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ASSESSMENT OF OXIDATIVE STRESS BIOMARKERS IN THE EQUINE BLOOD AFTER *IN VITRO* INCUBATION WITH LEAF EXTRACT OBTAINED FROM *DENDROBIUM PARISHII* RCHB.F.

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The main aim of the study was an assessment of the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification, total antioxidant capacity] in the plasma and equine erythrocytes after treatment with *Dendrobium parishii* Rchb. f. leaf extract. The leaves of *D. parishii* plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine. Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in proportion 1 : 19, w/w). The equine plasma and erythrocyte aliquots were used in the study. The pellet of blood was resuspended in phosphate buffer (pH 7.4). A volume of 0.1 ml of the *D. parishii* extract was added to 1.9 ml of clean equine erythrocytes or 1.9 mL of plasma. For positive control (blank), phosphate buffer was used. After incubation of the mixture at 37 °C for 60 min with continuous stirring, samples were used for the biochemical assays. The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives level, as well as total antioxidant capacity, was non-significantly altered in the erythrocytes' suspension after *in vitro* incubation with an extract obtained from *D. parishii*. More significant changes were observed in the plasma. The *D. parishii* extract caused to increase in the formation of intracellular aldehydic and ketonic derivatives of oxidatively modified proteins in the extract-treated plasma, but these results were non-significant. Total antioxidant capacity was non-significant decreased both in plasma and erythrocytes. Screening of *Dendrobium* plants for other biological activities including antioxidant activities is essential and may be effective for searching the preventive agents in the pathogenesis of some metabolic diseases.

Keywords: *Dendrobium parishii* Rchb. F., leaf extract, equine erythrocytes, plasma, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity

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Introduction

The genus *Dendrobium* Sw. is one of the largest genera in the family Orchidaceae, comprising with approximately 1,200–2,000 species, distributed in the tropical and subtropical regions of Asia and North Australia (Wood, 2006; Hou et al., 2017).

Dendrobium species are important commercial plants widely used as herbal medicines for hundreds of years in China due to their pharmacological properties (Lee et al., 2018; Cheng et al., 2019). Recently, within the *Dendrobium* genus, most studies have focused on the bioactivity and structural types of some small molecules, such as bibenzyl, phenanthrene, anthracene, fluorene, coumarin, flavone, cinnamate, sesquiterpene, sterol, fatty acid, and alkaloids (Honda and Yamaki, 2000; Zhao et al., 2017; Paudel et al., 2018; Ivannikov et al., 2019a,b). Among them, phenolic derivatives have been extensively studied for their biological activities, especially in the field of cancer, inflammation, and neurodegeneration (Lee et al., 2018; Sut et al., 2017). Numerous researchers have investigated polysaccharide, the major active constituents in *Dendrobium*. The polysaccharides in many plants are attracting worldwide attention because of their biological activities and medical properties, such as anti-viral, antioxidant, immune-stimulating and antitumor activities, anti-chronic inflammation, anti-hypertensive, neuron-protective effects (Lee et al., 2018). Polysaccharides extracted from some *Dendrobium* species, such as *Dendrobium officinale* Kimura & Migo (syn. *D. huoshanense* Z.Z. Tang & S.J. Cheng) and *Dendrobium chrysotoxum* Lindl., have been studied (Luo et al., 2009). Alkaloids exhibit antioxidant, anticancer, and neuroprotective activities. Other compounds manifest antioxidant, anticancer, and immunomodulatory (Ng et al., 2012).

Dendrobium parishii was studied for chemical constituents by solvent extraction, followed by column chromatography by Kongkatitham et al. (2018). Seven compounds including two new (1–2) and five known compounds (3–7) were isolated and screened for antioxidant activity using 2.2-Diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC) and deoxyribose assays. The new compound (compound 2) showed the highest antioxidant activities and was further evaluated for its antioxidant and anti-inflammatory effects, as well as mechanisms of action in RAW264.7 murine macrophage cells under oxidative stress and inflammation induced by hydrogen peroxide (H_2O_2) and lipopolysaccharide (LPS), respectively. Compound 2 at 12.5, 25 and 50 μg/mL significantly decreased ROS in H_2O_2 treated RAW264.7 cells in a dose-dependent manner and enhanced antioxidant enzyme [superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)] activities. For the anti-inflammatory activity, compound 2 reduced the expression of iNOS and COX-2 in LPS treated RAW264.7 cells. The results indicate that compound 2 has the potential to be developed as an antioxidant and anti-inflammatory agent (Kongkatitham et al., 2018).

Equine erythrocytes are more sensitive to oxidant-induced damage due to the use of inefficient mechanisms to correct and protect against oxidative damage, i.e. methemoglobin formation, alteration of aggregation, and reduction of cellular deformability (Baskurt and Meiselman, 1999). Erythrocytes from horses are slower than erythrocytes from other species studied in their ability to regenerate glutathione (GSH) after it has been oxidized *in vitro* (Harvey et al., 2003). These reduced abilities may be related to the fact that horse erythrocytes have lower

glutathione reductase (GR) activities than erythrocytes from humans and most domestic animal species, and the Michaelis-Menton constant (Km) of GSSG for GR is higher in horses than in three other species measured. Moreover, sulfhydryl groups in proteins and unsaturated lipids in membranes are especially susceptible to oxidation. Oxidative denaturation and the precipitation of the globin portion of hemoglobin into large aggregates result in the formation of Heinz bodies that can bind to and alter membranes. Membrane structure also is altered by the oxidation of sulfhydryl groups and by lipid peroxidation (Harvey, 1997).

The list of *Dendrobium* species used in Traditional Chinese Medicine comprises 41 species, including *D. parishii* Rchb. f., distributed in Yunnan, Guizhou, India, Myanmar, Thailand, Laos, Vietnam (Cheng et al., 2019).

Therefore, in the current study, the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification, total antioxidant capacity] in the equine plasma and erythrocytes was used for assessing the antioxidant activity of *Dendrobium parishii* Rchb. F. leaf extract, considering the tremendous medicinal importance of this endangered orchid species.

Material and methodology

Collection of Plant Materials

The leaves of *D. parishii* plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine (Figure 1). Since 1999, the whole collection of tropical and subtropical plants (including orchids) has the status of a National Heritage Collection of Ukraine. Besides that, the NBG collection of tropical orchids was registered at the Administrative Organ of CITES in Ukraine (Ministry of Environmental Protection, registration No. 6939/19/1-10 of 23 June 2004). 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 proportion 1 : 19, w/w) at room temperature. The extract was then filtered and used for analysis. The extract was stored at -20 °C until use.

Horses

Eighteen healthy adult horses from the central Pomeranian region in Poland (village Strzelinko, N 54º 30’ 48.0” E 16º 57’ 44.9”), aged 8.9 ±1.3 years old, including 6 Hucul pony, 5 Thoroughbred horses, 2 Anglo-Arabian horses and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical and vital parameters, which were within reference ranges. The females were non-pregnant.

Figure 1 *Dendrobium parishii* Lindl. plant, cultivated at M.M. Gryshko National Botanic Garden glasshouses (Kyiv, Ukraine). Photo: Lyudmyla Buyun

Collection of blood samples

Blood was drawn from the jugular vein of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM). Blood was stored in tubes with sodium citrate as the anticoagulant and held on the ice until centrifugation at 3,000 rpm for 5 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. For positive control (phosphate buffer) was used. After incubation of the mixture at 37 °C for 60 min with continuous stirring, it was centrifuged at 3,000 rpm for 5 min. Erythrocytes 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 malondialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with TBA under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The μmol of MDA per l L was calculated using $1.56 \cdot 10^5$ mM/cm as the extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay

To evaluate the protective effects of the extract obtained from leaves of *D. parishii* against free radical-induced protein damage in equine erythrocytes and plasma, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the erythrocyte suspension and plasma 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 and co-workers (1995). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehyde derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in the sample was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). 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.

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). All statistical calculation was performed on separate data from each individual with Statistica 8.0 software (StatSoft, Krakow, Poland).

Results and discussion

The data on the TBARS content as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine plasma and erythrocytes suspension after *in vitro* incubation with leaf extract obtained from *Dendrobium parishii* was presented in Figure 2.

As shown in Figure 2, treatment by extract caused to increase of the plasma TBARS level by 46.4% (p = 0.000), while erythrocyte TBARS level was non-significantly increased by 6.3% (p >0.05) when compared to untreated erythrocytes.

When equine erythrocytes were incubated with an extract obtained from *D. parishii*, the aldehydic and ketonic derivatives level, as well as total antioxidant capacity, was non-significantly altered. The *D. parishii* extract caused to increase in the formation of intracellular aldehydic and ketonic derivatives of OMP in the extract-treated plasma (by 2.3 and 17.4%, p >0.05). In the erythrocyte suspension, ketonic derivatives of OMP was decreased by 3.2% (p >0.05), but these results were non-significant. Total antioxidant capacity was non-

significantly decreased both in plasma (by 15.2%, p >0.05) and erythrocytes (by 14.1%, p >0.05) (Figure 2).

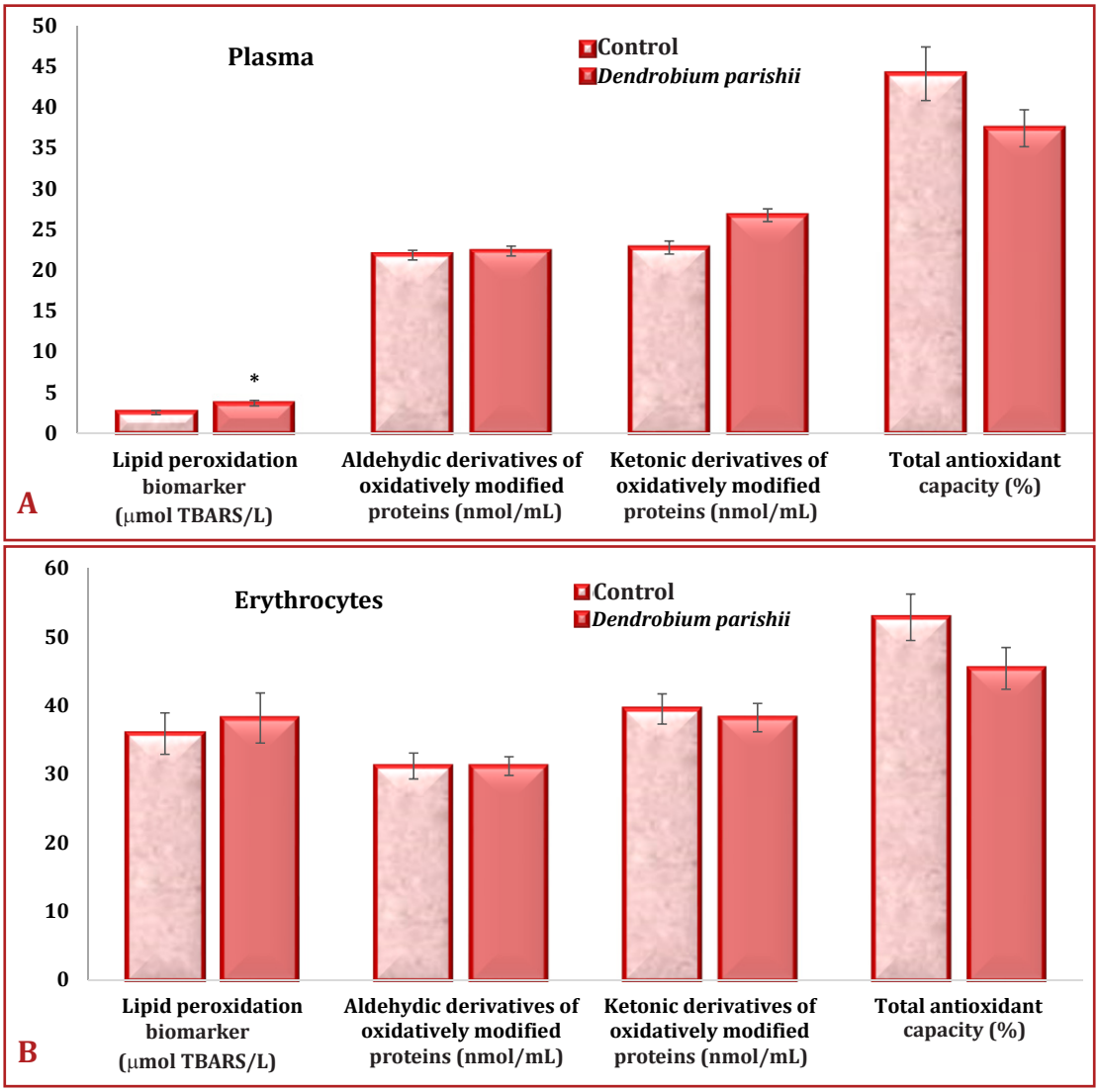

Figure 2 The TBARS content as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine plasma and erythrocytes suspension after *in vitro* incubation with leaf extract obtained from *Dendrobium parishii* (M ±m, n = 18)

Many studies have confirmed the antioxidant properties of *Dendrobium* plants. Antioxidant properties of crude extract, partition extract, and fermented medium from *Dendrobium* plant flower were investigated by Abu et al. (2017). The 100% methanolic crude extract showed the highest total phenolic content and the best antioxidant properties. A correlation relationship between antioxidant activity and total phenolic content showed

that phenolic compounds were the dominant antioxidant components in this flower extract. The microbial fermentation on the *D. sabin* flower medium showed potential in increasing the phenolic content and DPPH scavenging activity. The TPC of the final fermented medium showed approximately an 18% increment, while the DPPH of fermented medium increased significantly to approximately 80% at the end of the fermentation. The flower showed very good potential properties of antioxidants in crude extract and partition extract as well as better antioxidant activity in the flower fermented medium (Abu et al., 2017).

Phytochemical screening of the leaf extracts of *D. parishii* has been conducted by Ivannikov et al. (2019a). It was established that the phenolic acids and flavonoids in the glycoside form were the main groups of bioactive compounds revealed in the extracts. Moreover, these researches have employed nanotechnology to produce composites for delayed release of bioactive compounds using *D. parishii* leaf extracts and fumed silica (Ivannikov et al., 2019a).

The fact that plant extracts of *Dendrobium moniliforme* (L.) Sw. have a number of bioactive compounds (water-soluble polysaccharides, phenanthrenes, bibenzyl derivatives, and polyphenol compounds) that showed antioxidant and cytotoxic activities suggests the potential pharmacological importance of this plant. Paudel et al. (2018) have explored the antioxidant and cytotoxic activities of *D. moniliforme* extracts and detected their bioactive compounds. The total flavonoid content (TFC) content was highest (116.65 µg GAE/mg of extract) in *D. moniliforme* chloroform extract (DMC) and the total flavonoid content (TFC) content was highest (116.67 µg QE/mg of extract) in *D. moniliforme* acetone extract (DMA). *D. moniliforme* hexane extract (DMH) extract showed the highest percentage of DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging activity (94.48%), followed closely by *D. moniliforme* ethanol extract (DME) (94.45%), DMA (93.71%) and DMC (94.35%) at 800 µg/ml concentration. The antioxidant capacities of DMC, DMA, DMH and DME, which were measured in IC_{50} values, were much lower 42.39 µg/ml, 49.56 µg/ml, 52.68 µg/ml, and 58.77 µg/ml respectively than the IC_{50} of *D. moniliforme* methanol extract (DMM) (223.15 µg/ml). DMM at the concentration of 800 µg/ml most inhibited the growth of HeLa cells (78.68%) and DME at the same concentration most inhibited the growth of U251 cells (51.95%). The cytotoxic capacity (IC_{50}) of DMM against HeLa cells was 155.80 µg/ml of extract and that of DME against the U251 cells was 772.50 µg/ml of extract. A number of bioactive compounds were detected in both DME and DMM (Paudel et al., 2018). Chan and co-workers (2018) have investigated the polyphenol content, antioxidant activity, and inhibition ability of mushroom tyrosinase and melanogenesis of *Dendrobium officinale* Kimura & Migo (syn. D. tosaense Makino) (DT) extract.

Sukumaran and Yadav (2016) have determined *in vitro* free radical scavenging and anti-inflammatory activity of *D. macrostachyum* Lindl. Sequential stem and leaf extracts were assessed for its antioxidant and anti-inflammatory activity by *in vitro* methods. The stem ethanolic extracts exhibited significant IC_{50} value of 10.21, 31.54 and 142.97 µg/ml respectively for DPPH, ABTS radical scavenging and reducing power activity. The ethanol and water extract was highly effective as albumin denaturation inhibitors (IC_{50} = 114.13 and 135.818 µg/ml respectively) and proteinase inhibitors (IC_{50} = 72.49 and 129.681 µg/ml respectively). Membrane stabilization was also noticeably inhibited by the stem ethanolic

extract among other extracts (IC_{50} = 89.33 µg/ml) but comparatively lower to aspirin standard (IC_{50} = 83.926 µg/ml). The highest total phenol content was exhibited by the ethanolic stem and leaf extracts respectively at 20 and 16 mg of gallic acid equivalents of dry extract. On LCMS analysis 20 constituents were identified and it included a chemotaxonomic marker for *Dendrobium* species. The results of Sukumaran and Yadav (2016) showed a relatively high concentration of phenolics, high scavenger activity and high anti-inflammatory activity of the stem extract compared to the leaf extract.

In the study, conducted by Luo and Fan (2011), the polysaccharide DFHP (Mw 209.3 kDa) was first isolated from the stem of *Dendrobium fimbriatum* Hook. var. *oculatum* Hook. with the dominance of mannose, glucose, galactose. Free radicals scavenging activities *in vitro* indicated that DFHP has significant radicals scavenging abilities on ABTS and Hydroxyl radicals. The scavenging effects were powerful and close to the positive control (Vc). Therefore, the polysaccharide DFHP should be explored as a novel potential antioxidant. On the other hand, DFHP exhibited a weak scavenging effect on DPPH radical compared to the reference (Luo and Fan, 2011).

The protective effects of *Dendrobium officinale* polysaccharides against H_2O_2-induced injury in H9c2 cells were demonstrated. The results also indicated the anti-oxidative capability of *D. officinale* polysaccharides. Zhao et al. (2017) have studied the protective effect of *D. officinale* polysaccharides against H_2O_2-induced oxidative stress in H9c2 cells. MTT assay was carried out to determine the cell viability of H9c2 cells when pretreated with *D. officinale* polysaccharides. Fluorescent microscopy measurements were performed for evaluating the apoptosis in H9c2 cells. Furthermore, the effects of *D. officinale* polysaccharides on the activities of antioxidative indicators (malondialdehyde, superoxide dismutase), reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) levels were analyzed. *D. officinale* polysaccharides attenuated H_2O_2-induced cell death, as determined by the MTT assay. *D. officinale* polysaccharides decreased malondialdehyde levels, increased superoxide dismutase activities, and inhibited the generation of intracellular ROS. Moreover, pretreatment with *D. officinale* polysaccharides also inhibited apoptosis and increased the MMP levels in H9c2 cells (Zhao et al., 2017).

HPLC-profiling of metabolites extracted from *Dendrobium parishii* seeds was undertaken by Buyun and Grakhov (2014). It was revealed that seeds of this species comprise, together with phenolics, the low polar inhibitors, apparently, also terpenoid or steroid derivatives.

A polysaccharide is the main active ingredient in *D. officinale*; its antioxidant activity is a hot research topic nowadays. The antioxidant activities of the polysaccharide *in vitro* assay indicate that the *D. officinale* polysaccharide has a good scavenging activity of 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical, higher scavenging activity of hydroxyl radical, and metal chelating activities (Luo et al., 2016). Zhang et al. (2017) have adopted a model of H_2O_2-induced H9c2 cardiomyocytes apoptosis, aiming to study the effect of *D. officinale* Polysaccharide (DOP-GY) for cardiomyocyte apoptosis caused by oxidative stress and its possible mechanism. The pretreatment of DOP-GY (low dose: 6.25 µg/mL, medium-dose: 12.5 µg/mL, high dose: 25 µg/mL) followed by a 2h incubation with 200 µM H_2O_2 elevated the survival rate, cut the

lactate dehydrogenase (LDH) leakage, reduced lipid peroxidation damage, improved the activity of the endogenous antioxidant enzymes. In addition, the pretreatment of DOP-GY significantly inhibited the production of ROS, declined the mitochondrial membrane potential, down-regulated pro-apoptosis protein and up-regulated anti-apoptosis protein. The protective effect was correlated with the PI3K/Akt and MAPK signal pathway. The study of Zhang and co-workers (2017) suggests that DOY-GY has the potential to exert cardioprotective effects against H_2O_2-induced H9c2 cardiomyocyte apoptosis (Zhang et al., 2017).

The crude *D. officinale* polysaccharide (DOP) was obtained by hot water extraction- ethanol precipitation method, and four new polysaccharide fractions (DOP-40, DOP-50, DOP-60, and DOP-70) were further obtained from the crude DOP by fractional precipitation with ethanol method by Xing et al. (2018). The antioxidant activities of them were evaluated by the reducing power assay, and the superoxide anion, 2.2-diphenyl-1-picrylhydrazyl (DPPH), and hydroxyl free radicals scavenging assays, respectively. Finally, the anticancer activities of them were investigated via the MTT assay and the western blot analysis using HepG2 cells. Among these four purified fractions were mainly composed of D-mannose and D-glucose with different molar ratios, and their average molecular weights were 999, 657, 243 and 50.3 kDa, respectively. What's more, DOP-70 always exhibited the strongest antioxidant and anticancer activities, while DOP-40 and DOP-60 showed very close antioxidant and anticancer activities which were better than that of DOP-50. The western blotting analysis also showed that DOP-40, DOP-60, and DOP-70 induced apoptosis in HepG2 human liver cancer cells through the Bcl-2 and Bax-dependent pathway (Xing et al., 2018).

Liao et al. (2015) have compared the radical scavenging activity of five different acidic polysaccharides, and to find the correlation with the functional groups. Five kinds of *Dendrobium* polysaccharides had different abilities to scavenge ABTS+ free radicals and hydroxyl free radicals. Moreover, the study had shown that five kinds of antioxidant activity of acidic polysaccharides had an obvious correlation between uronic acid and sulfuric acid. The antioxidant activity of each sample was positively correlated with the content of uronic acid and negatively correlated with the content of sulfuric acid. Sulfuric acid can inhibit the antioxidant activity of acidic polysaccharide but uronic acid can enhance the free radical scavenging activity. By analyzing the structural characteristics of five acidic polysaccharides, all samples have similar structures, however, *D. denneanum* Kerr, *D. devonianum* Paxton and *D. officinale* which had β configuration have higher antioxidant activity than *D. nobile* and *D. fimbriatum* which had a configuration (Liao et al., 2015).

The anti-diabetic activity of *Dendrobium loddigesii* polyphenols (DJP) was evaluated by Li et al. (2018). In particular, the serum biochemical index and tissue appearance were evaluated. In order to gain an insight into the anti-diabetic mechanism, the oxidative stress index, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and gut microbiota modulation was determined by ELISA, immunohistochemistry or high throughput sequencing 16S rRNA gene. The results revealed that DJP had the effects to decrease the blood glucose, body weight, low-density lipoprotein cholesterol (LDL-C) levels and increase insulin (INS) levels in the mice. DJP improved the mice's fatty liver and diabetic nephropathy. DJP showed the anti-oxidative abilities to reduce the malondialdehyde (MDA) level and increase the contents of

superoxide dismutase (SOD), catalase (CAT) as well as glutathione (GSH). DJP exerted the anti-inflammatory effects of decreasing expression of IL-6 and TNF-α. After treatment of DJP, the intestinal flora balance of the mice was ameliorated, increasing *Bacteroidetes* to *Firmicutes* ratios as well as the relative abundance of *Prevotella*/*Akkermansia* and reducing the relative abundance of S24-7/*Rikenella*/*Escherichia coli*. The study of Li et al. (2018) revealed for the first time that DJP improves the diabetic db/db mice symptoms of diabetes and complications, which might be due to the effects that DJP induced the decrease of inflammation as well as oxidative stress and improvement of intestinal flora balance.

Pan et al. (2012) have studied the preventive effects of galactoglucomannan from *D. officinale* (syn. *D. huoshanense*) on sodium selenite-induced hepatic damage and fibrosis in rats. Oral administration of the galactoglucomannan brought about a decline in the activities of serum lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, and the levels of H_2O_2 and malonic dialdehyde. The level of glutathione was increased, hepatic plasma membrane fluidity was reinstated, and maintained the activities of antioxidative enzymes including catalase, glutathione *S*-transferase, and superoxide dismutase were maintained. The prevention of selenite-induced hepatic damage and fibrosis was in keeping with the attenuated expression of type I collagen and transforming growth factor-β1. Thus, the galactoglucomannan stands the chance of being developed into an antifibrotic agent for forestalling hepatic damage and fibrosis (Pan et al., 2012).

Conclusions

The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives level, as well as total antioxidant capacity, were non-significantly altered in the erythrocytes' suspension after *in vitro* incubation with an extract obtained from *D. parishii*. More significant changes were observed in the plasma. *D. parishii* leaf extract caused to increase in the formation of intracellular aldehydic and ketonic derivatives of OMP in the extract-treated plasma, but these results were non-significant. Total antioxidant capacity was non-significant decreased both in plasma and erythrocytes. The lack of clinical safety and toxicity data for *D. parishii* and many other herbs that are increasingly being used suggests the necessity of further investigations regarding their influence on organs and tissues function, including the evaluation of molecular mechanisms involved in order to exploit them for potential therapeutic benefits. Screening of *Dendrobium* plants for other biological activities including antioxidant activities is essential and may be effective for searching the preventive agents in the pathogenesis of some metabolic diseases.

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