g protein	Median ± SD	1.80 ± 1.28	1.91 ± 0.86	0.753
	P25/75	1.02/3.58	0.86/2.18	
GSH, μmol/L	Median ± SD	87.82 ± 8.12	89.81 ± 2.50	0.316
	P25/75	84.22/90.61	87.54/91.87	
TAC, μmol-equiv. quercetin/L	Median ± SD	374.13 ± 80.24	361.63 ± 82.08	0.753
	P25/75	335.62/427.97	358.44/449.23	
MDA-TBA complex, μmol/L	Median ± SD	7.63 ± 2.16	7.52 ± 1.68	0.430
	P25/75	6.14/7.85	6.55/10.0	
SOD, U/mg protein	Median ± SD	6.65 ± 1.58	7.89 ± 2.51	0.225
	P25/75	5.33/7.34	5.40/9.70	

*Significant difference as compared to control group (based on two-tailed Mann–Whitney *U* test).

3.3.3. Antioxidant status of rat liver

Long-term consumption of ethanolic onion husk extract increased the activity of the AOS in the liver of ageing rats (Table 5).

There was a statistically significant increase in SOD and CAT activities in the liver of the experimental group rats by 79.1% (P = 0.001) and 44.4% (P = 0.009), respectively, relative to the control group. Concentration of GSH in the liver of experimental group rats was also elevated by 88.3% (P = 0.002) compared to the control group.

Increased activity of enzymes as well as GSH content was reflected in a TAC increase in experimental rat liver by 59.1% (P = 0.001) compared to the control group.

Interestingly, the content of MDA-TBA complex in the liver of experimental group rats was increased by 82.0% (P = 0.059) compared to control group, but this was not statistically significant due to the wide variability of values within each group, as reflected in the value of the interquartile ranges.

3.3.4. Antioxidant status of rat brain

Long-term consumption of ethanolic onion husk extract increased the activity of the AOS in brain of ageing rats, mainly due to elevation of enzyme activity. Thus, there was a statistically significant increase in SOD and CAT activities in the brain of experimental group rats by 79.0% (P = 0.012) and three-fold (P = 0.002), respectively, relative to the control group (Table 6).

The elevation in GSH concentration in the brain of experimental group rats was not statistically significant, but noticable, and averaged 34.0% (P = 0.115) compared to the control group. Increased enzyme activity and GSH content led to a TAC increase in experimental rat brain by 47.7% (P = 0.059) compared to the control group.

As in the analysis of the AOS of the liver, the content of MDA-TBA complex in the liver of experimental group rats was increased by 18.9% (P = 0.074) compared to control group, but it was not statistically significant.

Table 5
Parameters of rat AOS in liver extracts.

Variables		Control group	Experimental group	P–value
CAT, U/g of protein	Median ± SD	204.34 ± 31.76	295.02 ± 70.85*	0.009
	P25/75	195.19/216.77	255.81/331.51	
GSH, μmol/g protein	Median ± SD	7.77 ± 1.57	14.63 ± 3.73*	0.002
	P25/75	7.58/10.45	12.14/18.50	
TAC, μmol-equiv. quercetin/g protein	Median ± SD	19.09 ± 3.19	30.38 ± 4.91*	0.001
	P25/75	16.79/23.06	28.57/33.55	
MDA-TBA complex, μmol/g protein	Median ± SD	0.89 ± 0.64	1.62 ± 0.44	0.059
	P25/75	0.40/1.59	1.26/2.01	
SOD, U/mg protein	Median ± SD	128.14 ± 24.58	229.56 ± 46.68*	0.001
	P25/75	112.40/163.61	191.70/248.30	

* Significant difference as compared to control group (based on two-tailed Mann–Whitney *U* test).

Table 6
Parameters of rat AOS in brain extracts.

Variables		Control group	Experimental group	P–value
CAT, U/g protein	Median ± SD	5.22 ± 2.65	15.91 ± 4.00*	0.002
	P25/75	4.44/9.03	15.17/20.5	
GSH,μmol/g protein	Median ± SD	25.04 ± 4.94	33.56 ± 12.10	0.115
	P25/75	22.27/31.15	25.09/41.11	
TAC, μmol-equiv. quercetin/g protein	Median ± SD	35.70 ± 9.28	52.73 ± 10.53	0.059
	P25/75	32.47/50.95	47.59/55.93	
MDA-TBA complex, μmol/g protein	Median ± SD	3.34 ± 0.67	3.97 ± 1.10	0.074
	P25/75	2.30/3.56	3.25/5.01	
SOD, U/mg protein	Median ± SD	242.71 ± 40.82	434.54 ± 113.60*	0.012
	P25/75	221.44/263.66	377.81/494.78	

* Significant difference as compared to control group (based on two-tailed Mann–Whitney *U* test).

4. Discussion

In recent years, numerous studies have confirmed that onions are a rich source of phytochemicals that promote health. It was suggested that consuming onion extract could decrease glucose levels in the blood, thereby contributing to the reduction in risk factors associated with diabetes (Akash et al., 2014). It also was confirmed that onion oil demonstrated an antioxidant effect against oxidative damage in rats caused by nicotine (Helen et al., 2000). A number of studies confirm that the use of onion husk extract can also have a positive effect on human health (Benítez et al., 2012; Kim, 2007; Ojieh et al., 2015). Thus, the extract of onion peels effect on lipid metabolism is a reduction in cholesterol levels (Helen et al., 2000). The ability of methanol extract of onion husk to inhibit the formation of melanin in melanoma cells was also reported (Arung et al., 2011) and the antispasmodic and anti-hypertensive activities of anion husk described (Gharib et al., 2008).

The main aim of our study was to investigate the effect of OHE on the AOS indicators of ageing laboratory rats. It was revealed that daily oral intake of onion peel extract for an extended time (188 days) can affect the indicators of the AOS of the liver (Table 5) and the brain (Table 6), but it does not affect the indicators of AOS in the blood and plasma (Table 4). It was also shown that OHE did not have an acute toxic effect. The IICI of the main organs of animals in the experimental and control groups did not differ significantly (Table 3). On the other hand, statistically insignificant increases in the level of MDA-TBA complex in animals that consumed the extract were observed: by 82% in the liver and by 18.0% in the brain. It is known that alcohol intake can stimulate ROS generation accompanied by a decrease in GSH (Ogbuagu et al., 2019); therefore the revealed stimulation of lipid peroxidation could be linked to chronic consumption of ethanol by animals despite its low concentration. The reference group consumed ethanolic water solution was not included in study according to welfare rules laboratory animals, because its chronic toxity was studied in numerous studies (Bondy, 1992; Hernández et al., 2016). However, the results of our work show that even with chronic ethanol intake and ageing, the concentrations of GSH, TAC and the activity of antioxidant enzymes in the liver and brain of animals consuming husk extract were increased. Based on the obtained data, it can be argued that the onion husk demonstrated a high protective potential due to antioxidant action, which corresponds to other studies (Ogbuagu et al., 2019; Saravanan and Pari, 2005; Zhao et al., 2016).

Ageing is accompanied by a decrease in the activity of the AOS and the development of chronic oxidative stress. The concentration of GSH is one of the biomarkers of oxidative stress, since GSH is an important intracellular non-enzymatic endogenous antioxidant that can neutralise FRs and is a cofactor for many antioxidant enzymes (Malfa et al., 2020). It was shown that the introduction of onion husk extract increased the concentration of GSH in the liver by 88.3%, but in the brain its elevation was not so noticeable and averaged only 34.0%. An increase in the level of GSHcould be explained by direct absorption of ROS by the antioxidants of the husk extract or by the stimulation of glutathione synthesis. Our observations corresponded to the results of another study (Zaki, 2019), in which an increase in the GSH concentration in the lungs of rats treated with onion extract was recorded.

Superoxide dismutase and catalase are two of the main enzymatic antioxidants. The main activity of SOD is to dismute the superoxide anion to hydrogen peroxide, which is reduced by CAT to water and molecular oxygen (Galadari et al., 2017), thereby preventing the formation of a highly reactive hydroxyl radical (HO•) (Lubrano, 2015). We revealed that intake of onion husk extract increased the activities of CAT and SOD in the liver by 44.4% and 79.1%, respectively, and in the brain three-fold and by 79.1%, respectively. Obtained data indicate that the alcoholic extract of onion husk possessed neuroprotective properties; our results were also comparable with the observations of Okoro et al. on the neuroprotective effect of onion extract on rats (Okoro et al., 2019). It was also reported that the introduction of an aqueous extract of *Allium cepa* to rats administered cadmium sulfate led to an increase in the activity of superoxide dismutase and catalase (Ige et al, 2020). An increase in the activity of enzymes and the concentration of GSH led to increases in TAC in the liver and brain of experimental animals by 59.1% and 47.7%, respectively. Obtained data demonstrated that yellow onion husks can be effective in AOS stimulation in the liver and brain of ageing rats.

ORAC and FRAP were used to determine the TAC of ethanolic onion husk extract. TAC_{ORAC} averaged 941.4 ± 32.7 μmol equiv. Trolox/g raw material, while TAC_{FRAP} amounted only 167.4 ± 16.4 μm ol-equiv. quercetin/g raw material. These values of TAC differ from those from other studies (Kim et al., 2010), which is explained by the significant influence of the species, place and conditions of growth and storage on the content of phenolic compounds (Benkeblia, 2005), as well as of the methods of extraction of target substances used. Marrelli et al. summarised a huge number of articles devoted to the study of the properties of onions and their husks, where authors had studied different species and used various extraction approaches and methods for determining antioxidant potential, and finally concluded that onion husk contained a greater amount of bioactive substances than the bulb (Marrelli et al., 2019).

The prospects of using fruit and vegetable wastes, in particular the by-products of onion processing, are actively studied by many scientists. Hollman et al. investigated more than 15 plants and showed that the quercetin content in onions is significantly higher than in each of the other studied samples (Hollman and Arts, 2000). Previously, we showed that the antioxidant potential of onion husks is not inferior to such well-studied and widely used plants such as basil and rosemary (Kotenkova and Kupaeva,

2019). We also revealed that aqueous extracts of the peel of quince, apple «Simirenko», feijoa, persimmon, Jerusalem artichoke, and white, red and yellow onion were more promising sources of natural antioxidants than the internal parts of the plants (Bedrníček et al., 2019). Moreover, a number of flavonoids were also found in various onion species, such as quercetin aglycon, quercetin-3, 4′-diglucoside, quercetin-4′-monoglucoside, quercetin-3-monoglucoside, quercetin 3-glycosides, delphinidin 3, 5-diglycosides, quercetin 3,7,4′-triglucoside, quercetin 7,4′-diglucoside, quercetin 3,4′-diglucoside, isorhamnetin 3,4′-diglucoside and others (Kwak et al., 2017; Teshika et al., 2019). Allium cepa contains a 5–10-fold higher quercetin content (300 mg/kg) than broccoli (100 mg/kg), apple (50 mg/kg) and blueberries (40 mg/kg) (Teshika et al., 2019). Based on published data, the content of phenolic compounds depends significantly on the species and place and conditions of growth and storage (Benkeblia, 2005). It is known that the level of flavonoids in the husk of onions varies from 2 to 10 g/kg, while the edible part of the onion contains only 0.03–1 g/kg (Piechowiak et al., 2020; Ren et al., 2020). Moreover, onion husk contains dietary fibre, fructooligosaccharides, alk(en)yl cystein sulphoxides and flavonoids (Benítez et al., 2011), as well as quercetin and its glycosides (Bedrníček et al., 2019). In our study, total phenols content in ethanolic extract of onion husk averaged 4.1 ± 0.2 mg-equiv. gallic acid /g plant material and was in 10 folds lower than in Burri et al. research results. This fact could be explained that authors used dry matter for investigation (Burri et al., 2017). Thus, the availability, cheapness and high antioxidant potential of onion waste attracts the attention of scientists to onion husk and its extract as a functional food ingredient that has already been used for fortifying a number of products such as wheat bread, gluten-free bread, meat and fish cutlets (Bedrnícek et al., 2020).

5. Conclusions

Long-term consumption of *Allium cepa* husk ethanolic extract did not affect status of the antioxidant system in the blood and plasma of ageing rats. Nevertheless, evident stimulation of the antioxidant system was observed in the liver and brain of animals, mainly due to elevated activity of enzymes such as catalase and superoxide dismutase, which led to increases in the total antioxidant capacity of internal organs. *Allium cepa* husk was also characterised by high antioxidant capacity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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