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BIOMARKERS OF OXIDATIVE STRESS IN THE EQUINE BLOOD AFTER *IN VITRO* TREATMENT WITH EXTRACTS DERIVED FROM PSEUDOBULBS OF *COELOGYNE PANDURATA* LINDL. (ORCHIDACEAE*)* PLANTS

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The *Coelogyne* genus (Orchidaceae) belongs to the group of orchids that possesses medical properties [1, 2]. The interesting species within the genus *Coelogyne*, comprising considerable interest for screening of biological activity of various parts of the plants, is *Coelogyne pandurata* Lindl. *Coelogyne pandurata* Lindl. is found in Malaysia, Sumatra, Borneo, and the Philippines as a large-sized, hot-growing epiphyte found on large trees near rivers or terrestrial with well-spaced, strongly compressed, oblong or suborbicular, sulcate pseudobulb carrying 2, apical, plicate, elliptic-lanceolate, leaves with a stout petiole that blooms in late spring-summer out of the center of newly emerging growths with up to 15 flowers on a terminal, arched to pendant, 15 to 30 cm long, racemose inflorescence. The simultaneously opening flowers are highly fragrant of honey to cinnamon but are short-lived (http://www.orchidspecies.com/).

The current study was conducted to investigate the antioxidant properties of biomarkers of oxidative stress [2-thiobarbituric acid reactive substances (TBARS) as biomarkers of lipid peroxidation, aldehydic and ketonic derivatives of oxidative modification of proteins (OMP), total antioxidant capacity TAC)] in the equine erythrocytes after in vitro treatment with the extract derived from pseudobulbs of *C. pandurata*. Our current scientific project was undertaken in the frame of the cooperation program between the Institute of Biology and Earth Sciences (Pomeranian University in Słupsk, Poland) and M.M. Gryshko National Botanic Gardens of the National Academy of Sciences of Ukraine, directed to assessment of medicinal properties of tropical plants has encompassed some tropical mega-diverse genera, including Orchidaceae.

The pseudobulbs of *C. pandurata*, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine. 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 Environment, registration No. 6939/19/1-10 of 23 June 2004). Freshly pseudobulbs of *C. pandurata* were washed, weighted, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in the ratio of 1:19, w/w) at room temperature.

Eighteen healthy adult horses from the central Pomeranian region in northern Poland (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. Blood was drawn from the jugular veins of the horses 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 and held on the ice until centrifugation at 3,000 rpm for 5 min. The plasma was removed. A pellet of blood was washed three times in sterile 4 mM phosphate buffer (pH 7.4). Erythrocyte aliquots were used in the study. The pellet of blood was re-suspended in sterile 4 mM phosphate buffer (pH 7.4). A volume of 0.1 ml of the extract obtained from pseudobulbs of *C. pandurata* was added to 1.9 ml of clean equine erythrocytes. After incubation of the mixture at 37°C for 60 min with continuous stirring, it was prepared for TBARS, OMP, and TAC assay. The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reactive substances (TBARS) by Kamyshnikov (2004). To evaluate the protective effects of the extracts against free radical-induced protein damage in the erythrocyte suspension, a content of carbonyl derivatives of protein oxidative modification (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 Levine and co-workers (1990) and as modified by Dubinina and co-workers (1995). 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).

Statistical analysis of the data obtained was performed by employing mean ± standard error of the mean (S.E.M.). All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p > 0.05$). In order to find significant differences (significance level, $p<0.05$) between groups, the Kruskal-Wallis test by ranks was applied to the data (Zar, 1999). All statistical analyses were performed using Statistica 13.3 software (StatSoft, Poland).

Our results revealed that extract derived from the pseudobulbs of *C. pandurata* after incubation with erythrocyte samples caused to increase in the TBARS level (45.05 ± 4.74 nmol·mL^{-1}) (by 25.6%, $p < 0.05$) compared to untreated samples (35.88 ± 3.02 nmol·mL^{-1}). On the other hand, the content of aldehydic derivatives of OMP in the erythrocyte samples after incubation with an extract derived from the pseudobulbs of *C. pandurata* was not altered (30.97 ± 1.23 nmol·mL^{-1} compared to the untreated samples 31.16 ± 1.89 nmol·mL^{-1}). Moreover, the content of ketonic derivatives of OMP in the erythrocyte samples after incubation with extracts derived from the pseudobulbs of *C. pandurata* was non-significantly decreased (by 10.9%, $p > 0.05$). A non-significant increase in the TAC level of the tested samples incubated with an extract derived from the pseudobulbs of *C. pandurata* was observed (54.68 ± 2.69 % compared to the untreated samples 52.83 ± 3.38 %).

In conclusion, the obtained results demonstrated the prooxidative activity of *C. pandurata* extract used in the studied dose (5 mg/mL) on the equine erythrocytes. Our results also showed that extract derived from the pseudobulbs of *C. pandurata* after incubation with erythrocyte samples caused to remaining the TAC level at a high level as compared to the group treated by phosphate buffer (controls), while levels of aldehydic and ketonic derivatives of OMP were un-changed. Future studies will be conducted to evaluate dose-dependent changes in the levels of oxidative stress biomarkers after incubation with extracts derived from *C. pandurata* using various cell models. Moreover, the plant compound profile characteristics and antioxidant activity of different *Coelogyne* plants may encourage the wider use of these orchids in the development of new medicinal substances in medicine and veterinary.

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