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In vitro antioxidant response of the equine blood treated by extract derived from leaves of *Ficus sagittata* Vahl (Moraceae)

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The current study aimed to investigate the oxidative stress biomarkers, such as 2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity, as well as antioxidant defenses (activity of superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin) in the equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from leaves of *Ficus sagittata* Vahl collected at two Botanic Gardens, i.e. M.M. Gryshko National Botanic Garden (Kyiv, Ukraine) and the Botanic Garden of Ivan Franko National University in Lviv (Lviv, Ukraine). Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in the proportion of 1 : 19, w/w) at room temperature. The extracts were then filtered and used for analysis. A volume of 0.1 mL of the plant extracts was added to 1.9 mL of clean equine erythrocytes or plasma (the final concentration of the extract was 5 $mg.mL^{-1}$). For positive control, 0.1 mL of phosphate buffer (pH 7.4) was used. The treatment of equine plasma and erythrocytes by extracts derived from leaves of *F. sagittata* resulted in reduced carbonyl derivatives of the oxidatively modified protein. When equine erythrocytes were incubated with the extract derived from leaves of *F. sagittata* collected in NBG (Kyiv), the TBARS levels were significantly increased compared to the untreated samples. The incubation of equine plasma with an extract derived from leaves of *F. sagittata* resulted in an increase in the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase with a simultaneous decrease of ceruloplasmin level. The level of total antioxidant capacity was significantly increased after the treatment by extract derived from leaves of *F. sagittata* collected in NBG. However, further detailed investigation, especially *in vivo* and *in vitro* antioxidant studies is needed to justify the use of extract derived from leaves of *F. sagittata* as a natural source of antioxidants.

Keywords: lipid peroxidation, oxidatively modified proteins, superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin, total antioxidant capacity

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Introduction

The mulberry family (Moraceae) is represented by mainly woody tropical or (more rarely) temperate species with specialized canals within their body containing milky latex, the feature most obviously distinguishing the family from other members of the order Urticales in which it is currently placed. The family comprises 37 genera and 1,050–1,100 species with a diversity of growth forms, including terrestrial trees, shrubs, climbers, hemi-epiphytes, subshrubs, and herbs. High variation is also observed in the morphology and arrangement of leaves. The small unisexual flowers of these plants are assembled into inflorescences varying considerably in structure and position on a plant among the species, although this variety can be reduced to their two basic types:

1. largely bisexual and circular in outline,
2. unisexual and elongate, racemose or spicate.

These features partly account for the subdivision of the family into five tribes: Moreae, Artocarpeae, Dorstenieae, Castilleae, and Ficeae (Berg, 2001; Datwyler and Weiblen, 2004; Clement and Weiblen, 2009).

Ficus L. is the only genus of the tribe Ficeae and the largest within the family, containing ca 750 species distributed in the tropics and subtropics worldwide. Despite the exceptionally large species diversity of *Ficus* unproportional to that of other moraceous taxa, its consideration as a single entity is well-grounded on several specific features, among which are the presence of waxy glands on vegetative plant parts, heterostyly, and anthesis of staminate flowers when the fruits are mature (Berg, 2001; Cook and Rasplus, 2003; Berg and Corner, 2005).

Ficus sagittata Vahl is a climbing shrub when young, often starting life as an epiphyte. As it grows older it can become a tree. It often starts life as an epiphyte in the branch of a tree and can eventually send down aerial roots that, once they reach the ground, provide extra nutrients that help the plant grow more vigorously. These aerial roots can completely encircle the trunk of the host tree, constricting its growth – this, coupled with the more vigorous top growth, can lead to the fig outcompeting and killing the tree in which it is growing. The plant is sometimes harvested from the wild for local medicinal use. It is cultivated for its ornamental value (https://tropical.theferns.info/viewtropical.php?id=Ficus+sagittata).

Ficus plants have a lot of pharmacological effects, being used both in traditional medicines and contemporary treatment of different disorders. Recent studies showed the therapeutic efficacy of *Ficus* spp., especially in respiratory, cardiovascular, and central nervous system disorders (Cagno et al., 2015; Alamgeer et al., 2017; Salehi et al., 2021). Also, *Ficus* plants are used for the treatment of diabetes (Deepa et al., 2018).

In our previous study (Tkachenko et al., 2018, 2019), we highlighted the antioxidant potential of an aqueous extract derived from leaves of other *Ficus* species using an equine erythrocyte suspension. In the study (Tkachenko et al., 2018), we have focused on the antioxidant effect of an extract derived from leaves of *F. religiosa* L. on oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of protein oxidative modification (OMP), total antioxidant capacity (TAC)] using the model of equine erythrocytes. Treatment by extract reduced the erythrocyte's TBARS level by 25.3% ($p = 0.009$), while plasma TBARS level was increased by 75.6% ($p = 0.000$), as compared to untreated erythrocytes. When equine plasma was incubated with extract, the level of ketonic derivatives was significantly increased by 22.8% ($p = 0.000$), while a non-significantly decrease in both aldehydic and ketonic derivatives of OMP was observed (by 1.6% and 8.9%, $p > 0.05$). Treatment by *F. religiosa* extract caused the increase of TAC in plasma and erythrocyte suspension when compared to untreated erythrocytes. However, these changes were statistically non-significant. All these data suggest that *F. religiosa* could be explored for its antioxidant potential using an equine erythrocyte suspension (Tkachenko et al., 2018).

Later, we investigated the *in vitro* antioxidant activity of aqueous extracts derived from the leaves developed on the shoots of various developmental stages (juvenile and mature/generative) of *F. pumila* L. using the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of protein oxidative modification, total antioxidant capacity] on the model of equine erythrocyte suspension (Tkachenko et al., 2019). The treatment with the extract derived from leaves of mature shoots reduced the erythrocyte's TBARS level by 22% ($p = 0.029$), while the TBARS level was increased by 15.5% ($p > 0.05$) when incubated with an extract derived from leaves of juvenile shoots as compared to untreated erythrocytes. When equine erythrocytes were incubated with the extract obtained from leaves of mature shoots, the ketonic derivatives level was significantly decreased by 6.9% ($p = 0.040$), while a non-significantly decrease in both aldehydic and ketonic derivatives of OMP was observed after incubation with an extract derived from juvenile shoots (by 8.18 and 12.5%, $p > 0.05$).

The treatment by *F. pumila* leaf extract (from juvenile and mature shoots) caused the increase of TAC in erythrocyte suspension as compared to untreated erythrocytes. Thus, extracts derived from both juvenile and mature shoots increased the total antioxidant capacity of equine erythrocytes (Tkachenko et al., 2019).

The current study aimed to investigate the oxidative stress biomarkers, such as 2-thiobarbituric acid reactive substances, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity, as well as antioxidant defenses (activity of superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin) in the equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from leaves of *Ficus sagittata*.

Materials and methodology

Collection of plant materials

The leaves of *Ficus sagittata* were collected at M.M. Gryshko National Botanic Garden (NBG), Kyiv(Ukraine) and the Botanic Garden of Ivan Franko National University in Lviv (BG) (Ukraine). The whole collection of tropical and subtropical plants at NBG and BG (including *Ficus* spp. plants) has the status of a National Heritage Collection of Ukraine. Plant samples were thoroughly washed to remove all the attached material and used to prepare extracts.

Preparation of plant extracts

Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in the proportion of 1 : 19, w/w) at room temperature. The extracts were then filtered and used for analysis. All extracts were stored at -25 °C until use.

Horses

Eighteen clinically healthy adult horses from the 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 ponies, 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*. Before sampling, all horses were thoroughly examined clinically by a veterinarian and screened for hematological, biochemical, and vital parameters, which were within reference ranges. The females were non-pregnant.

Collection of blood samples

Blood samples were collected in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM) by jugular venipuncture into tubes with sodium citrate as the anticoagulant and held on the ice until centrifugation at 3,000 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 prepared from the leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv) was added to 1.9 mL of clean equine erythrocytes or plasma (the final concentration of the extract was 5 $mg.mL^{-1}$). For positive control, 0.1 mL of phosphate buffer was used. After incubation of the mixture at 37 °C for 60 min with continuous stirring, biochemical assays were done. Erythrocytes and plasma aliquots were used in the study.

The 2-Thiobarbituric acid reactive substances 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 and described in the paper by Tkachenko et al. (2022). This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol per 1 mL was calculated using $1.56 \cdot 10^5$ $mM^{-1}. cm^{-1}$ as the extinction coefficient.

The carbonyl derivatives of oxidatively modified proteins assay

To evaluate the protective effects of the extract against free radical-induced protein damage in samples, a content of carbonyl derivatives of oxidatively modified proteins (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the samples 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 et al. (1990) and as modified by Dubinina et al. (1995) and described in the paper by Tkachenko et al. (2022). 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 (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity

The total antioxidant capacity (TAC) level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm by Galaktionova et al. (1998) and described in the paper by Tkachenko et al. (2022). The 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 the absorbance of the blank sample.

Superoxide dismutase activity assay

The activity of superoxide dismutase (SOD, E.C. 1.15.1.1) was assessed by its ability to dismutate superoxide generated in the process of quercetin auto-oxidation in an alkaline medium (pH 10.0), as proposed by Kostiuk et al. (1990) and described in the paper by Tkachenko et al. (2022). The activity was expressed in units of SOD per mL.

Catalase activity assay

The activity of catalase (CAT, E.C. 1.11.1.6) was determined by measurement of the decrease in H_2O_2 in the reaction mixture, using a spectrophotometer at the wavelength of 410 nm and the method described by Koroliuk and co-workers (1988) and described in the paper by Tkachenko et al. (2022). One unit of catalase activity was defined as the amount of enzyme necessary to decompose 1 μmol H_2O_2 per min per mL.

Glutathione peroxidase activity assay

The activity of glutathione peroxidase (GPx, EC 1.11.1.9) was determined by detecting the nonenzymatic utilization of GSH (reacting substrate) at an absorbance of 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB), as proposed by Moin (1986) and described in the paper by Tkachenko et al. (2022). GPx activity is expressed as μmol GSH per min per mL.

Ceruloplasmin level assay

Ceruloplasmin (CP, E.C. 1.16.3.1) level in the plasma was measured spectrophotometrically at the wavelength of 540 nm as described by Ravin (1961) and described in the paper by Tkachenko et al. (2022). The assay mixture contained 0.1 mL of plasma, 5 mL of 0.4 M sodium acetate buffer (pH 5.5), and 0.1 mL of 0.5% *p*-phenilendiamine. The mixture was incubated at 37 °C for 60 min. Before cooling at 4 °C for 30 min, the mixture was added to 3% sodium fluoride for inhibition. Ceruloplasmin is expressed as milligrams per dL of plasma.

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 (significance level, $p < 0.05$) was examined using the Kruskal-Wallis test by ranks (Zar, 1999). All statistical calculations were performed on separate data from each individual with Statistica 13.3 software (TIBCO Software Inc., USA).

Results and discussion

The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and the total antioxidant capacity (TAC) in the equine erythrocytes after *in vitro* incubation with extracts derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv) in was assessed and shown in Figure 1.

As can be seen in Figure 1, treatment by extracts derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv) resulted in non-significant changes in the TBARS level of (33.65 ±1.86 nmol.mL^{-1}) and significantly increased TBARS levels to (41.69 ±3.81 nmol.mL^{-1}) compared to the untreated samples (35.88 ±3.02 nmol.mL^{-1}) (Figure 1). When equine erythrocytes were incubated with the extract derived from leaves of *F. sagittata* collected in NBG (Kyiv), the TBARS levels were significantly increased by 16.2% ($p < 0.05$) compared to the untreated samples.

The levels of aldehydic derivatives of oxidatively modified proteins were not changed after treatment by extracts derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv). When equine erythrocytes were incubated with the extracts derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv), the levels of ketonic derivatives (34.82 ±1.66 nmol. mL^{-1} and 35.67 ±1.82 nmol.mL^{-1}) were significantly decreased by 11.8% ($p < 0.05$) and 9.6% ($p < 0.05$) compared to the untreated samples (39.47 ±2.20 nmol.mL^{-1}). Additionally, a non-significantly increased TAC level was observed after incubation with an extract derived from leaves of *F. sagittata* collected in BG (Lviv) (by 3.1%, $p > 0.05$). The TAC levels were

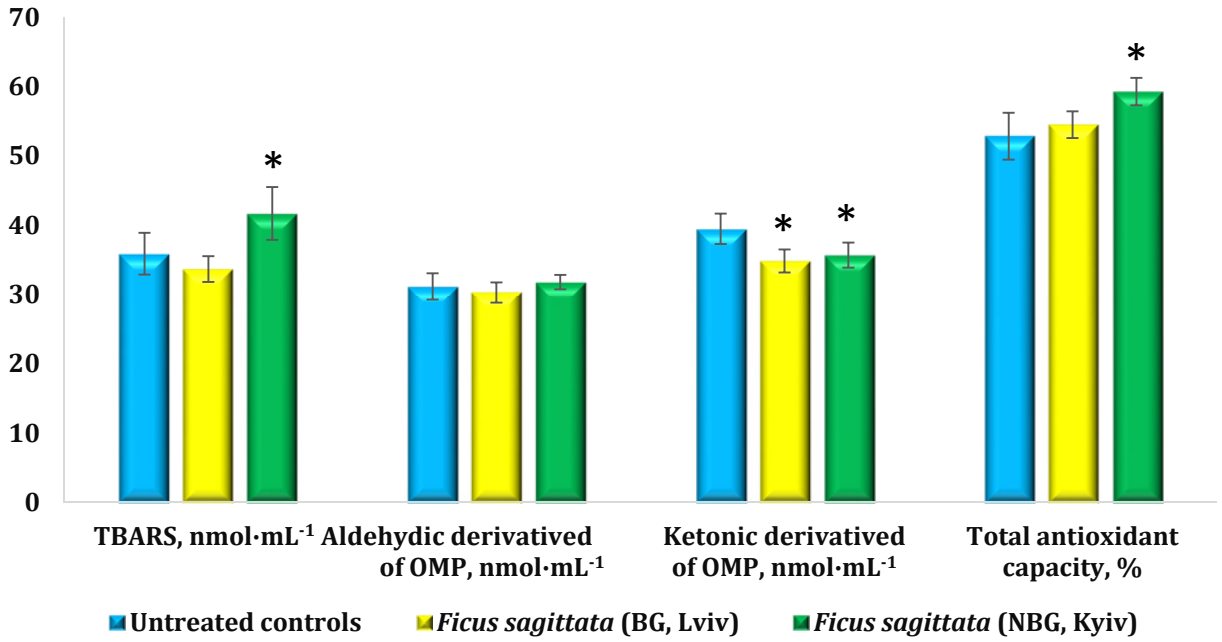

Figure 1 The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes after *in vitro* treatment by extracts derived from leaves of *Ficus sagittata* Vahl collected in BG (Lviv) or NBG (Kyiv) (M ±m, n = 18)
*– statistically significant differences between treated and untreated samples (p <0.05)

increased after treatment by extracts derived from leaves of *F. sagittata* collected in NBG (Kyiv) (by 12.2%, p <0.05) (Figure 1).

Activities of catalase and glutathione peroxidase, as well as ceruloplasmin levels in the equine plasma after *in vitro* incubation with extracts derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv) represented in Figure 2.

All cells have a complex antioxidant defense system, consisting of interacting low- and high-molecular

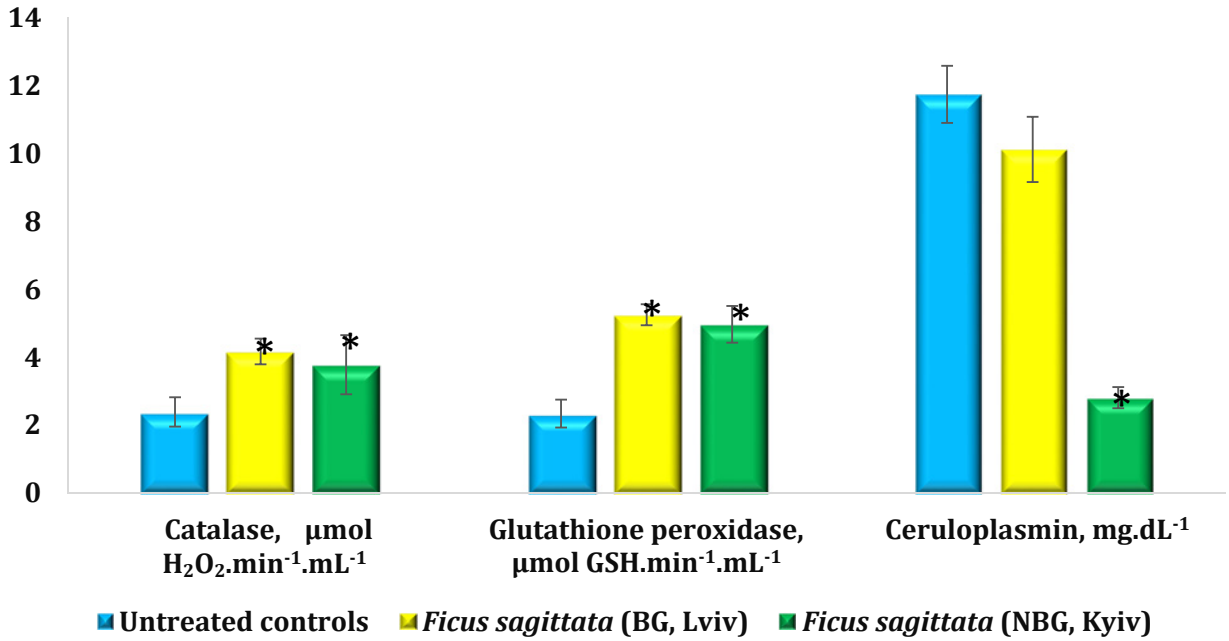

Figure 2 Activities of catalase and glutathione peroxidase, as well as ceruloplasmin level in the equine plasma after *in vitro* incubation with extracts derived from leaves of *Ficus sagittata* Vahl collected in BG (Lviv) or NBG (Kyiv) (M ± m, n = 18)
*– statistically significant differences between treated and untreated samples (p <0.05)

components. Among them, superoxide dismutase (SOD), glutathione peroxidases (GPx), and catalase (CAT) play a central role (Cerutti et al., 1994). In the current study, SOD activity was increased to (363.92 ±37.48 U.mL^{-1} and 365.31 ±37.78 U.mL^{-1}) in the equine plasma after *in vitro* incubation with extracts derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv) compared to the untreated samples (303.96 ±29.51 U.mL^{-1}). This was a 19.7% ($p > 0.05$) and 20.2% ($p > 0.05$) increase in SOD activity compared to the untreated samples. Catalase activity was non-significantly increased to values (4.17 ±0.38 μmol H_2O_2.min^{-1}.mL^{-1} and 3.78 ±0.87 μmol H_2O_2.min^{-1}.mL^{-1}) in the equine plasma after *in vitro* incubation with extracts derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv) compared to the untreated samples (2.38 ±0.43 μmol H_2O_2.min^{-1}.mL^{-1}). This was a 74.5% ($p < 0.05$) and 58.2% ($p < 0.05$) increase in CAT activity compared to the untreated samples (Figure 2).

Glutathione peroxidases are thiol-based enzymes that catalyze the reduction of H_2O_2 and hydroperoxides to H_2O or alcohols, they mitigate the toxicity of these compounds to the cell (Passaia and Margis-Pinheiro, 2015). Similarly to SOD and CAT activity, GPx activity was also increased to (5.25 ±0.31 μmol GSH.min^{-1}.mL^{-1} and 4.97 ±0.54 μmol GSH.min^{-1}.mL^{-1}) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv) compared to the untreated samples (2.34 ±0.41 μmol GSH.min^{-1}.mL^{-1}). This was a 124.4% ($p < 0.05$) and 112.4% ($p < 0.05$) increase in GPx activity compared to the untreated samples (Figure 2).

Ceruloplasmin (CP) is a copper-containing multifunctional oxidase of plasma, an antioxidant, an acute-phase protein, and a free radical scavenger (Samygina et al., 2017). It has been proposed to function in copper transport, oxidation of organic amines, iron(II) oxidation, and the regulation of cellular iron levels, catechols, radical scavenging, and other antioxidant processes (Healy and Tipton, 2007). In the current study, the CP level was decreased to (10.12 ±0.96 mg.dL^{-1} and 2.81 ±0.31 mg.dL^{-1}) in the equine plasma after *in vitro* incubation with extracts derived from leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv) compared to the untreated samples (11.74 ±0.84 mg.dL^{-1}). This was a 13.8% ($p > 0.05$) and 76.1% ($p < 0.05$) decrease in CP levels compared to the untreated samples (Figure 2).

In the current study, we used an *in vitro* model of equine plasma and erythrocytes to assess the antioxidant properties of an aqueous extract derived from the leaves of *F. sagittata* collected in BG (Lviv) or NBG (Kyiv). Many results also clearly suggest that treatment by herbal extracts *in vivo* and *in vitro* studies prevents organ damage through a decrease of lipid peroxidation and protection of the antioxidant defense system. On this basis, the current study was conducted to evaluate the antioxidant properties of an extract derived from the leaves of *F. sagittata*. The main finding of the current study was that this extract was able to decrease both aldehydic and ketonic derivatives of OMP, with a simultaneous increase in the activity of antioxidant enzymes (SOD, CAT, and GPx) in the equine erythrocytes and plasma after *in vitro* treatment.

Many studies *in vivo* revealed the antioxidant properties of extracts derived from different *Ficus* plants. For example, the study of Ahmed et al. (2013) evaluated the protective effects of sequential acetone extract of *Ficus racemosa* L. bark at two doses (FR250; 250 mg per kg and FR500; 500 mg per kg p.o.) against doxorubicin-induced renal and testicular toxicity in rats. Extract pretreatment (500 mg per kg) decreased TBARS and increased glutathione levels in the kidney and testis to control levels. These observations were substantiated by histopathological studies, wherein normal renal and testicular architecture was restored in FR500 group. Thus, administration of *F. racemosa* stem bark extract offers significant renal and testicular protection by inhibiting lipid peroxidation-mediated through scavenging free radicals (Ahmed et al., 2013). Also, *F. racemosa* extract was a potent chemopreventive agent and suppresses potassium bromate-induced nephrotoxicity in rats (Khan and Sultana, 2005). The results of Ahmed and Urooj (2010) indicate that *F. racemosa* possesses potent hepatoprotective effects against carbon tetrachloride-induced hepatic damage in albino rats. The acetone extract of *F. racemosa* bark possesses potential cardioprotective activity against doxorubicin-induced cardiotoxicity in rats by scavenging free radicals generated by the administration of the drug (Ahmed and Urooj, 2012).

Antidiabetic activity of *Ficus amplissima* Smith. bark extract in streptozotocin-induced diabetic rats was demonstrated by Arunachalam and Parimelazhagan (2013). Similarly, the antidiabetic effect of *Ficus religiosa* L. extract in streptozotocin-induced diabetic rats was revealed by Pandit et al. (2010). *F. religiosa* bark extract showed a significant anti-lipid peroxidative effect in the pancreas of streptozotocin-induced diabetic rats. The phenolic constituents of the aqueous-ethanolic extract of Tunisian *Ficus carica* L. fruit (FE) and its antihyperlipidemic and antioxidant

activities in high-fat diet-induced hyperlipidemic rats (HFD) were evaluated by Belguith-Hadriche et al. (2016). The FE has a significant hypocholesterolemic effect and antioxidant activity in HFD-fed rats. This beneficial effect may be partly due to phenolic constituents, especially vitexin, dihydroxybenzoic acid di-pentoside as well as rutin. The results indicate that properly dried figs can be used as a good source of phenolic compounds (Slatnar et al., 2011). Phytochemical studies on fruits and leaves of fig plants have explored that they are rich in phenolics, organic acids, and volatile compounds. Owing to the rich and diversified presence of biologically active compounds, they possess various biological activities such as antioxidant, anti-inflammatory, antibacterial, anticancer, hepatoprotective, antidiabetic, antifungal, antiviral, antimutagenic, antipyretic, antituberculosis, anti-angiogenic, antiparasitic, hematostatic, anticonstipation, and antiwarts activities (Hajam and Saleem, 2022).

Conclusions

In the current study, we investigated the changes in the levels of oxidative stress biomarkers and antioxidant defenses using the model of equine erythrocytes and plasma aimed to assess the antioxidant activities of the aqueous extract derived from the leaves of *Ficus sagittata* collected in BG (Lviv) or NBG (Kyiv). The treatment of equine plasma and erythrocytes by extracts derived from leaves of *F. sagittata* resulted in reduced carbonyl derivatives of the oxidatively modified protein. When equine erythrocytes were incubated with the extract derived from leaves of *F. sagittata* collected in NBG (Kyiv), the TBARS levels were significantly increased compared to the untreated samples. The incubation of equine plasma with an extract derived from leaves of *F. sagittata* resulted in an increase in the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase with a simultaneous decrease of ceruloplasmin level. The level of total antioxidant capacity was significantly increased after the treatment by extract derived from leaves of *F. sagittata* collected in NBG (Kyiv). However, further detailed investigation, especially *in vivo* and *in vitro* antioxidant studies is needed to justify the use of extract derived from leaves of *F. sagittata* as a natural source of antioxidants.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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