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EVALUATION OF OXIDATIVE STRESS BIOMARKERS IN EQUINE ERYTHROCYTES AFTER *IN VITRO* TREATMENT WITH LEAF EXTRACT OF *THYMUS PULEGIOIDES* L. (*LAMIACEAE*)

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In line with our previous study, we continue to evaluate the antioxidant potential of four species and one interspecific hybrid of the Thymus genus sampled in the Western part of Ukraine on the equine erythrocyte model. 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 (TAC)], as well as HCl-induced hemolysis in the equine erythrocytes, was used for assessing the antioxidant activity of extract obtained from the leaves of Thymus pulegioides L. in dose 5 mg/mL. Leaves of Th. pulegioides 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.). Equine erythrocyte aliquots were used in the study. For positive control (blank), phosphate buffer was used. After incubation of the mixture at 37°C for 60 mins with continuous stirring, samples were used for the biochemical assays. Results of the current study revealed that the extract obtained from leaves of Th. pulegioides (5 mg/mL) has a mild cytotoxic activity on the equine erythrocytes increasing the level of lipid peroxidation biomarker and hemolysis rate. The investigation also revealed that this extract exhibited hemolytic activity. These findings suggest the use of Th. pulegioides extract in dose 5 mg/mL as a source of prooxidant compounds and warrant further studies to evaluate their therapeutic potential. The aldehydic and ketonic derivatives level, as well as total antioxidant capacity, was non-significantly altered after in vitro incubation with an extract obtained from leaves of Th. pulegioides. 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 diseases as well as prevention and treatment of some disorders in medicine and veterinary.

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

The genus *Thymus* (Lamiaceae) comprises about 214 species throughout the world, mainly found in North Africa, Europe, and the temperate Asia zone. This genus of plants possesses a variety of activities including antimicrobial, antioxidant, anti-

inflammatory, cytotoxic, analgesic, and antidiabetic, traditionally used in folk medicines [16]. Several studies have evaluated the potential therapeutic uses of pharmaceuticals, nutraceuticals, and cosmeceuticals based on thymol 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 immunomodulatory [25]. The *Thymus* genus comprises numerous species that are particularly abundant in the West Mediterranean region. As Afonso and co-authors (2020) noted, many of these species are a rich source of bioactive compounds, including phenolic compounds, i.e. rosmarinic acid, salvianolic acids, and luteolin glycosides, able to render their potential applications in a range of industrial fields [1]. Thymol (10–64 %) is one of the major constituents of essential oils of thyme (*Thymus vulgaris* L., Lamiaceae), a medicinal plant with several therapeutic properties [25].

Thymol, chemically known as 2-isopropyl-5-methylphenol, has been used in traditional medicine for centuries exhibiting various pharmacological properties including antioxidant, free radical scavenging, anti-inflammatory, analgesic, antispasmodic, antibacterial, antifungal, antiseptic, and antitumor activities [22]. It is one of the main compounds of thyme essential oil. Both thymol and thyme essential oil have long been used in traditional medicine as an expectorant, anti-inflammatory, antiviral, antibacterial, and antiseptic agent, mainly in the treatment of the upper respiratory system [14]. The scientific literature reveals the pharmacological properties of thymol and its multiple therapeutic actions against various cardiovascular, neurological, rheumatological, gastrointestinal, metabolic, and malignant diseases at both biochemical and molecular levels [22].

The antimicrobial activity of *Thymus* species, as well as thymol, has been well studied. In our previous study [9, 10], the ethanolic extracts obtained from leaves of various *Thymus* representatives exhibited intermediate activity against β-lactamase-producing *Pseudomonas aeruginosa* and *Salmonella enteritidis* strains locally isolated. 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* Tausch ex A. Kern., 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* All. followed by *Th. serpyllum* L. and then by *Th. pulegioides* L. [10]. The ethanolic extract obtained from the leaves of *Th. pulegioides* were 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 [9].

Many *in vitro* studies confirmed the antioxidant properties of thyme extracts [8, 11, 22, 27]. 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 phenoxyl radicals, major transient species [22]. For example, the phenolic constituent profiles and the antioxidant, anti-proliferative, neuroprotective, anti-aging, and anti-diabetic activities of both *Th. pulegioides* aqueous decoctions (AD) and hydro-ethanolic extracts (HE) were studied by Taghouti et al. (2018). Rosmarinic acid was the main phenolic compound, accounting for 35.2 % or 47.8 % of total identified phenols in AD or HE, respectively. Furthermore, large amounts of luteolin-O-hexuronide, eriodictyol-O-hexuronide, and chrysoeriol hexoside were found. Both extracts showed significant *in vitro* antioxidant activity and anti-proliferative activity against Caco-2 cells and reduced hepatotoxicity (HepG2

cells). In general, both *Th. pulegioides* extracts showed poor anti-diabetic activity, moderate anti-aging effects and high neuroprotective activity with both AD and HE extracts, at 0.5 mg mL^{-1}, showing 80 % inhibition of the acetylcholinesterase activity and 94 % inhibition of the tyrosinase activity [27].

Six different assays were employed in the study of Kindl et al. (2015) to evaluate the antioxidant properties of the ethanolic extracts of selected *Thymus* species growing in Croatia (*Th. longicaulis, Th. praecox* subsp. *polytrichus, Th. pulegioides, Th. serpyllum* subsp. *serpyllum, Th. striatus*, and *Th. vulgaris*) as well as to elucidate its mode of action. The tested *Thymus* extracts and pure compounds at different concentrations (0.4–25 μg/mL) significantly inhibited $DPPH^{\cdot}$ in a concentration-dependent manner. The activities of plant extracts were 11–28 %, 23–52 %, and 52–85 % at 1.56 μg/mL, 3.13 μg/mL, and 6.25 μg/mL, respectively. At the mentioned concentrations, *Th. serpyllum* subsp. *serpyllum* as well as a commercial sample of *Th. vulgaris* were the least effective [13].

In line with our previous study, we continue to assess the antioxidant potential of four species and one interspecific hybrid of the *Thymus* genus sampled in the Western part of Ukraine on the equine erythrocytes' model. 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 (TAC)], as well as HCl-induced hemolysis in the equine erythrocytes, was used for assessing the antioxidant activity of extract obtained from the leaves of *Th. pulegioides* in dose 5 mg/mL.

Materials and methods. *Collection of Plant Materials.* Leaves of *Thymus 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.) (Photo 1). Identification of this species was made according to Nachychko (2014, 2015) and Nachychko and Honcharenko (2016) [18–20]. 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. This study was carried out during the Scholarship Program supported by The Visegrad Fund in the Department of Zoology and Animal Physiology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk (Poland).

Photo 1. ***Thymus pulegioides plant (Photo: Viktor Nachychko,*** **Vitaliy Honcharenko*)***

Preparation of Plant Extract. Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1M 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 the central Pomeranian region in Poland (Strzelinko, N54º30'48.0" E16º57'44.9"), 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 taken 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 3.000 rpm for 5 mins to remove plasma. The pellet of blood was resuspended 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 of the mixture at 37°C for 60 mins with continuous stirring, it was centrifuged at 3,000 rpm for 5 mins. Erythrocyte 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 malondialdehyde (MDA) concentration [12]. 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 L was calculated using $1.56 \cdot 10^5$ mM^{-1} 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. pulegioides* 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 erythrocyte 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-authors (1990) [15] and as modified by Dubinina and co-authors (1995) [5]. DNFH was used for determining carbonyl content in soluble and insoluble proteins. 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 [7]. Sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated concerning the absorbance of the blank sample.

Assay of Acid Resistance of Erythrocytes. The acid resistance of erythrocytes was measured spectrophotometrically with 0.1M HCl [28]. The assay is based on the measuring of the dynamics of erythrocytes disintegration into hemolytic reagent action.

The time of hemolytic reagent action serves as the measure of erythrocyte resistance. The assay mixture contained 5 mL of 1 % erythrocyte suspension and 0.05 mL of 0.1 M HCl. The absorbance was read at 540 nm every 30 seconds after HCl addition till the end of hemolysis. The difference of absorbance at the beginning and the end of hemolysis was determined as 100 % (total hemolysis). The disintegration of erythrocytes (%) at every 30 seconds was expressed as a curve.

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 Mann-Whitney *U* test [31]. All statistical calculation was performed on separate data from each individual with STATISTICA 8.0 software (StatSoft, Krakow, Poland).

Results and discussion. After incubation of equine erythrocytes with a leaf extract obtained from *Th. pulegioides*, the aldehydic and ketonic derivatives level, as well as total antioxidant capacity, were non-significantly altered. The *Th. pulegioides* extract caused to increase in TBARS content as a biomarker of lipid peroxidation in the extract-treated erythrocytes, and these results were statistically significant (by 51.2 %, $p<0.05$). The aldehydic and ketonic derivatives level was non-significantly decreased by 1.2 % and 5.6 % ($p>0.05$), respectively (Fig. 1).

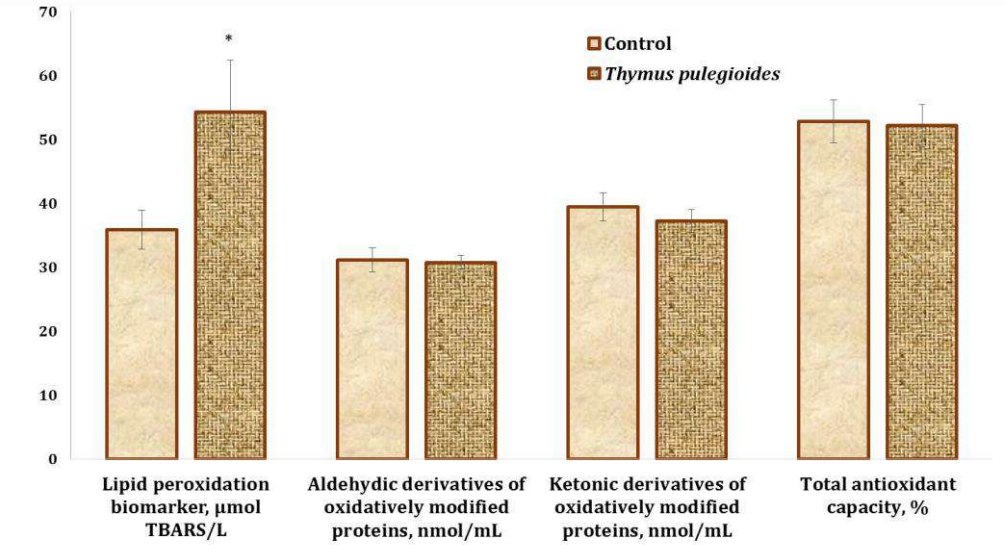

Fig. 1. **The TBARS content as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes suspension after *in vitro* incubation with leaf extract obtained from *Thymus pulegioides* (M ± m, n = 18).**

*– changes were statistically significant ($p < 0.05$).

The erythrocytes of mammals represent a good model to evaluate the cytotoxicity of molecules, organic and inorganic, natural or synthetic, by cellular damage measure [23]. Erythrocytes are especially vulnerable since they have no membrane repair and regenerative capacity [29] and red cell damages by free radicals would probably be associated with hemolysis [6]. Therefore, the next purpose of our study was to evaluate the effect of *Th. pulegioides* extract (5 mg/mL) on the *in vitro* HCl-induced hemolysis of the equine erythrocytes (Fig. 2).

In the control group (erythrocyte suspension), erythrocytes incubated with 0,1M HCl remained stable and demonstrated slight hemolysis. The maximum level of hemol-

ysis was (17.21±1.32) %; the total duration of hemolysis was 12 mins. When *Th. pulegioides* extract (5 mg/mL) was added to the erythrocyte suspension, the maximum level of hemolysis occurred after 6.5 mins of incubation with 0.1M HCl (17.27±1.55) %. The total duration of hemolysis after *Th. pulegioides* extract (5 mg/mL) incubation was 10.5 mins. Our results showed that HCl-induced hemolysis in a typical time-dependent manner. Therefore, *Th. pulegioides* extract at a concentration of 5 mg/mL induced the increase of hemolyzed erythrocytes and caused to decrease in hemolysis duration (Fig. 2).

Many both *in vitro* and *in vivo* studies have shown that plants belonging to the *Thymus* genus possess antioxidant properties. For instance, Afonso and co-authors (2017) have evaluated the phytochemical characterization and the potential antioxidant, anti-inflammatory, and antimicrobial activities of three *Thymus* species, i.e. *Thymus herba-barona*, *Thymus pseudolanuginosus*, and *Thymus caespititius*, which have been poorly explored. Results of these authors suggested that the *T. pseudolanuginosus* extract presented the best DPPH radical scavenging ability, a high reducing power, and effectively inhibited the oxidation of β-carotene. The extracts also showed NO scavenging activity close to that of ascorbic acid, and thus might be useful as anti-inflammatory agents. Also, they exhibited antibacterial activity against gram-negative and gram-positive bacteria. *Staphylococcus aureus* strains were the most sensitive bacteria to thyme extracts, with minimum inhibitory concentration and minimum bactericidal concentration values in the range of 0.6–3.5 mg/mL [2].

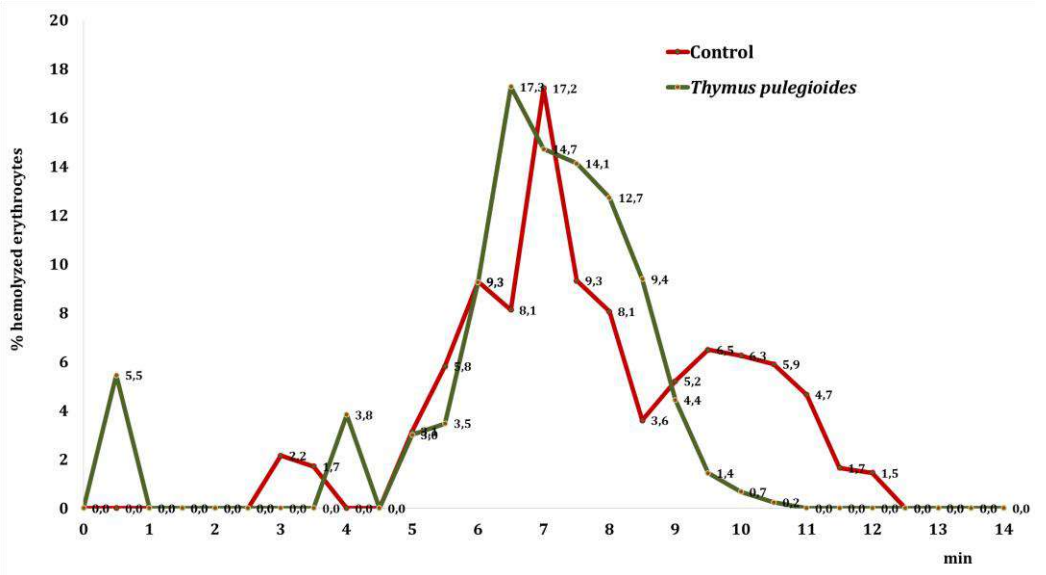

Fig. 2. **Effect of *Th. pulegioides* extract (5 mg/mL) on the HCl-induced hemolysis *in vitro* of the equine erythrocytes (M ± m, n = 18).**

On the other hand, Butt and co-authors (2019) have evaluated the anti-oxidant and anti-proliferative potential of Thymoquinone extracted from the essential oil of indigenous herbs of *Nigella sativa* and *Thymus vulgaris* [3]. Cell proliferation of HeLa cancer significantly decreased with a dose-response manner ($p<0.01$), showing the highest cell death in a high concentration of Thymoquinone. Inhibitory concentration 50 (IC_{50}) of cancer cell line treated with *Nigella sativa* oil was 0.5 µM and *Thymus vulgaris* was 18 µM compared to standard Thymoquinone, showing Inhibitory concentration (IC_{50}) of 6 µM. Researchers concluded that both *Nigella sativa* and *Thymus vulgaris* were found to be the best source of Thymoquinone as chemotherapeutic drugs expressed potent anti-oxidant and anti-proliferative activities [3].

The antioxidant and acetylcholinesterase (AChE) inhibitory activities of the ethanolic extracts of six selected *Thymus* species growing in Croatia (*T. longicaulis, T. praecox* subsp. *polytrichus, T. pulegioides, T. serpyllum* subsp. *serpyllum, T. striatus*, and *T. vulgaris*) were evaluated by Kindl and co-authors (2015) [13]. Antioxidant effectiveness was assessed using six different assays, in comparison with rosmarinic acid, luteolin, and reference antioxidants. All tested *Thymus* extracts possessed DPPH and nitric oxide free radical scavenging activities, strong reducing properties, ferrous ion chelating activity, ability to inhibit lipid peroxidation, and high total antioxidant capacities. All tested extracts showed anti-AChE activity in a dose-dependent manner. Additionally, the contents of total hydroxycinnamic derivatives, flavonoids, and tannins in dried plant samples were also determined by Kindl and co-authors (2015) [13]. Thus, *Thymus* species is a rich source of natural antioxidants and AChE inhibitors that could be useful in preventing and treating Alzheimer's disease and other neurodegenerative disorders.

Also, the *in vitro* cultures of many *Thymus* species represent a promising alternative for the production of valuable natural antioxidants. The phenolic metabolites and antioxidant activities of *Thymus lotocephalus* G. López & R. Morales wild plants and *in vitro* cultures using different extraction solvents were studied by Costa and co-authors (2012). HPLC-DAD analysis allowed the identification and quantification of phenolic (caffeic and rosmarinic acids) and flavones (luteolin and apigenin) in extracts from both sources. The *in vitro* cultures accumulated large amounts of rosmarinic acid. However, extracts from both sources were able to neutralize free radicals in different test systems (TEAC and ORAC assays), form complexes with Fe^{2+}, and protect mouse brains against Fe^{2+}-induced lipid peroxidation. The solvent significantly influenced the phenolic content and antioxidant activity of the extracts, water/ethanol being the most efficient for the extraction of antioxidant phytochemicals [4].

Thymus daenensis has also a potential herbal medicine that should be considered as an antibacterial and antioxidant with very low toxicity. Saidi and co-authors (2016) have investigated the antibacterial activity and subsequently, determine the antioxidant activity of *Thymus daenensis*. The association between phenolic compound and antioxidant activity was found for the ABTS·+ method (43.52 %) in the lowest level, while, for FRAD and DPPH· methods the opposite story occurred (70.5 % correlation for DPPH· and 50.9 % for FRAD) [24].

Thymol also increases 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 [17], and thereby the total antioxidant status *in vivo* [30]. In our previous study, the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification, total antioxidant capacity (TAC)], as well as HCl-induced hemolysis in the equine erythrocytes, was used for assessing the antioxidant activity of extract obtained from the leaves of *Th.* × *porcii* Borbás (a hybrid between *Th. pannonicus* and *Th. pulegioides*) was studied. The aldehydic and ketonic derivatives level, as well as total antioxidant capacity, was non-significantly altered after *in vitro* incubation with an extract obtained from leaves of *Th. x porcii*. The *Th. x porcii* extract caused to increase in TBARS content as a biomarker of lipid peroxidation in the extract-treated erythrocytes, and these results were statistically significant. Total antioxidant capacity was non-significantly increased. When *Th. x porcii* extract (5 mg/mL) was added to the erythrocyte suspension, the maximum level of hemolysis occurred after 8.5 mins of incubation with 0.1M HCl (17.91±1.87 %). The total duration of hemolysis after *Th. x porcii* extract (5 mg/mL)

incubation was 11.5 mins. Our results showed that HCl-induced hemolysis in a typical time-dependent manner. the extract obtained from leaves of *Th. x porcii* (5 mg/mL) has a mild cytotoxic activity on the equine erythrocytes increasing the level of lipid peroxidation biomarker and hemolysis rate. Investigation of the mechanism of action revealed that this extract has hemolytic activity. Therefore, *Th. x porcii* extract at a concentration of 5 mg/mL induced the increase of hemolyzed erythrocytes and caused to decrease in hemolysis duration [11]. Also, 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 *Thymus serpyllum* L. emend. Mill. [8]. 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.

The noteworthy effects of thymol are primarily 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 [22]. Thymol attenuates inflammation by inhibiting the release of lysosomal enzymes and downregulates the expressions of pro-inflammatory cytokines by its potent anti-inflammatory effect. The study conducted by Nagoor Meeran and co-authors (2015) revealed the protective effects of thymol on inflammation in isoproterenol (ISO) induced myocardial infarcted rats. ISO-induced myocardial infarcted rats showed increased levels of serum cardiac troponin-T, high sensitive C-reactive protein (hsCRP), lysosomal thiobarbituric acid reactive substances (TBARS), and elevated ST-segments. Also, the activities of lysosomal enzymes such as β-glucuronidase, β-galactosidase, cathepsin-B, and D, the stimulators of inflammatory mediators were increased in the serum and heart of ISO induced myocardial infarcted rats. Furthermore, ISO up-regulates the expressions of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) genes in the myocardium of rats analyzed by reverse transcription-polymerase chain reaction (RT-PCR). Pre and co-treatment with thymol (7.5 mg/kg body weight) near normalized the levels of lysosomal TBARS, activities of serum and heart lysosomal enzymes, and downregulates the expressions of pro-inflammatory cytokines in the myocardium of ISO induced myocardial infarcted rats [21].

The results of Youdim and Deans (2000) highlight the potential benefit of thyme oil as a dietary antioxidant. These researchers have evaluated changes in antioxidant enzyme activity and the phospholipid fatty acid composition of the aging rat brain and tested whether dietary supplementation with thyme oil or thymol could provide beneficial effects. There were significant declines in superoxide dismutase and glutathione peroxidase activities and the total antioxidant status in the untreated rats with age, while thyme-oil- and thymol-fed rats maintained significantly higher antioxidant enzyme activities and total antioxidant status. The proportions of 18:2n-6, 20:1n-9, 22:4n-6, and 22:5n-3 in the brain phospholipids resulting from all three dietary treatments were significantly higher in 28-month-old rats than in 7-month-old rats. Only 20:1n-9 levels in 28-month-old thyme-oil- and thymol-treated rats were significantly higher than in the age-matched control. The proportion of 22:6n-3 in brain phospholipids, which declined with age in control rats, was also significantly higher in rats given either supplement.

This latter finding is particularly important as optimum levels of 22:6n-3 are required for normal brain function [30].

Significant antioxidant activity of plants belonging to the *Thymus* genus is correlated with their polyphenol levels. For instance, the polyphenol compositions of *Thymus* × *citriodorus* and *Thymus vulgaris* extracts as obtained by exhaustive hydroethanolic (HE) extraction and aqueous decoction (AD) were compared in the study of Taghouti and co-authors (2020) [26]. Also, their compositions and bioactivities were compared to those of *Thymus pulegioides* and *Thymus mastichina*, grown under the same edaphoclimatic conditions, and *Thymus carnosus*. Rosmarinic acid was the most abundant polyphenol followed by luteolin-hexuronide, salvianolic acids I, and K. Cluster analysis suggests a similarity of the polyphenol composition of *T. citriodorus* and *T. vulgaris*. The same being observed for the higher anti-proliferative activity/cytotoxicity of HE extracts on Caco-2 and HepG2 cells as compared to AD extracts. A significant association between the total phenolic compounds with the anti-proliferative activity, for both cell lines, was observed. Results of Taghouti and co-authors (2020) supported the importance of salvianolic acid levels in *Thymus* extracts and their *in vitro* antiproliferative/cytotoxic activities [26].

Conclusions. The extract obtained from leaves of *Th. pulegioides* (5 mg/mL) has a mild cytotoxic activity on the equine erythrocytes increasing the level of lipid peroxidation biomarker and hemolysis rate. The investigation also revealed that this extract exhibited hemolytic activity. These findings suggest the use of *Th. pulegioides* extract in dose 5 mg/mL as a source of prooxidant compounds and warrant further studies to evaluate their therapeutic potential. The aldehydic and ketonic derivatives level, as well as total antioxidant capacity, was non-significantly altered after *in vitro* incubation with an extract obtained from leaves of *Th. pulegioides*. 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 diseases as well as prevention and treatment of some disorders in medicine and veterinary.

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ОЦЕНКА БИОМАРКЕРОВ ОКИСЛИТЕЛЬНОГО СТРЕССА В ЭРИТРОЦИТАХ ЛОШАДЕЙ ПОСЛЕ IN VITRO ОБРАБОТКИ ЭКСТРАКТОМ, ПОЛУЧЕННЫМ ИЗ ЛИСТЬЕВ THYMUS PULEGIOIDES L. (LAMIACEAE)

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В соответствии с нашим предыдущими исследованиями, мы продолжили оценку антиоксидантного потенциала четырех видов и одного межвидового гибрида рода Thymus, отобранных в западной части Украины на модели эритроцитов лошадей. В настоящем исследовании биомаркеры окислительного стресса [вещества, реагирующие с 2-тиобарбитуровой кислотой (ТБК-продукты), содержание карбонильных производных окислительно модифицированных белков, общая антиоксидантная активность (ТАС)], а также индуцированный HCl гемолиз в эритроцитах лошадей, использовали для оценки антиоксидантной активности экстракта, полученного из листьев Thymus pulegioides в дозе 5 мг/мл. Листья Th. pulegioides были собраны среди трав около земельных участков (с. Сывки Белогорского района Хмельницкой области, Украина; N50°02′02,8′′, E26°14′13,9′′, 306 м над у.м.). В исследовании использовали аликвоты эритроцитов лошадей. Для положительного контроля использовали фосфатный буфер (pH 7,4). После инкубации смеси при 37 °C в течение 60 мин при непрерывном перемешивании образцы использовали для биохимических анализов. Результаты настоящего исследования показали, что экстракт, полученный из листьев Th. pulegioides (5 мг/мл) обладает умеренной цитотоксической активностью в суспензии эритроцитов лошадей, повышая уровень биомаркера перекисного окисления липидов и скорость гемолиза. Исследование также показало, что этот экстракт проявлял гемолитическую активность. Эти данные предполагают использование Th. pulegioides в дозе 5 мг/мл в качестве источника прооксидантных соединений и требуют дальнейших исследований для оценки их терапевтического потенциала. Уровень альдегидных и кетоновых производных, а также общая антиоксидантная активность существенно не изменились после инкубации in vitro с экстрактом, полученным из листьев Th. pulegioides. Скрининг различных видов тимьяна относительно проявления других видов биологической активности, включая антиоксидантную, имеет важное значение и может быть эффективным для поиска профилактических агентов в патогенезе некоторых заболеваний, а также для профилактики и лечения некоторых нарушений в медицине и ветеринарии.

Ключевые слова: Thymus pulegioides L., экстракт листьев, эритроциты лошадей, перекисное окисление липидов, окислительно модифицированные белки, общая антиоксидантная способность, гемолиз

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Згідно до наших попередніх досліджень, ми продовжуємо оцінювати антиоксидантний потенціал чотирьох видів і одного міжвидового гібриду роду Thymus, відібраних в західній частині України на моделі еритроцитів коней. Таким чином, в цьому дослідженні біомаркери окисного стресу [речовини, що реагують з 2-тіобарбітурової кислотою (ТБК-продукти), вміст карбонільних похідних окиснювально модифікованих білків, загальна антиоксидантна активність (ТАС)], а також HCl-індукований гемоліз в еритроцитах коней, використовували для оцінки антиоксидантної активності екстракту, отриманого з листя Thymus pulegioides в дозі 5 мг/мл. Листя Th. pulegioides були зібрані серед трав близько земельних ділянок (с. Сивки Білогірського району Хмельницької області, Україна; N 50°02'02,8", E 26°14'13,9", 306 м р.м.). У дослідженні використовували аліквоти еритроцитів коней. Для позитивного контролю використовували фосфатний буфер (pH 7,4). Після інкубації суміші при 37 °C впродовж 60 хв при безперервному перемішуванні зразки використовували для біохімічних аналізів. Результати цього дослідження показали, що екстракт, отриманий з листя Th. pulegioides (5 мг/мл) володіють помірною цитотоксичною активністю щодо еритроцитів коней, підвищуючи рівень біомаркерів перекисного окиснення ліпідів і швидкість гемолізу. Дослідження також показало, що цей екстракт виявляв гемолітичну активність. Ці дані вимагають подальших досліджень для оцінки терапевтичного потенціалу Th. pulegioides. Рівень альдегідних і кетонових похідних, а також загальна антиоксидантна активність істотно не змінилися після інкубації in vitro з екстрактом, отриманим з листя Th. pulegioides. Скринінг різних видів чебрецю з метою виявлення інших видів біологічної активності, включаючи антиоксидантну активність, має важливе значення і може бути ефективним для пошуку профілактичних агентів в патогенезі деяких захворювань, а також для профілактики і лікування порушень в медицині і ветеринарії.

Ключові слова: Thymus pulegioides L., екстракт листя, еритроцити коней, перекисне окиснення ліпідів, окиснювально модифіковані білки, загальна антиоксидантна активность, гемоліз

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ПРОДУКТИВНІСТЬ ТА ВІДТВОРЮВАЛЬНА ЗДАТНІСТЬ КРОСБРЕДНИХ КОРІВ-ПЕРВІСТОК

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У статті наведено результати досліджень відтворювальної здатності, збереженості та середньодобових надоїв корів-первісток української червоно-рябої молочної породи, отриманих від монбельярдських і голштинських бугаїв-плідників у ДП ДГ „Гонтарівка" Харківської області.