UDC 611.018.51:615.451.1

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IN VITRO PROTECTIVE EFFECT OF LEAF EXTRACT OF *FICUS DELTOIDEA* JACK (*MORACEAE*) ON BIOMARKERS OF OXIDATIVE STRESS IN THE HUMAN ERYTHROCYTES

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ЗАХИСНИЙ ЕФЕКТ *IN VITRO* ЕКСТРАКТУ, ОТРИМАНОГО З ЛИСТЯ *FICUS DELTOIDEA* JACK (*MORACEAE*), НА БІОМАРКЕРИ ОКИСНЮВАЛЬНОГО СТРЕСУ В ЕРИТРОЦИТАХ ЛЮДИНИ

DOI: 10.58407/bht.3.22.7

ABSTRACT

Purpose: to investigate the antioxidant properties of the aqueous extracts derived from leaves of *Ficus deltoidea* using the model of human blood. For this purpose, oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), content of aldehydic and ketonic derivatives of oxidatively modified proteins, total antioxidant capacity (TAC)] in the human erythrocytes after *in vitro* incubation with aqueous extracts derived from leaves of *F. deltoidea* (at a final concentration of 5 mg/mL and 0.5 mg/mL) were used. Resistance of human erythrocytes after *in vitro* treatment by aqueous extracts derived from leaves of *F. deltoidea* (at a final concentration of 5 mg/mL and 0.5 mg/mL) was evaluated by HCl-induced hemolysis using a percentage of hemolyzed erythrocytes per each 30 sec. and a total time of hemolysis.

Methodology. The leaves of *F. deltoidea* were collected in M.M. Gryshko National Botanic Garden (Kyiv, Ukraine). Blood (10-20 mL) was obtained from normal volunteers via venipuncture (4 males and 5 females aged 28-53 years old). An erythrocyte suspension at 1% hematocrit was incubated with 4 mM phosphate buffer (pH 7.4) (control) and pre-incubated with the extract of *F. deltoidea* (at a final concentration of 5 mg/mL and 0.5 mg/mL, respectively) at 37 °C for 60 min. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS). 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). The total antioxidant capacity (TAC) in the samples was estimated by measuring the TBARS level after Tween 80 oxidation. The HCl-induced resistance of erythrocytes was measured spectrophotometrically with 0.1M HCl. The significance of differences (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test.

Scientific novelty. The treatment of human erythrocytes by extract derived from leaves of *F. deltoidea* at the final concentration of 5 mg/mL resulted in an increase of TBARS as biomarkers of lipid peroxidation and aldehydic derivatives of oxidatively modified proteins with a simultaneous decrease in the total antioxidant capacity compared to the untreated samples. On the other hand, *in vitro* treatment of human erythrocytes with an extract derived from leaves of *F. deltoidea* at the final concentration of 0.5 mg/mL resulted in the same values of TBARS level, aldehydic and ketonic derivatives of OMP, and total antioxidant capacity as the untreated samples. Extract of *F. deltoidea* at the final concentrations of 5 mg/mL and 0.5 mg/mL possess hemolytic properties to human erythrocyte suspension after 1-h incubation *in vitro*.

Conclusions. The changes in the oxidative stress biomarkers using the *in vitro* model of human erythrocytes to evaluate the antioxidant activities of the aqueous extract derived from the leaves of *F. deltoidea* at the two final concentrations (5 mg/mL and 0.5 mg/mL) revealed that high concentration of extract (5 mg/mL) resulted in the increase of lipid peroxidation and protein oxidation with a simultaneous decline in the total antioxidant capacity. HCl-induced hemolysis was activated after the treatment by extract derived from the leaves of *F. deltoidea* at the two final concentrations. More studies are warranted in the future, to illustrate the potential and mechanisms of *F. deltoidea* in preventing oxidative stress using different cell models *in vitro* and different final concentrations of the extract. Also, further studies are warranted to identify the bioactive components that contribute to this protective effect.

Key words: *Ficus deltoidea* Jack, human erythrocytes, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity, hemolysis.

АНОТАЦІЯ

Мета: дослідити антиоксидантні властивості водних екстрактів, отриманих із листя *Ficus deltoidea*, на моделі крові людини. Для цього досліджували біомаркери окиснювального стресу [реактивні речовини, які реагують з 2-тіобарбітуровою кислотою (ТБК-продукти), вміст альдегідних і кетонових похідних окиснювально модифікованих білків, загальна антиоксидантна активність (ЗАА)] в еритроцитах людини після інкубації *in vitro* з водними екстрактами, отриманими з листя *F. deltoidea* (у кінцевій концентрації 5 та 0,5 мг/мл). Стійкість еритроцитів після обробки *in vitro* водними екстрактами, отриманими з листя *F. deltoidea* (у кінцевій концентрації 5 та 0,5 мг/мл), оцінювали шляхом HCl-індукованого гемолізу, використовуючи відсоток гемолізованих еритроцитів на кожні 30 сек. гемолізу і загальний час гемолізу.

Методологія. Листки *F. deltoidea* були зібрані в Національному ботанічному саду імені М.М. Гришка (Київ, Україна). Кров (10-20 мл) була отримана від здорових добровольців шляхом венепункції (4 чоловіків і 5 жінок у віці 28-53 років). Суспензію еритроцитів при 1 % гематокриту інкубували з 4 мМ фосфатним буфером (pH 7,4) (контроль) і інкубували з екстрактом *F. deltoidea* (у кінцевій концентрації 5 і 0,5 мг/мл відповідно) при 37 °C впродовж 60 хв. Рівень перекисного окиснення ліпідів оцінювали за кількісним визначенням концентрації ТБК-продуктів. Швидкість окисної деструкції білка оцінювали за реакцією утворених карбонільних похідних амінокислот з 2,4-динітрофенілгідразином. Загальну антиоксидантну активність (ЗАА) у зразках оцінювали шляхом вимірювання рівня ТБК-продуктів після окиснення Tween-80. Стійкість еритроцитів до HCl вимірювали спектрофотометрично з 0,1 М HCl. Достовірність різниць (рівень значущості, $p < 0,05$) досліджували за *U*-критерієм Манна-Уітні.

Наукова новизна. Обробка еритроцитів людини екстрактом, отриманим із листя *F. deltoidea*, у кінцевій концентрації 5 мг/мл призвела до підвищення ТБК-продуктів як біомаркерів перекисного окиснення ліпідів та альдегідних похідних окиснювально модифікованих білків з одночасним зниженням загальної антиоксидантної активності порівняно з необробленими зразками. З іншого боку, обробка *in vitro* еритроцитів людини екстрактом, отриманим з листя *F. deltoidea*, у кінцевій концентрації 0,5 мг/мл призвела до подібних значень рівня ТБК-продуктів, альдегідних і кетонових похідних окиснювально модифікованих білків, а також загальної антиоксидантної активності як і у необроблених зразках. Екстракт *F. deltoidea* в кінцевих концентраціях 5 і 0,5 мг/мл проявляє гемолітичні властивості щодо суспензії еритроцитів людини після 1-годинної інкубації *in vitro*.

Висновки. Зміни біомаркерів окиснювального стресу з використанням моделі еритроцитів людини *in vitro* для оцінки антиоксидантної активності водного екстракту, отриманого з листя *F. deltoidea* у двох кінцевих концентраціях (5 та 0,5 мг/мл) показали, що вища концентрація екстракту (5 мг/мл) призводила до посилення перекисного окиснення ліпідів і окиснення білків з одночасним зниженням загальної антиоксидантної активності. Показано також активацію HCl-індукованого гемолізу після обробки екстрактом, отриманим з листя *F. deltoidei*, у двох кінцевих концентраціях. У майбутньому необхідні додаткові дослідження, щоб проілюструвати потенціал і механізми дії екстракту *F. deltoidea* у запобіганні окиснювальному стресу з використанням різних клітинних моделей *in vitro* та різних кінцевих концентрацій екстракту. Крім того, необхідні подальші дослідження для виявлення біоактивних компонентів, які сприяють цьому захисному ефекту.

Ключові слова: *Ficus deltoidea* Jack, еритроцити людини, перекисне окиснення ліпідів, окиснювально модифіковані білки, загальна антиоксидантна активність, гемоліз.

Introduction

The angiosperm family *Moraceae* represents morphologically quite diverse plant groups, including terrestrial and hemi-epiphytic trees, shrubs, lianas, subshrubs, and herbs. Its distribution range lies mostly within the tropics and subtropics with several taxa extending to the temperate zone [5; 10; 12]. Among 37 genera of *Moraceae* comprising 1050-1100 species in total, *Ficus* L. is the largest one with ca. 750 species of tropical and subtropical distribution worldwide. Its characteristic features include the presence of waxy glands on vegetative plant parts, heterostyly, and prolonged protogyny, i.e., the anthesis of staminate flowers in already mature fruits. These features are functionally linked to the unique pollination mode in *Ficus*, which involves mutualistic relationships with agaonid wasps (order *Hymenoptera*) and has attracted much research interest over the last two centuries [6; 11].

Ficus deltoidea Jack (*Moraceae*) is an evergreen shrub or a small tree that is widely distributed in Southeast Asian countries such as Thailand, Sumatra, Java, Kalimantan, Sulawesi, and Moluccas [28]. It is a well-known medicinal plant used in customary medication to reduce and mend sicknesses such as ulcers, psoriasis, cytotoxicity, cardioprotective, inflammation, jaundice, vitiligo, hemorrhage, diabetes, convulsion, hepatitis, dysentery injuries, wounds, and stiffness [4]. *F. deltoidea* contains a wide variety of bioactive compounds from different phytochemical groups such as alkaloids, phenols, flavonoids, saponins, sterols, terpenes, carbohydrates, and proteins [4; 8; 25]. The methanolic extract of *F. deltoidea* leaves has been reported to be rich in tannins, alkaloids, saponins, phenols, flavones,

isoflavones, and flavonoids [16]. The antioxidant property of the *F. deltoidea* extract was revealed through a total phenolic content and ferric reducing antioxidant potential (FRAP) assay by M.H. Omar and co-workers [25]. It was found that flavan-3-ol monomers and proanthocyanidins contributed 85 % of the antioxidant activity of the aqueous extract of *F. deltoidea* [8].

In our previous studies, we assessed *in vitro* antioxidant activities of aqueous extracts derived from leaves of juvenile and mature shoots of *Ficus pumila L.* [32] and leaf extract of *Ficus drupacea* Thunb. using equine blood as a biological model for cytotoxic studies [31]. The current study was designed to investigate the antioxidant properties of the aqueous extracts derived from leaves of *F. deltoidea* using the model of human blood. Erythrocytes are well equipped to degrade reactive oxygen species *via* the actions of superoxide dismutase that converts O_2 into H_2O_2, which is further catabolized by catalase and glutathione peroxidase [20]. As hemolysis is the cytotoxicity indicator, hemolytic assays are performed to check the hemolytic effect of compounds before establishing their pharmacological preparations [29]. In the current study, erythrocytes are used as a model system for studying oxidative damage induced by HCl and its pathophysiology.

Thus, the purpose of our study was to evaluate the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), content of aldehydic and ketonic derivatives of oxidatively modified proteins, total antioxidant capacity (TAC)] in the human erythrocytes after *in vitro* incubation with aqueous extracts derived from leaves of *F. deltoidea* (at a final concentration of 5 mg/mL and 0.5 mg/mL). Resistance of human erythrocytes after *in vitro* treatment by aqueous extracts derived from leaves of *F. deltoidea* (at a final concentration of 5 mg/mL and 0.5 mg/mL) was evaluated by HCl-induced hemolysis using a percentage of hemolyzed erythrocytes per each 30 sec. and a total time of hemolysis.

Material and methods

Collection of Plant Materials and Preparation of Plant Extracts. The leaves of *F. deltoidea* were collected in M.M. Gryshko National Botanic Garden (Kyiv, Ukraine). The whole collection of tropical and subtropical plants at M.M. Gryshko National Botanic Garden (Kyiv, 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. 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.

Collection of human blood samples. Blood (10-20 mL) was obtained from normal volunteers *via* venipuncture (4 males and 5 females aged 28-53 years old). The Research Ethics Committee of the Regional Medical Commission in Gdańsk (Poland) approved the study (KB-31/18). All patients provided written informed consent before the start of the study procedures. Human erythrocytes from citrated blood were isolated by centrifugation at 3,000 rpm for 10 min and washed two times with 4 mM phosphate buffer (pH 7.4) and then re-suspended using the same buffer to the desired hematocrit level. Cells stored at 4 °C were used within 6 h of sample preparation. An erythrocyte suspension at 1% hematocrit was incubated with 4 mM phosphate buffer (pH 7.4) (control) and pre-incubated with the extract of *F. deltoidea* (at a final concentration of 5 mg/mL and 0.5 mg/mL, respectively) at 37 °C for 60 min. This reaction mixture was shaken gently while being incubated for a fixed interval at 37 °C. Erythrocyte 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 V.S. Kamyshnikov [18] 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 nmol of per 1 mL was calculated using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as an extinction coefficient.

The carbonyl derivatives of protein oxidative modification (OMP) assay. To evaluate the protective effects of the extract against free radical-induced protein damage in erhytrocytes, carbonyl derivatives of oxidative modification of proteins (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives 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 R.L. Levine and co-workers [19] and as modified by Dubinina and co-workers [13]. 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 the 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 according to Galaktionova et al. [14]. 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.

Assay of HCl-induced Resistance of Erythrocytes. The HCl-induced resistance of erythrocytes was measured spectrophotometrically with 0.1M HCl [30]. The assay is based on measuring the dynamics of erythrocyte disintegration into hemolytic reagent action. The time of hemolytic reagent action serves as the measure of erythrocyte resistance. The assay mixture contained 5 mL of 1% erythrocyte suspension and 0.05 mL of 0.1M HCl. The absorbance was read at 540 nm every 30 seconds after HCl addition till the end of hemolysis. The difference in absorbance at the beginning and at the end of hemolysis was determined as 100% (total hemolysis). The disintegration of erythrocytes (%) at every 30 seconds was expressed as a curve.

Statistical analysis. The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test [37]. All statistical calculations were performed on separate data with Statistica v. 13.3 software (TIBCO Software Inc., Krakow, Poland).

Results and discussion

The data on TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins (OMP), and total antioxidant capacity (TAC) in the human erythrocytes after *in vitro* incubation with leaf extract of *F. deltoidea* at the final concentrations 5 mg/mL and 0.5 mg/mL were presented in Fig. 1.

In vitro incubation of human erythrocytes with an extract derived from leaves of *F. deltoidea* at the final concentration of 5 mg/mL resulted in a significant increase in TBARS level of (37.97 ± 1.76 nmol/mL) compared to the untreated samples (23.38 ± 1.08 nmol/mL). The increase in TBARS level was by 62.4% ($p < 0.05$) (Fig. 1A). On the other hand, *in vitro* incubation of human erythrocytes with an extract derived from leaves of *F. deltoidea* at the final concentration of 0.5 mg/mL resulted in the same values of TBARS level as untreated samples (26.24 ± 1.22 nmol/mL *vs.* 25.65 ± 1.19 nmol/mL) (Fig. 1B).

The levels of aldehydic and ketonic derivatives of oxidatively modified proteins were also changed in erythrocyte samples treated with an extract derived from leaves of *F. deltoidea* compared to the untreated samples. When erythrocytes were incubated with the extract derived from leaves of *F. deltoidea* at the final concentration of 5 mg/mL, the levels of aldehydic derivatives were statistically significantly increased by 21.2% ($p < 0.05$), i.e. (25.35 ± 0.94 nmol/mL) *vs.* (20.92 ± 0.97 nmol/mL) (Fig. 1A). On the other hand, when erythrocytes were incubated with the extract derived from leaves of *F. deltoidea* at the final concentrations 0.5 mg/mL, the levels of aldehydic derivatives were at the same level (19.70 ± 0.91 nmol/mL) as untreated samples (18.41 ± 0.85 nmol/mL). Levels of ketonic derivatives of oxidatively modified proteins were at the same level after treatment by extract derived from leaves of *F. deltoidea* both at the final concentrations 5 mg/mL and 0.5 mg/mL, (26.46 ± 1.23 nmol/mL) *vs.* (25.92 ± 1.20 nmol/mL) for extract at the final concentrations 5 mg/mL and (23.03 ± 1.07 nmol/mL) *vs.* (23.86 ± 1.11 nmol/mL) for extract at the final concentrations 0.5 mg/mL (Fig. 1).

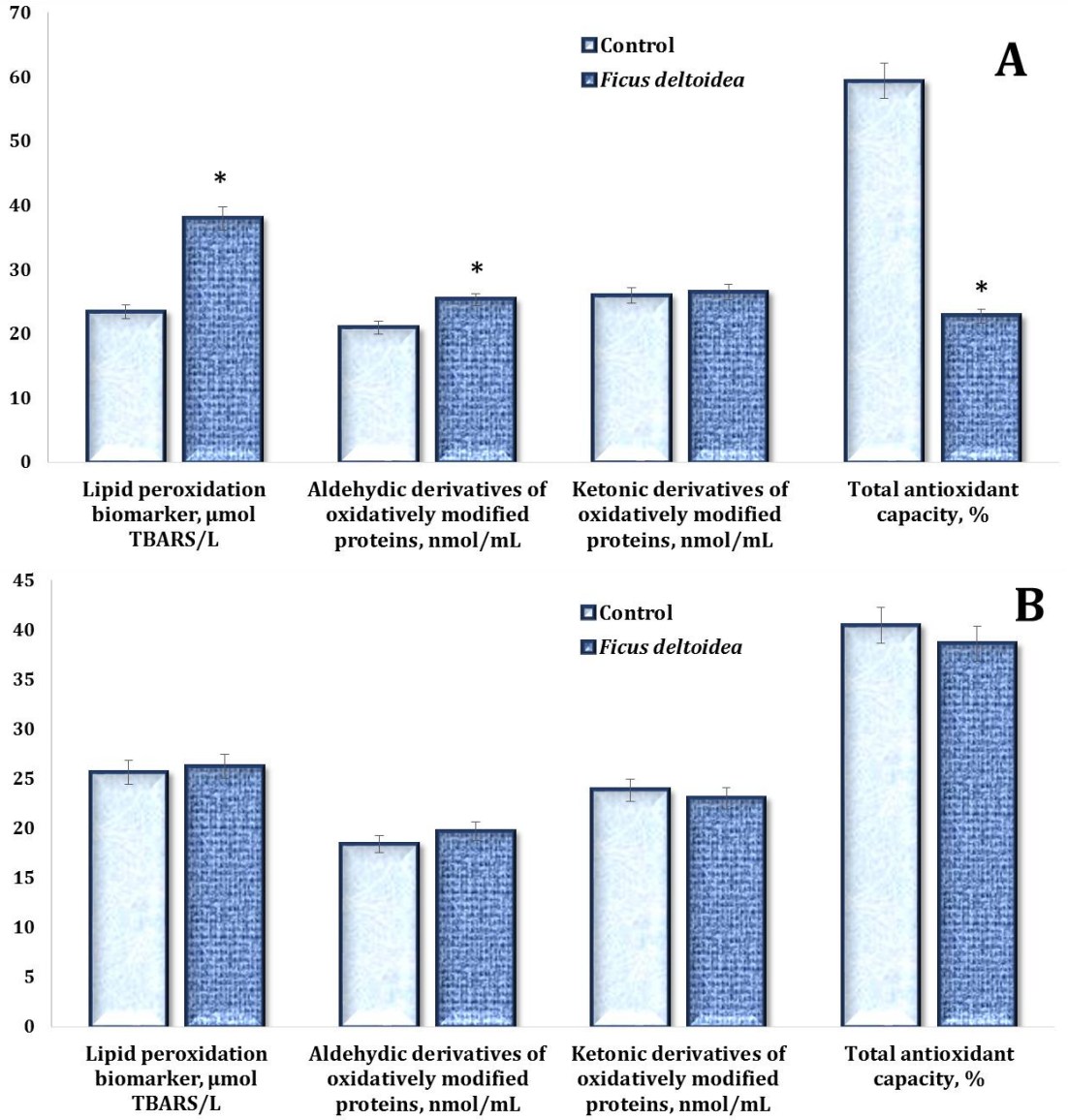

Fig. 1. The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins (OMP), and total antioxidant capacity (TAC) in the human erythrocytes after *in vitro* incubation with leaf extract of *F. deltoidea* at the final concentrations 5 mg/mL (A) and 0.5 mg/mL (B).
*** – changes were statistically significant ($p < 0.05$) compared to the untreated samples (n = 9)**

Also, a non-significantly decreased TAC level was observed after incubation with an extract derived from leaves of *F. deltoidea* at the final concentration of 0.5 mg/mL (by 4.6%, $p > 0.05$). On the other hand, when erythrocytes were incubated with the extract derived from leaves of *F. deltoidea* at the final concentration of 5 mg/mL, the levels of TAC were statistically significantly decreased by 61.6% ($p < 0.05$), i.e. (22.78 ± 1.06 nmol/mL) *vs.* (59.34 ± 2.75 nmol/mL) (Fig. 1A).

Thus, aqueous extracts derived from leaves of *F. deltoidea* (at the final concentration of 5 mg/mL) after incubation *in vitro* with the human erythrocytes exhibited prooxidant properties. On the other hand, when erythrocytes were incubated with the extract derived from leaves of *F. deltoidea* at the final concentration of 0.5 mg/mL, the levels of oxidative stress biomarkers were unchanged (Fig. 1B).

Results of HCl-induced hemolysis of the human erythrocytes after treatment by aqueous extracts derived from leaves of *F. deltoidea* at the final concentration of 5 mg/mL and 0.5 mg/mL were presented in Fig. 2.

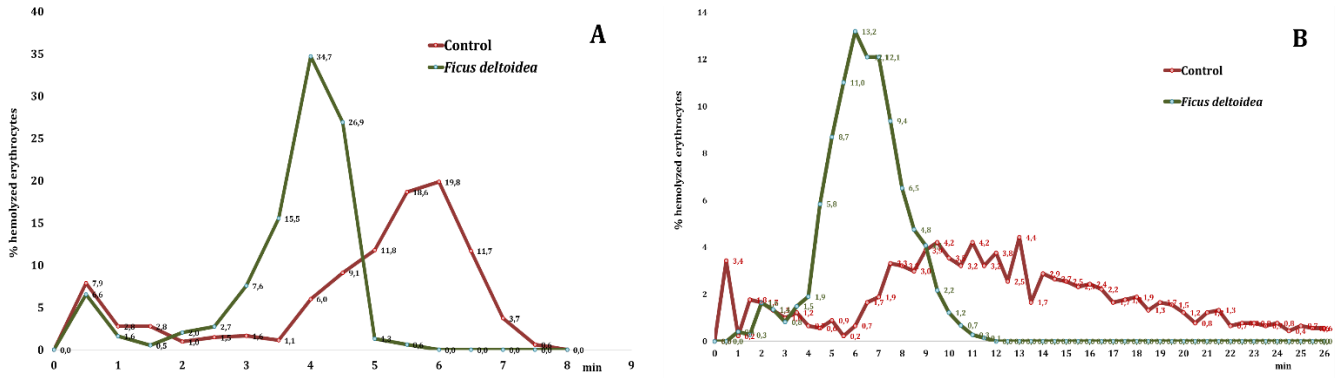

Fig. 2. HCl-induced hemolysis of the human erythrocytes after treatment by aqueous extracts derived from leaves of *F. deltoidea* at the final concentration of 5 mg/mL (A) and 0.5 mg/mL (B)

In the control group (untreated erythrocyte suspension), erythrocytes incubated with 0.1M HCl remained stable and demonstrated slight hemolysis. The maximum level of hemolyzed erythrocytes was (19.8 ± 1.1) %; the total duration of hemolysis was 8.0 min. When *F. deltoidea* extract at the final concentration of 5 mg/mL was added to the erythrocyte suspension, the maximum level of hemolysis occurred after 6.0 min of incubation with 0,1M HCl (34.7 ± 1.4 %). The total duration of hemolysis after incubation with *F. deltoidea* extract was 6.0 min. The results showed that HCl-induced hemolysis was increased by the treatment of *F. deltoidea* extract at the final concentration of 5 mg/mL (Fig. 2A).

Similar trends were observed in the case when *F. deltoidea* extract at the final concentration of 0.5 mg/mL was added to the erythrocyte suspension. The maximum level of hemolyzed erythrocytes in the control group (untreated erythrocyte suspension) was (4.4 ± 0.3 %); the total duration of hemolysis was above 26 min. When *F. deltoidea* extract at the final concentration of 0.5 mg/mL was added to the erythrocyte suspension, the maximum level of hemolysis occurred after 6.0 min of incubation with 0,1M HCl (13.2 ± 1.2 %). The total duration of hemolysis after incubation with *F. deltoidea* extract was 12.0 min. The results revealed that HCl-induced hemolysis was increased by the treatment of *F. deltoidea* extract at the final concentration of 0.5 mg/mL (Fig. 2A). Thus, extract of *F. deltoidea* at the final concentrations of 5 mg/mL and 0.5 mg/mL possess hemolytic properties to human erythrocyte suspension after 1-h incubation *in vitro*.

Our study is in the line of investigations carried out by other researchers which revealed the antioxidant properties of *F. deltoidea* plants. Previously, M. Hakiman and M. Maziah [15] described an experiment, where different aqueous extracts of *F. deltoidea* accessions were evaluated for their antioxidant activities using several assays such as FRAP, free radical scavenging assay, total polyphenol, flavonoid, phenolic acid, and vitamin C. Samsulrizal and co-workers [28] revealed that *F. deltoidea* promoted bone formation in streptozotocin-induced diabetic rats. *F. deltoidea* could prevent diabetic osteoporosis by enhancing osteogenesis and inhibiting bone oxidative stress. These findings support the potential use of *F. deltoidea* for osteoporosis therapy in diabetes [28]. *F. deltoidea* aqueous extract at all concentrations tested (12.5-100 µg/mL) exhibited antioxidant power, suggesting the cytoprotective effect of *F. deltoidea* aqueous extract could be partly attributed to its antioxidant properties [27]. The report of T.C. Ooi and co-workers [27] highlights that *F. deltoidea* may provide a chemopreventive effect on mutagenic and oxidative stress inducers. Maizatul H.O. and co-workers [21] have reported that 85% of the aqueous *F. deltoidea* showed good antioxidant activity due to the presence of flavan-3-ol monomers, proantho-cyanidins, and c-linked flavone glycosides. Adam Z. and co-workers [2] reported water-soluble insulin-secreting constituents in an aqueous extract of *F. deltoidea*, which gave better effects than glibenclamide. Vitexin and isovitexin are bioactive compounds abundantly found in the leaves of *F. deltoidea* that possessed many pharmacological properties including neuro-protection. Zolkiffly S.Z.I. and co-workers [38] revealed that the extract of *F. deltoidea* 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.

The antioxidant activities of *F. deltoidea* aqueous extract (FDD) on menadione-induced oxidative stress were determined in a V79 mouse lung fibroblast cell line by Ooi and co-workers [27]. The ferric-reducing antioxidant power (FRAP) assay was conducted to evaluate FDD antioxidant capacity. Results showed that 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 [27].

Mohd Dom N.S. and co-workers [24] evaluated 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. The antiglycation activity was analyzed based on the inhibition of AGEs, fructosamine, and thiol groups level followed by the inhibition of protein carbonyl formation. The antioxidant activity (DPPH radical scavenging activity and reducing power assay) and total phenolic contents were evaluated. After 28 days of induction, all varieties of *F. deltoidea* extracts significantly restrained the formation of fluorescence AGEs by 4.55-5.14 fold. The extracts also reduced the fructosamine levels by 47.0-86.5%, increased the thiol group levels by 64.3-83.7%, and inhibited the formation of protein carbonyl by 1.36-1.76 fold. DPPH radical scavenging activity showed an IC50 value of 66.81-288.04 µg/ml and reducing power activity depicted at 0.02-0.24 µg/ml. The extent of phenolic compounds present in the extracts ranged from 70.90 to 299.78 mg·GAE/g. Apart from that, correlation studies between the activities were observed [24].

F. deltoidea is traditionally used for regulating blood sugar, blood pressure, and cholesterol levels. *F. deltoidea* has been shown to have antidiabetic, anti-inflammatory, antinociceptive, and antioxidant properties [23]. 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 (2013). The crude extracts and fractions of *F. deltoidea* (var. *angustifolia* (SF) and var. *kunstleri* (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 (73.33 ± 4.99 µg/mg fraction). All the extracts and fractions exhibited antioxidant activities, with SF crude extract showing the highest antioxidant activity and phenolic content (121.62 ± 4.86 mg/g extract). 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 [22].

Yahaya N. and co-workers [34] assessed the insulin secretion activity induced by *F. deltoidea* standardized methanolic extracts from seven independent varieties and mechanisms that underlie the insulin secretion action of the extracts. The insulin secretion for *F. deltoidea* var. *deltoidea*, *F. deltoidea* var. *angustifolia* (Miq.) Corner, and *F. deltoidea* var. *motleyana* (Miq.) C.C. Berg was dose-dependent; further evaluation suggested that *F. deltoidea* var. *trengganuensis* Corner was involved in the K_{ATP}-independent pathway. It should be noted that all varieties of *Ficus deltoidea* screened by the cited authors are considered as synonyms of *F. deltoidea* in The Plant List, an international resource that is a working list of all land plant species, fundamental to understanding and documenting plant diversity and effective conservation of plants. Thus, standardized methanolic extracts of *F. deltoidea* varieties have an insulinotropic effect on the clonal BRIN BD11 cell line and can be utilized as a modern candidate for antidiabetic agents targeting the escalation of insulin secretion from pancreatic beta-cells [34]. *F. deltoidea* var. *deltoidea* could potentially improve insulin sensitivity, suppress hepatic glucose output and enhance glucose uptake in type 2 diabetes mellitus through the down-regulation of the protein tyrosine phosphatase 1B (PTP1B) [1].

The mechanisms that underlie the antihyperglycemic action of *F. deltoidea* were revealed by Adam Z. and co-workers [3]. 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. 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 [3]. The inhibition of the formation of mature adipocytes indicated that leaf extracts of *F. deltoidea* could have potential anti-obesity effects [33].

F. deltoidea is one of the well-known medicinal plants that is traditionally used to treat various ailments and for the maintenance of female reproductive health [35]. *F. deltoidea* can reverse symptoms of the polycystic ovarian syndrome (PCOS) in female rats by improving insulin sensitivity, antioxidant activities, hormonal imbalance, and histological changes. Insulin resistance and hormonal imbalances are key features in the pathophysiology of PCOS. Haslan M.A. and co-workers [17] investigated the biochemical, hormonal, and histomorphometric changes in letrozole (LTZ)-induced PCOS female rats following treatment with *F. deltoidea*. The results showed that treatment with *F. deltoidea* at the dose of 500 and 1000 mg/kg/day reduced insulin resistance, obesity indices, total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL), malonic dialdehyde (MDA), testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) to near-normal levels in PCOS rats. The levels of high-density lipoprotein cholesterol (HDL), estrogen, and superoxide dismutase (SOD) are also similar to those observed in normal control rats. Histomorphometric measurements confirmed that *F. deltoidea* increased the corpus luteum number and the endometrial thickness. These findings suggest the potential use of *F. deltoidea* as an adjuvant agent in the treatment program of PCOS [17].

This medicinal plant is beneficial as a daily dietary supplement for the maintenance of female reproductive health. Zaid S.S.M. and co-workers [35] evaluated the potential protective roles of *F. deltoidea* against bisphenol A (BPA)-induced toxicity of the pituitary-ovarian axis in pre-pubertal female rats. 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. Thus, *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 [35].

F. deltoidea, which has strong antioxidant properties, was selected as a potential protective agent to counter the detrimental effects of BPA in the rat uterus. As Zaid S.S.M. and co-workers [36] revealed, after 6 weeks of concurrent treatment with *F. deltoidea*, uterine abnormalities in the BPA-exposed rats showed a significant improvement. Specifically, the size of stromal cells increased; interstitial spaces between stromal cells expanded; the histology of the glandular epithelium and the myometrium appeared normal and mitotic figures were present. The suppressive effects of BPA on the expression levels of sex steroid receptors (ERα and ERβ) and the immunity gene C3 were significantly normalized by *F. deltoidea* treatment. The role of *F. deltoidea* as an antioxidant agent was proven by the significant reduction in malonic dialdehyde levels in BPA-exposed rats. Moreover, in BPA-exposed rats, concurrent treatment with ***F. deltoidea*** could normalize the level of the gonadotropin hormone, which could be associated with an increase in the percentage of rats with a normal estrous cycle [36].

F. deltoidea has been shown to possess antioxidant properties that could prevent the development of chronic inflammatory bone diseases [26]. The efficacy of *F. deltoidea* in preventing alveolar bone resorption in osteoporotic rats induced by ovariectomy (OVX) was investigated by Omar N.I. and co-workers [26]. The results showed that histologically, the OVXF group demonstrated a significantly lower number of osteoclasts and a higher number of osteoblasts compared with OVXN. Thus, *F. deltoidea* has the capacity to prevent alveolar bone loss in ovariectomy-

induced osteoporosis rats by potentially preserving trabecular bone microarchitecture and decreasing osteoclast, and increasing osteoblast cell count [26].

Osteoporosis (OP) and osteoarthritis (OA) are debilitating musculoskeletal diseases of the elderly. *F. deltoidea* was pre-clinically evaluated against OP- and OA-related bone alterations, in the postmenopausal OA rat model [9]. The *F. deltoidea* extract significantly mitigated these bone microstructural and biomarker changes by dose-dependently down-regulating pro-inflammatory NF-κβ, TNF-α, and IL-6 mRNA expressions. The *F. deltoidea* extract demonstrated good anti-osteoporotic properties in this OP/OA preclinical model by stimulating bone formation and suppressing bone resorption *via* anti-inflammatory pathways. A report by Che Ahmad Tantowi N.A. and co-workers [9] related the subchondral bone plate and trabecular thickening with the metaphyseal trabecular osteopenic bone loss under osteoporotic-osteoarthritis conditions, providing some insights on the debated inverse relationship between osteoporosis and osteoarthritis [9].

Conclusions

In the current study, we investigated the changes in the oxidative stress biomarkers using the *in vitro* model of human erythrocytes to evaluate the antioxidant activities of the aqueous extract derived from the leaves of *F. deltoidea* at the two final concentrations (5 mg/mL and 0.5 mg/mL). The treatment of human erythrocytes by extract derived from leaves of *F. deltoidea* at the final concentration of 5 mg/mL resulted in an increase of TBARS as biomarkers of lipid peroxidation and aldehydic derivatives of oxidatively modified proteins with a simultaneous decrease in the total antioxidant capacity compared to the untreated samples. On the other hand, *in vitro* treatment of human erythrocytes with an extract derived from leaves of *F. deltoidea* at the final concentration of 0.5 mg/mL resulted in the same values of TBARS level, aldehydic and ketonic derivatives of OMP, and total antioxidant capacity as the untreated samples. Extract of *F. deltoidea* at the final concentrations of 5 mg/mL and 0.5 mg/mL possess hemolytic properties to human erythrocyte suspension after 1-h incubation *in vitro*. More studies are warranted in the future, to illustrate the potential and mechanisms of *F. deltoidea* in preventing oxidative stress using different cell models *in vitro* and different final concentrations of the extract. Also, further studies are warranted to identify the bioactive components that contribute to this protective effect.

Acknowledgments

The authors wish to acknowledge The International Visegrad Fund for supporting our study.

References

1. Abdel-Rahman, R. F., Ezzat, S. M., Ogaly, H. A., Abd-Elsalam, R. M., Hessin, A. F., Fekry, M. I., Mansour, D. F., & Mohamed, S. O. (2020). *Ficus deltoidea* extract down-regulates protein tyrosine phosphatase 1B expression in a rat model of type 2 diabetes mellitus: a new insight into its antidiabetic mechanism. *Journal of Nutritional Science*, 9, e2. https://doi.org/10.1017/jns.2019.40.

2. Adam, Z., Ismail, A., Khamis, S., Mohd. Mokhtar, M.H., Hamid, M. (2011). Antihyperglycemic activity of *F. deltoidea* ethanolic extract in normal rats. *Sains Malaysiana*, *40*(5), 489–495.

3. Adam, Z., Khamis, S., Ismail, A., & Hamid, M. (2012). *Ficus deltoidea*: A potential alternative medicine for diabetes mellitus. *Evidence-based complementary and alternative medicine: eCAM, 2012*, 632763. https://doi.org/10.1155/2012/632763.

4. Ashraf, K., Haque, M. R., Amir, M., Ahmad, N., Ahmad, W., Sultan, S., Ali Shah, S. A., Mahmoud Alafeefy, A., Mujeeb, M., & Bin Shafie, M. F. (2021). An overview of phytochemical and biological activities: *Ficus deltoidea* Jack and other *Ficus* spp. *Journal of Pharmacy & Bioallied Sciences*, *13*(1), 11–25. https://doi.org/10.4103/jpbs.JPBS_232_19.

5. Berg, C.C. (2001). *Moreae*, *Artocarpeae*, and *Dorstenia* (*Moraceae*), with introductions to the family and *Ficus* and with additions and corrections to Flora Neotropica Monograph 7. *Flora Neotropica Monograph 83*. New York: The New York Botanical Garden.

6. 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

7. Berg, C.C., Wiebes, J.T. (1992). African fig trees and fig wasps. *Koninklijke Nederlandse Akademie van Wetenschappen, Verhandelingen Afdeling Natuurkunde*, 2de reeks, deel 89. North-Holland, Amsterdam

8. Bunawan, H., Amin, N. M., Bunawan, S. N., Baharum, S. N., & Mohd Noor, N. (2014). *Ficus deltoidea* Jack: A review on its phytochemical and pharmacological importance. *Evidence-Based Complementary and Alternative Medicine: eCAM*, 2014, 902734. https://doi.org/10.1155/2014/902734.

9. Che Ahmad Tantowi, N. A., Lau, S. F., & Mohamed, S. (2018). *Ficus deltoidea* Prevented Bone Loss in Preclinical Osteoporosis/Osteoarthritis Model by Suppressing Inflammation. *Calcified Tissue International*, *103*(4), 388–399. https://doi.org/10.1007/s00223-018-0433-1.

10. Clement, W.L., Weiblen, G.D. (2009). Morphological evolution in the mulberry family (*Moraceae*). *Systematic Botany*, *34*(3), 530-552.

11. Cook, J.M., Rasplus, J.-Y. 2003. Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology & Evolution*, *18*(5), 241–248.

12. Datwyler, S.L., Weiblen, G.D. (2004). On the origin of the fig: phylogenetic relationships of *Moraceae* from ndhF sequences. *American Journal of Botany*, *91*(5), 767-777.

13. Dubinina, E. E., Burmistrov, S. O., Khodov, D. A., & Porotov, I. G. (1995). Okislitel'naia modifikatsiia belkov syvorotki krovi cheloveka, metod ee opredeleniia [Oxidative modification of human serum proteins. A method of determining it]. *Voprosy meditsinskoi khimii, 41*(1), 24–26.

14. Galaktionova, L. P., Molchanov, A. V., El'chaninova, S. A., & Varshavskiĭ, B. I.a (1998). Sostoianie perekisnogo okisleniia u bol'nykh s iazvennoĭ bolezn'iu zheludka i dvenadtsatiperstnoĭ kishki [Lipid peroxidation in patients with gastric and duodenal peptic ulcers]. *Klinicheskaia laboratornaia diagnostika*, (6), 10–14.

15. Hakiman, M., Maziah, M. (2009). Non enzymatic and enzymatic antioxidant activities in aqueous extract of different *Ficus deltoidea* accessions. *Journal of Medicinal Plants Research*, *3*(3), 120-131.

16. Harun, H., Musapha, Z. (2015). Phytochemical constituents in leaves and callus of *Ficus deltoidea* Jack var. *Kunstleri* (King) Corner. *Walailak Journal of Science and Technology*, 12(5), 431–439.

17. Haslan, M. A., Samsulrizal, N., Hashim, N., Zin, N. S. N. M., Shirazi, F. H., & Goh, Y. M. (2021). *Ficus deltoidea* ameliorates biochemical, hormonal, and histomorphometric changes in letrozole-induced polycystic ovarian syndrome rats. *BMC Complementary Medicine and Therapies*, *21*(1), 291. https://doi.org/10.1186/s12906-021-03452-6.

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

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

20. Luqman, S., Kaushik, S., Srivastava, S., Kumar, R., Bawankule, D. U., Pal, A., Mahendra P. Darokar, M. P. & Khanuja, S. P. S. (2009). Protective effect of medicinal plant extracts on biomarkers of oxidative stress in erythrocytes. *Pharmaceutical Biology*, 47(6), 483–490. https://doi.org/10.1080/13880200902832900.

21. Maizatul, H.O., Mullen, W., Crozier, A. (2011). Identification of proanthocyanidin dimers and trimers, flavone C-Glycosides and antioxidants in *Ficus deltoidea*, a Malaysian herbal tea. *Journal of Agricultural and Food Chemistry*, 59, 1363-1369.

22. Misbah, H., Aziz, A. A., & Aminudin, N. (2013). Antidiabetic and antioxidant properties of *Ficus deltoidea* fruit extracts and fractions. *BMC Complementary and Alternative Medicine*, 13, 118. https://doi.org/10.1186/1472-6882-13-118.

23. Mohd Ariff, A., Abu Bakar, N. A., Abd Muid, S., Omar, E., Ismail, N. H., Ali, A. M., Mohd Kasim, N. A., & Mohd Nawawi, H. (2020). *Ficus deltoidea* suppresses endothelial activation, inflammation, monocytes adhesion and oxidative stress *via* NF-κB and eNOS pathways in stimulated human coronary artery endothelial cells. *BMC Complementary Medicine and Therapies*, *20*(1), 56. https://doi.org/10.1186/s12906-020-2844-6.

24. Mohd Dom, N. S., Yahaya, N., Adam, Z., Nik Abd Rahman, N. M. A., & Hamid, M. (2020). Antiglycation and antioxidant properties of *Ficus deltoidea* varieties. *Evidence-Based Complementary and Alternative Medicine: eCAM, 2020*, 6374632. https://doi.org/10.1155/2020/6374632.

25. Omar, M. H., Mullen, W., & Crozier, A. (2011). Identification of proanthocyanidin dimers and trimers, flavone C-Glycosides, and antioxidants in *Ficus deltoidea*, a Malaysian herbal tea. *Journal of Agricultural and Food Chemistry*, *59*(4), 1363–1369. https://doi.org/10.1021/jf1032729.

26. Omar, N. I., Baharin, B., Lau, S. F., Ibrahim, N., Mohd, N., Ahmad Fauzi, A., Muhammad, N., & Fernandez, N. M. (2020). The influence of *Ficus deltoidea* in preserving alveolar bone in ovariectomized Rats. *Veterinary Medicine International*, 2020, 8862489. https://doi.org/10.1155/2020/8862489.

27. Ooi, T. C., Ibrahim, F. W., Ahmad, S., Chan, K. M., Leong, L. M., Mohammad, N., Siew, E. L., & Rajab, N. F. (2021). Antimutagenic, cytoprotective and antioxidant properties of *Ficus deltoidea* aqueous extract *in vitro*. *Molecules (Basel, Switzerland), 26*(11), 3287. https://doi.org/10.3390/molecules 26113287.

28. Samsulrizal, N., Goh, Y. M., Ahmad, H., Md Dom, S., Azmi, N. S., NoorMohamad Zin, N. S., & Ebrahimi, M. (2021). *Ficus deltoidea* promotes bone formation in streptozotocin-induced diabetic rats. *Pharmaceutical Biology*, *59*(1), 66–73. https://doi.org/10.1080/13880209.2020.1865411.

29. Silihe, K. K., Zingue, S., Kemegne Sipping, M. T., Cazanevscaia, A. B., Dediu Botezatu, A. V., Njamen, D., Dinica, R. M. (2022). The Antioxidant Potential of *Ficus umbellata* Vahl (*Moraceae*) that accelerates *in vitro* and the *in vivo* anti-inflammatory protective effects. *Applied Sciences*, 12, 9070. https://doi.org/ 10.3390/ app12189070.

30. Terskov, I.A., Gitelson, I.I. (1957). Method of chemical (acid) erythrograms. *Biofizika*, 2, 259-266.

31. Tkachenko, H., Buyun, L., Kurhaluk, N., Honcharenko, V., Prokopiv, A. (2022). *In vitro* antioxidant response of the equine blood treated by leaf extract of *Ficus drupacea* Thunb. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, *6*(2), 292–300. https://doi.org/10.15414/ainhlq.2022.0030.

32. 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*). *Agrobiodiversity for Improving Nutrition, Health, and Life Quality*, (3), 1-13. https://doi.org/10.15414/agrobiodiversity.2019.2585-8246.001-013.

33. Woon, S. M., Seng, Y. W., Ling, A. P., Chye, S. M., & Koh, R. Y. (2014). Anti-adipogenic effects of extracts of *Ficus deltoidea* var. *deltoidea* and var. *angustifolia* on 3T3-L1 adipocytes. *Journal of Zhejiang University. Science. B*, *15*(3), 295–302. https://doi.org/10.1631/jzus.B1300123.

34. Yahaya, N., Mohd Dom, N. S., Adam, Z., & Hamid, M. (2018). Insulinotropic activity of standardized methanolic extracts of *Ficus deltoidea* from seven varieties. *Evidence-Based Complementary and Alternative Medicine: eCAM, 2018*, 3769874. https://doi.org/10.1155/2018/3769874.

35. Zaid, S. S. M., Othman, S., & Kassim, N. M. (2018). Protective role of *Ficus deltoidea* against BPA-induced impairments of the follicular development, estrous cycle, gonadotropin and sex steroid hormones level of prepubertal rats. *Journal of Ovarian Research*, *11*(1), 99. https://doi.org/10.1186/s13048-018-0466-0

36. Zaid, S. S. M., Othman, S., & Kassim, N. M. (2021). Protective role of Mas Cotek (*Ficus deltoidea*) against the toxic effects of bisphenol A on morphology and sex steroid receptor expression in the rat uterus. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, 140, 111757. https://doi.org/10.1016/j.biopha.2021.111757.

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

38. Zolkiffly, S. Z. I., Stanslas, J., Abdul Hamid, H., & Mehat, M. Z. (2021). *Ficus deltoidea*: Potential inhibitor of pro-inflammatory mediators in lipopolysaccharide-induced activation of microglial cells. *Journal of Ethnopharmacology*, 279, 114309. https://doi.org/10.1016/j.jep.2021.114309.

Received: 05.02.2023. **Accepted**: 25.02.2023. **Published**: 06.03.2023.

Cite this article in APA Style as:

Tkachenko, H., Kurhaluk, N., Buyun, L., Honcharenko, V., and Prokopiv, A. (2022). *In vitro* protective effect of leaf extract of *Ficus deltoidea* Jack (*Moraceae*) on biomarkers of oxidative stress in the human erythrocytes. *BHT: Biota. Human. Technology*, 3, 74–85. (in English)

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