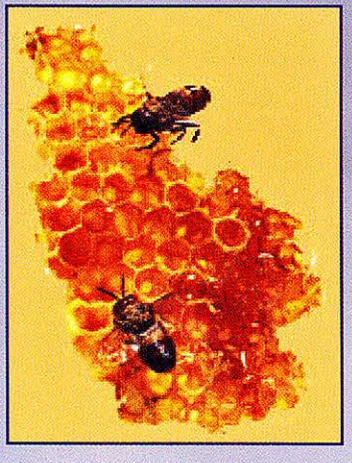
Volume 3

FUNCTIONAL FOODS FOR CHRONIC DISEASES



Advances in the Development of Functional Foods

Edited by Danik M. Martirosyan, Ph.D.

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FUNCTIONAL FOODS FOR CHRONIC DISEASES

Advances in the Development of Functional Foods

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ECDYSTEROIDS: USAGE IN MEDICINE, SOURCES, AND BIOLOGICAL ACTIVITY (REVIEW)

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ABSTRACT

In this paper, the actual level of scientific investigations on ecdysteroids is reviewed: fields of application, medical importance, major representatives, sources, and biological activity. Historical retrospection views and the achieved research levels are shown while the world flora screening, identification of most active formulas, and study of practical application possibilities. It is emphasized that chemically isolated ecdysteroids are extremely expensive and asked for mainly in science intensive investigations. To satisfy the mass demand for ecdysteroids in the pharmaceutical industry, non-purified or weakly purified plant formulations from super producer species with null toxicity rate that do not require very expensive processing technologies have good prospects.

In the case of Russia, cultivating Rhaponticum carthamoides (Willd.) Iljin and Serratula coronata L. plants. Regarding the former species, there has appeared an industrial growing technology and a new class of pharmaceutical preparations from its aboveground shoots is being developed. The effective biological activity rate of extracts from Rhaponticum carthamoides grown with a special technology in agropopulations accounts for 10-11…10-13 M, that is 3-4 orders of magnitude higher than the activity rate of highly purified individual ecdysteroids (0.5-10 microgram/kg against 5-50 milligram/kg).

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MEDICAL SIGNIFICANCE

One of the most important achievements of science in the past few years is providing

technologies on the plant-synthesized ecdysteroids' usage to control the growth and development parameters of different life organisms. In addition to well-known adaptogenic and immuno-modulating properties of ecdysteroid-containing preparations used in medicine (http://insectsciense.org/3.7), this last discovery becomes even more significant and urgent for people's health. Presenting ligands for intracell and membrane receptors, their regulating elements, ecdysteroids have an ability to change the homeostasis processes in an organism, influencing cell growth, differentiation, and programmed death, production of specific products of metabolism.

In practical medicine, ecdysteroid-containing formulations are applied to prevent illness and preserve the immune status of healthy people [1-3]; they take an important part in sport, cosmic, and military medicine as adapting and work efficiency increasing drugs under limiting factors, e.g. when overcoming extreme physical and psychical efforts [4-6]. They are usable for the acceleration of regeneration of tissues human organs and skin, to increase the hair growth, cure wounds, ulcers, and burns; they improve sexual function, stimulate libido and obviate difficulties in sexual life [7-12].

The ecdysteroid molecules (Fig. 1), presenting a group of lipophilic poly-hydroxylated steroids, participate in the life activity sustainability of practically all classes of organisms, while fulfilling numerous functions. The question on their role in living nature is still open. Certain is only the fact that one of the major ecdysteroid representatives, *20-hydroxyecdysone, makisterone C* and *25-deoxyecdysone*, is a true molting hormone of arthropoda (insects and crustaceans) and initiates changes that occur at different developing stages from larva to chrysalis and then to a grown-up insect [13, 14].

Since appeared several thousand million years ago, ecdysteroids have participated in a complex co-evolution pathway of ecosystems development and adaptation to the environment. The presence of ecdysteroids is characteristic, together with flowering plants, of such ancient organisms as ferns, mushrooms, mosses, algae, gymnosperms [15-16].

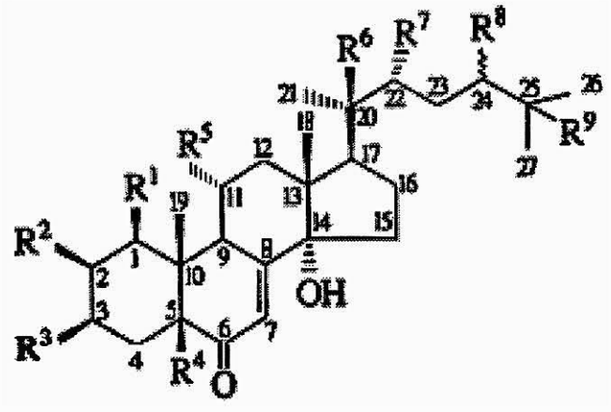

Figure 1. The structural formula of ecdysteroids

In the 60-ies years of XX century, the discovery of colossal amounts of molting hormones in plants (million times higher than in insects) caused a great scientific sensation. This discovery was proposed to contribute to an ecologically safe and quite effective method for the insect population control. Nevertheless, as the more detailed research has shown, most of insects are resistant to

phytoecdysteroids (http://www.sciteclibrary.ru/rus/catalog/pages/4723.html) or learnt how to detoxify the hormones [17-19] penetrating into their organisms through the mouth and started synthesis of their own zooecdysteroids (ecdysones) using other metabolic pathways differing from those of plants.

However, a 20-year-old investigation work in a field of cell and molecular biology, ecological genetics and physiological sciences has led to even more significant discoveries: ecdysteroids represent natural and absolutely safe ligands in molecular systems of gene switching [20-22]; mechanisms on ecdysone- (ecdysteroid-) induced gene expression systems like those existing in insect cells are applicable for mammals, including humans [23-26].

The importance of the last discoveries becomes greater in the post-genome medical era. Upon completion of the human genome library sequencing, it is proposed that switch genes will allow switching off cells which produce organism-destroying structures (e.g. cancerous growth) and bringing to a stop diseases, incurable by conventional treatment modes (many inherited diseases) [27-28]. Analogically, it will be possible to implant and point wise switch on genes not present in the host cells but responsible for producing target therapeutic agents, as well as to set off the regeneration factors of damaged tissues [29].

Though the new directions on ecdysteroid application seem to be quite unusual, ecdysone-induced systems are not only created and patented but also realized for commercial purposes (http://www.invitrogen.com). Moreover, important aspects on clinical application of ecdysteroids are their participation in numerous non-genome effects. Despite the mechanisms on interconnection of ecdysteroids with membrane receptors as signal molecules, which activate secondary messengers, have been just recently put under investigation [28, 30], ecdysteroid-containing preparations are broadly used in practical medicine when curing cardio-vascular, nervous, and reproduction system diseases, whatever disorders of the whole homeostasis processes [1-2, 31-32].

That is why, for today we need those sources of ecdysteroid molecules or ecdysteroid-related compounds that would act in small quantities, be highly active, non-toxic, resistant to decomposing influence, quickly removable from the organism, cost effective, and could be produced in large dimensions [3, 25, 33].

ALL-IMPORTANT ECDYSTEROIDS

The first investigations on ecdysones aimed at the isolation of insect hormones, which began in the early 30's, were conducted by German scientists. In 1954, they managed to isolate 25 mg of weakly purified substance from 500 kg of silkworm chrysalides (*Bombix mori*) and to crystallize it [34]. In 1963, as its general structure was discovered, *α-ecdysone* was related to steroids (with molecular weight M=464). In 1965, the molecular structure of *α-ecdysone* was determined by the X-ray structure analysis [35-36]. These studies themselves were familiar only to a limited number of specialists and, possibly, the things would not have changed for a long time, if it were not for the concurrence of circumstances elicited a great interest and big investments in the investigations dealing with the world flora screening and research of new molecules' properties.

The discovery of ecdysones in plants happened by a lucky chance when the scientist Karel Slama (Czecho-Slovakia) went to the USA to cultivate a soil insect *Pyrrhocoris apterus* L. on filter paper. Here he was surprised – the insect metamorphosis was disturbed, and he could not obtain puration in the last larval stage. The key to explaining this phenomenon lay in filter paper:

in that case it was produced from balsam fir (*Abies balsamea*). Other papers did not influence the metamorphism. The extraction procedure allowed for isolation of juvabione, structurally related to a juvenile hormone, which has selective effects on this single insect. Testing any other plants revealed that they contain numerous compounds with insect hormone activity. The biotest method was later modified in B_{II}-biotest and to date is broadly used for the primary screening of vegetal ecdysteroids, together with the radio-immune analysis (RIA).

Taking into account the economical and biological importance of phytoecdysones, the last three decades were characterized by making significant efforts by screening the world flora to discover species producing most ecdysones, identify most active formulations, and to study possibilities on practical usage of ecdysones in different biology and medicine fields.

Ponasterone was a first phytoecdysterone (fig. 2) isolated and described in 1966 by the Japanese scientists from the conifer *Podocarpus nakaii* [37]. Afterwards, it was identified in the affined species *Podocarpus macrophyllus* and *Podocarpus reichei* with concentration 100 g/kg dry wt, and in the yew-trees *Taxus canadasis, T. chinensis, T. cuspidata* (50-80 mg/kg). In the late 70s and early 80s, *ponasterone* was detected in cancroids, while in 1995 in flat-cap mushrooms (*Paxillaceae*) with concentration up to 50 mg/kg.

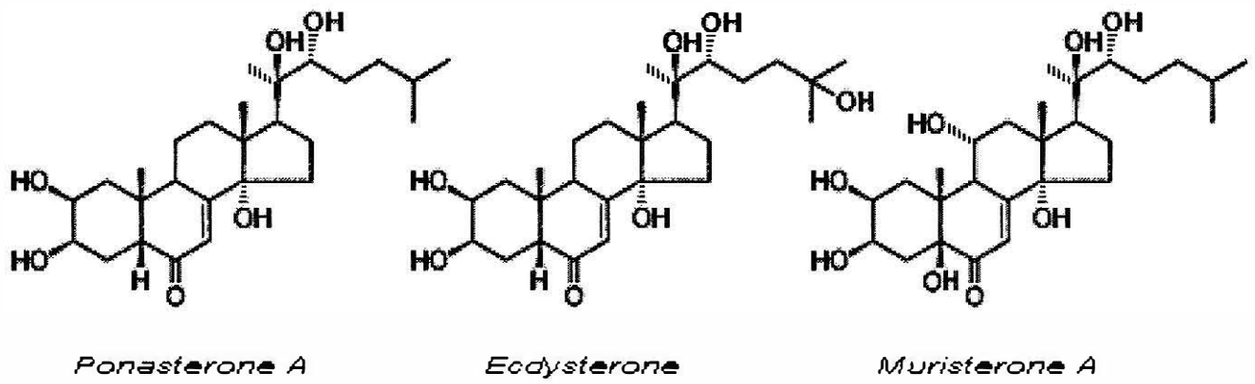

Figure 2. Ecdysteroids, found wide practical application

Ecdysterone (β-ecdysterone, 20-hydroxyecdysone, 20E) was first isolated in 1966 from the crustacean *Jasus calandei* with a quantity of 2 mg/t and, therefore, named *crustedysone* [38]. Then, it was found in insects, silkworms *Bombix mori* and *Antharea pernyi*, and extracted with 200 mg 31 kg chrysalides [39]. In the same year the structure was assigned, and *ecdysterone* was isolated from conifers and ferns: first 50 mg/kg of *Podocarpus elatus,* further 10 g/kg *Polipodium vulgare* rhizomes.

Lately, *ecdysterone* was found in most plants [40], including cereals (maize *Zea mays*) and crucifers (*Arabidopsis thaliana).* The concentrations differ by 1 million to 1 milliard times (20-300 ng to 20-30 g/kg). One of the main sources of reception on ecdysterone industrial production is the perennial plant *Rhaponticum carthamoides* (Willd.) Iljin (*Leuzea carthamoides* DC.) and *Serratula coronata* L., induced in different regions of Russia.

Muristerone A, the most active, rare and extremely expensive ecdysteroid for today, was found in 1972 by the German scientists in endemic plants' seeds, genus *Ipomoea* [41], which occupy southern slopes of the Himalayas. The *Ipomoea* presents the most mysterious ecdysteroid source. Since the discovery of *muristerone A*, more than 1.5 thousand articles have been published on various aspects of its research but only some very first papers mention the source. It

appeared because the *Ipomoea* nomenclature is highly looped: it can mean completely diverse plants, often endemic [42]. Only 30 years later, there occurred new messages on *muristerone* isolation from sequoia-trees, not scientifically proven yet (http://www.sequoiasciences.com).

ECDYSTEROID SOURCES

3.1. *Plants.* Actually, ecdysteroids were identified to compose not only higher flowering plants but also gymnosperms, ferns, mushrooms, algae, mosses, as well as insects, crustaceans, and nematodes. According to the last investigations, almost all terrestrial and water higher plants have ecdysteroid-synthesizing genes [15-16, 40-43].

Today we know the structure of more than 310 ecdysteroid molecules (www.ecdybase.org). Besides, angiosperms exceed in the diversity of kinds of ecdysteroids they contain. Among all various ecdysteroid molecules the mammals contain, the following three: *ponasterone A*, *muristerone A*, and *ecdysterone* are most active. The first two ecdysteroids do not occur in the higher flowering plants. *Ponasterone A* is present in single ferns (including eagle fern – *Pteridium aquilinum*), mushrooms of the *Paxillaceae* family (*Paxillus atrotomentosus*), and relict plants of the *Podocarpaceae* and *Taxaceae* families. *Muristerone A* is typical of the *Ipomoea* genus (morning-glory) from the *Conovolvulaceae* family. Less active *ecdysterone* is wide-spread among flowering plants.

Plants possess ecdysteroids in the form of water-soluble conjugates: conjugates with inorganic acids – sulphates, phosphates; conjugates with organic (carbon, fatty, phenolic) acids – acetates, benzoates, cumarates; conjugates with sugars – glucosides, galactosides, xylosides etc.

Apart from the above-mentioned main ecdysteroids, all the studied objects contain in trace amounts other structure analogues and their derivates (the so called minor ecdysteroids) numbering to 30-40 and more units. Some endemic and rare species, as well as those growing in specific ecological and geographical conditions, include ecdysteroids of unusual or abnormal structure, which do not occur in the most studied objects. The 90s are notable for isolation of ecdysteroids with new structures (*polyporusterone A...G*) from the Chinese bracket fungus *(Polyporus umbellatus)* in a quantity 0.1-3.0 mg/kg [44-45]. It was also in this period when a new type of ergostane ecdysteroids (*paxillosterone, atrotosterone, malakosterone)* and their derivates from the mushrooms *Tapinella panuoides* and *Paxillus atrotomentosus* were obtained [46-47].

None of the mammal species contains ecdysteroids. The artificial chemical synthesis is only possible in the case of the secondary, biologically inactive or weakly active products *via* chemical transformation of major ecdysteroids. For this purpose, *ecdysterone* is most often used [1]. Just recently the artificial photochemical transformation method was discovered allowing for structures being uncharacteristic of chemical transformation, e.g. dimers [48].

By the origin, it is accepted to divide ecdysteroid sources into phyto-, zoo-, and myco-ecdysteroids (i.e. plants; insects, cancroids, nematodes; mushrooms). Zooecdysteroids cannot be used for industrial production because of their utterly low concentrations in anthropoda. The value of this or that plant or mushroom species as a raw material source depends on its uniqueness grade formed by such indices as the biological activity, end use, concentration in biomass, availability, cost expediency [49].

The most important ecdysteroid sources for industrial isolation are plants, which, by their ability to biosynthesize ecdysteroids, can be classified as follows (on a dry weight basis):

I. 1-30 g/kg (0.1-3.0 %) – superconcentrator species;
II. 0.1-1 g/kg (0.01-0.1 %) – species with high ecdysteroid content;
III. 10-100 mg/kg (0.001-0.01 %) – species with mean ecdysteroid content;

IV. 0.5-10 mg/kg (0.00005-0.001 %) – species with low ecdysteroid content;
V. 0.1-0.5 mg/kg and less – species with trace ecdysteroid content.

In general, the differences among ecdysteroid concentration levels in plants are huge values – 8-9 orders (from 20-300 ng/kg to 20-30 g/kg). Normally, it is an extremely small value (thousandths and hundredths of a per cent on a dry weight basis). But there are plants which single organs in a restricted age and vegetation diapason can concentrate essential amounts of ecdysteroids. On average, among several thousands of other species there is one concentrator species. The species Rhaponticum carthamoides (Willd.) Iljin and Serratula coronata L. belong to the most important ecdysteroid-containing plants serving as industrial ecdysteroid sources. These species are considered to be highly promising in developing new classes of pharmaceutical preparations and biologically active food additives, and also ecologically safe products against pests [2].

Among angiosperms there is an insignificant number of other species with high content of ecdysteroids (mainly ecdysterone) in single organs, which are of interest for scientific purposes. Detailed investigations of the European northeast flora [43] showed the distribution of ecdysteroid-containing plants by taxons corresponding with the analogous distribution in other regions. The presence of these plants was identified in most species, whereas only 4% of them, represented by species with mean and high ecdysteroid content, developed a positive response in the radio-immune activity biotest. These data agree with the works done by other scientists where essential ecdysteroid concentrations belonged to 5-6% of plants.

The species of secondary importance in the Russian flora are: some Silene and Lychnis varieties; Coronata flos-cuculi L.; Helleborus purpurascens and Helleborus caucasicus; Paris guadrifolia L.; Ajuga reptans; Sagina procumbens L.; Potamogeton natans and Potamogeton perfoliatus; Pulmonaria officinalis; Butomus umbellatus; Androsace filiforms etc.

Unfortunately, all these plants have some negative features, which do not make their industrial use possible. The main limiting factor is that they are difficult of access, grow in scattered groups or lonely, only wild and cannot be cultivated. Often they are low-height, creeping, rosulate, forest, meadow or water plants, and poisonous or weakly toxic. They are met with in flood land thickets of meadow shrubs, forest edges and felled areas, peat-bogs, waste plots of land, along road and trench sides, at riverbanks and lake coasts or hill foots at high places. The life strategy of these plants is combined growing together with other species under the forest canopy, within meadows or as ruderal plants at cultivated fields. In most cases, introduction was not tried at all or represents serious difficulties.

3.2. ***Methods of biotechnology.*** Since isolation and purification of ecdysteroids from plant biomass is a complicated procedure heightening the prime cost of the end products, there have developed ecdysteroid production technologies using biotechnological methods (cultures of cells, tissues, and transformed roots). Since ecdysteroid-biosynthesizing genes are present in all plant organs, callose cultures (cultures multiplying in the artificial nutritional medium) can be obtained from every quick-growing tissue: seed-lobs, hypocotyl, leaves, shoots, buds, and roots. Ecdysterone and some other secondary important components of genera Ajuga, Serratula, Rhaponticum, Pteridium, Polypodium can be synthesized in cell culture. However, most active ecdysteroids, muristerone and ponasterone, cannot be synthesized in artificial conditions.

On the whole, ecdysteroid content in cell culture is significantly lower than that in nature. A long-term cultivation lowers total content and changes the proportion ratio between individual compounds. Moreover, not identified inactive ecdysteroids are synthesized. Ecdysteroid synthesis in suspension cultures has somewhat higher results than in cell culture, but these results are instable and ecdysterone

concentration increases very slowly [15].

The most promising biotechnology method is a transformed roots' culture. Inoculating sterile seedlings by the Agrobacterium rhizogenes strains causes an infection and agrobacterial transformation of roots to a hairy form. In case with Rhaponticum carthamoides the characteristic roots and swellings appear a month ago, they start spontaneous regeneration of modified plants. Within 4 weeks, hairy roots increase their mass by 4-6 times. However, ecdysteroid fraction in culture, 0.02-0.03 % in total, differs from that of natural plant roots [50].

The method of ecdysteroid production via hairy roots culture has its positive and negative features. Its advantage, compared to field culture, consists in a continuous reproduction source, high growth and regeneration rates of specimens; there is no need in external growth hormones as in the case with cell and tissue cultures. The nutrition source is saccharose. The method is applicable in respect to many cultures, modified roots are characterized by a high growth rate and genetic stability, large concentrations of secondary metabolites, comparable with that of natural plants. In addition to phytoecdysteroids, the secondary metabolites of hairy roots also involve alkaloids, polyacetilene compounds, glycosides, polyphenols, tannins, flavonoids, saponins etc.

However, the commercial use of such systems is restricted due to significant defects, which find a detailed treatment in the assay of Giri and Narasu [51]. The main restrictions arise from need for specially designed patterns of bioreactors with automatic control models to provide a free vertical culture development; these cultures need careful selection of optimal nutritional medium, temperature, and illumination conditions. One of the most important parameters, optimal roots morphology, influencing the density and aeration degrees of specimen is difficult to meet because many of various morphologies existing are in connection with different plasmid strains.

Uniform aeration and intermixing procedures also provide certain difficulties, which lead to stagnant zones and fermentation, tissues necrosis and vital capacity loss. The end product accumulation is inhibited by its concentration saturation. It is necessary to filtrate and renew the liquid medium constantly. Also there are some difficulties appearing by crop collection and treatment, i.e. partially root removing from the reservoir etc.

Generally, ecdysteroid production by the biotechnology methods did not find large acceptance. Modified secondary ecdysteroids obtained by methods of biotechnology comprise less ecdysteroid activity indices, compared to nature. Therefore, such systems are used only to get chemically pure ecdysteroids. Besides, as the world experience readily shows, it is not enough just to have an ecdysteroid, it is to be highly active already in minimal concentrations, as muristerone A and ponasterone A are. Otherwise, chemically pure substances do not meet a ready sale.

ACTIVITY

4.1. ***Chemically pure ecdysteroids.*** The activity rate of isolated ecdysteroids is evaluated by biotesting with insect cells containing natural ecdysteroid receptors (EcR). Ponasterone A, muristerone A, and ecdysterone are regarded to be most active ecdysteroids with large practical use possibilities. Each of them can show different results with different receptors, but their initial activity rates are generally the same comprising 10-9 (10-8...10-10) M [52]. There are other ecdysteroids with 5, 6, 7 or even 8 OH-groups but relatively less active in isolated form. The following decreasing activity series was obtained: muristerone A, ponasterone A, polypodine B, 20E, 22-acetate 20E, 2-deohy-20E [20]. Concerning minor components some derivatives from

muristerone (kaladasterone) and ajugasterone (dachryhainansterone) appeared active in biotests [53].

The activity rate of ecdysteroids in a real organism essentially differs from that in cell cultures, the doses are tissue-specific. Ecdysone-induced systems have effective doses of muristerone A and ponasterone A as single ligands in transgenic mice being equal to 10-5...10-7 M. Scientists give more preference to muristerone despite it is a rare and expensive substance ($ 120-135 for 1 mg). The ponasterone use is complicated because of its instability: after 3 hours the receptor complex decays by 50 % in buffer solution, whereas for muristerone the figures lie by 5 % [54].

Concerning ecdysterone, though the biotests on insect cells revealed a significantly high bioactivity rate of 10-8 M [20], the ecdysone-induced systems are 2-3 orders of magnitude less in results. The activity rate of other ecdysteroids, polypodine B, ecdysterone, inokosterone, makisterone, is even more less, while α-ecdysone, 2-deoxyecdysone, 20-deoxyecdysterone, 22-acetate-ecdysterone do not have it at all [25].

Apart from EcR, none of the steroid receptors can interact with isolated ecdysteroids as ligands in mammal cells [26]. This situation seems to be a satisfactory one because it allows for avoiding negative unforeseen side effects when using ecdysteroids as switch genes in the ecdysone-induced systems.

The anabolic activity of ecdysteroids, including the ability for protein synthesis inhibition or stimulation, was experimentally proven: a) in substances with insufficient purification degree (95 % and less); b) substances isolated from the producer plant Rhaponticum carthamoides; c) via unclear activation mechanisms with secondary agents [55-58]. Numerous experiments in the fields of cell and molecular biology with individual highly purified compounds (99 %) and other ecdysteroid sources, e.g. Serratula coronata [59-60], did not detected any signs of ecdysterone, muristerone, and ponasterone anabolic activity without secondary agents.

To activate gene transcription, hybrid ecdysteroid/retinoid receptors (EcR/RxR) and their modifications with other nuclear receptors are applied, where the RxR-partner is necessary to stabilize the heterodimer complex and to provide fixation of response elements before activation of gene expression mechanisms. In a living organism both ecdysteroid agonists and ligands of the heterodimer complex second partner (retinoid receptor) can act together, which enlarge their biological activity diapason significantly [74]. Moreover, the effective doses decrease to 10-9...10-10 M under condition of a local treating a target organ [24].

The aforesaid is true only for highly purified (more than 98-99 %) formulations isolated from the primary sources. The artificial systems on their basis are extremely expensive and used mainly for scientific purposes. Mass ecdysteroid production in pharmaceutical industry can use non-purified or weakly purified plant formulations from non-toxic superproducer species, which do not require high-expensive ecdysteroid-possessing technologies.

Note: Chemically pure ecdysteroids are obtained according to the following sequential procedures: raw material fining, extraction and extract's concentration by evaporation, watering, filtration, accompanying hydrophobic substances' re-extraction by hydrocarbon solvent, ballast compounds' precipitation, phytoecdysteroids extraction from water portion with evapoconcentration, chromatographic purification in column with aluminum oxide, eluate evapoconcentration, selective elution of ecdysteroids from sorbent material with solvent and resulted fractions' concentration, re-crystallization and vacuum drying after dissolution in metanole and evapoconcentration, or filtration after freezing at -40 to -70°C.

4.2. ***Non-purified Rhaponticum carthamoides formulations.*** Chemically isolated ecdysteroid fraction (91 %, including 75 % 20-hydroxyecdysone) extracted from the overground Serratula coronata shoots demonstrated a complex and ambiguous "dose-effect" modulation activity (also called two-phase operation) in the spontaneous E-rosette-forming biotest within the concentration limits 10-4...10-12 M [61]. The effective immuno-modulating activity rate of CD2+-rosette forming with human T-lymphocytes was achieved by a concentration value of 1 μM (10-6 M) and a stimulation index of 1.132 [62].

Natural ecdysteroid-containing substances can be significantly more active than chemically isolated ecdysteroids. Cultivating lymphocyte populations in vitro in presence of the Rhaponticum carthamoides extract [63] can cause proliferation of spleen cells in concentration 10-13...10-14 M, if 20-hydroxyecdysone. The nonspecifically activating ConA (T-mytogene) and LPS (B-mytogene) agents stimulate proliferation up to 10-15 M (Fig. 3).

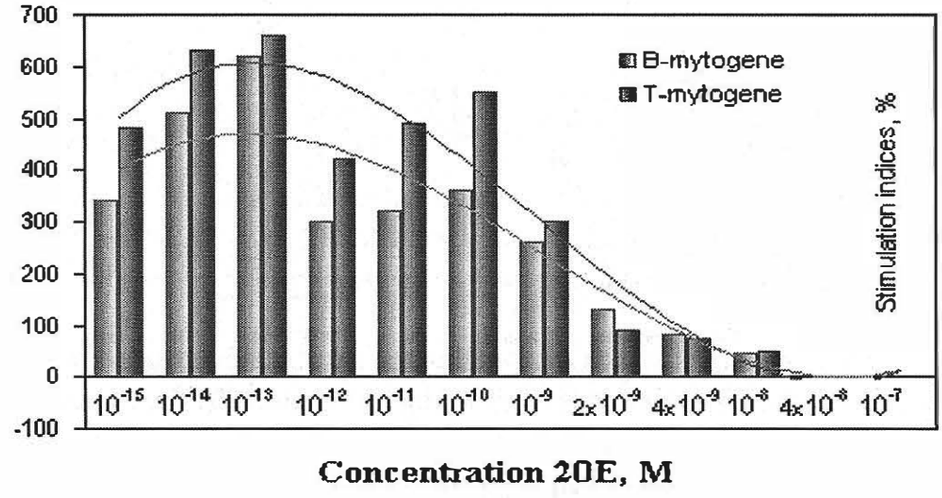

Figure 3. Cell proliferation stimulation by *Rhaponticum carthamoides* extract (according to [63] changed)

At present, a new class of ecdysteroid-containing pharmpreparations with super-low doses of active substances is being worked out. The new preparations are produced from the overground *Rhaponticum carthamoides* shoots grown according to a special technology in agropopulations [64-66]. The crude drug used in production allows decreasing today doses – by 3-4 orders of magnitude, if *20-hydroxyecdysone* [67]. For example, effective doses of the *Bioinfusin, BCL-PHYTO (BCL – BactoCelloLactin), Lipolite* and *Rapontik* pharmpreparations account for 0.5-10.0 microgram/kg biomass (10^{-12}...2×10^{-13} M), for *ecdysterone* [2, 68, 74]. This is not a mistake or misprint because an average daily dose of chemically pure *20-hydroxyecdysone* and preparations on its basis is 5-50 milligram/kg body weight [13, 55, 58-60, 69-71].

The action mechanism specificity of new preparations lies in a stimulating activity of small doses and inhibiting effects on proliferative organism processes in large doses. Even one-time introduction of such drugs can cause essential immuno-stimulating effects on cell and humoral levels [68-72]. The seven-day-long course of treatment allows for considerable immuno-stimulating aftereffects, which last for 30 days (Fig. 4). Besides, non-purified ecdysteroid formulations possess a stable industrial anabolic effect when used for mass industrial production [73] (Fig. 5).

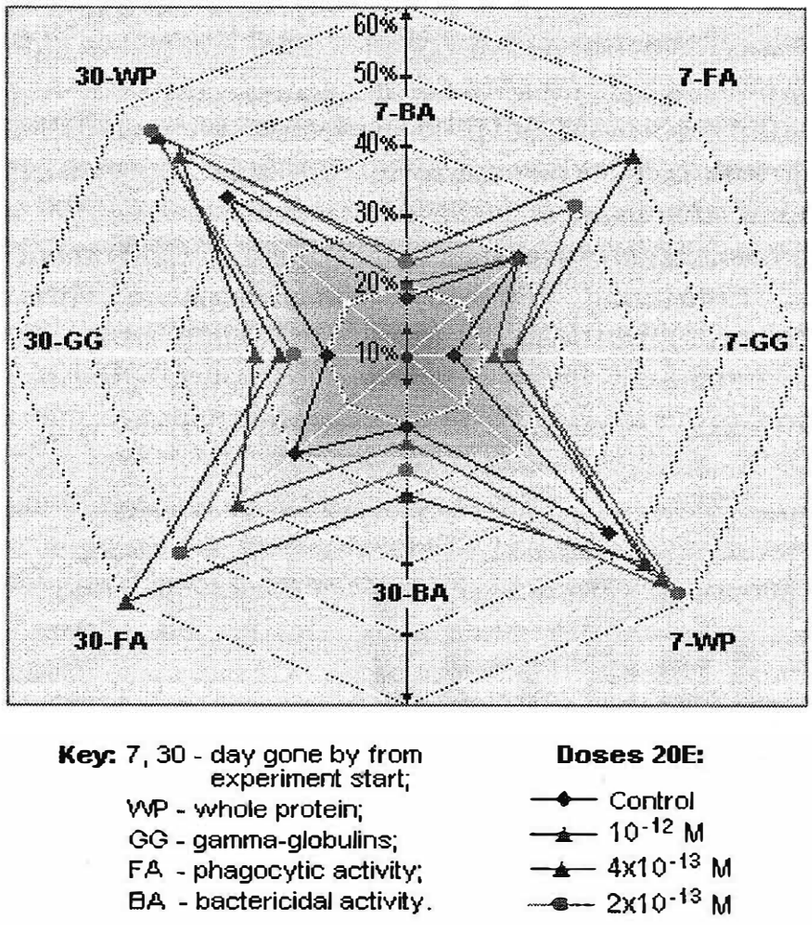

Figure 4. Immuno-modulating effect of the "Bioinfusin" preparation (course – sevenfold introduction; according to [68])

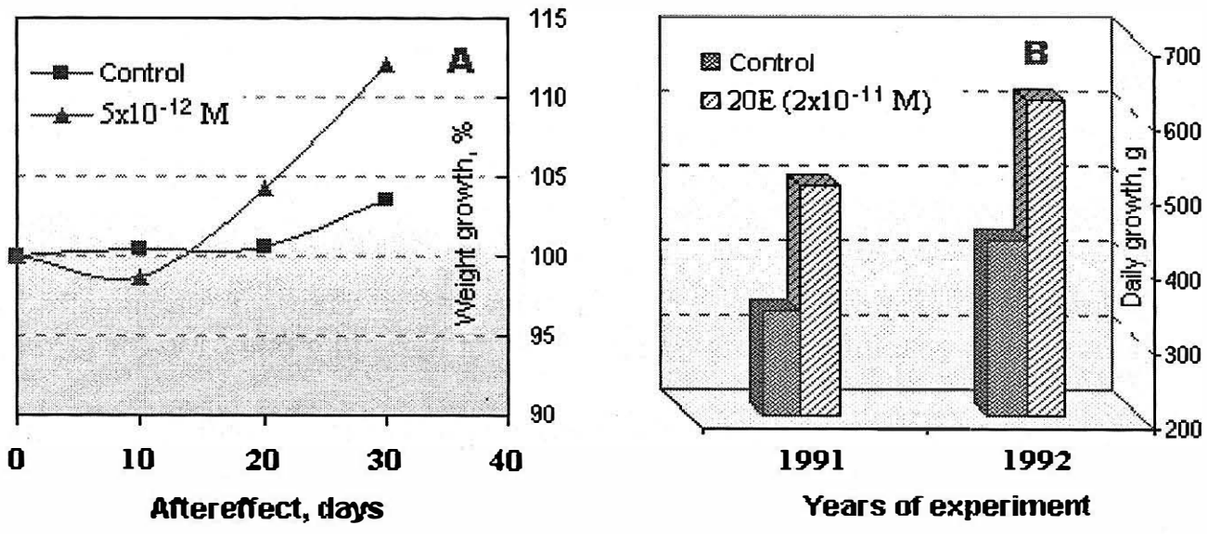

Figure 5. Anabolic effect of small ecdysteroid doses:
A –single intramuscular injection, according to [72];
B – production testing for 3 months, according to [73]

CONCLUSION

Research on ecdysteroids is a direction in biology offering rich possibilities for fundamental and practical scientific studies. Exploring the role and mechanisms of ecdysteroid biological activity allows for a real possibility to realize the boldest human projects, i.e. learn how to get control over the vital activity of different organisms manipulating the activity rate of the particular genes according to the "switch on-switch off" principle. Practically, it would help to get rid of whole number incurable diseases and refuse the chemical synthesis for the ecologically safe biological synthesis of many important substances.

Research on ecdysteroids including data on the genetics; cell and molecular biology; human, animal, and plant physiology, as well as commercial offers intended for solving real questions in chemistry, biotechnology, pharmacology, medicine, entology, and agriculture. Different states offer as their sources native plant species: ferns, convolvuluses, conifers, yew-trees, and amaranths.

In the case of Russia, cultivating Rhaponticum and Serratula plants representing super concentrator plant species is economically sound. The basic ecdysterone concentrations in plants of Rhaponticum carthamoides (Wlld.) Iljin (Leuzea carthamoides DC.) and Serratula coronata L. comprise 0.12-0.57 % and 0.31-1.15 % dry weight, respectively. Regarding the former species, there appeared an industrial growing technology and a new class of pharmaceutical preparations from its aboveground shoots is being developed. The latter is under introduction study in different Russian regions.

To satisfy the mass demand for ecdysteroids in the pharmaceutical industry, non-purified or weakly purified plant formulations from super producer species with a null toxicity rate that do not require high-expensive processing technologies have good prospects. The effective biological activity rate of extracts from Rhaponticum carthamoides grown with a special technology in agropopulations accounts for 10-11...10-13 M. It is about 3-4 orders of magnitude higher than the activity rate of highly purified individual ecdysteroids. Stable results of comparable doses were experimentally obtained in biotests, tests on laboratory animals, and in conditions of a large-scale production. What is particularly responsible for the unusually high activity rate of Rhaponticum carthamoides has to be looked for in its complex chemical composition causing the complex biological activity of ecdysteroids with other metabolites.

REFERENCES

1. Slama K., Lafont R. Insect hormones – ecdysteroids: their presence and actions invertebrates. Eur J Entomol., 1995; 92: 355-377.
2. Timofeev N.P. Leuzea carthamoides: Introduction questions and application prospects as biologically active components // Netraditsionnye prirodnye resursy, innovatsionnye tekhnologii i produkty. Collected proceedings. Is. 5. Moscow, RANS, 2001. P. 108-134.
3. Lafont R., Dinan L. Practical uses for ecdysteroids in mammals including humans: An update. Journal of Insect Science, 2003; 3(7): 30 pp.
4. Novikov V.S., Shamarin I.A., Bortnovskiy V.N. Experience on pharmacological correction of seamen sleep disturbances during navigation.Voenno-Meditsinskiy Zhurnal,1992;8:47-49.
5. Seyfulla R.D. Use of drugs by healthy people. Eksperimental'naya i Klinicheskaya Farmokologiya, 1994; 4: 3-6.

6. Seyfulla R.D. Sport pharmacology. Moscow: Sport-Pharma Press, 1999. 120 pp.
7. Mirzaev Yu.R, Syrov V.N, Krushev S.A, Iskanderova S.D. Study of the effects of ecdysten on the sexual function under experimental and clinical conditions. Eksperimental'naya i Klinicheskaya Farmakologiya, 2000; 63: 35-37.
8. Kibrik N.D., Reshetnyak J.A. Therapeutical approaches to sexual disadaption. European Neuropsychopharmacology, 1996; 6(4): 167.
9. Meybeck A., Bonte F., Redziniak G. Use of an ecdysteroid for the preparation of cosmetic or dermatological compositions intended, in particular, for strengthening the water barrier function of the skin or for the preparation of a skin cell culture medium, as well as to the compositions. US Patent 5,609,873. March 11, 1997.
10. Kovler L.A., Volodin V.V., Pshunetleva E.A. Ecdysteroid-containing liposomes and their description // Scientific reports of the Komi SC UrD RAS. Is. 407. Syktyvkar, 1998. 18 pp.
11. Tsuji K., Shibata J., Okada M., Inaoka Y. Blood flow amount-improving agent comprising steroid derivative and cosmetic using same. US Patent 5,976,515. November 2, 1999.
12. Darmograi V.N., Petrov V.K.,Yukhov Yu.I. Theoretical and clinical bases of a phytoecdysteroid action mechanism model // Biochemistry at the end of XX century. Interregional proceedings. Ryazan, 2000. P. 489-492.
13. Akhrem A.A., Kovganko V.V. Ecdysteroids: chemistry and biological activity. Minsk: Nauka i tekhnika, 1989. 327 pp.
14. Rees H.H. Ecdysteroid biosynthesis and inactivation in relation to function. Europ J Entomol., 1995; 92(1): 9-39.
15. Lafont R. Phytoecdysteroids and world flora: Diversity, distribution, biosynthesis, and evolution. Physiologiya Rasteniy, 1998; 3: 326-346.
16. Baltaev U.A. Phytoecdysteroids – structure, sources, and biosynthesis pathways in plants. Bioorganicheskaya Khimiya, 2000; 26(12): 892-925.
17. Zeleny J., Havelka J., Slama K. Hormonally mediated insect-plant relationships: Arthropod populations associated with ecdysteroid-containing plant, Leuzea carthamoides (Asteraceae). Eur J Entomol., 1997; 94(2): 183-198.
18. Dinan L. Strategy of valuating the role of ecdysteroids as deterrents in respect to invertebrates phytophagans. Phiziologiya Rasteniy, 1998; 3: 347-359.
19. Timofeev N.P. Whether protect phytoecdysones populations Rhaponticum and Serratula from damage by insects? // Chemistry and technology of plants substances. Saratov, Institute of Biochemistry and Physiology of Plants and Microorganisms RAS, 2004. P. 189-191.
20. Wang S.F, Ayer S, Segraves W.A, Williams D.R, Raikhel A.S. Molecular determinants of differential ligand sensitivities of insect ecdysteroid receptors. Mol Cell Biol., 2000; 20: 3870-3879.
21. Carlson G.R., Cress D.E., Dhadialla T.S., Hormann R.E., Le D.P. Ligands for modulating the expression of exogenous genes via an ecdysone receptor complex. US Patent 6,258,603. July 10, 2001.
22. Jepson I., Martinez A., Greenland A. J. Gene switch. US Patent 6,379,945. April 30, 2002.
23. Suhr S.T., Gil E.B., Senut M-C., Gage F.H. High level transactivation by a modified Bombyx ecdysone receptor in mammalian cells without exogenous retinoid X receptor. PNAS, 1998; 95: 7999-8004.
24. Albanese C., Reutens A.T., Bouzahzah B., Fu M., D'Amico M., Link T., Nicholson R., Depinho R.A., Pestel R.G. Sustained mammary gland-directed, ponasterone A-inducible expression in transgenic mice. FASEB J., 2000; 14: 877-884.

25. Saez E., Nelson M.C., Eshelman B., Banayo E., Koder A., Cho G.J., Evans R.M. Identification of ligands and coligands for the ecdysone-regulated gene switch. Proc Natl Acad Sci USA., 2000; 97: 14512-14517.
26. Evans R.M., Saez E. Formulations useful for modulating expression of exogenous genes in mammalian systems, and products related thereto. US Patent 6,333,318. December 25, 2001.
27. Vegeto E., McDonnell D.P., O'Malley B.W., Schrader W.T., Tsai M.J. Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy. US Patent 5,935,934. August 10, 1999.
28. Wolter S., Mushinski J.F., Saboori A.M., Resch K., Kracht M. Inducible expression of a constitutively active mutant of mitogen-activated protein kinase kinase 7 specifically activates c-JUN NH2-terminal protein kinase, alters expression of at least nine genes, and inhibits cell proliferation. J Biol Chem., 2002; 277(5): 3576-3584.
29. Patrick C.W., Zheng B., Wu X., Gurtner G., Barlow M., Koutz C., Chang D., Schmidt M., Evans G.R. Muristerone A induced nerve growth factor release from genetically engineered human dermal fibroblasts for peripheral nerve tissue engineering. J Tissue Eng., B, 2001; 7(3): 303-311.
30. Constantino S., Santos R., Gisselbrecht S., Gouilleux F. The ecdysone inducible gene expression system: unexpected effects of muristerone A and ponasterone A on cytokine signaling in mammalian cells. European Cytokine Network, 2001; 12(2): 365-367.
31. Syrov V.N. Phytoecdysteroids: biological effects in organism of higher animals and prospects of use in medicine. Experimentalnaya i Klinicheskaya Farmakologia, 1994; 5: 61-66.
32. Kholodova Yu.D. Phytoecdysteroids: biological effects, application in agriculture and complementary medicine. Ukrainskii Biokhimicheskii Zhurnal, 2001; 73: 21-29.
33. Rossant J., McMahon A. Meeting review "Cre"-ating mouse mutants a meeting review on conditional mouse genetics. Genes & development, 1999; 13(2): 142-145.
34. Butenandt A., Karlson P. Über die isolierung eines metamorphose-hormones der insekten in kristallisierten form. Z Naturforsch, 1954; 9b: 389-391.
35. Huber R., Hoppe W. Zur chemie des ecdysons. VII. Die kristall- und molekül-strukturanalyse des insektenverpuppung hormons ecdyson mit der automatisierten falt-molekülmethode. Chem Ber., 1965; 98: 2403-2424.
36. Karlson P., Hoffmeister H., Hummel H., Hocks P., Spiteller G. Zur chemie des ecdysons. VI. Reaktionen des ecdysonmoleküls. Chem Ber., 1965; 98: 2394-2402.
37. Nakanishi K., Koreeda M., Sasaki S., Chang M.L., Hsu H.Y. Insect hormones. The structure of ponasterone A, an insect-moulting hormone from the leaves of Podocarpus nakaii Hay. J Chem Soc Chem Commun., 1966; 24: 915-917.
38. Hampshire F., Horn D.H.S. Structure of crustecdysone, a crustacean moulting hormone. J Chem Soc Chem Commun., 1966; 37-38.
39. Hocks P., Wiechert R. 20-Hydroxyecdyson, isoliert aus insekten. Tetrahedron Lett., 1966; 26: 2989-2993.
40. Dinan L., Savchenko T., Whiting P. On the distribution of phytoecdysteroids in plants. Celluar and Molecular Life Sci., 2001; 58(8): 1121-1132.
41. Canonica L., Danieli B., Weisz-Vincze G., Ferrari G. Structure of muristerone A, a new phytoecdysone. Journal of the Chemical Society Chemical Communications, 1972; 1060-1061.
42. Austin D.F., Huaman Z. A synopsis of Ipomoea (Convolvulaceae) in the Americas. Taxon, 1996; 45: P. 3-38.
43. Volodin V., Chadin I., Whiting P., Dinan L. Screening plants of European North-East Russia for ecdysteroids. Biochemical Systematics and Ecology, 2002; 30(6): 525-578.

44. Ohsawa T., Yukawa M., Takao C., Muruyama M., Bando H. Studies on constituents of fruit body of Polyporus umbellatus and their cytotoxic activity. Chemical and Pharmaceutical Bulletin, 1992; 40(1): 143-147.
45. Ishida H., Inaoka Y., Shibatani J., Fukushima M., Tsuji K. Studies of the active substances in herbs used for hair treatment. II. Isolation of hair regrowth substances, acetosyringone and polyporusterone A and B, from Polyporus umbellatus Fries. Biol Pharm Bull., 1999; 22(11): 1189-1192.
46. Vokac K., Budesinsky M., Harmatha J., Pis J. New ergostane type ecdysteroids from fungi. Ecdysteroid constituents of mushroom Paxillus atrotomentosus. Tetrahedron, 1998; 54(8): 1657-1666.
47. Vokac K., Budesnsky M., Harmatha J., Kohoutova J. Ecdysteroid constituents of the mushroom Tapinella panuoides. Phytochemistry, 1998; 49(7): 2109-2114.
48. Harmatha J., Dinan L., Lafont R. Biological activities of a specific ecdysteroid dimer and of selected monomeric structural analogues in the B (II) bioassay. Insect Biochem Mol Biol., 2002; 32(2): 181-185.
49. Timofeev N.P. Industrial ecdysteroid sources: Part I. Ponasterone and muristerone. Netraditsionnye prirodnye resursy, innovatsionnye tekhnologii i produkty. Collected proceedings. Is. 9. Moscow, RANS, 2003. P. 64-86.
50. Orlova I.V., Zakharchenko N.S., Semenyuk E.G., Nosov A.M., Volodin V.V., Buryanov Ya.I. Development the Rhaponticum carthamoides transformed roots culture. Physiologiya Rasteniy, 1998; 45(3): 397-400.
51. Giri A., Narasu L.M. Transgenic hairy roots: recent trends and applications. Biotechnology Advances, 2000; 18: 1-22.
52. Harmata J., Dinan L. Biologocal activity of natural and synthetic ecdysteroids in the BII bioassy. Archives of Insect Biochemistry and Physiology, 1997; 35: 219-225.
53. Bourne C., Whiting P., Dhadialla T.S., Hormann R.E., Girault J-P., Harmatha J, Lafont R., Dinan L. Ecdysteroid 7,9(11)-dien-6-ones as potential photoaffinity labels for ecdysteroid binding protein. Journal of Insect Science, 2002; 2(11): 11 pp.
54. Landon T.M., Sage B.A., Seeler B.J., O'Connor J.D. Characterization and partial purification of the Drosophila Kc cell ecdysteroid receptor. J Biol Chem., 1988; 263(10): 4693-4697.
55. Syrov V.N., Ayzikov M.I., Kurmukov A.G. The influence of ecdysterone on protein, glycogene, and fat concentrations in the liver, heart, and muscle of white rats. Reports of the Uzbek SSR Academy of Sciences, 1975; 8: 37-38.
56. Slama K., Koudela K., Tenora J., Mathova A. Insect hormones in vertebrates: anabolic effects of 20-hydroxyecdysone in Japanese quail. Experientia, 1996; 52(7): 702-706.
57. Syrov V.N., Kurmukov A.G. To the anabolic activity rate of phytoecdysone-ecdysterone isolated from Rhaponticum carthamoides (Willd.) Iljin. Pharmokologiya i Toksikologiya, 1976; 6: 690-693.
58. Todorov I.N., Mitrokhin Yu. I., Efremova O.I., Sidorenko L.I. The impact degree of ecdysterone on protein and nucleic acid biosynthesis in mice organs. Khimiko-Pharmatsevticheskiy Zhurnal, 2000; 34(9): 3-5.
59. Pchelenko L.D., Metelkina L.V., Volodina S.O. The adaptogenic effect of Serratula coronata L. ecdysteroid fraction. Khimiya Rastitel'nogo Syrya, 2002; 1: 69-80.
60. Zaynullin V.V., Mishurov V.P. Punegov V.V., Starobor N.A., Bashlykova L.A., Babkina N. Yu. Biological efficiency rate of two folder components containing Serratula coronata L. ecdysteroids. Rastitel'nye Resursy, 2003; 39(2): 95-103.

61. Trenin D.S., Volodin V.V., Beykin Ya.B., Shlykova A.B. Ecdysteroid fraction of overground Serratula coronata L. in the spontaneous E-rosette-forming reaction and agaromigration test in vitro. Eksperimental'naya i Klinicheskaya Farmokologiya, 1996; 1: 55-57.
62. Trenin S., Volodin V.V. 20-hydroxyecdysone as a human lymphocyte and neutrophil modulator: in vitro evaluation. Archives of Insect Biochemistry and Physiology, 1999; 41: 156-161.
63. Zelenkov V.N., Timofeev N.P., Kolesnikova O.P., Kudaeva O.T. Revealing the biological activity rate of water extracts isolated from Rhaponticum carthamoides (Willd.) Iljin leaves in vitro // Topical questions on innovations concerning production of phytoproducts from untraditional plant resources and their use in phytotherapy. Moscow, RANS, 2001. P. 59-62.
64. Timofeev N.P., Volodin V.V., Frolov Yu.M. Some aspects of the production of ecdysteroid containing raw material from aerial part of Rhaponticum carthamoides (Willd.) Iljin // International Workshop on Phytoecdysteroids. Syktyvkar, Komi SC UrD RAS, 1996. P. 89.
65. Timofeev N.P., Volodin V.V., Frolov Yu.M. Distribution of 20-hydroxyecdysone in the structures of the above ground biomass of Rhaponticum carthamoides (Willd.) Iljin, under condition of agrocenosis in Komi Republic. Rastitel'nye Resursy, 1998; 34(3): 63-69.
66. Timofeev N.P. Growth and accumulation of ecdysteroids in the Rhaponticum carthamoides Iljin overground organs depending on the age and environmental conditions, Innovatsionnye tekhnologii i produkty. Collected proceedings. Is. 6. Moscow, RANS, 2002. P. 94-107.
67. Timofeev N.P. New preparations development from Rhaponticum carthamoides (Willd.) Iljin ("Bioinfusin" and "BCL-PHYTO") // Innovatsionnye tekhnologii i produkty. Collected proceedings. Is. 4. Novosibirsk, ARIS, 2000. P. 26-36.
68. Ivanovskiy A.A. Bioinfusin impact degree on several immunity indices. Veterinariya, 2000; 9: 43-46.
69. Kurakina I.O., Bulaev V.M. Ecdystene as a tonic in tablets 0.005 g each. Novye lekarstvennye preparaty, 1990; 6: 16-18.
70. Gadzhieva R.M., Portugalov S.N., Panyushkin V.V., Kondratyeva I.I. Anabolic effect comparative study on the plant preparations ecdystene, levetone, and "Prime-Plus". Eksperimental'naya i Klinicheskaya Farmokologiya, 1995; 5: 46-48.
71. Koudela K., Tenora J., Bajer J., Mathova A., Slama K. Simulation of growth and development in Japanese guails after oral administration of ecdysteroid-containing diet. Eur J Entomol., 1995; 92: 349-354.
72. Timofeev N.P., Ivanovskiy A.A. Anabolic effect of small doses Rhaponticum carthamoides preparations // International Workshop on Phytoecdysteroids. Syktyvkar, Komi SC UrD RAS, 1996. P. 132.
73. Timofeev N.P. Results on practical application Rhaponticum carthamoides to pig-breeding as ecdysteroid source // Untraditional plants selection, cultivation and processing technologies. Simferopol, 1994. P. 166-167.
74. Timofeev N.P. Phytoecdysteroids: Pharmacological use and activity. Medical sciences, 2005; 4:26-66