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In vitro antioxidant response of the equine blood treated by leaf extract of *Ficus drupacea* Thunb.

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The present study aimed to evaluate the antioxidant potential of the aqueous extract derived from the leaves of *Ficus drupacea* Thunb. using oxidative stress biomarkers (2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity) and antioxidant defences (activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase GPx), ceruloplasmin (CP)) on the model of equine erythrocytes and plasma after incubation *in vitro*. 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). The equine erythrocytes and plasma were used in the current study. A volume of 0.1 ml of the *F. drupacea* extract was added to 1.9 ml of equine erythrocytes or plasma. For positive control (blank), 0.1 ml of phosphate buffer was used. The treatment of equine plasma and erythrocytes by extract derived from leaves of *F. drupacea* resulted in reduced lipid peroxidation and oxidatively modified protein. Treatment by extract resulted in a reduced erythrocyte TBARS level of 21.9% ($p = 0.017$) compared to the untreated samples. The levels of aldehydic and ketonic derivatives of oxidatively modified proteins were non-significantly decreased. The incubation of equine plasma with an extract derived from leaves of *F. drupacea* increased antioxidant defences. The activity of SOD and GPx were increased by 41.6% ($p = 0.000$) and 61.5% ($p = 0.000$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples. The level of total antioxidant capacity was non-significantly increased. 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. drupacea* as a natural source of antioxidants.

Keywords: leaf extract, equine erythrocytes and plasma, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity

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Introduction

Ficus drupacea Thunb. (syn. *F. mysorensis*) is a monoecious evergreen tree growing to a height of up to 35 m, hemi-epiphytic or terrestrial, with glabrous to pale or rusty brown hairy leafy twigs, which naturally occurs in SE Asia to Australia and Solomon Islands. The leaves are 10–35 cm long and 4–16 cm wide, spirally arranged or subdistichous, coriaceous, elliptic to oblong or obovate with short-acuminate apex and cordate or rounded base. The lamina is glabrous to sparsely or densely brown tomentose or woolly mainly on the large veins. Figs are axillary, in pairs or solitary, sessile and ellipsoid, 2–3 cm in diameter and up to 4.0–4.5 cm long, glabrous, at maturity yellow to orange (Berg and Corner, 2005).

The leaves of *F. drupacea* are often used to treat malaria, paragonimiasis, nasosinusitis, sinusitis, andanasarca (Kiem et al., 2013). In the screening project carried out by Kiem et al. (2013) for α-glucosidase inhibition from natural sources, these researchers found *F. drupacea* to possess an α-glucosidase inhibitory effect with 39% at a concentration of 100 $\mu g.mL^{-1}$ (Kiem et al., 2013). Also, Manjuprasanna et al. (2021) tested 29 latices from the *Ficus* genus and revealed that *F. drupacea* exhibited potent pro-coagulant and thrombin-like activity. Drupin, a thrombin-like cysteine protease responsible for platelet aggregation was purified from *F. drupacea* latex. Drupin exhibits pro-coagulant activity and reduces the bleeding time in mice tails. It induces platelet aggregation by activating mitogen-activated protein kinases and the nuclear factor-κB and PI3K/Akt signaling cascade, which, in turn, phosphorylets, cytosolic phospholipase A2 leading to the release of thromboxane A2 from the granules to activate the nearby platelets to aggregate. The results of Manjuprasanna et al. (2021) confirmed that the drupin-induced platelet aggregation was mediated by both PAR1 and PAR4, synergistically. Overall, drupin reduces the bleeding time by exerting pro-coagulant activity and induces platelet aggregation by activating the intracellular signaling cascade (Manjuprasanna et al., 2021).

The study by Manjuprasanna et al. (2020) highlights the interference of drupin in wound healing by increased arginase 1 activity and collagen synthesis, and cell proliferation and migration. These authors revealed that cysteine protease is responsible for fibrinolysis purified from the *F. drupacea* latex named drupin, and tested for its wound healing efficacy. The accelerated wound healing was mediated by the downregulation of matrix metalloprotease (MMP)-9 without altering MMP-8 expression. Besides, drupin enhanced the rate of collagen synthesis at the wound site by increasing arginase 1 activity. And also, drupin increased the expression of arginase 1 in macrophages and was involved in cell proliferation and migration *via* MAP kinase and PI3K/Akt pathways (Manjuprasanna et al., 2020).

In our previous study (Tkachenko et al., 2018, 2019), we highlight 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 (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 was designed to investigate the oxidative stress biomarkers (2-thiobarbituric acid reactive substances, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity) and antioxidant defences (activity of superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin) using the model of equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from leaves of *Ficus drupacea*.

Material and methodology

Collection of plant materials

The leaves of *F. drupacea* were collected in M.M. Gryshko National Botanical Garden (Kyiv, Ukraine) and the Botanical Garden of Ivan Franko National University in Lviv (Lviv, Ukraine) in September 2016 (Figure 1). The whole collection of tropical and subtropical plants at these institutions (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. All biochemical assays were conducted at the Department of Biology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk (Poland).

Horses

Eighteen clinically healthy adult horses from the central Pomeranian region in Poland (village Strzelinko, N 54º 30’ 48.0” E 16º 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 haematological, 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 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 or plasma (at the final dose of an extract of 5 mg per mL). For positive control, 0.1 ml of phosphate buffer added to erythrocytes or plasma 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 (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 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a coloured adduct that is measured spectrophotometrically. The nmol of per 1 ml was calculated using $1.56 \cdot 10^5$ mM^{-1}. cm^{-1} as the extinction coefficient.

The carbonyl derivatives of oxidative modification of proteins (OMP) assay

To evaluate the protective effects of the extract 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 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). DNFH was used

Figure 1 General view of *Ficus drupacea* Thunb. specimen (A, C) and leaves of *F. drupacea* (B, D).
Photo: Yevhen Sosnovsky

for determining carbonyl derivatives 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 (TAC)

The 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 (Galaktionova et al., 1998). 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 concerning 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). 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). 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). 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). 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*-phenylenediamine. 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 between the total antioxidant capacity level (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test (Zar, 1999). In addition, the relationships between oxidative stress biomarkers were evaluated using Spearman's correlation analysis. All statistical calculations were performed on separate data from each individual with Statistica 13.3 software (TIBCO Software Inc., Krakow, Poland).

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 an extract derived from leaves of *F.drupacea* was assessed and shown in Figure 2.

Lipid peroxidation by reactive oxygen species (ROS) is known to be involved in the damaging mechanism of cell disorders. The most prominent and currently used assay as an index for lipid peroxidation products is the 2-thiobarbituric acid assay (TBARS test). It is based on the reactivity of an end product of lipid peroxidation, malonic dialdehyde (MDA) with 2-thiobarbituric acid to produce a red adduct (Garcia et al., 2005). As can be seen in Figure 2, treatment by extract resulted in a reduced erythrocyte TBARS level of (28.04 ±2.43 $nmol.mL^{-1}$) compared to the untreated samples (35.88 ±3.02 $nmol.mL^{-1}$). The decrease in TBARS level was by 21.9% ($p = 0.017$) (Figure 2).

Proteins are major targets for oxidation reactions (Kehm et al., 2021). As proteins are highly abundant in cells, extracellular tissues, and body fluids and react rapidly with many oxidants, they are highly susceptible to and are major targets of, oxidative damage (Hawkins and Davies, 2019). This can result in changes to protein structure, function, and turnover and loss or occasional gain of activity (Hawkins and Davies, 2019). Moreover, oxidative stress can degrade lipids and carbohydrates into highly reactive intermediates, which eventually attack proteins at various functional sites (Kehm et al., 2021). The levels of aldehydic and ketonic derivatives

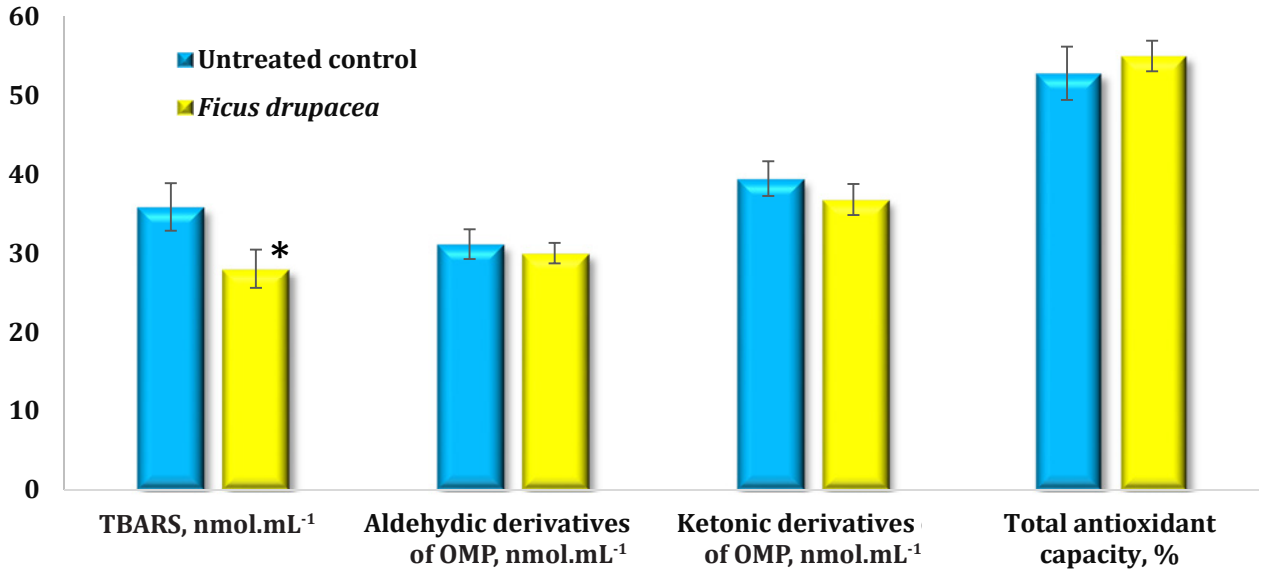

Figure 2 The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes after *in vitro* incubation with extract derived from leaves of *Ficus drupacea* Thunb. (M ±m, n = 18)
*– statistically significant differences between treated and untreated samples ($p < 0.05$)

of oxidatively modified proteins were also decreased in samples treated with an extract derived from leaves of *F. drupacea* compared to the untreated samples, but these decreases were statistically non-significant ($p > 0.05$). When equine erythrocytes were incubated with the extract derived from leaves of *F. drupacea*, the levels of aldehydic and ketonic derivatives were non-significantly decreased by 3.7% and 6.7% ($p > 0.05$). Also, a non-significantly increased TAC level was observed after incubation with an extract derived from leaves of *F. drupacea* (by 4.2%, $p > 0.05$) (Figure 2).

The activity of catalase, glutathione peroxidase, and ceruloplasmin level in the equine plasma after *in vitro* incubation with extract derived from leaves of *F. drupacea* was resented in Figure 3.

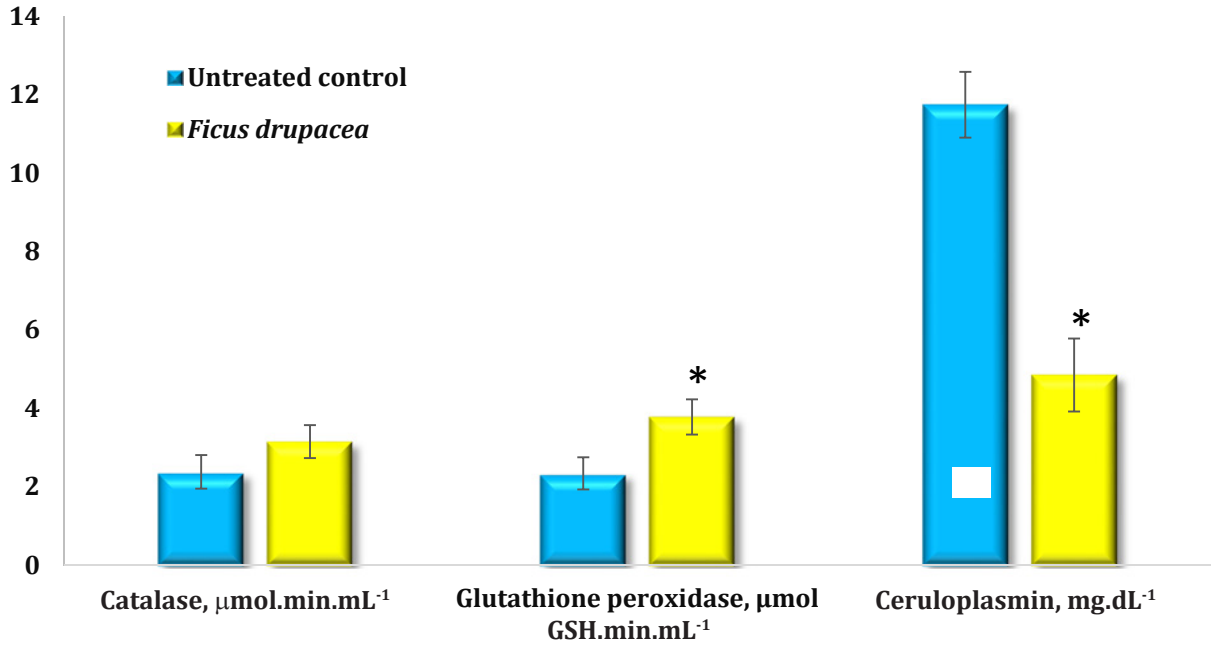

Figure 3 Activity of catalase, glutathione peroxidase, and ceruloplasmin level in the equine plasma after *in vitro* incubation with extract derived from leaves of *Ficus drupacea* Thunb. (M ±m, n = 18)
*– statistically significant differences between treated and untreated samples ($p < 0.05$)

Numerous short-lived and highly reactive oxygen species (ROS) such as superoxide ($O_{2\bullet}^{-}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) are continuously generated *in vivo* (Miao and St Clair, 2009). Superoxide dismutases (SODs), including copper-zinc superoxide dismutase (Cu, Zn-SOD), manganese superoxide dismutase(Mn-SOD), and extracellular (Ec-SOD) superoxide dismutase, play a crucial role in scavenging $O_{2\bullet}^{-}$ (Miao and St Clair, 2009). In the current study, SOD activity was increased to (430.39 ±34.15 $U.mL^{-1}$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples (303.96 ±29.51 $U.mL^{-1}$). This was a 41.6% (p = 0.000) increase in SOD activity compared to the untreated samples.

Catalases are well-studied enzymes that play critical roles in protecting cells against the toxic effects of hydrogen peroxide (Goyal and Basa, 2010). Catalase activity was no-significantly increased to value (3.15 ±0.42 μmol H_2O_2.min $.mL^{-1}$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples (2.38 ±0.43 μmol H_2O_2.$min.mL^{-1}$). This was a 32.4% (p >0.05) increase in CAT activity compared to the untreated samples (Figure 3).

Glutathione peroxidases (EC 1.11.1.9 and EC 1.11.1.12) catalyze the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols using reduced glutathione. Some glutathione peroxidase isozymes have a selenium-dependent glutathione peroxidase activity and present selenocysteine (Margis et al., 2008). Similarly to SOD and CAT activity, GPx activity was also increased to (3.78 ±0.45 μmol GSH.$min.mL^{-1}$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples (2.34 ±0.41 μmol GSH.min $.mL^{-1}$). This was a 61.5% (p = 0.000) increase in GPx activity compared to the untreated samples.

Ceruloplasmin (CP) is a serum ferroxidase that contains greater than 95% of the copper found in plasma. CP is a member of the multicopper oxidase family, an evolutionarily conserved group of proteins that utilize copper to couple substrate oxidation with the four-electron reduction of oxygen to water (Hellman and Gitlin, 2002). 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, CP level was decreased to (4.85 ±0.93 $mg.dL^{-1}$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples (11.74 ±0.84 $mg.dL^{-1}$). This was a 58.7% (p = 0.000) decrease in CP level compared to the untreated samples.

In the present 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. drupacea* leaves. 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 defence system. On this basis, the current study was conducted to evaluate the antioxidant properties of an extract derived from the leaves of *F. drupacea*. The main finding of the current study was that this extract was able to decrease both lipid peroxidation and protein damage, with a simultaneous increase in the activity of antioxidant enzymes (SOD, CAT, and GPx) in the equine erythrocytes and plasma after *in vitro* incubation.

The efficacy of *F. drupacea* on other *in vitro* and *in vivo* models was also investigated by other researchers. The efficacy of *Ficus* spp. on renal injury induced by hypercholesterolaemia was revealed by Awad et al. (2012a). The ethanol and hexane extracts of *F. microcarpa* L.f., *F. religiosa* L. and *F. mysorensis* B.Heyne ex Roth leaves were evaluated against renal injury induced by hypercholesterolaemia. For the *in vivo* study, all rats were orally given cholesterol (30 $mg.kg^{-1}$ body weight, BW) and leaf extract (500 $mg.kg^{-1}$ BW) five times per week for 9 weeks. Hypercholesterolaemic rats showed significant increases in urea nitrogen and creatinine while serum protein and albumin levels, nitric oxide (NO), Na^+, K^+-ATPase and phospholipids in kidney tissue were all decreased. Treatment with leaves extracts improved kidney function indices (urea nitrogen, creatinine, serum protein and albumin), kidney disorder biochemical parameters (NO, Na^+, K^+-ATPase and phospholipids), haematological profile (haemoglobin, RBCs and WBCs) and kidney histopathology. These researchers demonstrated that using *Ficus* spp. resulted in improving renal injury induced by hypercholesterolaemia, with the most potent effects seen while using *F. microcarpa* hexane extract (Awad et al., 2012a). Also, these researchers (Awad et al., 2012b) screened some *Ficus* and *Morus* spp. for hypolipidaemic and antioxidant activities and *in vivo* assessment of *F. mysorensis* and revealed that *F. mysorensis* demonstrated hypolipidaemic and antioxidant effects.

In an *in vitro* study, the ethanolic and hexane extracts of the investigated plants were evaluated against

hyperlipidaemia by estimating the rate-limiting enzyme of cholesterol biosynthesis; β-hydroxy-β-methylglutaryl coenzyme A reductase (HMG-CoA reductase). The antioxidant activity was evaluated by the reduction of DPPH(-) free radicals. Extra phytochemical screening of *Ficus* extracts was undertaken, which recorded potent hypolipidaemic and antioxidant activities. The more pronounced extract, *F. mysorensis* (hexane extract), was evaluated *in vivo* by estimation of the lipid profile and certain antioxidant parameters in hypercholesterolemic rats. The hexane fraction was chromatographed and six isolated compounds were identified (Awad et al., 2012b).

Yessoufou et al. (2015) investigated the antimicrobial (fungi and bacteria) and antiproliferative activities of crude extracts of the *F. drupacea* stem bark and isolated compounds from *F. drupacea*. Stem bark extracts of *F. drupacea* and the isolated compounds represent potential antibacterial and antifungal resources against a wide spectrum of microbes. Seven biochemical compounds from stem bark extracts including β-amyrin (1), β-sitosterol-3-O-β-D-glucopyranoside (2), 5-O-methyllatifolin (3), oleanolic acid (4), epifriedelanol (5), friedelin (6) and epilupeol acetate (7) were isolated and identified. Of all the seven compounds, compounds 3 and 7 exhibited the highest antifungal and antibacterial activities against screened microorganisms (Yessoufou et al., 2015).

Thus, in the current study, we have undertaken an attempt to investigate the *in vitro* antioxidant activity of an extract derived from the leaves of *F. drupacea* plants. The results obtained suggested that antioxidant compounds are dominant contributors to the antioxidant activity of the extract derived from the leaves of *F. drupacea* plants. Our future phytochemical screening of leaves also will reveal the presence of various classes of secondary metabolites which have great importance in medicinal chemistry and natural product research for their high antioxidant properties.

Conclusions

In the current study, we investigated the changes in the oxidative stress biomarkers and antioxidant defences using the model of equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from the leaves of *F. drupacea*. The treatment of equine erythrocytes by extract derived from leaves of *F. drupacea* resulted in reduced lipid peroxidation and oxidatively modified protein. The levels of aldehydic and ketonic derivatives of oxidatively modified proteins were non-significantly decreased. The incubation of equine plasma with an extract derived from leaves of *F. drupacea* increased antioxidant defences. The level of total antioxidant capacity was non-significantly increased. 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. drupacea* as a natural source of antioxidants.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

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

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References

Awad, N.E., Hamed, M.A., Seida, A.A., & Elbatanony, M.M. 2012a. Efficacy of *Ficus* spp. on renal injury induced by hypercholesterolaemia. In *Natural Product Research*, *26*(16), 1561–1564. https://doi.org/10.1080/14786419.2011.570760

Awad, N.E., Seida, A.A., Hamed, M.A., Mahmoud, A.H., & Elbatanony, M.M. 2012b. Phytochemical and *in vitro* screening of some *Ficus* and *Morus* spp. for hypolipidaemic and antioxidant activities and *in vivo* assessment of *Ficus mysorensis* (Roth). In *Natural Product Research*, *26*(12), 1101–1111. https://doi.org/10.1080/14786419.2010.545353

Berg, C.C., & Corner, E.J.H. 2005. Moraceae (*Ficus*). In: Noteboom, H.P. (ed.) *Flora Malesiana*, Ser. 1, Vol. 17, Part 2. National Herbarium Nederland, Leiden, pp. 1–730.

Dubinina, E.E., Burmistrov, S.O., Khodov, D.A., & Porotov, I.G. 1995. Oxidative modification of human serum proteins. A method of determining it. In *Voprosy Meditsinskoĭ Khimii*, *41*, 24–26. [In Russian]

Galaktionova, L.P., Molchanov, A.V., El'chaninova, S.A., & Varshavskiĭ, B. 1998. Lipid peroxidation in patients with gastric and duodenal ulcers. In *Klinicheskaia Labaratornaia Diagnostika*, *6*, 10–14. [In Russian]

Garcia, Y.J., Rodríguez-Malaver, A.J., & Peñaloza, N. 2005. Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. In *Journal of Neuroscience Methods*, *144*(1), 127–135.

https://doi.org/10.1016/j.jneumeth.2004.10.018

Goyal, M.M., & Basak, A. 2010. Human catalase: looking for complete identity. In *Protein Cell*, *1*(10), 888–897. https://doi.org/10.1007/s13238-010-0113-z

Hawkins, C.L., & Davies, M.J. 2019. Detection, identification, and quantification of oxidative protein modifications. In *Journal of Biological Chemistry*, *294*(51), 19683–19708. https://doi.org/10.1074/jbc.REV119.006217

Healy, J., & Tipton, K. 2007. Ceruloplasmin and what it might do. In *Journal of Neural Transmission* (*Vienna*), *114*(6), 777–781. https://doi.org/10.1007/s00702-007-0687-7

Hellman N.E., & Gitlin J.D. Ceruloplasmin metabolism and function. In *Annual Review of Nutrition*, *22*, 439–458. https://doi.org/10.1146/annurev.nutr.22.012502.114457

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

Kehm, R., Baldensperger, T., Raupbach, J., & Höhn, A. 2021. Protein oxidation – Formation mechanisms, detection and relevance as biomarkers in human diseases. In *Redox Biology*, *42*, 101901. https://doi.org/10.1016/j.redox.2021.101901

Kiem, P.V., Minh, C.V., Nhiem, N.X., Yen, P.H., Anh, H.L.T., Cuong, N.X., Tai, B.H., Quang, T.H., Hai, T.N., Kim, S.H., Kwon, S., Lee, Y., & Kim, Y.H. 2013. Chemical constituents of *Ficus drupacea*e leaves and their α-glucosidase inhibitory activities. In *Bulletin of the Korean Chemical Society*, *34*(1), 263–266. https://doi.org/10.5012/BKCS.2013.34.1.263

Koroliuk, M.A., Ivanova, L.I., Maĭorova, I.G., & Tokarev, V.E. 1988. Metod opredeleniia aktivnosti katalazy [A method of determining catalase activity]. In *Laboratornoe Delo*, (1), 16–19. [In Russian]

Kostiuk, V.A., Potapovich, A.I., & Kovaleva, Zh.V. 1990. Prostoĭ i chuvstvitel'nyĭ metod opredeleniia aktivnosti superoksid- dismutazy, osnovannyĭ na reaktsii okisleniia kvertsetina [A simple and sensitive method of determination of superoxide dismutase activity based on the reaction of quercetin oxidation]. In *Voprosy meditsinskoi khimii*, *36*(2), 88–91. [In Russian]

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

Manjuprasanna, V.N., Rudresha, G.V., Urs, A.P., Milan Gowda, M.D., Rajaiah, R., & Vishwanath, B.S. 2020. Drupin, a cysteine protease from *Ficus drupacea* latex accelerates excision wound healing in mice. In *International Journal of Biological Macromolecules*, *165*(Pt. A), 691–700. https://doi.org/10.1016/j.ijbiomac.2020.09.215

Manjuprasanna, V.N., Urs, A.P., Rudresha, G.V., Milan Gowda, M.D., Jayachandra, K., Hiremath, V., Rajaiah, R., & Vishwanath, B.S. 2021. Drupin, a thrombin-like protease prompts platelet activation and aggregation through protease-activated receptors. In *Journal of Cellular Biochemistry*, *122*(8), 870–881. https://doi.org/10.1002/jcb.29917

Margis, R., Dunand, C., Teixeira, F.K., & Margis-Pinheiro, M. 2008. Glutathione peroxidase family – an evolutionary overview. In *FEBS Journal*, *275*(15), 3959–3970. https://doi.org/10.1111/j.1742-4658.2008.06542.x

Miao, L., & St Clair, D.K. 2009. Regulation of superoxide dismutase genes: implications in disease. In *Free Radical Biology and Medicine*, *47*(4), 344–356. https://doi.org/10.1016/j.freeradbiomed.2009.05.018

Moin, V.M. 1986. Prostoĭ i spetsificheskiĭ metod opredeleniia aktivnosti glutationperoksidazy v éritrotsitakh [A simple and specific method for determining glutathione peroxidase activity in erythrocytes]. In *Laboratornoe Delo*, (12), 724–727. [In Russian]

Ravin, H.A. 1961. An improved colorimetric enzymatic assay of ceruloplasmin. In *Journal of Laboratory and Clinical Medicine*, *58*, 161–168.

Tkachenko, H., Buyun, L., Kurhaluk, N., Honcharenko, V., Prokopiv, A., & Osadowski, Z. 2019. *In Vitro* Antioxidant Activities of Aqueous Extracts Derived from Leaves of Juvenile and Mature Shoots of *Ficus pumila* L. (Moraceae). In *Agrobiodiversity for Improving Nutrition, Health, and Life Quality, 3*, 1–13. https://doi.org/10.15414/agrobiodiversity.2019.2585-8246.001-013

Tkachenko, H., Buyun, L., Osadowski, Z., Honcharenko, V., & Prokopiv, A. 2018. Oxidative stress biomarkers in the equine plasma and erythrocytes treated *in vitro* by leaf extract obtained from *Ficus religiosa* L. (Moraceae). In *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, *2*, 184–200. https://doi.org/10.15414/agrobiodiversity.2018.2585-8246.184-200

Yessoufou, K., Elansary, H.O., Mahmoud, E.A., & Skalicka-Woźniak, K. 2015. Antifungal, antibacterial and anticancer activities of *Ficus drupacea* L. stem bark extract and biologically active isolated compounds. In *Industrial Crops and Products*, 74, 752–758. http://dx.doi.org/10.1016/j.indcrop.2015.06.0110926-6690

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