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IN VITRO ANTIOXIDANT RESPONSE OF THE EQUINE BLOOD TREATED BY EXTRACT DERIVED FROM LEAVES OF *FICUS DELTOIDEA* JACK (MORACEAE)

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Ficus deltoidea Jack, which has strong antioxidant properties, is widely consumed in traditional medicine as a treatment for various diseases. 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 (SOD), catalase (CAT), glutathione peroxidase (GPx), ceruloplasmin (CP)) in the equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from leaves of Ficus deltoidea. The leaves of F. deltoidea were collected at 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 extract was added to 1.9 mL of clean equine erythrocytes or plasma (the final concentration of the extract was 5 mg/mL). For positive control, 0.1 mL of phosphate buffer (pH 7.4) was used. The treatment of equine plasma and erythrocytes by extract derived from leaves of F. deltoidea resulted in reduced carbonyl derivatives of the oxidatively modified protein. The levels of lipid peroxidation were non-significantly changed. The incubation of equine plasma with an extract derived from leaves of F. deltoidea 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 non-significantly increased. The results obtained suggested the antioxidant activity of the extract derived from the leaves of F. deltoidea plants exhibiting a decrease in the biomarkers of oxidative stress and enhancement of antioxidant defenses in the equine erythrocytes and plasma after in vitro treatment with the extract. 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. deltoidea as a natural source of antioxidants.

Keywords: *Ficus deltoidea Jack, lipid peroxidation, oxidatively modified proteins, superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin, total antioxidant capacity.*

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IN VITRO АНТИОКСИДАНТНА ВІДПОВІДЬ КРОВІ КОНЕЙ, ОБРОБЛЕНОЇ ЕКСТРАКТОМ З ЛИСТЯ *FICUS DELTOIDEA* JACK (MORACEAE)

Ficus deltoidea Jack, який володіє потужними антиоксидантними властивостями, широко використовується в традиційній медицині як засіб для лікування різних захворювань. Представлене дослідження мало на меті дослідити біомаркери окислювального стресу, а саме продукти перекисного окиснення 2-тіобарбітурової кислоти (TBARS), альдегідні та кетонові похідні окислювально модифікованих білків, загальну антиоксидантну здатність, а також антиоксидантний захист (активність супероксиддисмутази (SOD) , каталази (CAT), глутатіонпероксидази (GPx), церулоплазміну (CP)) в еритроцитах і плазмі коней для оцінки антиоксидантної активності водного екстракту, отриманого з листя F. deltoidea. Листки F. deltoidea зібрані у Національному ботанічному саду імені М.М. Гришка (Київ, Україна) та Ботанічному саду Львівського національного університету імені Івана Франка (Львів, Україна). Свіжозібране листя промивали, зважували, подрібнювали та гомогенізували в 0,1 М фосфатному буфері (pH 7,4) (у співвідношенні 1:19) при кімнатній температурі. Потім екстракти фільтрували та використовували для аналізу. Об'єм 0,1 мл рослинного екстракту додавали до 1,9 мл чистих еритроцитів або плазми коней (кінцева концентрація екстракту становила 5 мг/мл). Для позитивного контролю використовували 0,1 мл фосфатного буфера (pH 7,4). Обробка плазми та еритроцитів коней екстрактом, отриманим з листя F. deltoidea, призвела до відновлення карбонільних похідних окисно модифікованого білка. Рівні перекисного окиснення ліпідів були змінені незначно. Інкубація плазми коней з екстрактом, отриманим з листя F. deltoidea, призвела до підвищення активності антиоксидантних ферментів, таких як супероксиддисмутаза, каталаза та глутатіонпероксидаза, з одночасним зниженням рівня церулоплазміну. Рівень загальної антиоксидантної здатності підвищився незначно.

Отримані результати свідчать про антиоксидантну активність екстракту, отриманого з листя рослин F. deltoidea, що демонструє зниження рівня біомаркерів окислювального стресу та посилення антиоксидантного захисту в еритроцитах і плазмі коней після обробки екстрактом in vitro. Однак необхідні подальші детальні дослідження, особливо дослідження антиоксидантів in vivo та in vitro, щоб обґрунтувати використання екстракту, отриманого з листя F. deltoidea, як природного джерела антиоксидантів.

Ключові слова: *Ficus deltoidea Jack, перекисне окислення ліпідів, окислювально модифіковані білки, супероксиддисмутаза, каталаза, глутатіонпероксидаза, церулоплазмін, загальна антиоксидантна здатність.*

INTRODUCTION

The pantropical genus *Ficus* L., with its approximately 750 species, is the largest within the family and one of the most speciose genera of flowering plants. Among all Moraceae, it is characterized by the presence of waxy glands on vegetative organs, heterostyly, and prolonged protogyny, which is the anthesis of staminate flowers in already mature fruits. These features are functionally linked to the unique pollination mode in *Ficus* involving mutualistic relationships with agaonid wasps (order Hymenoptera). The closed urceolate inflorescences provide a shelter for the development of wasps, which, in turn, are the only pollinators of these plants ensuring their reproductive propagation [5, 9]. *Ficus* trees have a number of uses in various industries and fields of human activity. Virtually all parts of their body are utilized in ethnomedicine to cure disorders of the digestive and respiratory systems, skin diseases, parasitic infections, etc. Some species have been cited to have analgesic, tonic, and ecbolic effects [19].

The review paper of Ashraf and co-workers [4] provided information about the botanical description of the *Ficus deltoidea* Jack plant. *F. deltoidea* is an evergreen shrub or tiny tree that usually starts as an epiphyte in the form of a bush with aerial roots. It generally grows up to 22 feet, roughly 15–22 feet high and 3–10 feet wide in the fashion of zigzagging branches. The bark and trunk are usually gray and slender, respectively. The leaves are extensively spoon-formed, which are 1.5–3 inches (4–8 cm) long and intense. The leaves are dark, leathery, and succulent. The plant generated spherical to round figs that are roughly 1.5 cm in length, whereby during maturation, the coloring of figs turns from dull yellow to orange and purple that can be manufactured freely in pairs [4].

Modern pharmacological studies demonstrated that this plant has a wide variety of beneficial attributes for human health. *F. deltoidea* has been reported to have beneficial pharmaceutical uses as an antidiabetic, anti-inflammatory, antinociceptive, anti-melanogenic, antiphotoaging, antioxidant, antiulcerogenic, and antibacterial agent [6]. Many findings give scientific evidence for the traditional use of *F. deltoidea* as a chemopreventive and antidiabetic agent. For example, Ooi and co-workers [29] highlighted that *F. deltoidea* may provide a chemopreventive effect on mutagenic and oxidative stress inducers. The study by Mohd Dom and co-workers [25] aimed to evaluate the potential of standardized methanolic extracts from seven *F. deltoidea* varieties in inhibiting the formation of Advanced glycation end products (AGEs), protein oxidation, and their antioxidant effects. This study revealed that seven varieties of *F. deltoidea* have the potential to inhibit AGEs formation and possess antioxidant activity that might be attributed to the presence of phenolic compounds [25]. Moreover, *F. deltoidea* could prevent diabetic osteoporosis by enhancing osteogenesis and inhibiting bone oxidative stress [32].

F. deltoidea leaf extract mitigated postmenopausal osteoarthritic joint destruction by inhibiting inflammation and cartilage degradation enzymes, at an effective extract dose equivalent to about 60 mg/kg for humans. The main bioactive compounds are probably the antioxidative flavonoids vitexin and isovitexin [8]. Also, these bioactive compounds abundantly found in the leaves of *F. deltoidea* possess many pharmacological properties including neuroprotection. Zolkiffly and co-workers [38] determined the inhibitory properties of *F. deltoidea* aqueous extract on pro-inflammatory mediators involved in lipopolysaccharide (LPS)-induced microglial cells and revealed that this extract showed neuroprotective effects by attenuating the levels of pro-inflammatory and cytotoxic factors in LPS-induced microglial cells, possibly by mediating the nuclear factor-kappa B (NF-κB) signalling pathway [38].

The antioxidant property of the *F. deltoidea* extract was revealed through a total phenolic content and ferric reducing antioxidant potential (FRAP) assay by Omar and co-workers [28]. It was found that flavan-3-ol monomers and proanthocyanidins contributed 85% of the antioxidant activity of the aqueous extract of *F. deltoidea* [28]. Hakiman and Maziah [13] compared the polyphenol, phenolic acid, flavonoid, and compounds as nonenzymatic antioxidants, whereas ascorbate oxidase, peroxidase, catalase, and ascorbate peroxidase were presented as enzymatic antioxidants in *F. deltoidea* leaf extract. Interestingly, female leaves of *F. deltoidea* species gave higher antioxidant activity, and higher flavonoid, and phenolic content as compared to male leaves [3, 21].

In our previous study [33, 34], we highlight the antioxidant potential of an aqueous extract derived from leaves of other *Ficus* species using an equine erythrocyte suspension. In the study [34], we have focused on the antioxidant effect of 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 [34].

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 [33]. 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 resulted in an increase in the total antioxidant capacity of equine erythrocytes [33].

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 (SOD), catalase (CAT), glutathione peroxidase (GPx), ceruloplasmin (CP)] in the equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from leaves of *Ficus deltoidea*. The current study is a continuation of our cooperation with M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine (Kyiv, Ukraine) and Botanic Garden of Ivan Franko National University in Lviv (Lviv, Ukraine) concerning investigations of the antibacterial and antioxidant properties of extracts derived from leaves of some tropical and subtropical plants. The authors would like to acknowledge the International Visegrad Fund for providing support for this research.

MATERIALS AND METHODOLOGY

Collection of Plant Materials. The leaves of *F. deltoidea* were collected at M.M. Gryshko National Botanic Garden (Kyiv, Ukraine) and the Botanic Garden of Ivan Franko National University in Lviv (Lviv, Ukraine). The whole collection of tropical and subtropical plants at M.M. Gryshko National Botanic Garden (Kyiv, Ukraine) and Botanic Garden of Ivan Franko National University in Lviv (Lviv, Ukraine) (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 0.1M 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. All extracts were stored at -25°C until use.

Horses. Eighteen clinically healthy adult horses from the central Pomeranian region in Poland (village Strzelinko, N54º30’48.0” E16º57’44.9”), 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 was added to 1.9 mL of clean equine erythrocytes or plasma (the final concentration of the extract was 5 mg/mL). 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 (TBARS) assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov [15] 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 colored adduct that is measured spectrophotometrically. The μmol of per 1 L was calculated using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as the extinction coefficient.

The carbonyl derivatives of oxidatively modified proteins (OMP) 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. [20] and as modified by Dubinina et al. [10]. 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 (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 [11]. 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 with respect to 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 and co-workers [18]. 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 [17]. 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 [26]. 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 [30]. 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 Mann-Whitney *U* test [37]. 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. deltoidea* was assessed and shown in Figure 2.

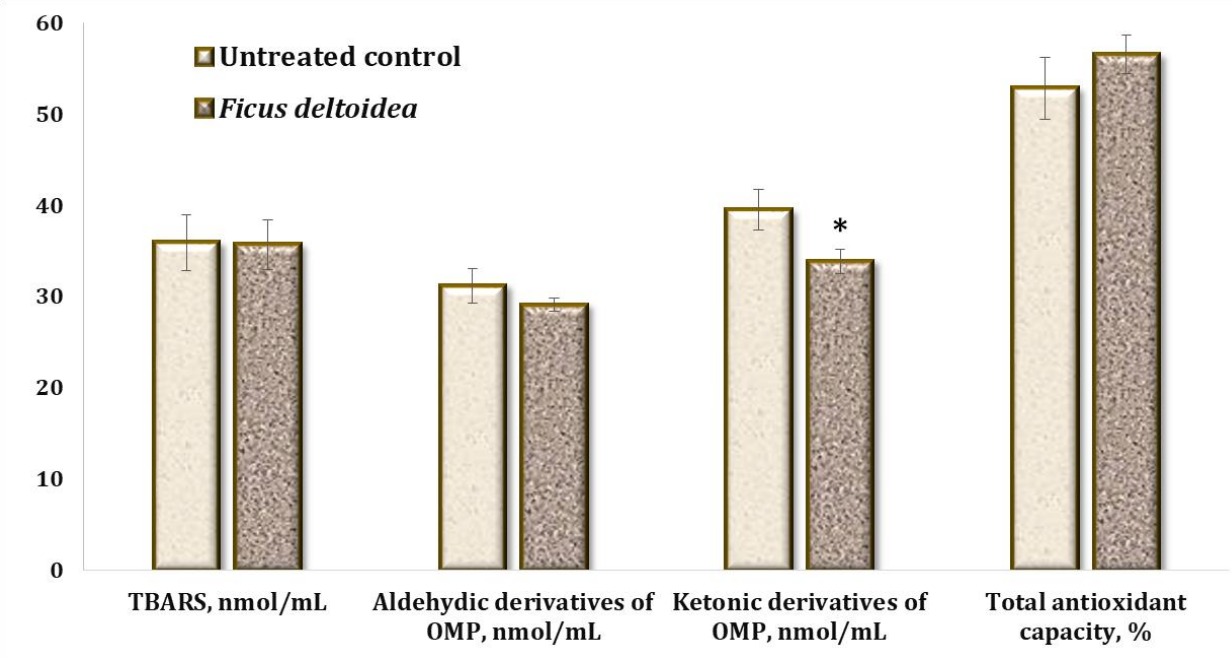

Fig. 1. The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes after *in vitro* treatment by extract derived from leaves of *Ficus deltoidea* (M ± m, n = 18)

*– statistically significant differences between treated and untreated samples ($p < 0.05$).

The most prominent and currently used assay as an index for lipid peroxidation products is the 2-thiobarbituric acid reactive substances (TBARS) assay. 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 [12]. As can be seen in Fig. 1, treatment by extract derived from leaves of *F. deltoidea* resulted in non-significant changes in the TBARS level of (35.66 ± 2.75 nmol/mL) compared to the untreated samples (35.88 ± 3.02 nmol/mL) (Fig. 1).

Measurement of protein oxidation products permits the degree of oxidative stress to be assessed and indicates that endogenous antioxidant defenses are overwhelmed [16]. The levels of aldehydic derivatives of oxidatively modified proteins were decreased in samples treated with an extract derived from leaves of *F. deltoidea* (29.05 ± 0.72 nmol/mL) compared to the untreated samples (31.16 ± 1.89 nmol/mL), but these decreases were statistically non-significant ($p > 0.05$). When equine erythrocytes were incubated with the extract derived from leaves of *F. deltoidea*, the levels of ketonic derivatives (33.82 ± 1.38 nmol/mL) were significantly decreased by 14.3% ($p <$

0.05) compared to the untreated samples (39.47 ± 2.20 nmol/mL). Additionally, a non-significantly increased TAC level was observed after incubation with an extract derived from leaves of *F. deltoidea* (by 6.95%, p > 0.05) (Fig. 1).

Activities of catalase and glutathione peroxidase, as well as ceruloplasmin level in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. deltoidea* represented in Fig. 2.

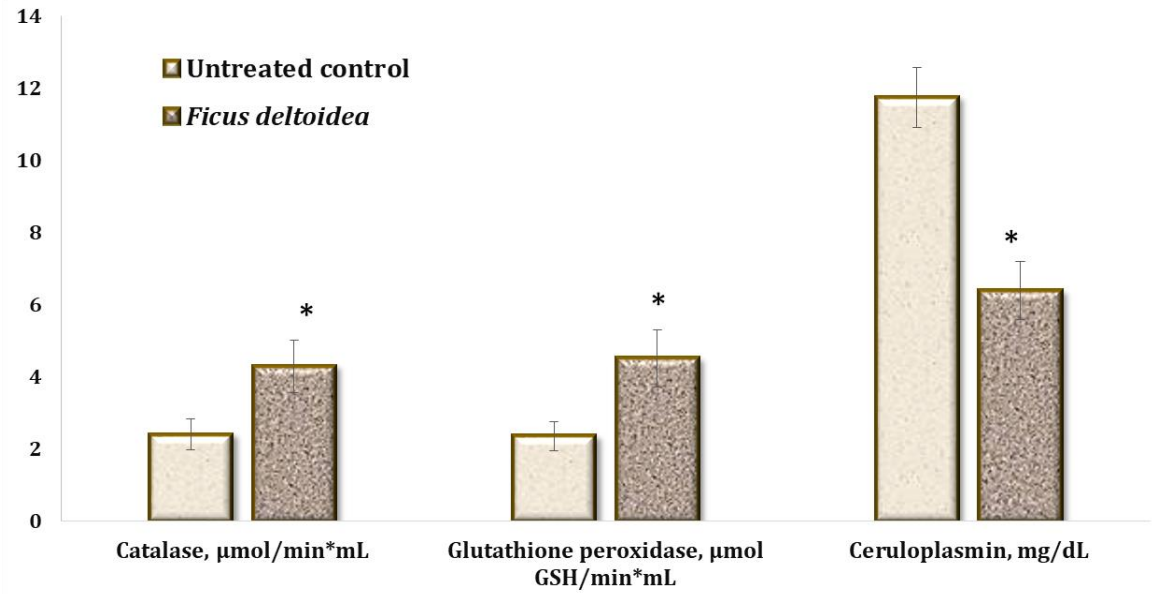

Fig. 2. Activities of catalase and glutathione peroxidase, as well as ceruloplasmin level in the equine plasma after *in vitro* incubation with extract derived from leaves of *Ficus deltoidea* (M ± m, n = 18).

*– statistically significant differences between treated and untreated samples (p < 0.05).

Antioxidant enzymes such as superoxide dismutase (SOD) play a key role in diminishing oxidative stress [7]. SOD catalyzes the conversion of the superoxide anion free radical ($^{\bullet}O_2^-$) to hydrogen peroxide (H_2O_2) and molecular oxygen O_2. Subsequently, H_2O_2 is reduced to water by the catalase (CAT) enzyme [31]. H_2O_2 may also generate another reactive oxygen species (ROS), the hydroxide ion ($^{\bullet}HO$) *via* the Fenton reaction in the presence of Fe^{2+} [31]. In the current study, SOD activity was increased to (456.62 ± 30.59 U/mL) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. deltoidea* compared to the untreated samples (303.96 ± 29.51 U/mL). This was a 50.2% (p = 0.000) increase in SOD activity compared to the untreated samples. Catalase activity was no-significantly increased to value (4.28 ± 0.72 μmol H_2O_2/min*mL) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. deltoidea* compared to the untreated samples (2.38 ± 0.43 μmol H_2O_2/min*mL). This was a 79.8% (p = 0.000) increase in CAT activity compared to the untreated samples (Fig. 2).

The glutathione peroxidases (GPx) are enzymes that are part of the cellular antioxidant system inhibiting the ROS-induced damage of membranes and proteins [22]. Similarly to SOD and CAT activity, GPx activity was also increased to (4.50 ± 0.80 μmol GSH/min*mL) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. deltoidea* compared to the untreated samples (2.34 ± 0.41 μmol GSH/min*mL). This was a 92.3% (p = 0.000) increase in GPx activity compared to the untreated samples (Fig. 2).

Ceruloplasmin (CP) is a copper-containing ferroxidase that functions as an antioxidant in part by oxidizing toxic ferrous iron to nontoxic ferric iron [35]. 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 [14]. In the current study, CP level was decreased to (6.38 ± 0.81 mg/dL) in the equine plasma after *in vitro* incubation with an

extract derived from leaves of *F. deltoidea* compared to the untreated samples (11.74 ± 0.84 mg/dL). This was a 45.7% ($p = 0.000$) decrease in CP level compared to the untreated samples (Fig. 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. deltoidea*. 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. deltoidea*. 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.

The different properties of *F. deltoidea* on other *in vitro* and *in vivo* models was also investigated by other researchers. For example, the antidiabetic and antioxidant activities of the fruits from different varieties of *F. deltoidea*, employing *in vitro* methods were investigated by Misbah and co-workers [23]. Two fruit varieties of *F. deltoidea* [var. *angustifolia* (SF) and var. *kunstleri* (BF)] were extracted separately using double-distilled water. The resulting aqueous extracts were partitioned using ethyl acetate to obtain the ethyl acetate and water fractions. The crude aqueous extracts and the corresponding fractions were evaluated for their phenolic, flavonoid, sugar, and protein contents. The crude extracts and fractions of SF and BF inhibited both yeast and rat intestinal α-glucosidases in a dose-dependent manner but did not inhibit porcine pancreatic α-amylase. The water fraction of BF showed the highest percentage of α-glucosidase inhibition while having the highest amount of protein. All the extracts and fractions exhibited antioxidant activities, with SF crude extract showing the highest antioxidant activity and phenolic content. Fractionation of the crude extracts resulted in the loss of antioxidant activities. There was no positive correlation between phenolic and flavonoid content with α-glucosidase inhibitory activities. However, phenolic content correlated well with the antioxidant activities of the crude extracts but not with the fractions. The antioxidant activities of the fruits of *F. deltoidea* might be asserted by the phenolic content but other polar plant components were possibly involved in the antidiabetic properties [23].

A study by Abrahim and co-workers [1] showed that *F. deltoidea* varieties have excellent antioxidant activity with no cytotoxic effects on normal liver cells. Leaves from three varieties of *F. deltoidea*, colloquially termed small- (FDS), medium- (FDM), and big-type leaf (FDB), were subjected to water extraction. The crude extracts were fractionated using water (WF) and ethyl acetate (EAF). The phenolic and flavonoid content, antioxidant activity, and cytotoxicity of the fractions were investigated. The EAF had the highest phenolic and flavonoid content compared to the other FDS fractions. Conversely, the FDM crude extract had the highest phenolic and flavonoid content compared to the other FDM samples. Antioxidant activity was highest in the FDB crude extract. Ultra-high-performance liquid chromatography showed that two compounds, vitexin, and coumaric acid, were present in the FDB crude extract. Additionally, the *F. deltoidea* leaves caused no signs of toxicity in a normal liver cell line [1].

Mohd Ariff and co-workers [24] have investigated the effects of four *F. deltoidea* varieties on endothelial activation, inflammation, monocytes binding to endothelial cells, and oxidative stress in human endothelial cells, as well as to identify possible underlying mechanisms in mediating those effects. Human coronary artery endothelial cells (HCAEC) were incubated with different concentrations of aqueous ethanolic extracts of *F. deltoidea* var. *trengganuensis* (FDT), var. *kunstleri* (FDK), var. *deltoidea* (FDD) and var. *intermedia* (FDI), together with LPS. This study demonstrated that *F. deltoidea* extracts of all four species up to 40 μg/ml did not exhibit any effects on the viability of HCAEC. All *F. deltoidea* varieties suppressed endothelial activation, inflammation, monocyte binding activity, and oxidative stress in stimulated HCAEC; with FDK, followed by FDT exhibiting the most potent effects. The anti-atherogenic actions were mediated *via* the nuclear factor-κB (NF-κB) and endothelial nitric oxide synthase (eNOS) pathways. These

findings suggest that *F. deltoidea* has anti-atherogenic properties and is a starting point for determining its potential use in humans as a novel, natural atheroprotective agent [24].

In the study of Ooi and co-workers [29], the mutagenic and antimutagenic activities of *F. deltoidea* aqueous extract (FDD) on both Salmonella typhimurium TA 98 and TA 100 strains were assessed using *Salmonella* mutagenicity assay (Ames test). Then, the cytoprotective potential of FDD on menadione-induced oxidative stress was determined in a V79 mouse lung fibroblast cell line. The ferric-reducing antioxidant power (FRAP) assay was conducted to evaluate FDD antioxidant capacity. Results showed that FDD (up to 50 mg/mL) did not exhibit a mutagenic effect on either TA 98 or TA 100 strains. Notably, FDD decreased the revertant colony count induced by 2-aminoanthracene in both strains in the presence of metabolic activation. Additionally, pretreatment of FDD (50 and 100 μg/mL) demonstrated remarkable protection against menadione-induced oxidative stress in V79 cells significantly by decreasing superoxide anion level. FDD at all concentrations tested (12.5-100 μg/mL) exhibited antioxidant power, suggesting the cytoprotective effect of FDD could be partly attributed to its antioxidant properties [29].

This medicinal plant is also beneficial as a daily dietary supplement for the maintenance of female reproductive health. This was supported by the study of Zaid and co-workers [36], in which the potential protective roles of *F. deltoidea* against bisphenol A (BPA)-induced toxicity of the pituitary-ovarian axis in pre-pubertal female rats was evaluated. The findings showed that *F. deltoidea* demonstrated a preventive role against BPA-induced toxicity in the ovaries. This was evident by the increased percentage of rats with normal estrous cycle, qualitatively reduced number of atretic follicles (as observed in histopathological examination), and normalization of the gonadotropins hormone (FSH) and sex steroid hormone (progesterone) levels. In conclusion, *F. deltoidea* has the capability to prevent the effects of BPA toxicity in the hypothalamus-pituitary-gonadal axis of the prepubertal female reproductive system, possibly due to its variety of phytochemical properties [36].

It is suggested that *F. deltoidea* has the potential to be developed as a future oral antidiabetic agent. Adam and co-workers [2] evaluated the mechanisms that underlie the antihyperglycemic action of *F. deltoidea*. The results had shown that a hot aqueous extract of *F. deltoidea* stimulated insulin secretion significantly with the highest magnitude of stimulation being 7.31-fold ($P < 0.001$). The insulin secretory actions of the hot aqueous extract involved K_{ATP} channel-dependent and K_{ATP}-channel-independent pathways. The extract also has the ability to induce the usage of intracellular Ca^{2+} to trigger insulin release. The ethanolic and methanolic extracts enhanced basal and insulin-mediated glucose uptake into adipocyte cells. The extracts possess either insulin-mimetic or insulin-sensitizing properties or a combination of both properties during enhancing glucose uptake into such cells. Meanwhile, the hot aqueous and methanolic extracts augmented basal and insulin-stimulated adiponectin secretion from adipocyte cells [2]. *F. deltoidea* and vitexin increased pancreatic antioxidant enzymes and promoted islet regeneration. However, a significant increase in insulin secretion was observed only in rats treated with *F. deltoidea*. These results accentuate that *F. deltoidea* and vitexin could be potential agents to attenuate pancreatic oxidative damage and advocate their therapeutic potential for treating DM [27].

Thus, in the current study, we have attempted to investigate the in vitro antioxidant activity of an extract derived from the leaves of *F. deltoidea* plants. The results obtained suggested the antioxidant activity of the extract derived from the leaves of *F. deltoidea* plants exhibiting a decrease in the biomarkers of oxidative stress and enhancement of antioxidant defenses in the equine erythrocytes and plasma after *in vitro* treatment with the extract. 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 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 *F. deltoidea*. The treatment of equine plasma and erythrocytes by extract derived from leaves of *F. deltoidea* resulted in reduced carbonyl derivatives of the oxidatively modified protein. The levels of lipid peroxidation were non-significantly changed. The incubation of equine plasma with an extract derived from leaves of *F. deltoidea* 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 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. deltoidea* as a natural source of antioxidants.

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