The influence of pharmaceutical vermipreparations on the test-reaction of organisms of the different levels of the organization

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Submitted: 24-12-2013 Revised: 23-04-2014 Published: 16-05-2014

ABSTRACT

The efficiency of vermipreparations has been investigated with the help of the test-reactions on various invertebrate organisms, plants and microorganisms. The principal possibility of the use of biotest on the basis of yeast, fungi, algae, water and ground-based plants, sponges, protozoan, small crustaceans, oligochaetes for an estimation of biological activity of vermipreparations and in further and for definition of quality, selection of dose of medicinal complex preparations is shown.

Key words: Algae, earthworms, fungi, oligochaetes, plants, protozoan, small crustaceans, sponge, test-reaction, vermipreparations, yeast

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.132594

Quick Response Code:

INTRODUCTION

Today in all over the world the demand for pharmaceutical earthworm-based preparations is growing. The interest in vermipreparations is due to their antitumor, antibacterial, antioxidant, immunostimulatory and many other effects. There are a large number of different vermipreparations. But the most widely used vermipreparations are "Lyumbricus", "Funaykan", fibrinolytic complex "Lumbrokinase", enzyme activator of heart "Long tong", antimicrobial peptides lyumbritsin, lysenin etc.[1]

The investigations of biological effects of vermipreparations are done basically on the laboratory animals and in clinical groups. Thus, there is not enough information about the influence of vermipreparations on biochemical, physiological and behavioral reactions of more lower organisms, for example, such as the protozoa, fungi, algae, higher plant, oligochaetes and crustaceans.

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Besides the theoretical value and ethical aspects same researches would have also the obvious and applied importance. These researches are extremely important by way of development of express, cheap and simple methods of an assessment of activity of vermipreparation. For preparation of vermipreparation as raw material, animal materials are used. The quality and quantity of biologically active agents involving in its structure, depends on many factors. In particular value can have an initial physiological condition of organisms, conditions of their cultivation, etc.

Existing methods of assessment of vermipreparations and other medical products quality are labor-consuming. They as a rule are directed on measurement of activity of only separate components, for example, hemolytic and fibrinolytic enzymes. These methods often do not give an integrated assessment of efficiency of pharmaceutical means.[2] Therefore, the availability of technically simple methods of an expressed assessment of their total pharmaceutical value is so actual. Unfortunately, now it is not enough same approaches. The availability of such methods would allow us to overcome the basic aspect constraining the application in official medicine of the most various complex multicomponent animals and vegetative preparations, which is a difficult at a dosage of their active

beginning. In this context, objective of the present work was: Checking of the possibility of the use for the decision of these problems of a wide range the test-reactions various invertebrate organisms, plants and microorganisms.

MATERIALS AND METHODS

As the test-objects used cellulose-digesting strain of fungi *Trichoderma viride*; culture of yeast *Saccharomyces cerevisiae*; unicellular green algae *Scenedesmus quadricauda*, multicellular filamentous blue-green alga *Oscillatoria* sp., charophyte *Nitella* sp., higher aquatic plant *Elodea canadensis*, seeds of a garden radish and cress; the protozoan: Fresh-water infusorians *Paramecium caudatum* and *Euglena caudata*, branchy sponge *Lubomirski baicalensis*, crustaceans *Daphnia magna*, *Moina* sp., *Simocephalus vetulus, Cyclops kolensis* and *Epischura baicalensis*, oligohaetes *Mesenchytraeus bungei* and *Tubifex tubifex*.

The vermipreparations were obtained of zooids of red Californian hybrid *Eisenia fetida* Andrei boche by the traditional method.[1] In the laboratory the worms were bred in trays with soil at 25°C and a humidity of 80-85%. The animals were fed by soaked buckwheat. In experiments adult worms with a belt zone and length of 8-10 cm were used. Worms were kept for 2 days in the acidified water without food for cleansing of the bowel and to cover from the soil. Then live worms were crushed and dried for 10 h at 50°C. Preparations with reduced activity prepared by heating of vermipulvis at 100°C for 30 minutes. For further experiments aqueous suspensions of vermipreparations used. With this end in the view, a weighed portion of vermipreparation mixed with a magnetic stirrer and water in mass units of 1:100 for 1 hour. Then the suspension was centrifuged for 5 min at 3000 rpm. The protein concentration in the supernatant was estimated by the Lowry method.[3] The test solution was stored in an airtight container at +5°C for up to a day. The dechlorinated tap water served as the control.

The reaction of organisms of different organization levels on influence of the active and weaked by heating vermipreparations was estimated by method stated below.

The new method based on estimation of foaming in the yeast suspension was used for evaluation of pharmaceutical vermipreparations activity.[4] For these purposes a dry baker's yeast *S. cerevisiae* with vermipreparations were suspended in a solution of *D*-glucose for 25 min at 30 ºC. Thereafter the volume of the formed yeast foam was determined and the speed of its rise was calculated by the formula V = h/t, where V is the speed of the foam rise in ml/min, h - volume of the foam, ml, t - time, min. The degree of inhibitive or stimulating influence of tested compound on the yeast was estimated on this parameter.[4]

The germination of fungal spores observed after an irrigation of spores sown of nutrient mediums by water suspensions of vermipreparations.[5]

The disk-diffusion method included the paper disks impregnation by vermipreparations' suspensions and its imposing on the lawns of the prorated algae.[6]

The registration of change of a level of fluorescence of a chlorophyll and tempus of growth of *S. quadricauda* under the influence of vermipreparations' solutions spent with the help of the device «Fluorat 02-3» on Federal Register of Russian Federation. 1.39.2007.03223.[7]

The activity of vermipreparations were evaluated with the help of such test-reactions as cyclosis stop and change of the intensity and nature of luminescence in cells of *Nitella* sp. and *E. canadensis*. The green leaves of elodea and cells of stoneworts were exposed in the suspensions of vermipreparations for this purpose. The changing of luminescence of chlorophyll's granulas and motion of cytoplasm of their cells were estimated in the fluorescence microscope "LUMAM I-1".[8,9]

The method of estimation of the vermipreparation's activity by germination of seeds involved the put of radish or watercress' seeds on a filter paper impregnated with suspensions of vermipreparations. The seeds were germinated in the dark at room temperature for three days. Then their germinating ability was analyzed.[10,11]

The ability of sponge to gather in conglomerates of the dissociated fragments of the animal body was used as a test reaction at the work.[12] In the absence of vermipreparations within two days of the cells formed well-defined aggregates. Some clusters of cells looked like balls with a diameter of 1-3 mm, while others, like intricately branched figures. The suspensions of vermipreparations prevented the cell aggregation.

The influence of vermipreparations' suspensions was estimated by time of immobilization of zooids of *P. caudatum* and *E. caudata*.[13] Working with Daphnia like an indicator of biological activity of vermiprepartions was served the survival of crustaceans.[14]

The method of estimation of vermiprepartions' activity with the help of oligochaetes *M. bungei and T. tubifex* is based on the infringement of such behavioral reaction as slipping of oligochaetes in a tangle at their placement in pure water.[15,16]

The obtained results were statistically processed using the software package Microsoft Excel 2010. All experiments were performed in 5 independent experiments in three

parallel replicates. Difference reliability was determined by Student *t*-test. Conclusions are made at $P < 0.05$.[17]

RESULTS AND DISCUSSION

The test responses of the organisms of different organization level to the influence of pharmaceutical vermipreparations presented on the Table 1.

On the basis of the experiments conducted the tested bioassays can build the following series of sensitivity to vermipreparations influence: Speed of foaming in the suspension of yeast > slipping of oligochaetes in a tangle > death of Daphnia > inhibition of growth of the *Oscillatoria* sp.'s lawn > stop time of cyclosis in *E. canadensis*'s cells > stimulation of growth of *S. quadricauda* and *D. salina* > start of characteristics' changing of the chlorophyll's luminescence in *Nitella* sp.'s cells > start of characteristics' changing of the chlorophyll's luminescence in *E. canadensis*' cells > stop time of cyclosis in *Nitella* sp.'s cells > germination of cress seeds > germination of *T. viride*'s spores > gathering of dissociated cells of sponge *L. baikalensis* > stimulation of *S. quadricauda's* fluorescence > time of immobilization of *Euglena* sp.' s cells > germination of radish seeds > time of immobilization of *P. caudatum*' s cells > time of death of *C. collensis* and *E. baicalensis*.

Accordingly to the speed of receiving the answer, the bioassays can be arranged in the following order: Time of death of *C. collensis* and *E. baicalensis* > time of immobilization of *P. caudatum*' s cells > time of immobilization of *Euglena* sp.' s cells > speed of foaming in the suspension of yeast > slipping of oligochaetes in a tangle > death of Daphnia > start of characteristics' changing of the chlorophyll's luminescence in *E. canadensis*' cells > stop time of cyclosis in *E. canadensis*'s cells > stop time of cyclosis in *Nitella* sp.'s cells > changing of the chlorophyll's luminescence in *Nitella* sp.'s cells > gathering of dissociated cells of sponge *L. baikalensis* > stimulation of *S. quadricauda's* fluorescence > germination of cress seeds > germination of *T. viride*'s spores > germination of radish seeds > inhibition of growth of the *Oscillatoria* sp.'s lawn > stimulation of growth of *S. quadricauda* and *D. salina*.

Thus, vermipreparations and coelomic fluid stimulate the growth of green (*S. quadricauda and D. salina*) but inhibit the growth processes of the blue-green (*Oscillatoria sp.)* algae. Vermipreparations suppressed the cyclosis in the cells of aquatic macrophytes (*Nitella* sp., *E. canadensis*), and then to change the intensity of the luminescence of chloroplasts. The vermipreparation debilitated by heating inhibited movement of the cytoplasm and chloroplasts luminescence hydrophytes less than intact ones. The cyclosis and luminescence are more

Table 1: Test-responses of organisms of different organization level on the pharmaceutical vermipreparations' influence

The criterion for estimation of the vermipreparation's activity (test-reaction), unit of measurement	The intact vermipreparation, 1%	The vermipreparation debilitated by heating, 1%	The control
The speed of foaming in suspension of yeast, in % to the control	20±15	60±9	100
The germination of *T. viride*'s spores	–	+	+
The disc-diffusion method. The inhibition of growth of the *Oscillatoria* sp.'s lawn, in % to the control	40±11	90±16	0
The disc-diffusion method. The stimulation of growth of *S. quadricauda* and *D. salina*	+++	+	0
The stimulation of *S. quadricauda*'s fluorescence, in % to the control	160±10	140±5	100
The stop time of cyclosis in *Nitella* sp.'s cells, hrs	After 2.0±0.5	After 4.0±0.9	Without stop
The stop time of cyclosis in *E. canadensis*'s cells, hrs	After 4.0±0.8	More than a day	Without stop
The start of characteristics' changing of the chlorophyll's luminescence in *Nitella* sp.'s cells, hrs	After 20±5 (orange-pink color)	After two days (orange-pink color)	Without change (red glow)
The start of characteristics' changing of the chlorophyll's luminescence in *E. canadensis*' cells, hrs	After 3.0±0.6 (The occurrence of grey sites)	After 5.0± (The occurrence of grey sites)	Without change (red glow)
The germination of radish seeds, in % to the control	40±10	30±8	100
The germination of cress seeds, in % to the control	70±13	40±7	100
The gathering of dissociated cells of sponge *L. baikalensis*, a day	+	++	+++
Time of immobilization of *Euglena* sp.' s cells, after min	5.0±1.3	15.0±3.5	Not immobilize
Time of immobilization of *P. caudatum*' s cells, after min	5.0±0.8	5.0±0.6	Not immobilize
Time of death of *C. collensis*, after min	10±1	10±2	-
Time of death of *E. baicalensis*, after min	10±2	10±1	-
Time of death of *D. moina*, after min	30±5	210±30	-
Time of death of *D. magna*, after min	30±4	200±35	-
Time of death of *S. vetulus*, after min	30±3	195±28	-
The slipping of oligochaetes *M. bungei* in a tangle	–	+	+
The slipping of oligochaetes *T. tubifex* in a tangle	–	+	+

sensitive to the influence of tested preparations in cells of *Elodea* in comparison with to the cells of *Nitella*. The seed germination of cress was suppressed by intact vermipreparations more than vermipreparations debilitated by heating. Intact vermipreparation caused immobilization of oligochaetes *M. bungei* and *T. tubifex*, as well as to prevent their gathering into the tangle in distinction from vermipreparation debilitated by heating.

The *E. fetida* preparations lower heartbeats of crustaceans *D. magna* and lead to their death. Intact vermipreparations of *E. fetida* suppress *Daphnia*'s heart stronger than vermipreparations debilitated by heating. Intact vermipreparations lead to the death of *Cladocera Moina* sp. and *S. vetulus* faster than ones debilitated by heating. Intact and debilitated by heating vermipreparations cause immobilization of *Euglena* sp. and *P. caudatum*'s cells.

Deserves special attention the fact of the ability of earthworms' preparations active influence on living organisms, such as plants and algae.

CONCLUSION

Thus a prinicipal possibility of using of bioassays based on yeast, fungi, algae, aquatic and terrestrial plants, sponges, protozoa, crustaceans, oligochaetes to estimate the biological activity of vermipreparations. However, the results conducted allow supposing that with further refinement described test reactions can be applied to estimate the quality and selection of the drug doses of complex products based on plant and animal materials.

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Cite this article as: Stom DI, Bybin VA, Balayan AE, Saksonov MN, Salovarova VP. The influence of pharmaceutical vermipreparations on the test-reaction of organisms of the different levels of the organization. Phcog Res 2014;6:195-8.

Source of Support: The work was carried out with the partial support of the Russian Foundation for Basic Research (№ I-140 122 090 159), Strategic Development Program of ISU and Project P211-PF-016. **Conflict of Interest:** None declared.