



OXIDATIVE STRESS BIOMARKERS IN THE EQUINE ERYTHROCYTE SUSPENSION AFTER *IN VITRO* INCUBATION WITH LEAF EXTRACT OBTAINED FROM *THYMUS SERPYLLUM* L. EMEND. MILL. (LAMIACEAE)

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The main aim of the study was an assessment of 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. emend. Mill. extract. Leaves of *Th. serpyllum* were collected among grass on sandy soil in the edge of a pine forest (Baymaky village, Bilohirya district, Khmelnytsky region, Ukraine). Freshly collected leaves were washed, weighted, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in proportion 1 : 19, w/w). The equine erythrocyte aliquots were used in the study. The pellet of blood was re-suspended in phosphate buffer (pH 7.4). A volume of 0.1 ml of the *Th. serpyllum* extract was added to 1.9 ml of clean equine erythrocytes. For positive control (blank), phosphate buffer was used. After incubation the mixture at 37 °C for 60 min with continuous stirring, samples were used for the biochemical assays. 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*. Screening of *Thymus* species for other biological activities including antioxidant activities is essential and may be effective for searching the preventive agents in the pathogenesis of some metabolic diseases.

Keywords: *Thymus*, leaf extract, equine erythrocytes, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity

Introduction

Thymus serpyllum L. emend. Mill., known as Breckland thyme, wild thyme, or creeping thyme, is a perennial shrub, native to regions of northern and central Europe. It has a long stem; leaves are oval (rounded at the top, tapered at the base), and glabrous on the face and underside,

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while at the base along the edge they have long trichomes, a prominent central vein, and less prominent lateral veins. Inflorescences are 4–7 cm tall and form in a series along a low-lying stem, with a uniform layer of trichomes on all sides. Flowers are located at the top of the stems and form spherical (or more rarely elongated) verticillaster. Wild thyme grows best on dry, stony ground, open sandy heaths, and grasslands (Diklić, 1974; Jarić et al., 2015). *Th. serpyllum* is a medicinal plant with antioxidant, antimicrobial, antitumor, and cytotoxic properties with effective medicinal application in pharmaceutical, food, and cosmetic industries as an anthelmintic, a strong antiseptic, an antispasmodic, a carminative, deodorant, diaphoretic, disinfectant, expectorant, sedative, tonic, anticholesterolemic and immunostimulant plant (Jarić et al., 2015).

The chief component of the essential oil of *Th. serpyllum* is carvacrol, while it also contains borneol, isobutyl acetate, caryophyllene, 1,8-cineole, citral, citronellal, citronellol, *p*-cymene, geraniol, linalool, α -pinene, γ -terpinene, α -terpineol, terpinyl acetate, and thymol in relatively high concentrations (Thomson, 2004). In addition to essential oil, wild thyme also contains flavonoids, phenol carboxylic acids, and their derivatives, triterpenes, and tannins (Thomson, 2004). 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. Moreover, this compound also exhibits antimicrobial, antioxidant, anticarcinogenesis, anti-inflammatory, and antispasmodic activities, as well as a potential as a growth enhancer and immunomodulator (Salehi et al., 2018). The noteworthy effects of thymol are largely attributed to its anti-inflammatory (*via* inhibiting recruitment of cytokines and chemokines), antioxidant (*via* scavenging of free radicals, enhancing the endogenous enzymatic and non-enzymatic antioxidants and chelation of metal ions), antihyperlipidemic (*via* increasing the levels of high density lipoprotein cholesterol and decreasing the levels of low density lipoprotein cholesterol and low density lipoprotein cholesterol in the circulation and membrane stabilization) (*via* maintaining ionic homeostasis) effects (Nagoor Meeran et al., 2017).

Thymol exhibits *in vitro* antioxidant activity on high-fat-diet-induced hyperlipidemia and atherosclerosis. The antioxidant properties may be related to its phenolic structure, which may adsorb and neutralize free radicals and exhibit redox properties (Yu et al., 2016). Moreover, it may suppress the progression of high-fat-diet-induced hyperlipidemia and atherosclerosis by reducing aortic intimal lipid lesion, lowering serum lipids and oxidative stress, and alleviating inflammation-related responses (Yu et al., 2016). This compound scavenges hydroxyl free radicals and produces phenoxyl radicals, major transient species (Nagoor Meeran et al., 2017).

An earlier study in our laboratory showed no toxic effects in terms of hemolysis or increased methemoglobin content linked with leaf extracts of various *Thymus* species on equine erythrocytes. Moreover, Nagoor Meeran and Prince (2012) also confirmed the antioxidative effect of thymol *via* increase of the activity of endogenous antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and the level of other non-enzymatic antioxidants such as vitamin C, vitamin E, and reduced glutathione (Nagoor Meeran and Prince, 2012), and thereby the total antioxidant status *in*

vivo (Youdim and Deans, 2000). In our study, the extracts actually reduced hemolysis and hemoglobin oxidation. Ethanol-based extracts obtained from various *Thymus* species revealed pronounced antibacterial ability. A number of reports concerning thymol revealed its antioxidant activity *via* scavenging of free radicals, enhancing the endogenous enzymatic and non-enzymatic antioxidants and chelation of metal ions (Youdim and Deans, 2000; Nagoor Meeran and Prince, 2012; Jarić et al., 2015; Nagoor Meeran et al., 2017).

Equine erythrocytes are more sensitive to oxidant-induced damage due to the use of inefficient mechanisms to correct and protect against oxidative damage, i.e. methemoglobin formation, alteration of aggregation, and reduction of cellular deformability (Baskurt and Meiselman, 1999). It was shown, that horses have a greater risk than other mammalian species of developing methemoglobinemia and hemolytic anemia following ingestion of oxidizing toxins, due to deficiencies in the mechanisms that protect against oxidative damage in erythrocytes. Erythrocytes from horses are slower than erythrocytes from other species studied in their ability to regenerate GSH after it has been oxidized *in vitro* (Harvey et al., 2003). These reduced abilities may be related to the fact that horse erythrocytes have lower glutathione reductase (GR) activities than erythrocytes from humans and most domestic animal species, and the Michaelis-Menton constant (Km) of GSSG for GR is higher in horses than in three other species measured. Moreover, sulfhydryl groups in proteins and unsaturated lipids in membranes are especially susceptible to oxidation. Oxidative denaturation and the precipitation of the globin portion of hemoglobin into large aggregates result in the formation of Heinz bodies that can bind to and alter membranes. Membrane structure also is altered by the oxidation of sulfhydryl groups and by lipid peroxidation (Harvey, 1997).

Erythrocytes were proved to be a good tool for analyzing the oxidative stress and lipid peroxidation as a mechanism of toxic action in various studies (Baskurt and Meiselman, 1999). Erythrocytes help in assessing the toxicity of various extracts as well. Oxidative damage to erythrocytes after exposure to extracts induced alterations in the morphology of cells, membrane protein conformation, protein cross-linking, lipid peroxidation and consequently hemolysis of erythrocytes (Frag and Alagawany, 2018). Therefore, in the present study, the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification, total antioxidant capacity] in the equine erythrocytes was used for assessing the antioxidant activity of *Th. serpyllum* extract.

Material and methodology

Collection of plant materials

Plants were harvested in June-August, 2016. Leaves of *Th. serpyllum* were collected among 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.).

Identification of this species was made according to Nachychko (2015). 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 attached material and used to prepare extracts.



Figure 1 Plant of *Thymus serpyllum* used in our study. Photo by Viktor Nachychko

Preparation of plant extracts

Freshly collected leaves were washed, weighted, 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 extract was stored at -20 °C until use.

Horses

Eighteen healthy adult horses from central Pomeranian region in Poland (village Strzelinko, N 54° 30' 48.0" E 16° 57' 44.9"), aged 8.9 ± 1.3 years old, including 6 Hucul pony, 5 Thoroughbred horses, 2 Anglo-Arabian horses and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. The females were non-pregnant.

Collection of blood samples

Blood was drawn from the jugular vein of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM). Blood was stored

in tubes with sodium citrate as the anticoagulant and held on the ice until centrifugation at 3000 rpm for 5 min to remove plasma. The pellet of blood was re-suspended in 4 mM phosphate buffer (pH 7.4). A volume of 0.1 ml of the plant extract was added to 1.9 ml of clean equine erythrocytes. For positive control (phosphate buffer) was used. After incubation the mixture at 37 °C for 60 min with continuous stirring, it was centrifuged at 3000 rpm for 5 min. Erythrocytes aliquots were used in the study.

The 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 malondialdehyde (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. Briefly, 0.1 mL of sample (blood, plasma, and erythrocytes' suspension) was added to 2 mL of distilled water, 1 mL of 20% TCA and 1 mL of 0.8% TBA. The mixture was heated in a boiling water bath for 10 minutes. After cooling, the mixture was centrifuged at 3.000 g for 10 minutes. The μmol of MDA per l L was calculated using $1.56 \cdot 10^5 \text{ mM}^{-1} \text{ cm}^{-1}$ as the extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay

To evaluate the protective effects of extracts obtained from leaves of *Th. serpyllum* against free radical-induced protein damage in equine erythrocytes, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the erythrocytes' suspension was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) and as modified by Dubinina et al. (1995). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Briefly, 1 mL of 0.1 M DNPH (dissolved in 2M HCl) was added to 0.1 mL of the sample after denaturation of proteins by 20% trichloroacetic acid (TCA). After addition of the DNPH solution (or 2M HCl to the blanks), the tubes were incubated for a period of 1 h at 37 °C. The tubes were spun in a centrifuge for 20 min at 3,000 g. After centrifugation, the supernatant was decanted and 1 mL of ethanol-ethylacetate solution was added to each tube. Following the mechanical disruption of the pellet, the tubes were allowed to stand for 10 min and then spun again (20 min at 3,000 g). The supernatant was decanted and the pellet washed thrice with ethanol-ethylacetate. After the final wash, the protein was solubilized in 2.5 mL of 8M urea solution. To speed up the solubilization process, the samples were incubated in a 90 °C water bath for 10–15 min. The final solution was centrifuged to remove any insoluble material. The carbonyl content was calculated from the absorbance measurement at 370 nm and 430 nm, and an absorption coefficient $22.000 \text{ M}^{-1} \cdot \text{cm}^{-1}$. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehyde derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in the sample was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). Sample inhibits the Fe²⁺/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. Briefly, 0.1 mL of sample was added to 2 mL of 1% Tween 80 reagent, 0.2 mL of 1 mM FeSO₄, and 0.2 mL of 10 mM ascorbic acid. In the blank assay, 0.1 mL of distilled water was used instead of the sample. The mixture was heated in a water bath for 48 hrs at 37 °C. After cooling, 1 mL of 20% trichloroacetic acid was added. The mixture was centrifuged at 3000 g for 10 min. After centrifugation, 2 mL of supernatant and 2 mL of 0.25% 2-thiobarbituric acid were mixed. The mixture was heated in a water bath at 95 °C for 15 min. The absorbance of the obtained solution was measured at 532 nm. The absorbance of the blank was defined as 100%. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

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 total antioxidant capacity level (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* 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

Many lipid peroxidation (LPO) products exert cytotoxicity, but sublethal concentrations of LPO products induce cellular adaptive responses and enhance tolerance against subsequent oxidative stress through upregulation of antioxidant compounds and enzymes (Niki, 2009). This adaptive response is observed not only for chemically reactive carbonyl compounds but also for chemically stable compounds. On the other hand, LPO, as well as reactive oxygen and nitrogen species, have been shown to play an important role as a regulator of gene expression and cellular signaling messenger (Niki, 2009).

When equine erythrocytes were incubated with an extract obtained from *Th. serpyllum*, the TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives level, as well as total antioxidant capacity, was non-significantly altered. The *Th. serpyllum* extract reduced the formation of intracellular aldehydic and ketonic derivatives of OMP in the extract-treated erythrocytes (by 8.8 and 6.3%, $p > 0.05$), but these results were non-significant. Total antioxidant capacity was non-significantly increased by 8.1% ($p > 0.05$) (Figure 2).

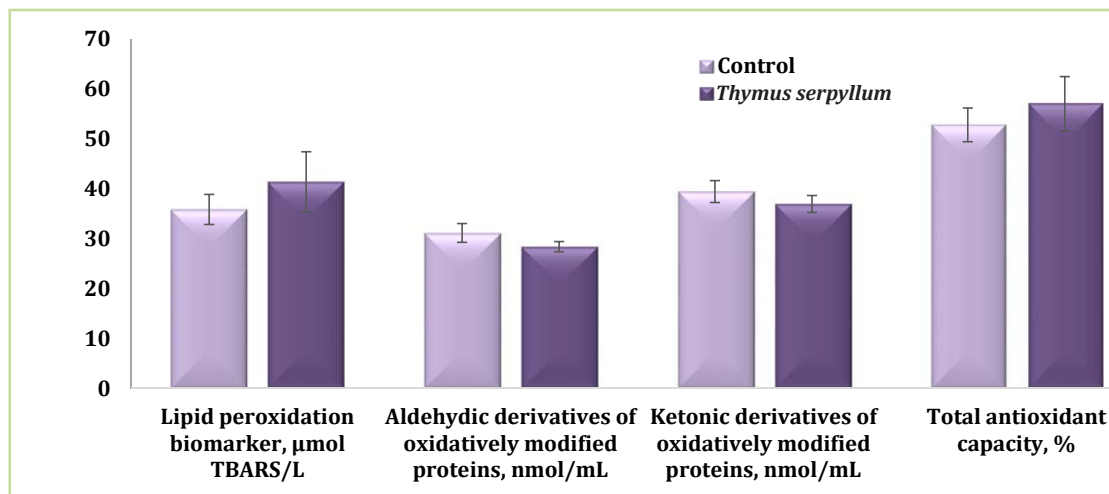


Figure 2 The TBARS content as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes suspension after *in vitro* incubation with leaf extract obtained from *Thymus serpyllum* ($M \pm m$, $n = 18$)

Many *in vitro* studies confirmed antioxidant properties of thyme extracts. 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). For example, Petrović et al. (2014) studied the antioxidant capacity of wild thyme essential oil in terms of its ability to neutralize DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals, that is, the ability of the components of the essential oil to donate hydrogen atoms and transform DPPH into its reduced form DPPH-H. Their results showed that the essential oil exhibited significantly better antioxidant activity when compared to synthetic antioxidants like butylated hydroxyanisole (BHA) and in particular butylated hydroxytoluene (BHT) (Petrović et al., 2014). The essential oil of *Th. serpyllum* growing in Croatia revealed poorer ability to neutralise DPPH radicals than BHA, BHT, tocopherol, ascorbic acid compared to the essential oil of *Th. vulgaris* L. Hussain et al. (2013) also demonstrated that the essential oil of *Th. serpyllum* exhibited less ability to neutralize DPPH radicals than BHT and thymol.

Six different assays were employed in the study of Kindl et al. (2015) in order to evaluate the antioxidant properties of the ethanolic extracts of selected *Thymus* species growing in Croatia (*Th. longicaulis* C. Presl., *Th. praecox* Opiz subsp. *polytrichus* (A.Kern. ex Borbás) Jalas, *Th. pulegioides* L., *Th. serpyllum* subsp. *serpyllum*, *Th. striatus* Vahl, and *Th. vulgaris*) as well as elucidate its mode of action. The tested *Thymus* extracts and pure compounds at different concentrations ($0.4\text{--}25 \mu\text{g}\cdot\text{mL}^{-1}$) significantly inhibited DPPH• in a concentration-dependent manner. The activities of plant extracts were 11–28, 23–52, and 52–85% at 1.56 , 3.13 , and $6.25 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. At the mentioned concentrations, *Th. serpyllum* subsp. *serpyllum* as well as a commercial sample of *Th. vulgaris* were the least effective. Rosmarinic acid and luteolin at concentrations up to $3.13 \mu\text{g}\cdot\text{mL}^{-1}$ showed the highest radical

scavenging effectiveness (56 and 50% at $0.8 \mu\text{g}\cdot\text{mL}^{-1}$, resp.). Interestingly, at concentrations $\geq 12.5 \mu\text{g}\cdot\text{mL}^{-1}$, activities of most *Thymus* species were comparable to that of luteolin. DPPH radical scavenging activities of the tested samples were assessed using IC_{50} values which are inversely related to their antioxidant abilities. The obtained IC_{50} values of studied *Thymus* extracts were in the range $3.01\text{--}6.01 \mu\text{g}\cdot\text{mL}^{-1}$. The scavenging effects of the extracts decreased in the order of *Th. longicaulis* > *Th. praecox* subsp. *polytrichus* > *Th. pulegioides*, *Th. striatus* > *Th. vulgaris* > *Th. serpyllum* subsp. *serpyllum* (Kindl et al., 2015). Moreover, all tested *Thymus* extracts inhibited nitrite formation in a concentration-dependent manner. They scavenged $\text{NO}\bullet$ by 16–45, 37–58, and 54–72% at 50, 100, and $200 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. At these concentrations, *Th. longicaulis* and *Th. pulegioides* showed the highest activity, while *Th. serpyllum* subsp. *serpyllum* demonstrated the weakest effect. Rosmarinic acid and luteolin inhibited the formation of $\text{NO}\bullet$ by 58% already at $25 \mu\text{g}\cdot\text{mL}^{-1}$. Comparing obtained IC_{50} values, the effectiveness of plant extracts as $\text{NO}\bullet$ scavengers were in the following descending order: *Th. longicaulis*, *Th. pulegioides* > *Th. striatus*, and *Th. vulgaris* > *Th. praecox* subsp. *polytrichus* > *Th. serpyllum* subsp. *serpyllum*. The IC_{50} values of the most potent *Th. longicaulis* and *Th. pulegioides* were 71.57 and $69.77 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. Rosmarinic acid ($\text{IC}_{50} = 15.67 \mu\text{g}\cdot\text{mL}^{-1}$) and luteolin ($\text{IC}_{50} = 18.31 \mu\text{g}\cdot\text{mL}^{-1}$) demonstrated the greatest $\text{NO}\bullet$ scavenging activity, even significantly higher ($p < 0.001$) than Trolox ($\text{IC}_{50} = 53.91 \mu\text{g}\cdot\text{mL}^{-1}$), and these results were in accordance with the findings obtained by DPPH assay (Kindl et al., 2015). All investigated *Thymus* extracts inhibited lipid peroxidation in a concentration-dependent manner. The activities of plant extracts at concentrations of $10 \mu\text{g}\cdot\text{mL}^{-1}$ and $100 \mu\text{g}\cdot\text{mL}^{-1}$ were in the ranges 32–40 and 56–76%, respectively. *Th. longicaulis* ($\text{IC}_{50} = 34.30 \mu\text{g}\cdot\text{mL}^{-1}$) and *Th. pulegioides* ($\text{IC}_{50} = 34.83 \mu\text{g}\cdot\text{mL}^{-1}$) exhibited once again the most powerful antioxidant effect, comparable to that of rosmarinic acid ($\text{IC}_{50} = 21.07 \mu\text{g}\cdot\text{mL}^{-1}$). The IC_{50} values obtained for the other four extracts were in the range $63.01\text{--}80.00 \mu\text{g}\cdot\text{mL}^{-1}$, without significant difference between them. All investigated plant samples were active in a concentration-dependent manner with total antioxidant capacities ranging between 238.16 and 293.82 mg equivalents of ascorbic acid (AAE)/g. Their effectiveness decreased in the following order: *Th. longicaulis*, *Th. praecox* subsp. *polytrichus* \geq *Th. striatus* > *Th. pulegioides*, *Th. vulgaris* \geq *Th. serpyllum* subsp. *serpyllum*. The activity of *Th. longicaulis* was comparable to that of Trolox. Rosmarinic acid was found to have the much higher total antioxidant capacity ($598.34 \text{ mg AAE}\cdot\text{g}^{-1}$) than Trolox ($307.89 \text{ mg AAE}\cdot\text{g}^{-1}$). Luteolin showed the lowest activity in comparison to all tested samples ($67.72 \text{ mg AAE}\cdot\text{g}^{-1}$) (Kindl et al., 2015).

Promising results in terms of the antihypertensive effect of thyme have been observed in Mihailovic-Stanojevic et al. (2013) study. They have evaluated total phenol and flavonoid contents, antioxidant capacity, free radical scavenging activity and potential antihypertensive effect of aqueous extract obtained from *Th. serpyllum* in spontaneously hypertensive rats and in normotensive Wistar rats. Total phenol content of *Th. serpyllum* was $2008.33 \pm 10.6 \text{ mg}\cdot\text{L}^{-1}$ gallic acid equivalents, and rosmarinic and caffeic acids were predominant phenolic compounds. The rosmarinic and caffeic acids as predominant phenols presented in the *Th. serpyllum*. The ferric reducing/antioxidant power and antioxidant capacity analysis revealed strong antioxidative properties of *Th. serpyllum*. *In vitro* nitric oxide-scavenging activity of $1 \text{ mg}\cdot\text{L}^{-1}$ *Th. serpyllum* was 63.43% with the IC_{50} value of $122.36 \mu\text{g}\cdot\text{mL}^{-1}$. Because

the spontaneously hypertensive rats are a useful model to investigate human essential hypertension, and compounds which lower blood pressure in rats also lower blood pressure in hypertensive humans, results obtained in a study of Mihailovic-Stanojevic et al. (2013) could be promising in using *Th. serpyllum* in hypertensive patients.

Moreover, *Th. serpyllum* may be a promising candidate in the development of novel therapeutic drugs for breast cancer treatment. Bozkurt et al. (2012) have evaluated the effects of *Th. serpyllum* on apoptosis and epigenetic events in breast cancer cells. XTT cell viability assay was used to determine cytotoxicity, while the DNA fragmentation and caspase 3/7 activity assays were used in the assessment of apoptosis. DNA methyltransferase (DNMT) and histone deacetylase (HDAC) activities were evaluated by ELISA and verified by qRT-PCR. *Th. serpyllum* extract induced significant cytotoxicity in breast cancer cells (MCF-7 and MDA-MB-231) but not in normal cells. It also induced apoptosis and inhibited the DNMT and HDAC activities in MDA-MB-231 cells (Bozkurt et al., 2012).

Essential oil of *Th. serpyllum* also showed the strongest inhibitory effect on the growth and mycotoxin production of *Aspergillus ochraceus*, *A. carbonarius*, and *A. niger* which may have been related to the synergistic or cumulative effects of its components. Minimal inhibitory concentration (MIC) determined for the essential oil and thymol, and selected concentration of the total phenolic content in extract inhibited fungal growth and ochratoxin A biosynthesis by more than 60%, depending on the conditions and duration of incubation with the fungi (Sokolić-Mihalak et al., 2012).

The aqueous extract of *Th. serpyllum* also might be used alone or in combination with insulin to manage diabetes and its associated complications. Alamgeer and et al. (2016) have evaluated and compare the hypoglycemic activity of different solvents extracts of *Th. serpyllum* in rabbits. Diabetes was induced with a single intravenous injection of alloxan monohydrate (150 mg.kg⁻¹). The crude powder of *Th. serpyllum* (500 mg.kg⁻¹ b.w.) significantly reduced the blood glucose level in both normal and diabetic rabbits. Ether and aqueous extracts of *Th. serpyllum* significantly reduced the blood glucose level with maximum effect ($p < 0.001$) produced by aqueous extract, which was selected for further study. Aqueous extract significantly inhibited the rise in glucose level in oral glucose tolerance test. The extract showed a synergistic effect with different doses of insulin; however serum insulin level of the diabetic rabbits was not significantly increased by the extract. The HbA1c level was significantly ($p < 0.05$) reduced whereas hemoglobin level was significantly increased in three months of study. Phytochemical screening of the aqueous extract showed the presence of alkaloids, flavonoids, tannins, terpenoids, reducing sugar and cardiac glycosides (Alamgeer et al., 2016). According to many supporting documents, it can be assumed that secondary plant metabolites, i.e. polyphenolic compounds in extracts of various species from *Thymus* genus extract may contribute to the antioxidant activity.

Conclusions

The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives level, as well as total antioxidant capacity, was non-significantly altered after *in vitro* incubation with an extract obtained from *Th. serpyllum*. The *Th. serpyllum* extract reduced the formation

of intracellular aldehydic and ketonic derivatives of OMP in the extract-treated erythrocytes, but these results were non-significant. Total antioxidant capacity was non-significantly increased. The lack of clinical safety and toxicity data for thyme, and many other herbs that are increasingly being used, suggests the necessity of further investigations regarding their influence on organs and tissues function, including the evaluation of molecular mechanisms involved in order to exploit them for potential therapeutic benefits (Rašković et al., 2015). Screening of *Thymus* species for other biological activities including antioxidant activities is essential and may be effective for searching the preventive agents in the pathogenesis of some metabolic diseases.

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