hydrophila, Aeromonas salmonicida subsp. salmonicida). Зразки листків було зібрано в Національному ботанічному саду імені М. М. Гришка (НБС, Київ, Україна) та Ботанічному саду Львівського національного університету імені Івана Франка (Львів, Україна). Свіжозібрані листки зважували та гомогенізували в 96%-ному етанолі (у співвідношенні 1:10) при кімнатній температурі. Три штами Aeromonas: Aeromonas sobria (K825), Aeromonas hydrophila (K886), а також Aeromonas salmonicida subsp. salmonicida (St30), виділені з видів прісноводних риб, таких як короп звичайний (Cyprinus carpio L.) та райдужная форель (Oncorhynchus mykiss Walbaum), відповідно, були отримані у Відділі захворювань риб Національного науково-дослідного ветеринарного інституту в Пулавах (Польща). Матеріал для бактеріологічних досліджень було виділено з риб із вираженими клінічними проявами захворювання. Наші результати свідчать про те, що три штами Aeromonas (Aeromonas sobria, Aeromonas hydrophila, Aeromonas salmonicida subsp. salmonicida) виявили резистентність до етанольних екстрактів листків F. lyrata. Діаметр зони інгібування становив (9,50±0,33 мм), (9,38±0,38 мм) і (9,5±0,50 мм) для Aeromonas sobria, Aeromonas hydrophila та Aeromonas salmonicida subsp. salmonicida (St30), відповідно. Екстракт листків F. lyrata 'Bambino' виявив проміжну активність стосовно Aeromonas sobria (діаметр зони інгібування становив 12±0,73 мм); натомість штами Aeromonas hydrophila і Aeromonas salmonicida subsp. salmonicida (St30) виявили резистентність до дії екстракту (діаметр зони інгібування становив 9,18±0,54 мм і 9,13±0,44 мм). Отримані результати є передумовою для створення природних добавок, здатних замінити синтетичні. Відповідно, подільші дослідження, спрямовані на виділення активних сполук та їх фармакологічне дослідження як in vitro, так і in vivo, є, на наш погляд, вкрай необхідними.

Ключові слова: Ficus lyrata, Ficus lyrata 'Bambino', Aeromonas sobria, Aeromonas hydrophila, Aeromonas salmonicida subsp. salmonicida, антимікробна активність, диско-дифузійний метод, етанольні екстракти.

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EVALUATION OF OXIDATIVE STRESS BIOMARKERS LEVELS IN THE EQUINE BLOOD AFTER *IN VITRO* TREATMENT WITH *SANSEVIERIA CAULESCENS* N.E.BR. EXTRACT

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The main goal of present study was to evaluate the level of the 2-thiobarbituric acid reactive substances (TBARS) as lipid peroxidation biomarker, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes' suspension induced by treatment of leaf extracts obtained from

Sansevieriacaulescens N.E.Br. There were no significant changes for TBARS level between the value in the control group and in the equine erythrocytes' suspension after incubation with extracts derived from leaves of S.caulescens(35.88±3.02 μmol/L vs. 35.04±2.31 μmol/L). The level of aldehydic and ketonic derivatives of oxidatively modified proteins was non-significantly changed in the equine erythrocytes' suspension incubated with an extract obtained from the leaves of S. caulescens(31.16±1.89 nmol/mL vs. 29.77±1.17 nmol/mL for aldehydic derivatives, 39.47±2.20 nmol/mL vs. 36.75±1.73 nmol/ mL for ketonic derivatives of oxidatively modified proteins). The antioxidative and prooxidative mechanism of various Sansevieria species in equine erythrocyte suspension will be further studied in detail. Our study suggests that there were no significant changes for TBARS level as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity between values in control group and in the muscle tissue of rainbow trout after incubation with extracts from leaves of S. caulescens. Taking into account existing experimental evidence, it is reasonable to assume that secondary plant metabolites, i.e. polyphenolic compounds in the extract of S. caulescens may contribute to the antioxidant activity. In conclusion, the antioxidative and prooxidative mechanism of various Sansevieria species in equine erythrocyte suspension will be further studied in detail. The obtained information may be useful in the clinical usage of plants in medicine and veterinary. Finally, these findings justify the traditional uses of Sansevieriaplants for therapeutic purposes.

Keywords: **Sansevieriacaulescens N.E.Br., horses, erythrocytes, 2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins, total antioxidant capacity.**

In recent years, the use of natural antioxidants present in various biological materials [i.e. phenolic compounds (flavonoids, phenolic acids, and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids] has attracted considerable interest due to their presumed safety, nutritional and therapeutic value [1]. Antioxidants derived from plant materials, i.e. leaves, fruits, vegetables, spices, and cereals are very effective in the reduction of oxidative stress, increase the body's ability to use free radicals constructively to reduce the risks of chronic diseases and prevent disease progression [12, 31]. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase. Many medicinal plants have been investigated for their beneficial use as antioxidants or source of antioxidants using presently available experimental techniques [21].

In the current study, attention was focused on*Sansevieria*Thunb., a genus with diverse ethnobotanical uses in its geographical distribution range, which occupies an important place among plant genera applied for treatment of a broad spectrum of diseases and disorders [7, 15, 16, 23, 24, 30]. Our previous study [5, 6, 26-28] have highlighted the antibacterial capacity of ten species of *Sansevieria*genus against *Staphylococcus aureus.* These plants have beenscreened in order to validate scientifically the inhibitory activity for microbial growth attributed to their popular use and to propose new sources of antimicrobial agents. Theleavesof*Sansevieriacanaliculata*Carrière, *S. trifasciata*Prain, *S. cylindrica*Bojer ex Hook., *S. parva* N.E.Br. (syn. *S. dooneri* N.E.Br.), *S. fischeri* (Baker) Marais, *S. kirkii* Baker, *S. aethiopica*Thunb., *S. metallica*-Gérôme&Labroy, *S. caulescens* N.E.Br., *S. francisii*Chahin were used. Our results proved that the zones of inhibition ranged from 16 to 34 mm. Extracts from the leaves of *S. fischeri* and *S. francisii* were particularly active against tested organism (inhibition

zones comprise up to 34 mm in diameter). This was followed by the activities of extracts from the *S. parva, S. kirkii, S. aethiopica, S. caulescens, S. metallica* leaves (diameters of inhibition zones ranged from 25 to 31 mm). The ethanolic extracts of *S. canaliculata* and *S. trifasciata* showed less antimicrobial activities (diameters of inhibition zones ranged between 16 and16.5 mm). The results proved that the ethanolic extracts from *S. fischeri, S. francisii, S. parva, S. kirkii, S. aethiopica, S. caulescens, S. metallica* exhibit a favorable antibacterial activity against *S. aureus* [5, 6, 26, 27].

In our previous study, we also studied the antioxidant activity of extracts obtained from leaves of selected species from *Sansevieria* species against oxidative stress using equine erythrocyte suspension [28]. When erythrocytes were incubated with leaf extracts of various *Sansevieria* species, the aldehydic derivatives level was significantly reduced by 13.6 % ($p<0.05$) for *S. forskaliana* extract. Moreover, all extracts (except *S. francisii* extract) reduced the formation of intracellular aldehydic derivatives of oxidatively modified proteins (OMP) in the extracts-treated erythrocytes, but these results were non-significant. Treatment by extracts of various *Sansevieria* species reduced the concentration of ketonic derivatives of OMP when compared to untreated erythrocytes. The most potent effect was demonstrated by the *S. canaliculata, S. forskaliana, S. aethiopica, S. cylindrica, S. metallica, S. hyacinthoides,* and *S. kirkii* compared to control samples (phosphate buffer) (16.1, 14.7, 13.4, 12.9, 12.9, 12.7, 12.1 %, respectively). However, there were no significant changes in other extracts. The experimental evidence obtained in our previous study indicated that various species of *Sansevieria* genus are a rich source of compounds that manifest antioxidant activity and can effectively protect erythrocytes against oxidative-induced damage. Thus, *S. canaliculata, S. forskaliana, S. aethiopica, S. cylindrica, S. metallica, S. hyacinthoides,* and *S. kirkii*may be a valuable source of natural antioxidants that may potentially be recommended for applications in medicine and veterinary practice. According to the above-mentioned antioxidant mechanisms, extracts of various species from *Sansevieria* genus may inhibit the formation of protein carbonyl by scavenging free radicals formed *in vitro*. According to many supporting documents, it can be assumed that secondary plant metabolites, i.e. polyphenolic compounds in extracts of various species from *Sansevieria*genus extract may contribute to the antioxidant activity [28].

The erythrocytes of mammals represent a good model to evaluate the cytotoxicity of molecules, organic and inorganic, natural or synthetic, by cellular damage measure [22]. The erythrocyte could be isolated and handled easily so that they could provide a good model for many assays [2, 9]. Additionally, the high concentration of polyunsaturated fatty acids in RBCs membrane, the high oxygen tension, and redox active hemoglobin molecules [the source of reactive oxygen species in erythrocyte] make them a good biological lipid membrane model especially for screening the oxidative stress conditions induced by various substances [9].

Therefore, the main goal of present study was to evaluate the level of the 2-thiobarbituric acid reactive substances (TBARS) as lipid peroxidation biomarker, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes' suspension induced by treatment of leaf extracts obtained from *Sansevieriacaulescens* N.E.Br.

Materials and methods. ***Collection of plant material.*** The leaves of *Sansevieriacaulescens* plants, cultivated under glasshouse conditions, were sampled at M. M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine (Fig. 1).

Fig. 1. General view of *Sansevieriacaulescens* N.E.Br. plant (Photo by Myroslava Maryniuk)

Preparation of plant extract. Freshly collected leaves were washed, weighted, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in proportion 1:19, w/w) at room temperature. The extract was then filtered and investigated for its antioxidant capacity. The extract was stored at -20°C until use.

Horses. Eighteen healthy adult horses from central Pomeranian region in Poland (village Strzelinko, N54º30'48.0" E16º57'44.9"), 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.

Collection of blood samples. Blood sampleswere taken simultaneously in all horses from the jugular vein in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM). Whole blood was stored insterile tubes with sodium citrate as the anticoagulant and held on the ice until centrifugation at 3000 rpm for 5 min at 4°C using a refrigerated centrifuge to remove plasma. The separated erythrocytes were washed three times in 4 mM phosphate buffer saline (PBS), pH=7.4. After centrifugation, the supernatant and the buffy coat were carefully removed with each wash. Washed erythrocytes were finally re-suspended to the desired hematocrit level in 4mM PBS. The erythrocytes were stored at 4°C and used within 2h of sample preparation. A volume of 0.1 mL of the various extracts was added to 1.9 mL of clean equine erythrocyte suspension. For positive control, PBS was used. After incubation the mixture at 37°C for 60 min with continuous stirring, it was centrifuged at 3000 rpm for 5 min. Erythrocytes aliquots were used in the study.

Quantitative estimation of lipid peroxidation by determination of the 2-thiobarbituric acid reactive substances (TBARS). The most important product of lipid peroxidation reacting with thiobarbituric acid (TBA) is malondialdehyde (MDA) [18]. Therefore, the lipid peroxidation was determined by quantifying the concentration of TBARS by Kamyshnikov (2004) for determining the malonic dialdehyde (MDA) concentration [13]. Briefly, 0.1 mL of erythrocyte suspension was added to 1 mL of 20 % of trichloroacetic acid (TCA) and 1 mL of 0.8 % of 2-thiobarbituric acid (TBA). The mixture was heated in a boiling water bath for 10 min. After cooling, the mixture was centrifuged at 3,000 g for 10 min. The absorbance of the supernatant was measured at 540 nm. The concentration of MDA (μmol per L) was calculated using $1.56\ 10^5\ mM^{-1}\ cm^{-1}$ as the extinction coefficient.

The ***carbonyl derivatives*** content of protein oxidative modification ***(OMP)*** as-

say. To evaluate the protective effects of the extract against free radical-induced protein damage, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the samples was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) [17] and as modified by Dubinina and co-workers (1995) [8].DNFH was used for determining carbonyl content in soluble and insoluble proteins. Briefly, 1 mL of 0.1M DNPH (dissolved in 2M HCl) was added to 0.1 mL of the sample after denaturation of proteins by 20 % trichloroacetic acid (TCA). After addition of the DNPH solution (or 2M HCl to the blanks), the tubes were incubated for a period of 1 h at 37 ^{o}C. The tubes were spun in a centrifuge for 20 min at 3000 g. After centrifugation, the supernatant was decanted and 1 mL of ethanol-ethylacetate solution was added to each tube. Following the mechanical disruption of the pellet, the tubes were allowed to stand for 10 min and then spun again (20 min at 3,000 g). The supernatant was decanted and the pellet washed thrice with ethanol-ethylacetate. After the final wash, the protein was solubilized in 2.5 mL of 8 M urea solution. To speed up the solubilization process, the samples were incubated in a 90^{o}C water bath for 10–15 min. The final solution was centrifuged to remove any insoluble material. The carbonyl content was calculated from the absorbance measurement at 370 nm and 430 nm, and an absorption coefficient 22.000 $M^{-1}\cdot cm^{-1}$. Carbonyl groups (nmol per mg of protein) 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 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 [10]. Sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. Briefly, 0.1 mL of sample was added to 2 mL of 1 % Tween 80 reagent, 0.2 mL of 1 mM $FeSO_4$, and 0.2 mL of 10 mM ascorbic acid. In the blank assay, 0.1 mL of distilled water was used instead of the sample. The mixture was heated in a water bath for 48 hrs at 37^{o}C. After cooling, 1 mL of 20 % trichloroacetic acid was added. The mixture was centrifuged at 3000 g for 10 min. After centrifugation, 2 mL of supernatant and 2 mL of 0.25 % 2-thiobarbituric acid were mixed. The mixture was heated in a water bath at 95^{o}C for 15 min. The absorbance of the obtained solution was measured at 532 nm. The absorbance of the blank was defined as 100 %. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis. The mean ± the standard error of 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 ($p>0.05$).In order to find significant differences (significance level, $p<0.05$) between groups, the the Mann–Whitney U test by ranks was applied to the data [32]. All statistical analyses were performed using Statistica 8.0 software (StatSoft, Krakow, Poland).

Results and discussion. In a present study, we have studied the influence of extracts derived from leaves of *S.caulescens* on the TBARS level as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins and the total antioxidant capacity in the equine erythrocytes' suspension after incubation with extract obtained from leaves of *S. caulescens*in*in vitro* culture. There were no significant changes for TBARS level between the value in the control group and in the equine

erythrocytes' suspension after incubation with extracts derived from leaves of *S. caulescens* (35.88±3.02 μmol/L *vs.* 35.04±2.31 μmol/L) (Fig. 2).

Similarly, the level of aldehydic and ketonic derivatives of oxidatively modified proteins was non-significantly changed in the equine erythrocytes' suspension incubated with an extract obtained from the leaves of *S. caulescens* (31.16±1.89 nmol/mL *vs.* 29.77±1.17 nmol/mL for aldehydic derivatives, 39.47±2.20 nmol/mL *vs.* 36.75±1.73 nmol/ mL for ketonic derivatives of oxidatively modified proteins) (Fig. 2).

The total antioxidant capacity (TAC) determines the ability of a tested material to neutralize oxygen-free radical specific form, irrespectively to the specific antioxidant activity of present antioxidants [29]. Our results showed that extract of *S. caulescens* efficiently increased the TAC level by 1 % ($p>0.05$) due to inhibited the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level (Fig. 2).

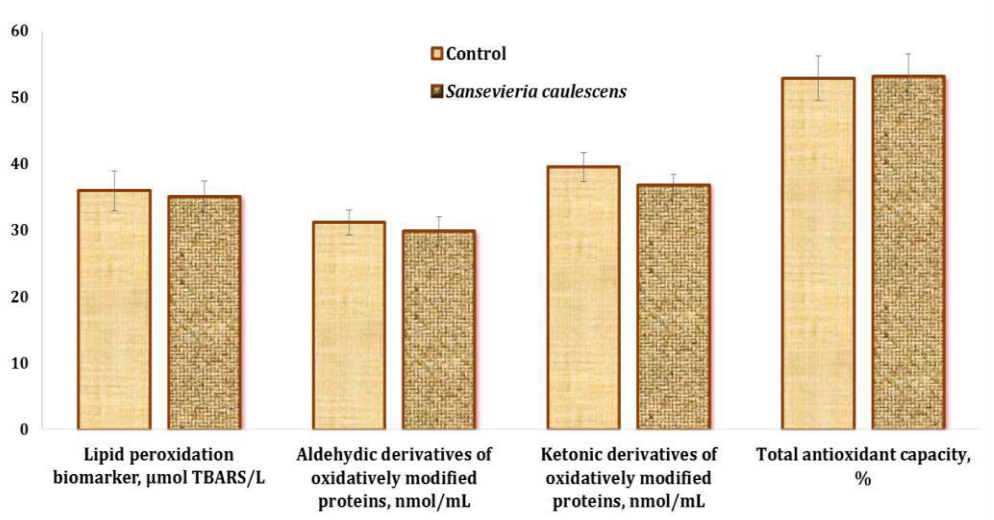

Fig. 2. The level of 2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins andtotal antioxidant capacity in the equine erythrocytes' suspension induced by treatment of leaf extracts obtained from *Sansevieriacaulescens* (M ±m, n=18).

Our similar study suggests that the *S.caulescens* leaf extract has shown good antioxidant potential *invitro* study after incubation with muscle tissue homogenate of rainbow trout [20]. There were no significant changes for TBARS level as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins between values in control group and in the muscle tissue of rainbow trout after incubation with extracts from leaves of *S.caulescens.* Nevertheless, our results showed that extract of *S. caulescens* efficiently increased the total antioxidant capacity in muscle tissue by 46.6 % ($p<0.05$) due to inhibited the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. ***Taking into account existing***experimental***evidence***, it is reasonable to assume that secondary plant metabolites, i.e. polyphenolic compounds in the extract of *S.caulescens* may contribute to the antioxidant activity. In conclusion, the results of this study provide a new perspective on the use of various *Sansevieria*species as a medicinal plant to improve the antioxidant response of rainbow trout. Further studies including the use of other medicinal plants as food additives in aquaculture, the assessment of its antioxidant effects on various tissues are in progress. Finally, research needs to be focused on subjecting fish to these compounds to determine their effectiveness, stability, and impact both on the host and on the environment [20].

In our previous study we have evaluated the lipid peroxidation biomarkers and total antioxidant capacity in the muscle tissue of rainbow trout (*Oncorhynchus mykiss*-Walbaum) under incubation with extracts derived from the leaves of various *Sansevieria* species, aimed at the further improving methods for preventing and treating fish diseases by increasing the natural resistance of fish organism using antibacterial and antioxidant agents in aquaculture [20]. The most potent antioxidant effect was demonstrated for the extracts of *S. caulescens, S. suffruticosa, S. hyacinthoides, S. canaliculata, S. aethiopica, S. gracilis,* and *S. parva* as compared to phosphate buffer control (46.6, 66.8, 77.3, 49.8, 71.1, 63.4, 39.4 %, respectively). The results showed that extracts of *S. hyacinthoides* and *S. aethiopica* efficiently increased the total antioxidant capacity in rainbow trout muscle tissue (Maryniuk et al., 2017). Among plant extracts screened for *in vitro* antioxidant properties in rainbow trout muscle tissue, the strongest toxicity responses were exhibited by *S. cylindrica, S. canaliculata, S. trifasciata, S. metallica* extracts [20].

On the other hand, many studies clearly demonstrate that various plants of *Sansevieria* genus are effective agents in the treatment and prevention of many diseases and disorders. For instance, Maheshwari and co-workers (2017) have studied the antioxidant and antiproliferative activities of *Sansevieriaroxburghiana*Schult. and Schult. f. methanol extract and its fractions [19]. Significant antioxidant and anti-proliferate activity were detected in the ethyl acetate fraction. Ethyl acetate fraction showed prominent scavenging activity in 1,1-diphenyl-2-picrylhydrazyl, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and nitric oxide antioxidant assays with a concentration yielding 50 % inhibition (IC_{50}) 15.33 ± 1.45, 45.3 ± 1.93 and 48.43 ± 0.46 mg/ml, respectively. Cytotoxicity of ethyl acetate fraction was the highest among other fractions against HCT-116, HeLa, and MCF-7cancer cell lines with IC50 values 16.55 ± 1.28, 12.38 ± 1.36, and 8.03 ± 1.9 μg/ml, respectively, by MTT assay and 15.57 ± 0.70, 13.19 ± 0.49, and 10.34 ± 0.9 μg/ml, respectively, by SRB assay. The presence of gallic acid in the ethyl acetate fraction of *S. roxburghiana* rhizomes was confirmed by HPLC and HPTLC analysis. Results of Maheshwari and co-workers (2017) suggested that the ethyl acetate fraction exhibited effective antioxidant and antiproliferative activities. The phenolic compounds identified in the ethyl acetate fraction could be responsible for the activities [19].

The antioxidative and antimutagenic capacities of the methanolic extracts derived from the leaves and rhizomes of*S. cylindrica*and*S. trifasciata*have been assessed by Karamovaand co-workers (2016). This study was focused of the following parameters: inhibitory activity onlipid peroxidation, suppressing ability on direct-acting mutagensodium azide-induced mutagenesis in*Salmonella typhimurium*cells [14].The preliminary phytochemical screening of leaf extracts of *S. cylindrica* revealed the presence of various compounds such as phenols, alkaloids, saponins, flavonoids, fatty acids and coumarins [25].

Sansevieriaroxburghiana rhizome has been claimed to possess antidiabetic activity in the ethnomedicinal literature in India. Therefore, experiments of Bhattacharjee and co-workers (2016) were carried out to explore the protective role of an edible (aqueous) extract of *S. roxburghiana* rhizome (SR) against experimentally induced type 2 diabetes mellitus (T2DM) and its associated cardiomyopathy in Wistar rats [4]. SR was chemically characterized by GC-MS analysis. Antidiabetic activity of SR (50 and 100 mg/kg, orally) was measured in high-fat diets (ad libitum) + low-single dose of streptozotocin (35 mg/kg, intraperitoneal) induced type 2 diabetic (T2D) rat. Phytochemical screening of the crude extract revealed the presence of phenolic compounds, sugar alcohols, sterols, amino acids, saturated fatty acids within SR. T2D rats exhibited

significantly ($p<0.01$) higher fasting blood glucose level with respect to control. Alteration in serum lipid profile ($p<0.01$) and increased levels of lactate dehydrogenase ($p<0.01$) and creatine kinase ($p<0.01$) in the sera revealed the occurrence of hyperlipidemia and cell destruction in T2D rats. T2DM caused significant ($p<0.05$-0.01) alteration in the biochemical markers in the sera. T2DM altered the redox status ($p<0.05$-0.01), decreased ($p<0.01$) the intracellular NAD and ATP concentrations in the myocardial tissues of experimental rats. While investigating the molecular mechanism, activation PKC isoforms were observed in the selected tissues. T2D rats also exhibited an upregulation in nuclear NF-κB (p65) in the cardiac tissues. So, oral administration of SR (50 and 500 mg/kg) could reduce hyperglycemia, hyperlipidemia, membrane disintegration, oxidative stress, vascular inflammation and prevented the activation of oxidative stress-induced signaling cascades leading to cell death. Histological and ultrastructural studies of cardiac tissues supported the protective characteristics of SR. Therefore, findings of Bhattacharjee and co-workers (2016) suggest that SR could offer protection against T2DM and its associated cardiotoxicity *via* multiple mechanisms viz. hypoglycemic, antioxidant and anti-inflammatory actions [4].

Moreover, *S. roxburghiana* rhizome exhibited remarkable antitumor activity in Swiss mice that are plausibly attributable to its augmenting endogenous antioxidant mechanisms. Haldar and co-workers (2010) have evaluated the hydroalcoholic extract of *S. roxburghiana* rhizome (HASR) for antitumor activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice [11]. Twenty-Four hours after intraperitoneal inoculation of tumor (EAC) cells in mice, HASR was administered at 50 and 100 mg/kg body weight for nine consecutive days. On day 10 half of the mice were sacrificed and rest were kept alive for assessment of the increase in life-span. The antitumor effect of HASR was assessed by evaluating tumor volume, packed cell count, viable and non-viable tumor cell count, median survival time and increase in life-span of EAC bearing hosts. Hematological profiles and serum biochemical parameters were estimated. Further, antioxidant properties were assessed by estimating lipid peroxidation, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). HASR showed a significant ($p<0.001$) decrease in tumor volume, packed cell volume and viable cell count and increased the life span of EAC bearing mice. Hematological and serum biochemical profiles were restored to normal levels in HASR treated mice as compared to EAC control. HASR treatment significantly ($p<0.001$) decreased lipid peroxidation and recovered GSH, SOD, and CAT towards normal as compared to EAC control [11].

Andhare and co-workers (2012) have studied the antiallergic activity of ethanolic extract of *Sansevieriatrifasciata* (EEST), the plant eliciting analgesic, anti-inflammatory, and antipyretic activity, on various animal models as well as *in vitro* conditions [3]. Ethanolic extract of *S. trifasciata* leaves (EEST) was prepared by cold maceration followed by concentration and evaporation under reduced pressure on a rotary evaporator to obtain semisolid mass. The various phytoconstituents were analyzed. The acute toxicity study of EEST was carried out in mice. The antiallergic and anaphylactic activities were evaluated using animal models *viz.* milk induced eosinophilia and leukocytosis, compound 48/80 induced mast cell degranulation, active and passive cutaneous anaphylaxis, and histamine-induced pedal edema. In addition, EEST effect on Shultz-Dale reaction in sensitized guinea pig ileum in ex vivo and antioxidant activity by free radical scavenging by DPPH method (*in vitro*) were also studied. EEST treatment at 100 mg/kg and 200mg/kg p.o. inhibited (a) milk-induced increased eosinophilia, leukocytosis, monocytes, and neutrophils. (b) Prevented passive cutaneous and active anaphylactoid reactions. (c) Prevented compound 48/80 induced degranulation of sensitized mesenteric mast cells. (d) Inhibited histamine-induced pedal edema formation

significantly. EEST pretreatment inhibited Shultz-Dale reaction in guinea pig ileum and also elicited potent antioxidant activity. Experimental findings of Andhare and co-workers (2012) have demonstrated promising antiallergic and anti-anaphylactic activity of EEST and also elicited potent antioxidant activity. The antiallergic and anti-anaphylactic activity might be due to inhibition of release of chemical mediators from mast cells largely by phytoconstituents like steroidal saponins, triterpenoids, and flavonoids present in EEST [3].

Conclusions. Our study suggests that there were no significant changes for TBARS level as biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity between values in control group and in the muscle tissue of rainbow trout after incubation with extracts from leaves of *S. caulescens*. Taking into account existing experimental evidence, it is reasonable to assume that secondary plant metabolites, i.e. polyphenolic compounds in the extract of *S. caulescens* may contribute to the antioxidant activity. In conclusion, the antioxidative and prooxidative mechanism of various *Sansevieria* species in equine erythrocyte suspension will be further studied in detail. The obtained information may be useful in the clinical usage of plants in medicine and veterinary. Finally, these findings justify the traditional uses of *Sansevieria*plants for therapeutic purposes.

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ОПРЕДЕЛЕНИЕ IN VITRO УРОВНЯ БИОМАРКЕРОВ ОКИСЛИТЕЛЬНОГО СТРЕССА В СУСПЕНЗИИ КРОВИЛОШАДЕЙ ПОСЛЕ ВОЗДЕЙСТВИЯ ЭКСТРАКТА SANSEVIERIA CAULESCENSN. E.BR.

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Основная цель данного исследования состояла в том, чтобы на экспериментальной модели окислительного стресса (суспензии эритроцитов лошадей) оценить уровень веществ, реагирующих с 2-тиобарбитуровой кислотой (TBARS), в качестве маркеров липидной пероксидации, альдегидных и кетоновых производных окислительно-модифицированных белков, общую антиоксидантную способность после воздействия in vitro экстракта листьев Sansevieria caulescens N.E.Br. Существенных отличий в значениях уровней TBARS в контроле и в суспензии эритроцитов лошадей после инкубации с экстрактами листьев S. caulescens (35,88±3,02 мкмоль/л vs. 35,04±2,31 мкмоль/л) отмечено не было. Уровень альдегидных и кетоновых производных окислительно-модифицированных белков в суспензии эритроцитов лошадей, инкубированной с экстрактом листьев S. caulescens, также существенно не изменился (31,16±1,89 нмоль/мл vs. 29,77±1,17 нмоль/мл для альдегидных производных, 39,47±2,20 нмоль/мл vs.

36,75±1,73 нмоль/мл для кетоновых производных окислительно-модифицированных белков). Антиоксидантные и прооксидантные механизмы действия экстрактов листьев различных видов Sansevieria в суспензии эритроцитов лошадей требуют дальнейшего тщательного исследования. Проведенное исследование свидетельствует об отсутствии достоверных изменений уровня ТБАР как биомаркера перекисного окисления липидов, альдегидных и кетоновых производных окислительно модифицированных белков, а также общей антиоксидантной емкости между значениями в контрольной группе и в мышечной ткани радужной форели после инкубации с экстрактами из листьев S. caulescens. Принимая во внимание имеющиеся экспериментальные данные, можно предположить, что вторичные растительные метаболиты, т. е. полифенольные соединения в экстракте S. caulescens, могут вносить вклад в антиоксидантную активность. В заключение следует отметить, что антиоксидантный и прооксидантный механизм различных видов Sansevieria в суспензии эритроцитов лошадей будет подробно изучен в дальнейшем. Полученные сведения могут быть полезны при клиническом использовании растений в медицине и ветеринарии. Наконец, эти результаты оправдывают традиционное использование Sansevieriaplants в терапевтических целях.

Ключевые слова: Sansevieria caulescens N.E.Br., лошади, эритроциты, вещества, реагирующие с 2-тиобарбитуровой кислотой (TBARS), альдегидные и кетоновые производные окислительно-модифицированных белков, общая антиоксидантная способность

ВИЗНАЧЕННЯ IN VITRO РІВНІВ БІОМАРКЕРІВ ОКСИДАЦІЙНОГО СТРЕСУ В СУСПЕНЗІЇ КРОВІ КОНЕЙ ПІСЛЯ ОБРОБКИ ЕКСТРАКТОМ SANSEVIERIACAULESCENSN.E.BR.

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Основна мета цього дослідження полягала в тому, щоб на експериментальній моделі окиснювального стресу (суспензії еритроцитів коней) визначити рівень речовин, що реагують з 2-тіобарбітуровою кислотою (TBARS), як маркерів ліпідної пероксидації, альдегідних та кетонових похідних окиснювально-модифікованих білків, загальну антиоксидантну здатність, індуковану обработкою екстрактом листків Sansevieriacaulescens N.E.Br. Істотних відмінностей у значеннях рівнів TBARS у контролі та в суспензії еритроцитів коней після інкубування з екстрактами листків S. caulescens (35,88±3,02 мкмоль/л vs. 35,04±2,31 мкмоль/л) виявлено не було. Рівень альдегідних та кетонових похідних окиснювально-модифікованих білків в суспензії еритроцитів коней, інкубованій з екстрактом листків S. caulescens, також істотно не змінився (31,16±1,89 нмоль/мл vs. 29,77±1,17 нмоль/мл для альдегідних похідних, 39,47±2,20 нмоль/мл vs. 36,75±1,73 нмоль/мл для кетонових похідних окиснювально-модифікованих білків). Антиоксидантні та прооксидантні механізми дії екстрактів листків різних видів Sansevieria в суспензії еритроцитів коней потребують подальшого ретельного дослідження. Проведене дослідження свідчить про відсутність достовірних змін рівня ТБАР як биомаркера перекисного окислення ліпідів, альдегідних і кетонових похідних окисно модифікованих білків, а також загальної антиоксидантної ємності між значеннями в контрольній групі і в м'язовій тканині райдужної форелі після інкубації з екстрактами з листків S.

caulescens. Беручи до уваги наявні експериментальні дані, можна припустити, що вторинні рослинні метаболіти, тобто поліфенольні сполуки в екстракті S. caulescens, можуть вносити вклад в антиоксидантну активність. На закінчення слід зазначити, що антиоксидантний і прооксидантний механізм різних видів Sansevieria в суспензії еритроцитів коней буде детально вивчений у подальшому. Отримані відомості можуть бути корисні при клінічному використанні рослин в медицині і ветеринарії. Нарешті, ці результати виправдовують традиційне використання Сансевієріаплантатів в терапевтичних цілях.

Ключові слова: Sansevieria caulescens N.E.Br., коні, еритроцити, речовини, що реагують з 2-тіобарбітуровою кислотою (TBARS), альдегідні та кетонові похідні окиснювально-модифікованих білків, загальна антиоксидантна здатність.

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ФОРМУВАННЯ ФУНКЦІОНАЛЬНИХ ОЗНАК МОЛОДНЯКУ УКРАЇНСЬКОЇ ЧОРНО-РЯБОЇ МОЛОЧНОЇ ПОРОДИ ЗА РІЗНИХ ТЕХНОЛОГІЙ УТРИМАННЯ ТА СЕЗОНУ НАРОДЖЕННЯ

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Технологія вирощування ремонтного молодняку великої рогатої худоби полягає у створенні таких умов утримання, які б сприяли розвитку бажаних якостей, кращому використанню тваринами поживних речовин корму більш інтенсивному росту і розвитку організму.

У статті наведено результати дослідження до удосконалення вирощування теличок української чорно-рябої молочної породи в ДП ДГ «Гонтарівка» Інституту тваринництва НААН Вовчанського району Харківської області. Було сформовано дві групи теличок зимового (по 12 голів) і весняного сезону народження (по 14 голів) у кожній, вік тварин 4 місяці. Першу групу утримували безприв'язно (дослідна група), другу (контрольна) – прив'язно.

Встановлено, що фактичне споживання кормосуміші залежно від періоду досліджень і групи теличок варіювало незначно. Телички дослідної групи, за їх утримання у групових секціях безприв'язно мали більшу живу масу за перший місяць досліду на 6,0 кг або 5,1 % ($p \leq 0,05$), за другий та третій місяць на 10 кг або 7,3 % та 13 кг або 8,2 % ($p \leq 0,05$), щодо одноліток, яких утримували прив'язно.

Подібна закономірність спостерігалась і в межах по другого періоду досліджень, за впливу весняного сезону народження теличок і системи утримання на живу масу.

Врахування фактичного споживання кормів та їх залишків дало змогу розрахувати витрати корму на 1 кг приросту живої маси, які в дослідній групі в перший період досліду варіювали від 4,14 до 4,17 кг корм. од. другий – від 3,32 до 3,74 кг корм. од., у контрольній групі відповідно – від 4,83 до 5,12 кг корм. од (перший період) та від 4,32 до 4,74 кг корм. од. (другий період).