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RESEARCH ARTICLE

Medicinal plants and their role for the production of effective biological preparations to reduce the ecotoxicity of the animal body by heavy metals using the example of a plant *Eleutherococcus senticosus*

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Abstract

Contamination of the biosphere and its components of air, soil, water, plants and animals by heavy metals has become widespread. In Ukraine, the war led to the destruction of oil depots, hydraulic structures, solid waste landfills, sewage treatment plants, testing of various types of weapons, which only exacerbated the environmental problem of heavy metal pollution. In the post-war period, agricultural enterprises will concentrate their attention on the production of ecologically safe cow's milk on cattle farms, the agro-ecosystems of which are subject not only to increased technogenic influence, but also to mines and explosions. Heavy metals incorporated with feed cause chronic intoxication, affect the kidneys, liver and other organs and systems of the body. Medicinal plants with proven antidote properties and new biotechnological methods of their introduction into the body are a way to solve the problem that scientists are working on. The aim of the research was to evaluate the antidote properties of medicinal plants as part of a phytopreparation using the example of *Eleutherococcus senticosus* to reduce the intoxication of the body of dairy cows with cadmium and lead. Experiments were conducted on dairy cows with different types of feeding. Twins of analogues selected by the method were divided into 3 groups. The first control and two experimental ones. The diet included feed with a high concentration of cadmium and lead. In the second experimental group, the animals were fed only the antitoxic premix, and in the third, the effect of the premix was enhanced by the injection of a phytopreparation from the extract of medicinal plants, among which *Eleutherococcus senticosus* 15 ml in 100 ml of the drug. Biometric data processing was carried out in the STATISTICA software package version 10.0. For each sample, the average value of the trait in the sample (M), Standard Deviation (SD) was calculated. The estimate is given as M ± SD. Differences between mean values were considered statistically significant at $p<0.05$. The animals of all experimental groups were fed with feed that exceeded the permissible standards of environmental safety in terms of cadmium and lead content. The use of the extract of the medicinal plant *Eleutherococcus senticosus* in the composition of the phytopreparation enhanced the antidotic properties of both the herbal preparation itself and the fed mineral-vitamin premix in the cows of the third experimental group. The concentration of toxic metals in milk, blood and internal organs of animals of this group has decreased significantly. The effect of pollutant accumulation was clearly visible in the body of the cows of the first control groups. Upon completion of the experiment, it was possible to produce milk that met both domestic quality and environmental safety standards, as well as the requirements of Regulation

(EC) No. 853/2004 and No. 1881/2006). The extract of the medicinal plant *Eleutherococcus senticosus* as part of the phytopreparation acts as an antidote, protects the animal body from the toxic effects of heavy metals Cd, Pb, Cu and Zn, enhancing the elimination of xenobiotics from the body. A complex of antidotes, a premix and a phytopreparation contribute to the production of environmentally safe milk with a concentration of heavy metals within the permissible norms of eco-safety. Further research is aimed at the analysis of other medicinal plants included in the phytopreparation for their antidote, protective, phytomorphological and biochemical properties in relation to the toxic effects of heavy metals.

Keywords: Extract, *Eleutherococcus senticosus*, Ecotoxicant, Antidote, Pollutants

Introduction

The ecological situation regarding environmental pollution of atmospheric air, soil, and water resources by heavy metals in various countries of the world has not improved in recent years, but on the contrary has worsened and continues to sharpen the attention of scientists. Scientists (Yajuan Tang, et al., 2024) are investigating changes in the physical and chemical properties of the soil, enzymatic activity, the structure of microbocinosis and the chemical composition, concentration of chromium, cadmium, copper, and zinc due to pollution with polyethylene terephthalate. Other researchers (Zhiwei Liang, et al., 2024) pay considerable attention to phytoremediation, which is recognized as a sustainable solution to mitigate soil pollution by heavy metals, but faces problems in the transition from laboratory experiments to practical field applications. Human activities have led to numerous pollution problems, among which heavy metal pollution poses significant problems for the natural environment. In Ukraine, the situation has been complicated by hostilities, the use by the aggressor of various types of weapons, toxic rocket fuel, the destruction of industrial infrastructure, oil depots, solid waste landfills, hydroelectric dams, mining of agricultural land, so after the war, the state of agro-ecosystems will require special attention, including ecological production safe milk at livestock enterprises for keeping cattle, conducting organic and biological agriculture (Portiannyk, 2023; Mamenko & Portiannyk, 2021).

In response to these challenges, the interest of scientists in finding sustainable solutions has increased. Phytoremediation is a promising approach due to its environmental friendliness, economic efficiency, ability to immobilize pollutants. Plants with high biomass are hyperaccumulators of heavy metals and are used for various purposes, such as the purification of wastewater from pig farms (Eder Carlos Lopes Coimbra et al., 2023). A certain amount of heavy metals with feed or drinking water will still get into the body of dairy cows, then into milk products, then into the human body, which poses a risk to the health of both animals and humans. Chronic cadmium intoxication (nephrotoxicity, hepatotoxicity, immunotoxicity, osteotoxicity) causes oxidative stress in liver and kidney cells, damage to them and DNA, which can lead to carcinogenesis and cancer (Liu et al., 2009). So we chose the path of finding effective medicinal plants, the biogenic substances of which, in the composition of phytopreparations made from their extracts or in the composition of feed additives, acted as antidotes, which could be used on a permanent basis to prevent the occurrence of cadmium and lead toxicosis in animals.

The purpose of the research is to evaluate the antidote properties of medicinal plants as part of a herbal preparation using the example of *Eleutherococcus senticosus*.

Literature Review

Scientific and economic experiments were carried out in livestock enterprises of the biogeocenosis, which are subject to increased anthropogenic load not far from the industrial city, places of extraction and processing of oil and gas, large motor highways. Experiments were conducted on dairy cows. 126 cows were selected with silage-hay-concentrate type of feeding, 63 with silage-hay, 36 with silage-root and 195 with silage-hay type of feeding, respectively. The animals, which were selected by the analog method, were divided into three groups: the first control group and the second and third experimental groups. The main ration of feeding animals of all groups contained feed with an increased concentration of heavy metals cadmium and lead. The animal feed of the second group included a mineral-vitamin premix of antitoxic effect, and in the third experimental group, the effect of the premix was enhanced with an antidote phytopreparation called "BP-9", which means a biological preparation made from the extract of 9 medicinal plants, including Eleutherococcus senticosus. 100 ml of herbal preparation contains 15 ml of *Eleutherococcus senticosus*. The dose

of herbal preparation "BP-9" is 20 ml/day. The frequency of administration of the drug is 5 times a month (once every 6 days). The biological preparation was made in sterile conditions of the production laboratory according to the method (Hmel'nic'kij et al., 1994; Sokolov et al., 2002). The duration of the experimental period is 120 days. Cows were in the same conditions of feeding and maintenance.

Laboratory analysis of samples of plant and animal origin: fodder, blood, internal organs and tissues, urine, milk for the content of macro- and microelements, including heavy metals, was carried out by the method of atomic absorption spectrophotometry (spectrophotometer AAS-30) (Pryce, 1972). Quality and ecological safety control of milk was carried out according to DSTU 3662-97, as well as taking into account the requirements of international quality standards (Regulation (EU) No. 853/2004 and No. 1881/2006) (Mamenko et al., 1997).

All animal manipulations were performed in accordance with the European convention for the protection of vertebrate animals used for experimental and scientific purposes (Strasbourg, 1986).

The analysis of biometric data was performed taking into account the characteristics of the obtained results: the size of the sample, the type of data distribution, the nature of variances. For each sample, the average value of the trait in the sample (M) and Standard Deviation (SD) were calculated, the assessment is given as M ± SD. Differences between mean values were considered statistically significant at $p<0.05$. The calculation was carried out in the STATISTICA program package version 10.0 for the Windows 7 operating system.

The content of Pb in cows with a silage-root type of feeding exceeded the permissible concentrations and amounted to 28.59 mg/kg in cereal-legume hay, 13.62 in wheat straw, 13.51 in corn silage, 16.78 in alfalfa hay, 12.22 in fodder beet, and 20.51 mg/kg in corn grits, respectively, Cd -0.96 mg/kg, 0.71 mg/kg, 0.69 mg/kg, 0.75 mg/kg, 0.64 mg/kg, 0.96 mg/kg, respectively. In cows fed with silage and hay, the concentration of Pb in alfalfa hay was 11.22 mg/kg, wheat straw 12.5, corn silage 9.54, fodder beet 17.21, and barley straw 11.6, respectively, Cd –0 .57 mg/kg, 0.51 mg/kg, 0.39 mg/kg , 0.45 mg/kg, 0.75 mg/kg, 0.54 mg/kg, respectively. The content of Pb in cows with silage-hay type feeding was 16.51 mg/kg, cereal-legume hay 18.5, corn silage 15.5, oat straw 23.12, pea straw 23.51, respectively, Cd –0 .63 mg/kg, 0.6 6 mg/kg , 0.48 mg/kg, 0.54 mg/kg, 0.75 mg/kg, 0.78 mg/kg, respectively. The concentration of Pb in alfalfa hay, 36.51 in corn silage, 14.23 in alfalfa hay, 18.51 in corn grits, and 20.53 in pea grits, respectively, in cows with silage-hay concentrate feeding was 16.52 mg/kg, 69 mg/kg , 0.75 mg/kg, 0.42 mg/kg, 0.48 mg/kg, 0.54 mg/kg, 0.63 mg/kg, respectively.

Fodder beets had the highest level of contamination compared to all other fodder, as they accumulate heavy metals from the soil mainly in the root part of the plant. In fodder that was part of the silage-hay type diet, in addition to exceeding the maximum permissible concentrations of Cd, Pb, Cu, and Zn, a high zinc content in oat and pea grains was recorded, on average 6.3 times-6.8 times. The highest content of Cd and Pb among the rest of the fodder was distinguished by pea grit, and Cu by cereal-legume hay (3.9 times). The fodder used in feeding cows with silage-hay-concentrate type of feeding had the highest contamination, which exceeded the permissible norm for Pb by 7.3 times, Zn by 7.8 times, and Cu by 4.1 times. In terms of Cd contamination of fodder, this ration takes the last place together with the ration where the animals were fed fodder according to the silage-hay type of feeding. Cereal-legume hay had the highest content of Cd, Pb, and Cu among the forages in the diet, and corn grain accumulated the most Zn.

Heavy metals from the gastrointestinal tract were absorbed into the blood. The content of Cd in the blood of cows of the first control group with silage-root type of feeding was 98.34 nmol/l ± 1.03 nmol/l. The antitoxic mineral-vitamin premix added to the diet of the cows of the second experimental group blocked the absorption of the element, as indicated by a decrease in the concentration to 79.11 ± 2.90 according to $p<0.01$ in comparison with the control group (n=5). Subcutaneous injection of a plant phytopreparation with an extract of *Eleutherococcus senticosus* in the third experimental group enhanced the antidote effect of the premix, blood analysis recorded the concentration of Cd in the blood at the level of 49.19 ± 2.41 $p<0.01$. A similar situation was observed for Pb – 8.32 μmol/l ± 1.65 μmol/l, 3.02 ± 0.99 ($P<0.01$) and 1.98 ± 0.16, respectively, for $p<0.01$ in comparison with the first control group (n=5). We obtained similar results regarding the content of Cd and Pb in the blood, against the background of chronic intoxication of the body of dairy cows with toxic heavy metals and the use of a premix and a plant biological preparation in experiments with other types of animal feeding. The concentration of Cd in the blood of cows of the first control group fed with silage and hay was 101.20 nmol/l

± 3.17 nmol/l, the second – 54.29 ± 2.64 p<0.01 and the third – 40.72 nmol/l ± 1.98 nmol/l p<0.01, respectively, and lead – 6.54 μmol/l ± 0.45 μmol/l, 4.01 ± 0.64 p<0.01, 1.38 ± 0.28, p<0.01, respectively; silage-hay - the concentration of Cd in the blood of the animals of the first control group was 81.17 nmol/l ± 0.60 nmol/l, the second – 48.19 ± 0.73 p<0.01 and the third – 41.61 ± 1.05 p<0.01, respectively, the concentration of Pb in the blood was 5.74 μmol/l ± 0.32 μmol/l, 2.07 ± 0.16 p<0.01, 1.87 ± 0.09 p<0.01, respectively, and by the silage-hay-concentrate type of feeding, respectively, the first – 77.94 nmol/l ± 0.99 nmol/l, the second – 40.64 ± 0.54 p<0.01 and the third – 32.14 nmol/l ± 0.55 nmol/l p<0.01 (Cd) and the first – 4.63 μmol/l ± 0.37 μmol/l, the second – 1.72 ± 0.17 p<0.01, the third – 1.27 ± 0.22 p<0.01 (Pb) respectively, under the physiological norm of Cd in the blood at the level of 20 nmol/l-50 nmol/l, and Pu up to 2 μmol/l. The sample of animals is the same five. All comparisons of blood test results obtained in the second and third experimental groups with the first control group.

Thus, the concentration of toxic metals Cd and Pb in the blood of cows of only the third experimental group, where the antidote effect of the premix was enhanced by the injection of a herbal preparation with an extract of Eleutherococcus senticosus, was within the physiological norm, regardless of the type of feeding. The silage-hay-concentrate diet contributed to the normalization of Cd and Pb in the blood of animals and the second experimental group to the limits of physiological norms. Positive anti-toxic, the protective effect of the biological preparation and its plant components is obvious.

The mammary gland does not perform a barrier function with regard to heavy metals.

Chemical analysis of selected milk samples (n=5) showed an increased content of Cd in the milk of animals of the first control groups with different types of feeding 0.087 mg/kg ± 0.008 mg/kg silage-root type of feeding, 0.09 ± 0.085 silage-hay, 0.068 ± 0.017 silage-hay and 0.053 ± 0.019 silage-hay-concentrate at the rate of 0.03 mg/kg. Similarly, according to the content of Pb - 1.835 mg/kg ± 0.093 mg/kg silage-root, 1.641 ± 0.253 silage-hay, 1.734 ± 0.148 silage-hay and 1.794 ± 0.165 silage-hay-concentrate at the norm of 0.02 mg/kg The antitoxic effect of the herbal preparation with *Eleutherococcus senticosus* extract in the third experimental groups of cows enhanced the antitoxic effect of the premix fed to the animals, the protective functions of the body improved, the concentration of toxic heavy metals decreased not only in the blood of the animals of these groups, but also in the milk. It was possible to produce milk from cows of only the third experimental groups with silage-root 0.018 mg/kg ± 0.002 mg/kg (Cd), 0.014 ± 0.003 (Pb) and silage-hay 0.012 mg/kg ± 0.002 mg/kg (Cd), 0.014 mg/kg ± 0.004 mg/kg (Pb) type of feed that met domestic and international environmental safety standards. The degree of probability compared to the data of the control group is P<0.01.

The results obtained in the experiment show that the toxic effect of Pb and especially Cd on the body of dairy cows is difficult to reduce without the use of additional biotechnological methods. The antitoxic premix and phytopreparation with *Eleutherococcus senticosus* extract developed by us and comprehensively applied in scientific research gave a good result as a whole, acting as antidotes. A significant role belongs not only to the mineral elements and vitamins included in the antidote premix, but also to the well-chosen medicinal plants from which the extract was made, in particular, an important role, in our opinion, belongs to the medicinal plant *Eleutherococcus senticosus*. An acute experiment (n=3) confirmed the accumulation of pollutants in the animals of the first control groups, where antidotes were not used P<0.01, which is generally characteristic of heavy metals and a significantly lower concentration of cadmium, lead, copper, zinc in the kidneys and liver of cows of the second and third experimental groups groups p<0.01. The best indicators were recorded in experimental animals of the third experimental groups, where the herbal preparation was used. It is obvious that the antidote effect of the biological preparation from the extract of medicinal plants increased the elimination of pollutants with excrement and in particular with urine, restoring the homeostasis of the body of cows with different types of feeding.

Thus, the use of a biotechnological technique based on two antidotes, a specially developed premix and a biological preparation from 9 medicinal plants, one of which is an extract of *Eleutherococcus senticosus*, ensured the production of ecologically safe cow's milk, which met the environmental safety parameters of both the domestic standard DSTU 6262-97 and the Regulation (EU) No. 1881/2006 by the content of Cd and Pb (<0.01), contributed to the improvement of physiological functions and the state of health of the intoxicated organism of animals as a whole.

Discussion

Golan scientists found that the root of *Eleutherococcus senticosus* (*Acanthopanax senticosus*) protects living organisms from temperature shock and the toxic effects of some heavy metals, including cadmium and copper. (Gutyj et al., 2016) prove the toxic effect of cadmium on a number of organs and systems. The cardiovascular, reproductive, excretory, especially kidneys and liver, respiratory, musculoskeletal system are affected. Toxicants cause osteodystrophy, hematopoiesis. Researchers indicate the most dangerous carcinogenic and mutagenic effects of cadmium (Gutyj et al., 2016). We also checked in an experiment the antidote effect to toxic metals of the water extract of this plant in scientific and economic experiments on milk-producing cows. The results show the high efficiency of the developed herbal preparation with the extract of this plant, which was manifested in a stable decrease in the content of cadmium and lead in the milk of cows of the third experimental groups, where the effect of the mineral-vitamin premix was enhanced by the injection of the herbal preparation, and the improvement of the quality of the ecological safety of the milk as a whole $p<0.01$. The root of *Eleutherococcus* thorn contains polysaccharides, heteroglycans, which have an immunostimulating effect, eleuterosides A-G (daukosterol, coumarin glycoside, syringaresinol, etc.), I-M (saponins), etc. substances, and the plant itself is derived from coumarin, flavonoids, more than seven *eleuterosides*. The active biogenic substances of the plant cause a multifaceted effect on the body: they stimulate the central nervous system, increase motor activity and conditioned reflex activity, increase the basic metabolism, adaptogenic properties of the body, reduce blood sugar, increase appetite, have gonadotropic properties, stimulate the adrenal glands. *Eleutherococcus senticosus* as part of the biological preparation "BP-9" had a positive effect on geodynamic indicators, helped the body to adapt to stress, cold, as well as the influence of incorporated ecotoxicants of heavy metals, as evidenced by a decrease in their concentration in the liver and kidneys at the end of the experiment in the animals of the third experimental groups $p<0.01$. Like other plants, the extract of the herbal preparation *Eleutherococcus senticosus* stimulates the immune system, especially its lymphocytic link. *Eleutherococcus senticosus* is recommended for use at low blood pressure, as well as prevention of viral infections, which is important in the minds of epidemics or pandemics, the outbreak of which has recently increased. In order to ensure the safe use of *Eleutherococcus senticosus* extract as part of a herbal preparation, a professional approach from technologists and veterinary specialists, scientists is necessary, since the plant has many contraindications. The extract should not be used during animal corpulence, high blood pressure, obesity, rapid pulse or excitement. These contraindications were taken into account during the preparation of the herbal preparation and when using it, but the antidote (protective) effect of this plant against the toxic metals cadmium, lead, copper and zinc is not easy to replace with another type of plant. Such tasks of the biotechnological development of the drug with the selection of more effective antidote medicinal plants are facing us now, as well as many other scientists, including the methods of their introduction into the body. In particular, scientists (Svetlana Ivanova et al., 2024) analyzing global trends note the interest of scientists in phytogenic feed additives, phytobiotics, which are economically effective due to their positive impact on animal health. Plant feed additives can be added to feed in dried form or in the form of extracts, which affects the reduction of the use of synthetic antibiotics and growth stimulants, we also consider and do not exclude the possibility of using such biotechnological methods, but for the antidote, antitoxic effect in the experiment, injection is more effective. For prolonged preventive purposes, adding extract or dried plants to the diet is also possible, but requires additional research. Additional research will be required and the introduction of new medicinal plants into the composition of the phytopreparation with the verification of antidote properties in a new experiment. Scientists (Svetlana Ivanova et al., 2024) also emphasize that the introduction of phytobiotics has a positive effect on the body of farm animals, protecting them from pathogenic microorganisms, antitumor and immunostimulating effects, and improving productivity. The market of phytobiotics is rapidly developing, and the search for alternatives to antibiotics and hormonal drugs, and in our case, the presence of antitoxic action necessitates the development of new phytobiotic formulations and the search for new plant sources of such additives. The nature of Ukraine is rich in various types of medicinal plants, the problem is to quickly check their biogenic effect in the conditions of a complex scientific and economic experiment on farm animals.

The use of feed additives based on phytobiotics without restrictions has significant prospects, that is, farmers can use them in the diets of animals on a permanent basis, throughout the entire period of production of the corresponding type of agricultural products. Such phytobiopreparations must be well studied by scientists and guarantee safety for animals, consumers of animal husbandry products, and the natural environment, which is also what our research is aimed

at. (Svetlana Ivanova et al., 2024) emphasize the phytobiotics of the future when there is a need to combine phytobiotics with other means to improve bioavailability, prolong the action of active substances, we do not exclude the combination with antitoxic properties of phytobiopreparations (phytobiotics) with mineral-vitamin premixes for the production of environmentally friendly safe livestock products and maintenance of healthy cows in livestock enterprises. Studies (Donghun Lee et al., 2012) on rats prove the effectiveness of oral administration of *Eleutherococcus senticosus* to protect against the death of neurons in the hippocampus region of the brain, explaining this effect by the anti-inflammatory properties of the active substances of the plant and define the effect of the drug as neuroprotective. Enhances removal of cadmium and lead pollutants from blood, milk, and internal organs. Normalizes blood pressure, which is very important for dairy cows that are exposed to the toxic impact of pollutants, xenobiotics, and the immunostimulating effect, resistance to stress and stimulating effect on the adrenal glands contributed to the restoration of the metabolism of mineral substances in the body at the same time as strengthening resistance to diseases, including of viral origin, which the body of a productive animal is quite often subjected to. The results of the experiment showed a high antidote role in the composition of the phytopreparation of this plant *Eleutherococcus senticosus*.

Conclusions

The extract of the medicinal plant *Eleutherococcus senticosus* as part of the phytopreparation acts as an antidote, helps protect the body of dairy cows from the harmful effects of toxic heavy metals, not only Cd and Cu, but also Pb. Increases the elimination of xenobiotics from the body. Enhancing the effect of the premix, it contributes to the production of environmentally safe milk with a concentration of pollutants within the permissible norms of environmental safety.

Further research is aimed at an in-depth analysis of other medicinal plants included in the phytopreparation for their anti-toxic, protective, phytomorphological and biochemical properties against the action of toxic metals, improvement of the plant composition of the preparation and techniques for its effective use in animal husbandry.

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