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CYTOTOXIC EFFECTS OF LEAF EXTRACTS OF SOME *THYMUS* L. (LAMIACEAE) REPRESENTATIVES USING *IN VITRO* HUMAN BLOOD MODEL

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In the course of *in vitro* systems search for the toxicity screening of plant extracts, different cellular models have been applied to examine their adverse effects in our previous studies. In this study, the main aim was to assess the dose-dependent pro- and antioxidant potential of four species and one interspecific hybrid of *Thymus* genus sampled in the western part of Ukraine on human erythrocytes' model. For this purpose, we used 2-thiobarbituric acid reactive substances (TBARS) as a biomarker of lipid peroxidation used as an assessment of oxidative stress in erythrocytes' suspension after incubation with plant extracts in two doses (5 mg/mL and 0.5 mg/mL). An erythrocytes' suspension at 1% hematocrit was incubated with 4 mM phosphate buffer (pH 7.4) (control) and pre-incubated with the extracts (5 mg/mL and 0.5 mg/mL, respectively) at 37 °C for 60 min. The treatment by extracts obtained from various plants belonging to the *Thymus* genus in dose 5 mg/mL increased the TBARS level as a biomarker of lipid peroxidation in the human erythrocyte suspension when compared to untreated erythrocytes. The most potent prooxidative effect was demonstrated by the *Th. alpestris* Tausch ex A. Kern., *Th. serpyllum* L., *Th.* × *porcii* Borbás, and *Th. pannonicus* All. compared to phosphate buffer as a control samples. The minimum increase of TBARS content in human erythrocyte suspension was induced by *Th. pulegioides* L. extract. In the case of dose 0.5 mg/mL, *Th. alpestris*, *Th. pannonicus*, *Th.* × *porcii* caused also increased the TBARS level (by 58%, 51%, 43.1%, p <0.05, respectively) compared to untreated erythrocytes. On the other hand, *Th. serpyllum* and *Th. pulegioides* extracts decreased the TBARS level (by 16.5 and 2.7%, respectively), but these changes were no significant. Moreover, *Th. serpyllum* extract in dose 0.5 mg/mL caused the increase of TBARS level (by 48.8%, p <0.05) compared to those in dose 5 mg/mL. The extracts obtained from leaves of *Th. alpestris*, *Th. pannonicus*, and *Th.* × *porcii* in both doses (5 and 0.5 mg/mL) has a mild cytotoxic activity on the human erythrocytes increasing the level of lipid peroxidation biomarker. These findings suggest that *Th. alpestris*, *Th. pannonicus*, *Th.* × *porcii* extracts possessed prooxidant effects in both doses, while effects of *Th. serpyllum* and *Th. pulegioides* extracts to erythrocyte suspension were

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dose-dependent. Further research is needed to determine the effects of the active compounds of various plants belonging to the *Thymus* genus on erythrocytes' metabolism.

Keywords: *Thymus*, leaf extracts, human erythrocytes, lipid peroxidation, *Th. alpestris*, *Th. serpyllum*, *Th.* × *porcii*, *Th. pannonicus*, *Th. pulegioides*

Introduction

Plants from the genus *Thymus* are important medicinal herbs, which are known are rich in different active substances such as thymol, carvacrol, p-cymene, and terpinene (Morales, 2002; Nabavi et al., 2015). It is one of the most widely used genera in folk medicine, consisting of approximately 215 species currently recognized. It is popular for its stimulatory action on all organism functions (Viuda-Martos et al., 2011).

Nowadays, thymol and thyme present a wide range of functional possibilities in the medicine, pharmacy, food, and cosmetic industry (Salehi et al., 2018). Thymol, which is a monocyclic monoterpene, chemically known as 2-isopropyl-5-methylphenol found in oil of thyme various other kinds of plants (Aydın et al., 2017). For centuries, it has been used in traditional medicine and has been shown to possess various pharmacological properties, i.e. antioxidant, free radical scavenging, anti-inflammatory, analgesic, antispasmodic, antibacterial, antifungal, antiseptic and antitumor activities with multiple therapeutic actions against various cardiovascular, neurological, rheumatological, gastrointestinal, metabolic and malignant diseases (Nagoor Meeran and Prince, 2012; Nagoor Meeran et al., 2017). The interest in the formulation of pharmaceuticals, nutraceuticals, and cosmeceuticals based on thymol is due to several studies that have evaluated the potential therapeutic uses of this compound for the treatment of disorders affecting the respiratory, nervous, and cardiovascular systems (Salehi et al., 2018). Thymol has also been reported as an anti-cancer agent, but its anti-cancer mechanism has not yet been fully elucidated (Deb et al., 2011).

In the course of *in vitro* systems search for the toxicity screening of plant extracts, different cellular models have been applied to examine their adverse effects in our previous studies. For example, we have assessed the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification, total antioxidant capacity] in the equine erythrocytes after treatment with *Thymus serpyllum* L. extract. Lipid peroxidation biomarker, aldehydic and ketonic derivatives of oxidatively modified proteins, total antioxidant capacity was non-significantly altered after *in vitro* incubation with an extract obtained from *Th. serpyllum* (Honcharenko et al., 2018c). According to the results obtained, we addressed the hypothesis that by-products in the leaf extracts obtained from various *Thymus* representatives may be a major contributor to an increase of the antioxidant capacity of muscle tissue of rainbow trout after incubation *in vitro*. We have investigated the influence of leaf extracts obtained from various *Thymus* representatives on the total antioxidant capacity (TAC) in the muscle tissue of rainbow trout after incubation with extracts under *in vitro* conditions. The most potent antioxidant effect was demonstrated for the extracts of *Th. alpestris* Tausch ex A. Kern., *Th.* × *porcii* Borbás, *Th. pannonicus* All.,

Th. serpyllum L., and *Th. pulegioides* L. compared to phosphate buffer as a control samples (59.5, 48, 45.9, 43.9, and 38.8%, p <0.05, respectively). The results showed that the leaf extract of *Th. alpestris* efficiently increased the TAC level in muscle tissue. Our results provide a new perspective on the use of various *Thymus* species as a medicinal plant to improve the antioxidant response of rainbow trout (Honcharenko et al., 2018b).

Moreover, we also demonstrated the antibacterial activity of some *Thymus* representatives against β-lactamase-producing *Pseudomonas aeruginosa* and *Salmonella enteriditis* strains locally isolated (Honcharenko et al., 2018a, d). The effects varied significantly according to the *Thymus* taxa. It should be noted that the most antimicrobial effective plant against β-lactamase-producing *P. aeruginosa* was *Th. alpestris*, being highly active with the ethanolic extract (mean diameter of inhibition zone was 12.8 ±0.8 mm). The antibacterial activity of extracts was greatest for *Th. alpestris* followed by *Th. pannonicus* followed by *Th. serpyllum* and then by *Th. pulegioides* (Honcharenko et al., 2018d). The ethanolic extract obtained from the leaves of *Th. pulegioides* was the most effective plant extracts against *S. enteritidis*. The antibacterial activity of extracts was greatest for *Th. pulegioides* followed by *Th. pannonicus* followed by *Th. alpestris*, *Th.* × *porcii*, and then by *Th. serpyllum*. These plant extracts could be a potential source of new antibacterial agents (Honcharenko et al., 2018a).

This study describes the interaction of aqueous extracts of leaf extracts obtained from various *Thymus* representatives with human erythrocytes. The cell membrane is a diffusion barrier which protects the cell interior. Therefore, its structure and functions are susceptible to alterations as a consequence of interactions with foreign species. Erythrocytes were chosen because although less specialized than many other cell membranes they carry on enough functions in common with them, i.e. active and passive transport, the production of ionic and electric gradients, etc. Therefore, their structure can be considered representative of the plasma membrane in general (Suwalsky et al., 2006, 2008). The erythrocytes could be isolated and handled easily so that they could provide a good model for many assays (Alagawany et al., 2016; Farag and Alagawany, 2018). Additionally, the high concentration of polyunsaturated fatty acids in the membrane, the high oxygen tension, and redox-active hemoglobin molecules [the source of reactive oxygen species in erythrocyte] make them a good biological lipid membrane model especially for screening the oxidative stress conditions induced by various substances (Farag and Alagawany, 2018).

We continue to assess the dose-dependent pro- and antioxidant potential of four species and one interspecific hybrid of *Thymus* genus sampled in the western part of Ukraine on human erythrocytes‘ model. Thus, the main aim of our study was to assess the dose-dependent pro- and antioxidant potential of four species and one interspecific hybrid of *Thymus* genus sampled in the western part of Ukraine on human erythrocytes‘ model. For this purpose, we used 2-thiobarbituric acid reactive substances (TBARS) as a biomarker of lipid peroxidation used as an assessment of oxidative stress in erythrocytes‘ suspension after incubation with plant extracts in two doses (5 mg/mL and 0.5 mg/mL).

Material and methodology

Collection of Plant Materials

Leaves of *Thymus serpyllum* L. were collected among the grass on sandy soil in the edge of a pine forest (Baymaky village, Bilohirya district, Khmelnytsky region, Ukraine; N 50° 03′ 58,9′′, E 26° 13′ 37,5′′, 257 m a.s.l.). Leaves of *Th. pannonicus* All. were harvested among grass in the roadside between the two cultivated fields (Syvky village, Bilohirya district, Khmelnytsky region, Ukraine; N 50° 02′ 09,6′′, E 26° 13′ 19,2′′, 283 m a.s.l.). Leaves of *Th. pulegioides* L. were collected among grass nearby land parcels (Syvky village, Bilohirya district, Khmelnytsky region, Ukraine; N 50° 02′ 02,8′′, E 26° 14′ 13,9′′, 306 m a.s.l.). Leaves of *Th.* × *porcii* Borbás (a hybrid between *Th. pannonicus* and *Th. pulegioides*) were sampled in the grass stand, on the side of the footpath of the race track (Medovoi Pechery Str., Lviv, Ukraine; N 49° 49‘ 15.1“, E 24˚ 05‘ 12.5“, 348 m a.s.l.). Leaves of *Th. alpestris* Tausch ex A. Kern. were harvested on the side of the road below the stream, in mountain valley Shumneska (Kvasy village, Rakhiv district, Zakarpattia region, Ukraine; N 48° 09‘ 32.3“, E 24° 21‘ 26.4“, 1259 m a.s.l.). Identification of these five taxa was made according to Nachychko (2014, 2015) and Nachychko and Honcharenko (2016). The voucher herbarium specimens of plants used in this study were deposited at the Herbarium of M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (KW). Plant samples were thoroughly washed to remove all the attached material and used to prepare the extract.

Preparation of Plants Extracts

Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in proportion 1 : 19, w/w) at room temperature. The extracts were then filtered and used for analysis. The extracts were stored at -20 °C until use.

Human blood samples

Blood (10–20 ml) was obtained from normal volunteers via venipuncture (4 males and 5 females aged 28–53-years old). The Research Ethics Committee of Regional Medical Chamber in Gdańsk (Poland) approved the study (KB-31/18). All patients provided written informed consent before the start of the study procedures. Human erythrocytes from citrated blood were isolated by centrifugation at 3,000 rpm for 10 min and washed two times with 4 mM phosphate buffer (pH 7.4) and then re-suspended using the same buffer to the desired hematocrit level. Cells stored at 4 °C were used within 6 h of sample preparation. An erythrocytes' suspension at 1% hematocrit was incubated with 4 mM phosphate buffer (pH 7.4) (control) and pre-incubated with the extracts (5 mg/mL and 0.5 mg/mL, respectively) at 37 °C for 60 min. This reaction mixture was shaken gently while being incubated for a fixed interval at 37 °C. For positive control (phosphate buffer) was used. Erythrocytes' aliquots were used in the study.

2-Thiobarbituric Acid Reactive Substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method

for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with TBA under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The μmol of MDA per L was calculated using $1.56 \cdot 10^5$ mM/cm as the extinction coefficient.

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test (p >0.05). The significance of differences between the values (significance level, p <0.05) was examined using the Kruskal-Wallis H-test (Zar, 1999). All statistical calculation was performed on separate data from each individual with Statistica 8.0 software (StatSoft, Krakow, Poland).

Results and discussion

As shown in Figure 1, treatment by extracts obtained from various plants belonging to the *Thymus* genus in dose 5 mg/mL increased the TBARS level, when compared to untreated erythrocytes. The most potent prooxidative effect was demonstrated by the *Th. alpestris*, *Th. serpyllum*, *Th.* × *porcii*, and *Th. pannonicus* compared to phosphate buffer as control samples (an increase of TBARS content by 82.0, 78.7, 53.7, and 34.1%, p <0.05, respectively). The minimum increase of TBARS content in human erythrocytes' suspension was induced by *Th. pulegioides* extract (by 11.2%, p <0.05) (Figure 1).

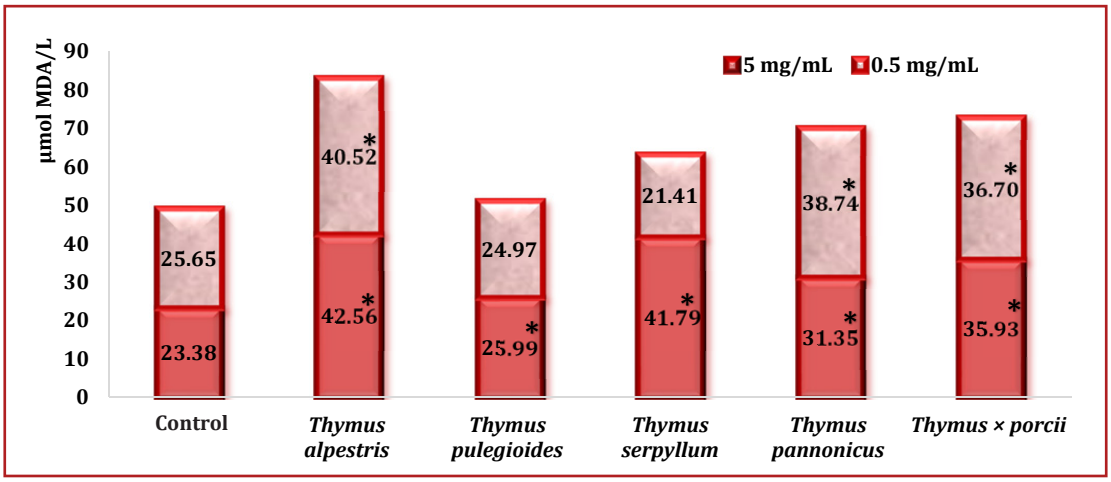

Figure 1 The TBARS content as a biomarker of lipid peroxidation in the human erythrocytes suspension after *in vitro* incubation with leaf extract obtained from *Thymus alpestris*, *Th. pulegioides*, *Th. serpyllum*, *Th. pannonicus*, and *Th.* × *porcii* (n = 9). * – changes were statistically significant (p <0.05) compared to the untreated control (phosphate buffer as a control sample)

Extracts obtained from various plants belonging to the *Thymus* genus in dose 0.5 mg/mL caused increased the TBARS level, i.e. in the case of *Th. alpestris* (by 58%, p <0.05), *Th. pannonicus* (by 51%, p <0.05), *Th.* × *porcii* (by 43.1%, p <0.05) compared to untreated

erythrocytes. *Th. serpyllum* and *Th. pulegioides* extracts decreased the TBARS level (by 16.5 and 2.7%, respectively), but these changes were no significant (Figure 1). Moreover, *Th. serpyllum* extract in dose 0.5 mg/mL caused the increase of TBARS level (by 48.8%, p <0.05) compared to those in dose 5 mg/mL (Figure 1).

Many results also clearly suggest that treatment by *Thymus* extracts *in vivo* and *in vitro* prevents organ damage via protection of the antioxidant defense system and scavenge of hydroxyl free radicals by producing of phenoxyl radicals, major transient species (Nagoor Meeran et al., 2017). Thymol is also a potent antioxidant and anti-inflammatory agent in human cells. For example, Braga et al. (2006) have investigated whether thymol can interfere with the production of reactive oxygen species (ROS), nitric oxide and nitric oxide-derived peroxynitrite released by human neutrophils after activation by formyl methionyl-leucyl-phenylalanine (fMLP) and paramethoxyamphetamine (PMA) with and without the addition of the L-arginine (L-Arg) nitric oxide donor to the medium. In cell-free systems using H_2O_2/ $HOCl^-$ and 3-morpholinosydnonimine (SIN-1) as radical producers, significant scavenging activity of thymol was present already at 0.08 and 0.68 μg/ml respectively, and these are very low concentrations. These findings can be related to the phenolic structure of thymol, because phenolic compounds have redox properties and play an important role in absorbing and neutralizing free radicals and peroxynitrite, and in decomposing peroxides (Braga et al., 2006).

Many studies using human cells model were conducted for assessment of the antioxidative activity, chemopreventive efficacy and cytotoxicity of *Thymus* extracts. For instance, Kozics et al. (2013) have investigated the composition and the quantitative estimation of *Salvia officinalis* L. and *Thymus vulgaris* L. extracts, the protective effects of plant extracts against hydrogen peroxide- and 2,3-dimethoxy-1,4-naphthoquinone-induced DNA damage, and levels of enzymatic and non-enzymatic antioxidants [superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione] in human HepG2 cells. To measure the antioxidative activity of plant extracts three assays were used, i.e. 1.1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and 2.2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The results of Kozics et al. (2013) showed that the oxidant-induced DNA lesions were significantly reduced in cells pre-treated with the plant extracts studied. The observed DNA-protective activity could be explained by both elevations of GPx activity in cells pre-treated with *S. officinalis* and *Th. vulgaris* and antioxidant activity of those extracts (Kozics et al., 2013). Moreover, the results of Horváthová et al. (2016) have indicated that the consumption of *S. officinalis* and *Th. vulgaris* extracts positively affect the resistance of rat liver cells against oxidative stress and may have hepatoprotective potential. Intake of sage and thyme had no effect either on the basal level of DNA damage or on the activity of SOD in rat hepatocytes and did not change the biochemical parameters of blood plasma. Simultaneously, the activity of GPx was significantly increased and the level of DNA damage induced by oxidants was decreased. Moreover, the sage extract was able to start up the antioxidant protection expressed by an increased content of glutathione (Horváthová et al., 2016).

Galasso et al. (2014) have assessed the chemopreventive efficacy of the *Th. longicaulis* C. Presl. extracts collected at different phases during its life cycle, by means of their anti-

inflammatory, cytotoxic and antioxidant activities. To this purpose, each extract underwent an extensive screening towards five human cell lines: CCRF-CEM (leukemia); U251 (glioblastoma); MDA-MB-231 (breast cancer); HCT-116 (colon cancer) and MRC-5 (lung fibroblasts) through [2.3bis(2-metoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H tetrazolium hydroxide] test. The ability of the extracts to counteract cyclooxygenase-2 (COX-2) expression was also evaluated by COX-2 expression assay in human THP-1 monocyte-derived macrophages. COX-2 inhibition could represent a valuable anticancer strategy as it is associated with carcinogenesis and over-expressed in a variety of human malignancies. Extract, which was particularly rich in rosmarinic acid and methylapigenin, exhibited a strong antioxidant and anti-inflammatory effectiveness (Galasso et al., 2014).

The study of Caprioli et al. (2018) confirms the very low cytotoxicity of the *Th. lanceolatus* Desf. ethanolic extract and highlights its nutraceutical properties as an antioxidative and preservative agent and its possible use as an ingredient in functional foods. The biological activity of *Th. lanceolatus* ethanolic extract on proliferation, viability and ROS protection was investigated towards K562, CaCo-2, and SH-SY5Y human cancer cell lines. Cell proliferation was inhibited in K562 and SH-SY5Y cells in the first 48 h at 500 μg/mL but slowly resumed after 72 h. A weak cytotoxic effect can be observed at 24, 48 and 72 hours: 12.8, 14.9 and 24.2% reduction in K562 viability, and 11, 15 and 12.7% in SH-SY5Y. No cytotoxicity was observed in CaCo-2 cells under the same experimental conditions. Even at the lowest concentrations (50 μg/mL), the extract was efficiently able to protect human cells against t-BHP-induced oxidative damage. For instance, the highest concentration of the extract (100 μg/mL) decreases ROS generation by about 30% in SH-SY5Y and 70% in CaCo-2 and K562 cells (Caprioli et al., 2018).

Thymol may have antiproliferative potential against brain tumor cells involving oxidative alteration. *In vitro* antiproliferative [by 3-(4.5 dimetylthiazol-2-yl)-2.5 diphenlytetrazolium bromide (MTT) test], genotoxic [by single-cell gel electrophoresis (SCGE)] and oxidative effects [by total antioxidant capacity (TAC) and total oxidative status (TOS) analysis] of thymol (0–400 mg/L) were assessed on cultured primary rat neurons (CPRNs) and N2a neuroblastoma cells by Aydın et al. (2017). The obtained data from MTT analysis revealed that thymol (only at 400 mg/L) led to significant decreases in the cell viability in cultured primary rat neurons. Moreover, thymol inhibited cell growth in N2a cells at concentrations of 200 and 400 mg/L. DNA damage rates were statistically no-significant in both treated cell types as compared to the control group. The results of Aydın et al. (2017) also showed that 10, 25 and 50 mg/L of thymol application into the cell cultures supported antioxidant capacity in primary rat neurons but not in N2a cells.

Thymol also induces mitochondrial dysfunction and apoptosis and may be efficacious against multiple cancers. De La Chapa et al. (2018) have evaluated the effects of thymol against oral squamous cell carcinoma (OSCC) and its anticancer mechanism-of-action. Thymol had significant, long-lasting antiproliferative effects *in vitro*. *In vivo*, thymol displayed significant antitumor effects in Cal27-derived tumors. Thymol‘s anticancer effects were confirmed in HeLa-derived xenografts demonstrating that thymol effects are not tumor-type specific. Thymol induces significant ΔΨm depolarization and apoptosis (De La Chapa et al., 2018).

Deb et al. (2011) have investigated the anticancer activity of thymol on HL-60 (acute promyelocytic leukemia) cells. Thymol demonstrated dose-dependent cytotoxic effects on HL-60 cells after 24h of exposure. However, thymol did not show any cytotoxic effect in normal human peripheral blood mononuclear cells. The cytotoxic effect of thymol on HL-60 cells appears to be associated with the induction of cell cycle arrest at sub G0/G1 phase, and apoptotic cell death based on genomic DNA fragmentation pattern. Thymol also showed a significant increase in the production of ROS activity, an increase in mitochondrial H_2O_2 production and depolarization of mitochondrial membrane potential. On performing Western Blot analysis, thymol showed an increase in Bax protein level with a concomitant decrease in Bcl2 protein expression in a dose-dependent manner. Activation of caspase-9, -8 and -3 and concomitant PARP cleavage, which is the hallmark of caspase-dependent apoptosis was also shown by Deb et al. (2011). Moreover, to rule out the involvement of other mechanisms in apoptosis induction by thymol, these researchers also studied its effect on the apoptosis-inducing factor (AIF). Thymol induced AIF translocation from mitochondria to cytosol and to the nucleus, thus indicating its ability to induce caspase-independent apoptosis. Thymol-induced apoptosis in HL-60 cells involves both caspase-dependent and caspase-independent pathways (Deb et al., 2011).

The *Th. serpyllum* may protect against hypertension in an experimental model of essential hypertension. Mihailovic-Stanojevic et al. (2013) have evaluated the total phenol and flavonoid contents, antioxidant capacity, free radical scavenging activity and potential antihypertensive effect of the aqueous extract obtained from *Th. serpyllum* (wild thyme, TE) in spontaneously hypertensive rats (SHR) and in normotensive Wistar rats. The ferric reducing/antioxidant power and antioxidant capacity analysis revealed strong antioxidative properties of wild thyme. Bolus injection of wild thyme (100 mg/kg body weight i.v.) induced a significant decrease of systolic and diastolic blood pressure and total peripheral resistance in SHR, without effects on these parameters in normotensive Wistar rats. The cardiac index remained unchanged after *Th. serpyllum* treatment in all experimental rats. A given dose of *Th. serpyllum* did not show significant nitric oxide-scavenging activity *in vivo* (Mihailovic-Stanojevic et al., 2013).

In our study, treatment by extracts obtained from various plants belonging to the *Thymus* genus in dose 5 mg/mL increased the TBARS level, when compared to untreated erythrocytes. On the other hand, extracts obtained in dose 0.5 mg/mL caused the less increased the TBARS level compared to untreated erythrocytes. Moreover, *Th. serpyllum* and *Th. pulegioides* extracts decreased the TBARS level (by 16.5 and 2.7%, respectively), but these changes were no significant.

In conclusion, the extracts obtained from leaves of *Th. alpestris*, *Th. pannonicus*, and *Th.* × *porcii* in both doses (5 and 0.5 mg/mL) has a mild cytotoxic activity on the human erythrocytes increasing the level of lipid peroxidation biomarker (TBARS level). These findings suggest that *Th. alpestris*, *Th. pannonicus*, *Th.* × *porcii* extracts possessed prooxidant effects in both doses, while effects of *Th. serpyllum* and *Th. pulegioides* extracts to erythrocyte suspension were dose-dependent.

Conclusions

In conclusion, the treatment by extracts obtained from various plants belonging to the *Thymus* genus in dose 5 mg/mL increased the TBARS level as a biomarker of lipid peroxidation in the human erythrocyte suspension, when compared to untreated erythrocytes. The most potent prooxidative effect was demonstrated by the *Th. alpestris*, *Th. serpyllum*, *Th.* × *porcii*, and *Th. pannonicus* compared to phosphate buffer as a control sample. The minimum increase of TBARS content in human erythrocyte suspension was induced by *Th. pulegioides* extract. In the case of dose 0.5 mg/mL, *Th. alpestris*, *Th. pannonicus*, *Th.* × *porcii* caused also increased the TBARS level (by 58, 51, 43.1%, p <0.05, respectively) compared to untreated erythrocytes. On the other hand, *Th. serpyllum* and *Th. pulegioides* extracts decreased the TBARS level (by 16.5 and 2.7%, respectively), but these changes were no significant. Moreover, *Th. serpyllum* extract in dose 0.5 mg/mL caused the increase of TBARS level (by 48.8%, p <0.05) compared to those in dose 5 mg/mL. Further research is needed to determine the effects of the active compounds of various plants belonging to the *Thymus* genus on erythrocytes' metabolism.

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References

ALAGAWANY, M., FARAG, M.R., EL-KHOLY, M.S., EL-SAYED, S.A.A., DHAMA, K. 2016. Effect of resveratrol, cinnamaldehyde and their combinations on the antioxidant defense system and ATP release of rabbit erythrocytes: *in vitro* study. In *Asian J. Anim. Sci. Vet. Adv.*, vol. 12, p. 1–9. https://doi.org/10.3923/ajava.2017.1.9

AYDIN, E., TURKEZ, H., TASDEMIR, S., HACIMUFTUOGLU, F. 2017. Anticancer, Antioxidant and Cytotoxic Potential of Thymol *in vitro* Brain Tumor Cell Model. In *Cent. Nerv. Syst. Agents Med. Chem.*, vol. 17(2), p. 116–122. https://doi.org/10.2174/1871524916666160823121854

BRAGA, P.C., DAL SASSO, M., CULICI, M., GALASTRI, L., MARCECA, M.T., GUFFANTI, E.E. 2006. Antioxidant potential of thymol determined by chemiluminescence inhibition in human neutrophils and cell-free systems. In *Pharmacology*, vol. 76(2), p. 61–68. https://doi.org/10.1159/000089719

CAPRIOLI, G., MAGGI, F., BENDIF, H., MIARA, M.D., CINQUE, B., LIZZI, A.R., BRISDELLI, F., CELENZA, G. 2018. *Thymus lanceolatus* ethanolic extract protects human cells from t-BHP induced oxidative damage. In *Food Funct.*, vol. 9(7), p. 3665–3672. https://doi.org/10.1039/c8fo00568k

DE LA CHAPA, J.J., SINGHA, P.K., LEE, D.R., GONZALES, C.B. 2018. Thymol inhibits oral squamous cell carcinoma growth via mitochondria-mediated apoptosis. In *J. Oral Pathol. Med.*, vol. 47(7), p. 674–682. https://doi.org/10.1111/jop.12735

DEB, D.D., PARIMALA, G., SARAVANA DEVI, S., CHAKRABORTY, T. 2011. Effect of thymol on peripheral blood mononuclear cell PBMC and acute promyelotic cancer cell line HL-60. In *Chem. Biol. Interact.*, vol. 193(1), p. 97–106. https://doi.org/10.1016/j.cbi.2011.05.009

FARAG, M.R., ALAGAWANY, M. 2018. Erythrocytes as a biological model for screening of xenobiotics toxicity. In *Chem. Biol. Interact.*, vol. 279, p. 73–83. https://doi.org/10.1016/j.cbi.2017.11.007

GALASSO, S., PACIFICO, S., KRETSCHMER, N., PAN, S.P., MARCIANO, S., PICCOLELLA, S., MONACO, P., BAUER, R. 2014. Influence of seasonal variation on *Thymus longicaulis* C. Presl. chemical composition and its antioxidant and anti-inflammatory properties. In *Phytochemistry*, vol. 107, p. 80–90. https://doi.org/10.1016/j.phytochem.2014.08.015

HONCHARENKO, V., TKACHENKO, H., NACHYCHKO, V., PROKOPIV, A., OSADOWSKI, Z. 2018a. The antibacterial activities of some *Thymus* (Lamiaceae) representatives against *Salmonella enteriditis* strain locally isolated. In *Agrobiodiversity for Improving Nutrition, Health, and Life Quality*, vol. 2, p. 212–222. https://doi.org/10.15414/agrobiodiversity.2018.2585-8246.212-222

HONCHARENKO, V., TKACHENKO, H., NACHYCHKO, V., PROKOPIV, A., OSADOWSKI, Z. 2018b. Total antioxidant capacity in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) under *in vitro* incubation with leaf extracts of some *Thymus* (Lamiaceae) representatives. In *Agrobiodiversity for Improving Nutrition, Health, and Life Quality*, vol. 2, p. 223–233. https://doi.org/10.15414/agrobiodiversity.2018.2585-8246.223-233

HONCHARENKO, V., TKACHENKO, H., NACHYCHKO, V., PROKOPIV, A., OSADOWSKI, Z. 2018. Oxidative stress biomarkers in the equine erythrocyte suspension after *in vitro* incubation with leaf extract obtained from *Thymus serpyllum* L. emend. Mill. (Lamiaceae). In *Agrobiodiversity for Improving Nutrition, Health, and Life Quality*, vol. 2, p. 201–211. https://doi.org/10.15414/agrobiodiversity.2018.2585-8246.201-211

HONCHARENKO, V., TKACHENKO, H., OSADOWSKI, Z., NACHYCHKO, V., PROKOPIV, A. 2018. The antibacterial activities of ethanolic extracts obtained from leaves of some *Thymus* (Lamiaceae) representatives against β-lactamase producing *Pseudomonas aeruginosa* strain. In *Słupskie Prace Biologiczne*, vol. 15, p. 59–78.

HORVÁTHOVÁ, E., SRANČÍKOVÁ, A., REGENDOVÁ-SEDLÁČKOVÁ, E., MELUŠOVÁ, M., MELUŠ, V., NETRIOVÁ, J., KRAJČOVIČOVÁ, Z., SLAMEŇOVÁ, D., PASTOREK, M., KOZICS, K. 2016. Enriching the drinking water of rats with extracts of *Salvia officinalis* and *Thymus vulgaris* increases their resistance to oxidative stress. In *Mutagenesis*, vol. 31(1), p. 51–59. https://doi.org/10.1093/mutage/gev056

KAMYSHNIKOV, V.S. 2004. *A reference book on the clinic and biochemical researches and laboratory diagnostics*. MEDpress-inform, Moscow.

KOZICS, K., KLUSOVÁ, V., SRANČÍKOVÁ, A., MUČAJI, P., SLAMEŇOVÁ, D., HUNÁKOVÁ, L., KUSZNIEREWICZ, B., HORVÁTHOVÁ, E. 2013. Effects of *Salvia officinalis* and *Thymus vulgaris* on oxidant-induced DNA damage and antioxidant status in HepG2 cells. In *Food Chem.*, vol. 141(3), p. 2198–2206. https://doi.org/10.1016/j.foodchem.2013.04.089

MIHAILOVIC-STANOJEVIC, N., BELŠČAK-CVITANOVIĆ, A., GRUJIĆ-MILANOVIĆ, J., IVANOV, M., JOVOVIĆ, DJ., BUGARSKI, D., MILORADOVIĆ Z. 2013. Antioxidant and antihypertensive activity of extract from *Thymus serpyllum* L. in experimental hypertension. In *Plant Foods Hum. Nutr.*, vol. 68(3), p. 235–240. https://doi.org/10.1007/s11130-013-0368-7

MORALES, R. 2002. *The history, botany, and taxonomy of the genus Thymus*. In Stahl-Biskup E., Sáez F. (Eds.) Thyme. The genus *Thymus*. Taylor & Francis, London, New York, pp. 1–43.

NABAVI, S.M., MARCHESE, A., IZADI, M., CURTI, V., DAGLIA, M., NABAVI, S.F. 2015. Plants belonging to the genus *Thymus* as antibacterial agents: from farm to pharmacy. In *Food Chem.*, vol. 173, p. 339–347. https://doi.org/10.1016/j.foodchem.2014.10.042

NACHYCHKO, V. 2014. The genus *Thymus* L. (Labiatae Juss.) in the Ukrainian Carpathians' flora: systematics and taxonomic problems. In *Visnyk of Lviv University. Biological Series*, vol. 64, p. 159–169 (In Ukrainian).

NACHYCHKO, V.O. 2015. Diagnostic features of representatives of *Thymus* sect. *serpyllum* and *Th.* sect. *marginati* (Lamiaceae) and guidance for their herborization. In *The Journal of V. N. Karazin Kharkiv National University. Series: Biology,* vol. 25, p. 77–89 (In Ukrainian).

NACHYCHKO, V.O., HONCHARENKO, V.I. 2016. Hybrids of *Thymus* L. (Lamiaceae) genus in flora of the western regions of Ukraine: taxonomic composition and distribution. In *Studia Biologica*, vol. 10(1), p. 163–186. (In Ukrainian). https://doi.org/10.30970/sbi.1001.442

NAGOOR MEERAN, M.F., JAVED, H., AL TAEE, H., AZIMULLAH, S., OJHA, S.K. 2017. Pharmacological Properties and Molecular Mechanisms of Thymol: Prospects for Its Therapeutic Potential and

Pharmaceutical Development. In *Front Pharmacol*., vol. 8, p. 380. https://doi.org/10.3389/fphar.2017.00380

NAGOOR MEERAN, M.F., PRINCE, P.S. 2012. Protective effects of thymol on altered plasma lipid peroxidation and nonenzymic antioxidants in isoproterenol-induced myocardial infarcted rats. In *J. Biochem. Mol. Toxicol.*, vol. 26(9), p. 368–373. https://doi.org/10.1002/jbt.21431

SALEHI, B., MISHRA, A.P., SHUKLA, I., SHARIFI-RAD, M., CONTRERAS, M.DM., SEGURA-CARRETERO, A., FATHI, H., NASRABADI, N.N., KOBARFARD, F., SHARIFI-RAD, J. 2018. Thymol, thyme, and other plant sources: Health and potential uses. In *Phytother Res.*, vol. 32(9), p. 1688–1706. https://doi.org/10.1002/ptr.6109

SUWALSKY, M., ORELLANA, P., AVELLO, M., VILLENA, F., SOTOMAYOR, C.P. 2006. Human erythrocytes are affected *in vitro* by extracts of Ugni molinae leaves. In *Food Chem. Toxicol*., vol. 44(8), p. 1393–1398. https://doi.org/10.1016/j.fct.2006.03.003

SUWALSKY, M., VARGAS, P., AVELLO, M., VILLENA, F., SOTOMAYOR, C.P. 2008. Human erythrocytes are affected *in vitro* by flavonoids of *Aristotelia chilensis* (Maqui) leaves. In *Int. J. Pharm.*, vol. 363(1-2), p. 85–90. https://doi.org/10.1016/j.ijpharm.2008.07.005

VIUDA-MARTOS, M., RUIZ-NAVAJAS, Y., FERNÁNDEZ-LÓPEZ, J., PÉREZ-ÁLVAREZ, J.A. 2011. Spices as functional foods. In *Critical Review in Food Science and Nutrition*, vol. 51(1), p. 13–28. https://doi.org/10.1080/10408390903044271

ZAR, J.H. 1999. *Biostatistical Analysis*. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.