Ukrainian Journal of Natural Sciences
№ 7
Український журнал природничих наук
№ 7

ISSN: 2786-6335 print
ISSN: 2786-6343 online

БІОЛОГІЯ

УДК 58.085: 612.111: 577.112.4: 577.115.4
DOI https://doi.org/10.32782/naturaljournal.7.2024.1

DOSE-DEPENDENT CHANGES IN BIOMARKERS OF OXIDATIVE STRESS IN HUMAN ERYTHROCYTES FOLLOWING *IN VITRO* TREATMENT WITH EXTRACTS FROM BERRIES OF EUROPEAN MISTLETOE (*VISCUM ALBUM* L.)

H. M. Tkaczenko[1], O. V. Tverdokhlib[2], V. I. Honcharenko[3], N. M. Kurhaluk[4]

Radical scavenging activity and protective effects against oxidative stress caused by free radicals, nitric oxide and superoxide anion have been demonstrated for a number of mistletoe extracts and isolated lectins. The aim of the present study was to determine the antioxidant activity of extracts from the berries of mistletoe (Viscum album L.). For this purpose, biomarkers of oxidative stress [2-thiobarbituric acid reactive substances (TBARS) as a biomarker of lipid peroxidation, carbonyl derivatives of oxidative modification of proteins, total antioxidant capacity (TAC)] were used in human blood after in vitro incubation with extracts derived from mistletoe berries at two final concentrations (5 and 2.5 mg/mL). The results of our study showed that treatment with mistletoe berry extracts resulted

[1] Professor, Ph.D., D.Sc.
Deputy Director of the Faculty of Biology,
Department of Zoology,
(Pomeranian University of Słupsk, Słupsk, Poland)
e-mail: halina.tkaczenko@upsl.edu.pl
ORCID: 0000-0003-3951-9005
[2] Candidate of Biological Sciences (Ph.D. in Biology)
Senior Researcher, Associate Professor at the
Department of Botany
(H.S. Skovoroda Kharkiv National Pedagogical University, Kharkiv)
e-mail: etverd@hnpu.edu.ua
ORCID: 0000-0002-7209-1808
[3] Head of the Department of Botany,
Deputy Dean of the Faculty of Biology for educational and methodological work,
Candidate of biological sciences,
Associate Professor, art. of science employee
Faculty of Biology
(Ivan Franko National University in Lviv, Lviv)
e-mail: vitaliy.honcharenko@lnu.edu.ua
ORCID: 0000-0001-6888-2124
[4] Ph.D., D.Sc.,
Head of the Department of Animal Physiology
(Pomeranian University of Słupsk, Słupsk, Poland)
e-mail: natalia.kurhaluk@upsl.edu.pl
ORCID: 0000-0002-4669-1092

in a significant increase in TBARS levels in human erythrocytes after in vitro treatment with extracts at final concentrations of 5 mg/mL compared to untreated samples. On the other hand, a statistically non-significant decrease in TBARS levels was observed for the extract at a final concentration of 2.5 mg/mL. The levels of aldehydic derivatives of oxidatively modified proteins were statistically significantly increased in samples treated in vitro with mistletoe berry extracts at a final concentration of 5 mg/mL compared to untreated samples, and this increase was statistically significant. Treatment of human erythrocytes with mistletoe berry extracts at a final concentration of 2.5 mg/mL resulted in a statistically significant decrease in the levels of aldehydic derivatives of oxidatively modified proteins. The reduction was 24.1% ($p < 0.05$). When human erythrocytes were incubated with mistletoe berry extracts, the levels of ketonic derivatives of OMP were at the same level as in untreated samples. Treatment of human erythrocytes with mistletoe berry extracts at a final concentration of 2.5 mg/ml resulted in a statistically non-significant decrease in the levels of ketonic derivatives of oxidatively modified proteins. TAC levels in human erythrocytes were increased after in vitro incubation with mistletoe berry extracts (final concentration of 5 mg/mL) compared to untreated samples. This represented a 29% ($p < 0.05$) increase in TAC levels compared to untreated samples. TAC levels in human erythrocytes after in vitro incubation with mistletoe berry extracts (final concentration 2.5 mg/mL) were at the same level as in untreated samples. Future studies can add to the current findings to better understand the antioxidant properties of mistletoe berry extracts, with the potential to develop treatments and products using these extracts.

***Key words:** European mistletoe (Viscum album L.), human blood, extracts, 2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of oxidative modification of proteins, total antioxidant capacity (TAC)*

ДОЗОЗАЛЕЖНІ ЗМІНИ БІОМАРКЕРІВ ОКИСНЮВАЛЬНОГО СТРЕСУ В СУСПЕНЗІЇ ЕРИТРОЦИТІВ ЛЮДИНИ ПІСЛЯ ІНКУБАЦІЇ *IN VITRO* З ЕКСТРАКАТАМИ ЯГІД ОМЕЛИ ЗВИЧАЙНОЇ (*VISCUM ALBUM* L.)

Г. М. Ткаченко, О. В. Твердохліб, В. І. Гончаренко, Н. М. Кургалюк

Активність поглинання радикалів і захисні ефекти проти окислювального стресу, спричиненого вільними радикалами, оксидом азоту та супероксидним аніоном, були продемонстровані для ряду екстрактів омели та окремих її лектинів. Метою цієї роботи було визначення антиоксидантної активності екстрактів ягід омели звичайної (Viscum album L.). Для цього інкубували кров людини in vitro з екстрактом ягід омели у двох концентраціях (5 та 2,5 мг/мл). Результати нашого дослідження показали, що інкубація суспензії еритроцитів з екстрактами ягід омели призвело до значного підвищення рівня речовин, які реагують з 2-тіобарбітурової кислоти (TBARS) як біомаркерів перекисного окиснення ліпідів в еритроцитах людини після обробки in vitro екстрактами в кінцевих концентраціях 5 мг/мл порівняно з необробленими зразками. З іншого боку, статистично неістотне зниження рівня TBARS в суспензії еритроцитів спостерігалося після використання екстракту при кінцевій концентрації 2,5 мг/мл. Рівні альдегідних похідних окиснювально модифікованих білків були статистично істотно підвищені у зразках, оброблених in vitro екстрактами ягід омели звичайної в кінцевій концентрації 5 мг/мл порівняно з необробленими зразками. Обробка еритроцитів людини екстрактами ягід омели в кінцевій концентрації 2,5 мг/мл призвела до статистично істотного зниження рівнів альдегідних похідних окиснювально модифікованих білків. Зниження становило 24,1% ($p < 0,05$). Після інкубації еритроцитів з екстрактами ягід омели, рівні кетонових похідних були на тому ж рівні, що і в необроблених зразках. Обробка еритроцитів людини екстрактами ягід омели білої в кінцевій концентрації 2,5 мг/мл призвела до статистично неістотного зниження рівнів кетонових похідних окиснювально модифікованих білків. Рівні загальної антиоксидантної активності (TAC) в еритроцитах були підвищені (на 29%, $p < 0,05$) після інкубації in vitro з екстрактами ягід омели (кінцева концентрація 5 мг/мл) порівняно з необробленими зразками. Рівні TAC в еритроцитах людини після інкубації in vitro з екстрактами ягід омели (кінцева концентрація 2,5 мг/мл) були на тому ж рівні, що й у необроблених зразках. Майбутні дослідження можуть доповнити поточні результати, щоб краще зрозуміти антиоксидантні властивості екстрактів ягід омели, з потенціалом для розробки засобів лікування та продуктів із використанням цих екстрактів.

***Ключові слова:** омела звичайна (Viscum album L.), кров людини, екстракти, реактивні речовини, які взаємодіють з 2-тіобарбітуровою кислотою (TBARS), карбонільні похідні окиснювально модифікованих білків, загальна антиоксидантна активність (TAC).*

Introduction

Oxidative stress, or the uncontrolled increase in free radical reactions, is a pressing problem in medicine and biology. Its development is the main pathogenetic mechanism in the development of many human diseases (Luo et al., 2020; Hajam et al., 2022; Teleanu et al., 2022). As a result of the activation of free radical processes, oxidative modification of biomolecules (proteins, lipids, nucleic acids) occurs, ultimately leading to cell and organ damage and death (Liu et al., 2022). From birth, the human body has an antioxidant defence system that protects cell membranes from potentially dangerous reactions that cause them to oxidise. However, this protection weakens over time and needs to be constantly replenished and supported (Benzie, 2000; Tiberi et al., 2023).

The problems of chemical regulation of oxidative stress and the search for biologically active substances with antioxidant activity are the focus of many researchers. At present, there is a constant search for new drugs that are alternatives to synthetic substances, with high biological activity, low toxicity and no side effects (Valko et al., 2007; Demirci-Çekiç et al., 2022). Antioxidants of plant origin and natural composition have a number of advantages over synthetic antioxidants; they provide a fairly wide range of beneficial physiological effects on the body. The chemically similar structure of plant biologically active substances (BAS) to the structure of metabolites in the human body increases the availability of drugs of natural origin for the effective influence of human enzyme systems, which influences the efficacy of such drugs and makes them quite safe (Amarowicz & Pegg, 2019; Lourenço et al., 2019). Plant antioxidants bind free radicals, suppress free radical oxidation reactions and thus create normal conditions for metabolism (Liu, 2013). A number of biologically active plant compounds (polyphenols: flavonoids, tannins, ascorbic acid, etc.) have pronounced antioxidant properties (Carlsen et al., 2010; Kozłowska & Szostak-Wegierek, 2014). Currently, the introduction of natural antioxidants into products of the food, pharmacological and especially cosmetic industries of natural components is actively used, with special attention being paid to local plant raw materials due to their easy availability and renewability (Halvorsen et al., 2002; Tapsell et al., 2006).

Radical scavenging activity and protective effects against oxidative stress caused by free radicals, nitric oxide and superoxide anion have been demonstrated for a number of mistletoe extracts and isolated lectins (Kim et al., 2010; Patil et al., 2011). As a medicinal plant, mistletoe (*Viscum album* L.) has a long history of use in both formal and folk medicine, and has become an indispensable ingredient in many remedies (Nazaruk & Orlikowski, 2016). Tea made from mistletoe leaves is used to treat high blood pressure, menopausal symptoms, heavy menstrual periods, uterine bleeding, gastrointestinal bleeding, haemorrhoids, chronic joint problems and bronchial asthma (Kim et al., 2015). Mistletoe has excellent hypotensive, nervous system calming, analgesic, astringent, antitumour and haemostatic properties (Sunjic et al., 2015; Nazaruk & Orlikowski, 2016; Thronicke et al., 2022). By increasing the tone of the blood vessels, mistletoe tea also helps with poor health, loss of strength and dizziness (especially in old age) (Nazaruk & Orlikowski, 2016; Nicoletti, 2023). Mistletoe also stimulates the appetite, increases the secretion of gastric juices, improves metabolism and helps to remove cholesterol from the body. It has a detoxifying, choleretic and tonic effect (Kienle & Kiene, 2010).

Mistletoe is an evergreen parasitic plant that has leaves and the ability to photosynthesise, but no root system of its own, so it cannot feed on soil substrates (Mistletoe, 1988). Mistletoe is fed by donor plants to whose branches it is attached. It parasitises almost 40 species of trees, including maple, pine, willow, birch, false acacia, rowan, lime, chestnut, pine and fir. The most susceptible species are poplar, birch, lime and some fruit trees such as apple and pear. The most common trees on which mistletoe is found are poplar, apple and pear. After settling on the top of a tree or its branches, it grows into a dense green shrub (Kienle et al., 2011).

Mistletoe contains a number of biologically active substances. Among the best described and most active phytochemicals identified in *V. album* are lectins and viscotoxins, which play an important role in cancer treatment due to their apoptotic and cytotoxic effects (Nazaruk and Orlikowski, 2016). Preclinical studies have demonstrated cytotoxic, apoptosis-inducing and immunomodulatory effects of *V. album* lectins and viscotoxins (Melzer et al., 2009; Rostock, 2020). Another group of compounds found in mistletoe are phenolic acids, phenylpropanoids and flavonoids, which have antioxidant and anti-inflammatory activities and reduce blood pressure (Radenkovic et al.,

2009; Nazaruk & Orlikowski, 2016; Nicoletti, 2023). Other mistletoe constituents include triterpenes with cytotoxic and apoptotic properties, phytosterols, oligo- and polysaccharides (Khwaja et al., 2008; Nazaruk & Orlikowski, 2016; Beztsinna et al., 2018).

The aim of the present study was to determine the antioxidant activity of extracts derived from the berries of mistletoe (*Viscum album*). For this purpose, biomarkers of oxidative stress [2-thiobarbituric acid reactive substances (TBARS) as a biomarker of lipid peroxidation, carbonyl derivatives of oxidative modification of proteins, total antioxidant capacity (TAC)] were used in the human blood after *in vitro* incubation with extracts derived from the berries of *Viscum album* at two final concentrations (5 and 2.5 mg/mL).

Material and methodology

Collection of Plant Material. The plant material of apple tree (*Malus* Mill.) was collected in Dubno (50°23′35″N 25°44′06″E), a town and village on the Ikva River in Rivne Oblast (province), western Ukraine. It serves as the administrative centre of Dubno Raion (district). Freshly picked fruits were washed, weighed, crushed and homogenised in 0.1 M phosphate buffer (pH 7.4) (at a ratio of 1:19, w/w) at room temperature and centrifuged at 3,000 rpm for 5 min. The extracts were then filtered and used for analysis. The supernatants were stored at -20°C in bottles protected with laminated paper until needed. This work was supported by the Pomeranian University in Słupsk (Poland) in collaboration with H. S. Skovoroda Kharkiv National Pedagogical University (Kharkiv, Ukraine) and Ivan Franko National University in Lviv (Lviv, Ukraine). The authors acknowledge and are grateful for the support of the International Visegrad Fund.

Collection of blood samples. Blood (10 mL) was collected from healthy volunteers by venipuncture. The study was approved by the Regional Research Ethics Committee of the Medical University of Gdansk, Poland (KB-31/18). All patients gave written informed consent before the start of the study procedures. Blood samples were drawn into commercially available tubes after overnight fasting. Venous blood samples (10 mL) were obtained from the antecubital vein of each participant using sterile disposable plastic syringes. Samples were collected at the same standardised time to minimise the effect of diurnal variation. Blood was kept on ice until centrifuged at 3,000 rpm for 5 minutes. The plasma was removed. The erythrocyte suspension (one volume) was washed three times with five volumes of saline and centrifuged at 3,000 rpm for 5 minutes.

Experimental design. The erythrocyte suspensions were used for incubation with mistletoe berry extracts, followed by determination of 2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of protein oxidative modification, and total antioxidant capacity (TAC). The erythrocyte suspension samples were pre-incubated with 4 mM phosphate buffer (pH 7.4) (control) and with mistletoe berry extracts (at final concentrations of 5 and 2.5 mg/mL) at 37°C for 60 minutes. This reaction mixture was gently shaken at fixed intervals during incubation at 37°C. A 4 mM phosphate buffer (pH 7.4) was used as a positive control.

The 2-Thiobarbituric acid reactive substances (TBARS) assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reactive substances (TBARS) using the method of Buege and Aust (1978) for the determination of malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation product of lipid peroxidation, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to form a coloured adduct which is measured spectrophotometrically. The nmol of MDA per mL was calculated using an extinction coefficient of $1.56 \cdot 10^5$ $mM^{-1} \cdot cm^{-1}$.

Carbonyl groups of the oxidatively modified proteins assay. Carbonyl groups were measured as an indication of oxidative damage to proteins according to the method of Levine and co-workers (1990) with some modification. Carbonyl content was measured spectrophotometrically at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}) (molar extinction coefficient 22,000 $M^{-1} \cdot cm^{-1}$) and expressed as nmol per mL.

Total antioxidant capacity (TAC) assay. Blood TAC levels were estimated by measuring TBARS levels after oxidation of Tween 80 as described previously (Kurhaluk et al., 2023). Blood inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The absorbance of the solution obtained was measured at 532 nm. The absorbance of the blank was defined as 100%. The content of TAC in the sample (%) was calculated from the absorbance of the blank.

Statistical analysis. The mean ± S.E.M. values were calculated for each group to determine the significance of the difference between the groups. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors tests ($p > 0.05$). The significance of differences between the levels of oxidative stress biomarkers (significance level, $p < 0.05$) was tested using the Kruskal–Wallis one-way analysis of variance (Zar, 1999). All statistical calculations were performed on separate data from each individual using STATISTICA 13.3 software (TIBCO Software Inc., USA).

Results and discussion

The TBARS assay measures malondialdehyde (MDA) present in a sample as well as malondialdehyde formed from lipid hydroperoxides under hydrolytic reaction conditions. Malondialdehyde (MDA) is one of many low molecular weight end products of LPO (Khalili & Biloklytska, 2008). The levels of TBARS as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins (OMP), and total antioxidant capacity (TAC) in human erythrocytes after *in vitro* treatment with extracts derived from the berries of mistletoe (*Viscum album*) at two final concentrations (5 and 2.5 mg/mL) were assessed and shown in Figures 1-3.

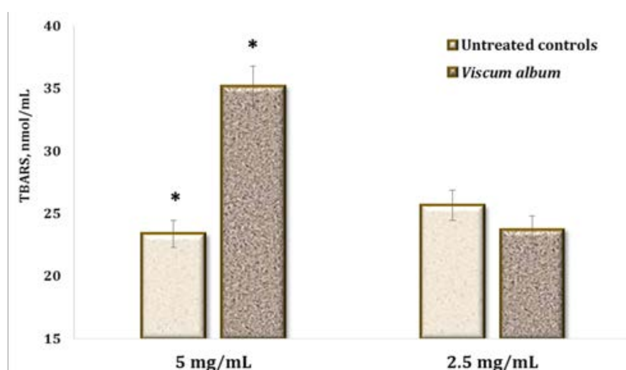

Fig. 1. TBARS content, as a biomarker of lipid peroxidation, in human erythrocytes after *in vitro* treatment with extracts derived from the berries of mistletoe (*Viscum album*) at two final concentrations (5 and 2.5 mg/mL) (M ± m, n = 8). *– statistically significant differences between treated and untreated samples ($p < 0.05$).

As shown in Figure 1, treatment with mistletoe berry extracts resulted in a significant increase in TBARS levels (35.17 ± 1.63 nmol/mL) in human erythrocytes after *in vitro* treatment with extracts at final concentrations of 5 mg/mL compared to untreated samples (23.38 ± 1.08 nmol/mL). A statistically significant increase in TBARS levels (by 50.4% $p < 0.05$) was observed for the extract at final concentrations of 5 mg/mL. On the other hand, a statistically non-significant decrease in TBARS levels (by 7.6% $p > 0.05$) was observed for the extract at a final concentration of 2.5 mg/mL (Fig. 1).

Under the influence of ROS, carbonyl groups (aldehyde or ketone) are formed at the ends of the protein chain, particularly at proline, arginine, lysine and threonine residues, forming 2-pyrrolidone, glutamic semialdehyde, α-aminoadipic semialdehyde and 2-amino-3-ketobutyric acid, respectively (Dalle-Donne et al., 2003). These fragments are chemically stable, which is useful for their assay. In addition, protein carbonyl derivatives can be obtained by oxidative cleavage of the protein chain, either by α-amidation or by oxidation of the glutamyl end of the protein chain, resulting in a peptide in which the N-terminus is blocked by a carbonyl derivative (Berlett & Stadtman, 1997). Carbonyl groups can be introduced into the protein chain by secondary exposure to both lipid peroxidation products (4-hydroxy-2-nonenal, malondialdehyde, 2-propenal or acrolein) and reactive carbonyl compounds (ketoamines, ketoaldehydes) formed during the metabolism of carbohydrates or their oxidised products that are tropic to lysine residues (glycation and glycosylation reactions). Glycosylation reactions produce carboxymethyllysine and pentosidine. LPO-induced oxidative modification of proteins produces malondialdehyde-lysine and 4-hydroxynonenal-peptide products (Berlett & Stadtman, 1997; Squier, 2001).

The levels of aldehydic and ketonic derivatives of oxidatively modified proteins in in human erythrocytes after *in vitro* treatment with extracts derived from the berries of mistletoe (*Viscum album*) at two final concentrations (5 and 2.5 mg/mL) are shown in Figure 2.

The levels of aldehydic derivatives of oxidatively modified proteins were increased in samples treated *in vitro* with mistletoe berry extracts at a final concentration of 5 mg/mL (12.84 ± 0.60 nmol/mL) compared to untreated samples (9.89 ± 0.46 nmol/mL), and this increase was statistically significant (by 29.8%, $p < 0.05$). Treatment of human erythrocytes with mistletoe berry extracts at a final concentration of 2.5 mg/mL resulted in a statistically significant decrease in the levels of aldehydic derivatives of oxidatively modified

proteins to (5.85 ± 0.27 nmol/mL) compared with untreated samples (7.71 ± 0.36 nmol/mL). The reduction was 24.1% ($p < 0.05$) (Fig. 2).

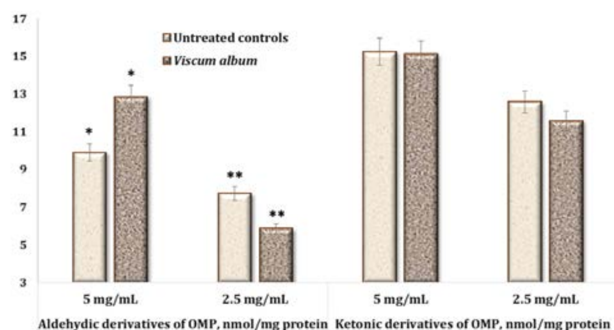

Fig. 2. Levels of aldehydic and ketonic derivatives of oxidatively modified proteins in human erythrocytes after *in vitro* treatment with extracts derived from the berries of mistletoe (*Viscum album*) at two final concentrations (5 and 2.5 mg/mL) (M ± m, n = 8).
*– statistically significant differences between treated and untreated samples at final concentration 5 mg/mL ($p < 0.05$);
**– statistically significant differences between treated and untreated samples at final concentration 2.5 mg/mL ($p < 0.05$)

When human erythrocytes were incubated with mistletoe berry extracts, the levels of ketonic derivatives of OMP (15.12 ± 0.70 nmol/mL) were at the same level as untreated samples (15.23 ± 0.71 nmol/mL). Treatment of human erythrocytes with mistletoe berry extracts at a final concentration of 2.5 mg/ml resulted in a statistically non-significant decrease in the levels of ketonic derivatives of oxidatively modified proteins to (11.57 ± 0.54 nmol/mL) compared to untreated samples (12.54 ± 0.58 nmol/mL). The reduction was 7.7% ($p > 0.05$) (Fig. 2).

Total antioxidant capacity is an indicator of the body's antioxidant system, which protects the body from the toxic effects of a number of oxygen compounds produced in the body, such as oxygen ions, peroxides and free radicals (Bartosz, 2003). Total antioxidant capacity (TAC) levels in human erythrocytes after *in vitro* treatment with extracts from mistletoe berries at two final concentrations (5 and 2.5 mg/ml) are shown in Fig. 3.

In the current study, TAC levels in human erythrocytes were increased to (85.75 ± 3.98%) after *in vitro* incubation with mistletoe berry extracts (final concentration 5 mg/mL) compared to untreated samples (66.48 ± 3.08%). This represented a 29% ($p < 0.05$) increase in TAC levels compared to untreated samples. TAC levels in human erythrocytes after *in vitro* incubation with mistletoe berry extracts (final concentration 2.5 mg/ml) were at the same level (70.16 ± 3.25 %) compared to untreated samples (70.55 ± 3.27 %) (Fig. 3).

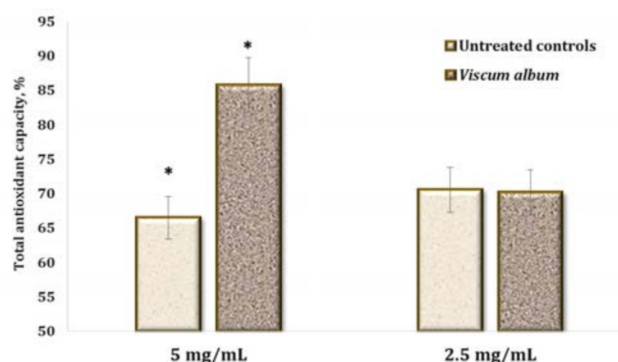

Fig. 3. Total antioxidant capacity (TAC) levels in human erythrocytes after *in vitro* treatment with extracts derived from the berries of mistletoe (*Viscum album*) at two final concentrations (5 and 2.5 mg/mL) (M ± m, n = 8).
*– statistically significant differences between treated and untreated samples at final concentration 5 mg/mL ($p < 0.05$)

The results of the current study demonstrated the antioxidant properties of mistletoe berry extracts at final concentration of 2.5 mg/mL after incubation with human erythrocytes. The results of the current study are similar to those of other researchers who have demonstrated the antioxidant capacity of numerous mistletoe extracts. For example, the epiphyte *V. album* was shown to vary with the type of host plant and extraction solvent. Majeed and co-workers (2021) evaluated the antioxidant profile of the medicinal epiphyte *V. album* harvested from three tree species, namely *Populus ciliata* L., *Ulmus villosa* L., and *Juglans regia* L. The crude extracts were obtained with ethanol, methanol and water and were evaluated for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities using total reducing power (TRP), ferric reducing antioxidant power (FRAP), 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH), superoxide radical scavenging (SOR) and hydroxyl radical scavenging (-OH) assays. The results of these researchers showed that crude leaf extracts of plants harvested from the host *J. regia* exhibited higher yields of phytochemical constituents and significant antioxidant properties. The ethanolic leaf samples showed the highest levels of phenolics

(13.46 ± 0.87 mg/g), flavonoids (2.38 ± 0.04 mg/g), FRAP (500.63 ± 12.58 µM Fe II/g DW) and DPPH (87.26% ± 0.30 mg/mL). In addition, the highest values for TRP (4.24 ± 0.26 µg/mL), SOR (89.79% ± 0.73 mg/mL) and OH (67.16% ± 1.15 mg/mL) were obtained from aqueous leaf extracts. Furthermore, Pearson correlation was used to quantify the relationship between TPC, TFC and antioxidant (FRAP, DPPH, SOR, OH) activities in *V. album* compared to their hosts (Majeed et al., 2021).

Kleszken and co-workers (2022) studied the influence of mistletoe on the content of chlorophylls, proline, total phenolics, flavonoids and antioxidant capacity of leaves of host trees (*Malus domestica*, *Prunus domestica* and *Populus alba*) growing in north-western Romania. Based on HPLC chromatographic analysis, the leaves of mistletoe growing on apple (VAM) had the highest content of phenolic acids (7.833 mg/g dw), followed by poplar (VAO) and plum (VAP) mistletoe leaves (7.033 mg/g dw and 5.559 mg/g dw, respectively). Among the flavonols, rhamnazin glucosides were the predominant component in VAO (1.025 ± 0.08 mg/g dw), followed by VAP and VAM (0.514 ± 0.04 and 0.478 ± 0.04 mg/g dw, respectively) (Kleszken et al., 2022).

The enhanced antioxidant activity of fermented Korean mistletoe (KM) is due to an increase in the levels of caffeic acid and lyoniresinol, as demonstrated by Kim and co-workers (2016). The KM extract showed enhanced antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl, Trolox equivalent antioxidant capacity and 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester assays after fermentation with a crude enzyme extract from a soybean paste fungus, *Aspergillus kawachii*. High-performance liquid chromatography analysis revealed four elevated peaks in the enzyme-treated KM. The elevated peaks were isolated and identified as caffeic acid (1), hesperetin (2), syringaldehyde (3) and lyoniresinol (4). Of the four compounds, only 1 and 4 showed strong antioxidant activity. Therefore, fermentation increased the content of 1 and 4, which consequently increased the antioxidant activity of KM (Kim et al., 2016).

Stefanucci and co-workers (2020) described the chemical profiles and biological activities of homogeniser-assisted extract (HAE) and ultrasound-assisted extract (UAE) of *V. album* parts (leaf, fruit and seeds). Antioxidant (radical scavenging, reducing power, metal chelation and phosphomolybdenum assays) and enzyme inhibitory (cholinesterases, amylase, glucosidase and tyrosinase) properties were selected for biological evaluation. Chemical profiles were studied by HPLC-MS/MS and 32 compounds were identified in the extracts; caffeoylquinic acids and their derivatives, dimethylated flavonoids were the most significant compounds. In general, the leaf extracts showed the best antioxidant and enzyme inhibitory effects in tests. Strong correlations were observed between total bioactive compounds and the parameters tested (Stefanucci et al., 2020).

A conventional experimental rat model of streptozotocin (STZ)-induced diabetes was used to evaluate the effect of mistletoe on lipid peroxidation and the antioxidant system. Orhan and co-workers (2005) investigated the hypoglycaemic effect and antioxidant activity of three subspecies of European mistletoe in streptozotocin-induced diabetic rats. The antioxidant activity of these extracts was also investigated in liver, kidney and heart tissues of streptozotocin-induced diabetic rats after subacute administration. To determine the antioxidant activity of the extracts, tissue MDA and GSH levels were measured using spectrophotometric methods. The results of the experiments showed that European mistletoe subspecies possess potent antihyperglycaemic and antioxidant activity depending on the host plant (Orhan et al., 2005). Also, Turkkan and co-workers (2016) studied the prophylactic effect of *V. album* in streptozotocin-induced diabetic rats. A total of 32 adult male Sprague-Dawley rats were divided into 4 groups of 8 rats each: Control group, STZ group, mistletoe group, and mistletoe + STZ group. VA extract was 100 mg/kg preparation administered once a day by oral gavage for 10 days. A single dose of 55 mg/kg STZ citrate buffer (0.1 M, pH 4.5) was administered intraperitoneally to induce diabetes. Fasting blood glucose was measured and recorded. Animals were sacrificed and catalase (CAT), malondialdehyde (MDA) and protein were measured in liver and kidney tissue samples. Mistletoe given to diabetic rats reduced oxidative stress and improved their general condition (Turkkan et al., 2016).

The antioxidant properties of mistletoe extract administration significantly reduced acute oxidative stress and hepatocellular damage in rats with hepatocellular injury. The protective effect of a mistletoe extract (Helixor®, HLX) on itraconazole (ITZ)-induced hepatocellular injury and acute oxidative stress in rats was investigated by Çetin and co-workers (2023) using histological,

biochemical and comet assay methods. Four groups, a control group, an HLX group (5mg/kg/14days/i.p.), an ITZ group (100mg/kg/14days/oral) and an HLX plus ITZ group (5mg/kg/14days/ip+100mg/kg/14days/oral) were created from 32 female Wistar albino rats. At the end of the experiment, AST and ALT liver enzymes, total oxidant status (TOS) and total antioxidant status (TAS) levels, histopathological analysis and comet assay were performed. The highest genotoxicity, higher levels of plasma AST and ALT, higher TOS, more degenerative liver histopathology including hepatocyte degeneration, hepatocyte apoptosis and necrosis, portal/periportal inflammation, bile duct hyperplasia and multinucleated giant cell formation were observed in the ITZ group ($p<0.05$). In contrast, administration of HLX plus ITZ improved histopathological changes and DNA damage and showed a dramatic decrease in AST, ALT and TOS levels ($p<0.05$) and an increase in TAS level ($p<0.001$) compared to the ITZ group (Çetin et al., 2023).

Conclusions

In the present study, we determined the antioxidant activity of extracts derived from the berries of mistletoe (*Viscum album*). For this purpose, biomarkers of oxidative stress [2-thiobarbituric acid reactive substances (TBARS) as a biomarker of lipid peroxidation, carbonyl derivatives of oxidative modification of proteins, total antioxidant capacity (TAC)] were used in human blood after *in vitro* incubation with extracts derived from mistletoe berries of at two final concentrations (5 and 2.5 mg/mL). The results of our study showed that treatment with mistletoe berry extracts resulted in a significant increase in TBARS levels in human erythrocytes after *in vitro* treatment with extracts at final concentrations of 5 mg/mL compared to untreated samples. On the other hand, a statistically non-significant decrease in TBARS levels was observed for the extract at a final concentration of 2.5 mg/mL.

The levels of aldehydic derivatives of oxidatively modified proteins were statistically significantly increased in samples treated *in vitro* with mistletoe berry extracts at a final concentration of 5 mg/mL compared to untreated samples. Treatment of human erythrocytes with mistletoe berry extracts at a final concentration of 2.5 mg/mL resulted in a statistically significant decrease in the levels of aldehydic derivatives of oxidatively modified proteins. The reduction was 24.1% ($p < 0.05$). When human erythrocytes were incubated with mistletoe berry extracts, the levels of ketonic derivatives of OMP were at the same level as in untreated samples. Treatment of human erythrocytes with mistletoe berry extracts at a final concentration of 2.5 mg/ml resulted in a statistically non-significant decrease in the levels of ketonic derivatives of oxidatively modified proteins. TAC levels in human erythrocytes were increased after *in vitro* incubation with mistletoe berry extracts (final concentration 5 mg/mL) compared to untreated samples. This represented a 29% ($p < 0.05$) increase in TAC levels compared to untreated samples. TAC levels in human erythrocytes after *in vitro* incubation with mistletoe berry extracts (final concentration 2.5 mg/mL) were at the same level as in untreated samples. Future studies can add to the current findings to better understand the antioxidant properties of mistletoe berry extracts, with the potential to develop treatments and products using these extracts.

Acknowledgments. The authors would like to thank the International Visegrad Fund for supporting this study.

References

Amarowicz, R., & Pegg, R.B. (2019). Natural antioxidants of plant origin. *Advances in food and nutrition research*, 90, 1–81. https://doi.org/10.1016/bs.afnr.2019.02.011 [in English].

Bartosz, G. (2003). Total antioxidant capacity. *Advances in clinical chemistry*, 37, 219–292. https://doi.org/10.1016/s0065-2423(03)37010-6 [in English].

Benzie, I.F. (2000). Evolution of antioxidant defence mechanisms. *European journal of nutrition,* 39 (2), 53–61. https://doi.org/10.1007/s003940070030 [in English].

Berlett, B.S., & Stadtman, E.R. (1997). Protein oxidation in aging, disease, and oxidative stress. *The Journal of biological chemistry,* 272 (33), 20313–20316. https://doi.org/10.1074/jbc.272.33.20313 [in English].

Beztsinna, N., de Matos, M.B.C., Walther, J., Heyder, C., Hildebrandt, E., Leneweit, G., Mastrobattista, E., & Kok, R.J. (2018). Quantitative analysis of receptor-mediated uptake and pro-apoptotic activity of mistletoe lectin-1 by high content imaging. *Scientific reports,* 8 (1), 2768. https://doi.org/10.1038/s41598-018-20915-y [in English].

Buege, J.A., & Aust, S.D. (1978). Microsomal lipid peroxidation. *Methods Enzymol,* 52, 302-310. https://doi.org/10.1016/s0076-6879(78)52032-6 [in English].

Carlsen, M.H., Halvorsen, B.L., Holte, K., Bøhn, S.K., Dragland, S., Sampson, L., Willey, C., Senoo, H., Umezono, Y., Sanada, C., Barikmo, I., Berhe, N., Willett, W. C., Phillips, K. M., Jacobs, D.R., Jr, & Blomhoff, R. (2010). The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutrition journal*, 9, 3. https://doi.org/10.1186/1475-2891-9-3 [in English].

Çetin, E.S., Sozen, H., Celik, O.I., Cigerci, I.H., & Yılmaz, N. (2023). Mistletoe (*Viscum album* L.) extract attenuates ıtraconazole-ınduced acute oxidative stress and hepatocellular ınjury in rats. *Pakistan journal of pharmaceutical sciences,* 36 (1), 9–16 [in English].

Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., & Colombo, R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clinica chimica acta; international journal of clinical chemistry,* 329 (1-2), 23–38. https://doi.org/10.1016/s0009-8981(03)00003-2 [in English].

Demirci-Çekiç, S., Özkan, G., Avan, A.N., Uzunboy, S., Çapanoğlu, E., & Apak, R. (2022). Biomarkers of Oxidative Stress and Antioxidant Defense. *Journal of pharmaceutical and biomedical analysis,* 209, 114477. https://doi.org/10.1016/j.jpba.2021.114477 [in English].

Hajam, Y.A., Rani, R., Ganie, S.Y., Sheikh, T.A., Javaid, D., Qadri, S.S., Pramodh, S., Alsulimani, A., Alkhanani, M.F., Harakeh, S., Hussain, A., Haque, S., & Reshi, M.S. (2022). Oxidative Stress in Human Pathology and Aging: Molecular Mechanisms and Perspectives. *Cells,* 11 (3), 552. https://doi.org/10.3390/cells11030552 [in English].

Halvorsen, B.L., Holte, K., Myhrstad, M.C., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A.B., Haffner, K., Baugerød, H., Andersen, L.F., Moskaug, Ø., Jacobs, D.R., Jr, & Blomhoff, R. (2002). A systematic screening of total antioxidants in dietary plants. *The Journal of nutrition,* 132 (3), 461–471. https://doi.org/10.1093/jn/132.3.461 [in English].

Khalili, J., & Biloklytska, H.F. (2008). Salivary malondialdehyde levels in clinically healthy and periodontal diseased individuals. *Oral diseases,* 14 (8), 754–760. https://doi.org/10.1111/j.1601-0825.2008.01464.x [in English].

Khwaja, T.A., Wajahat, T., Ahmad, I., Hoessli, D.C., Walker-Nasir, E., Kaleem, A., Qazi, W.M., Shakoori, A.R., & Din, N.U. (2008). *In silico* modulation of apoptotic Bcl-2 proteins by mistletoe lectin-1: functional consequences of protein modifications. *Journal of cellular biochemistry,* 103 (2), 479–491. https://doi.org/10.1002/jcb.21412 [in English].

Kienle, G.S., & Kiene, H. (2010). Review article: Influence of *Viscum album* L (European mistletoe) extracts on quality of life in cancer patients: a systematic review of controlled clinical studies. *Integrative cancer therapies,* 9 (2), 142–157. https://doi.org/10.1177/1534735410369673 [in English].

Kienle, G.S., Grugel, R., & Kiene, H. (2011). Safety of higher dosages of *Viscum album* L. in animals and humans – systematic review of immune changes and safety parameters. *BMC complementary and alternative medicine,* 11, 72. https://doi.org/10.1186/1472-6882-11-72 [in English].

Kim, B.K., Choi, M.J., Park, K.Y., & Cho, E.J. (2010). Protective effects of Korean mistletoe lectin on radical-induced oxidative stress. *Biological & pharmaceutical bulletin,* 33 (7), 1152–1158. https://doi.org/10.1248/bpb.33.1152 [in English].

Kim, M.J., Park, J.H., Kwon, D.Y., Yang, H.J., Kim, D.S., Kang, S., Shin, B.K., Moon, N.R., Song, B.S., Kim, J.H., & Park, S. (2015). The supplementation of Korean mistletoe water extracts reduces hot flushes, dyslipidemia, hepatic steatosis, and muscle loss in ovariectomized rats. *Experimental biology and medicine (Maywood, N.J.),* 240 (4), 477–487. https://doi.org/10.1177/1535370214551693 [in English].

Kim, S.Y., Yang, E.J., Son, Y.K., Yeo, J.H., & Song, K.S. (2016). Enhanced anti-oxidative effect of fermented Korean mistletoe is originated from an increase in the contents of caffeic acid and lyoniresinol. *Food & function,* 7 (5), 2270–2277. https://doi.org/10.1039/c6fo00138f [in English].

Kleszken, E., Purcarea, C., Pallag, A., Ranga, F., Memete, A.R., Miere Groza, F., & Vicas, S.I. (2022). Phytochemical Profile and Antioxidant Capacity of *Viscum album* L. subsp. *album* and *Effects on Its Host Trees. Plants (Basel, Switzerland),* 11 (22), 3021. https://doi.org/10.3390/plants11223021 [in English].

Kozłowska, A., & Szostak-Wegierek, D. (2014). Flavonoids – food sources and health benefits. *Roczniki Panstwowego Zakladu Higieny,* 65 (2), 79–85 [in English].

Kurhaluk, N., Tkachenko, H., & Tomin, V. (2023). *In vitro* impact of a combination of red and infrared LEDs, infrared laser and magnetic field on biomarkers of oxidative stress and hemolysis

of erythrocytes sampled from healthy individuals and diabetes patients. *Journal of photochemistry and photobiology. B, Biology,* 242, 112685. https://doi.org/10.1016/j.jphotobiol.2023.112685 [in English].

Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S., & Stadtman, E.R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in enzymology,* 186, 464–478. https://doi.org/10.1016/0076-6879(90)86141-h [in English].

Liu, R.H. (2013). Dietary bioactive compounds and their health implications. *Journal of food science,* 78 Suppl 1, A18–A25. https://doi.org/10.1111/1750-3841.12101 [in English].

Liu, X., Hussain, R., Mehmood, K., Tang, Z., Zhang, H., & Li, Y. (2022). Mitochondrial-Endoplasmic Reticulum Communication-Mediated Oxidative Stress and Autophagy. BioMed research international https://doi.org/10.1155/2022/6459585 [in English].

Lourenço, S.C., Moldão-Martins, M., & Alves, V.D. (2019). Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules (Basel, Switzerland),* 24 (22), 4132. https://doi.org/10.3390/molecules24224132 [in English].

Luo, J., Mills, K., le Cessie, S., Noordam, R., & van Heemst, D. (2020). Ageing, age-related diseases and oxidative stress: What to do next? *Ageing research reviews,* 57, 100982. https://doi.org/10.1016/j.arr.2019.100982 [in English].

Majeed, M., Pirzadah, T.B., Mir, M.A., Hakeem, K.R., Alharby, H.F., Alsamadany, H., Bamagoos, A.A., & Rehman, R.U. (2021). Comparative Study on Phytochemical Profile and Antioxidant Activity of an Epiphyte, *Viscum album* L. (White Berry Mistletoe), *Derived from Different Host Trees. Plants (Basel, Switzerland),* 10 (6), 1191. https://doi.org/10.3390/plants10061191 [in English].

Melzer, J., Iten, F., Hostanska, K., & Saller, R. (2009). Efficacy and safety of mistletoe preparations (*Viscum album*) for patients with cancer diseases. A systematic review. *Forschende Komplementarmedizin (2006)*, 16 (4), 217–226. https://doi.org/10.1159/000226249 [in English].

Mistletoe. (1987). Nursing standard (Royal College of Nursing (Great Britain), 2(13), 28. https://doi.org/10.7748/ns.2.13.28.s62 [in English].

Nazaruk, J., & Orlikowski, P. (2016). Phytochemical profile and therapeutic potential of *Viscum album* L. *Natural product research*, 30 (4), 373–385. https://doi.org/10.1080/14786419.2015.1022776 [in English].

Nicoletti, M. (2023). The Anti-Inflammatory Activity of *Viscum album*. *Plants (Basel, Switzerland)*, 12 (7), 1460. https://doi.org/10.3390/plants12071460 [in English].

Orhan, D.D., Aslan, M., Sendogdu, N., Ergun, F., & Yesilada, E. (2005). Evaluation of the hypoglycemic effect and antioxidant activity of three *Viscum album* subspecies (European mistletoe) in streptozotocin-diabetic rats. *Journal of ethnopharmacology*, 98 (1-2), 95–102. https://doi.org/10.1016/j.jep.2004.12.033 [in English].

Patil, S., Anarthe, S., Jadhav, R., & Surana, S. (2011). Evaluation of Anti-Inflammatory Activity and *In - vitro* Antioxidant Activity of Indian Mistletoe, the Hemiparasite *Dendrophthoe falcate* L. F. (Loranthaceae). *Iranian journal of pharmaceutical research: IJPR,* 10 (2), 253–259 [in English].

Radenkovic, M., Ivetic, V., Popovic, M., Brankovic, S., & Gvozdenovic, L. (2009). Effects of mistletoe (*Viscum album* L., Loranthaceae) extracts on arterial blood pressure in rats treated with atropine sulfate and hexocycline. *Clinical and experimental hypertension (New York, N.Y.: 1993),* 31 (1), 11–19. https://doi.org/10.1080/10641960802409820 [in English].

Rostock, M. (2020). Die Misteltherapie in der Behandlung von Patienten mit einer Krebserkrankung [Mistletoe in the treatment of cancer patients]. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz,* 63 (5), 535–540. https://doi.org/10.1007/s00103-020-03122-x [in English].

Squier, T.C. (2001). Oxidative stress and protein aggregation during biological aging. *Experimental gerontology*, 36 (9), 1539–1550. https://doi.org/10.1016/s0531-5565(01)00139-5 [in English].

Stefanucci, A., Zengin, G., Llorent-Martinez, E.J., Dimmito, M.P., Della Valle, A., Pieretti, S., Ak, G., Sinan, K.I., & Mollica, A. (2020). *Viscum album* L. homogenizer-assisted and ultrasound-assisted extracts as potential sources of bioactive compounds. *Journal of food biochemistry,* 44 (9), e13377. https://doi.org/10.1111/jfbc.13377 [in English].

Sunjic, S.B., Gasparovic, A.C., Vukovic, T., Weiss, T., Weiss, E.S., Soldo, I., Djakovic, N., Zarkovic, T., & Zarkovic, N. (2015). Adjuvant Cancer Biotherapy by *Viscum Album* Extract Isorel: Overview of Evidence Based Medicine Findings. *Collegium antropologicum*, 39 (3), 701–708 [in English].

Tapsell, L.C., Hemphill, I., Cobiac, L., Patch, C.S., Sullivan, D.R., Fenech, M., Roodenrys, S., Keogh, J.B., Clifton, P.M., Williams, P.G., Fazio, V.A., & Inge, K.E. (2006). Health benefits of herbs

and spices: the past, the present, the future. *The Medical journal of Australia*, 185 (S4), S1–S24. https://doi.org/10.5694/j.1326-5377.2006.tb00548.x [in English].

Teleanu, D.M., Niculescu, A.G., Lungu, I.I., Radu, C.I., Vladâcenco, O., Roza, E., Costăchescu, B., Grumezescu, A.M., & Teleanu, R.I. (2022). An Overview of Oxidative Stress, Neuroinflammation, and Neurodegenerative Diseases. *International journal of molecular sciences,* 23 (11), 5938. https://doi.org/10.3390/ijms23115938 [in English].

Thronicke, A., Schad, F., Debus, M., Grabowski, J., & Soldner, G. (2022). *Viscum album* L. Therapy in Oncology: An Update on Current Evidence. *Viscum album* L. Therapie in der Onkologie: Ein Update zur bestehenden Evidenz. *Complementary medicine research*, 29 (4), 362–368. https://doi.org/10.1159/000524184 [in English].

Tiberi, J., Cesarini, V., Stefanelli, R., Canterini, S., Fiorenza, M.T., & La Rosa, P. (2023). Sex differences in antioxidant defence and the regulation of redox homeostasis in physiology and pathology. *Mechanisms of ageing and development,* 211, 111802. https://doi.org/10.1016/j.mad.2023.111802 [in English].

Turkkan, A., Savas, H.B., Yavuz, B., Yigit, A., Uz, E., Bayram, N.A., & Kale, B. (2016). The prophylactic effect of *Viscum album* in streptozotocin-induced diabetic rats. *Northern clinics of Istanbul*, 3 (2), 83–89. https://doi.org/10.14744/nci.2016.22932 [in English].

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, 39 (1), 44–84. https://doi.org/10.1016/j.biocel.2006.07.001 [in English].

Zar, J.H. (1999). Biostatistical Analysis. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey [in English].

Отримано: 26.01.2024
Прийнято: 20.02.2024