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

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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 “Lyumbiricus”, “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.

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.[3] 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

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

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.

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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 *Sieneedsmus 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 *Englena caudata*, branchy
sponge *Labomirski baicalensis*, crustaceans *Daphnia magna*,
*Maina* sp., *Simocphalus 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.[2] 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.[3] 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 °C.
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 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.[8,9]

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.[10] 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*. Working with Daphnia like
an indicator of biological activity of vermipreparations was
served the survival of crustaceans.[11]

The method of estimation of vermiprepartions’ activity
with the help of oligohaetes *M. bungei* and *T. tubifex* is
based on the infringement of such behavioral reaction as
slipping of oligohaetes in a tangle at their placement in
pure water.[12,13]

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 $P < 0.05$.\cite{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 > skipping 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 > germination of cress seeds > germination of T. viride's spores > gathering of dissociated cells of sponge L. baikalensis > stimulation of S. quadracippida'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 > skipping of oligochaetes in a tangle > death of Daphnia > start of characteristics' changing of the chlorophyll's luminescence in E. canadensis's 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. quadracippida'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. quadracippida and D. salina.

Thus, vermipreparations and coelomic fluid stimulate the growth of green (S. quadracippida 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. quadracippida and D. salina | +++ | + | 0 |
| The stimulation of S. quadracippida'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 | After two days | Without change |
| The start of characteristics' changing of the chlorophyll's luminescence in E. canadensis's cells, hrs | (orange-pink color) | (red glow) | (red glow) |
| The start of characteristics' changing of the chlorophyll's luminescence in E. canadensis's cells, hrs | After 3.0±0.6 | (The occurrence of grey sites) | (The occurrence of grey sites) |
| 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. bungii 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 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 *Englona* 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 principal 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.

**REFERENCES**

1. Sun Z. Vermiculture and vermiprotein/ Z. Sun. Beijing, People's Republic of China: China Agricultural University 2003. p. 367.
2. Xu L. Dictionary of materia medica. In: Li Xu, Wei W, editors. Shanghai: Shanghai Sci. and Tech. Press; 1985. p. 2111.
3. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent J Biol Chem 1951;193:265-75.
4. Vyatchina OF, Zhdanov GO, Stom DI. Express acceptance of biological water quality analysis using Saccharomyces. Nat Sci 2009;3:86-8.
5. Chitarra GS, Dijkstra J. The germinating spore as a contaminating vehicle. In: Dijkstra J, Samson RA, editors. Food Mycology: A multifaceted approach to fungi and food. Mycology. Vol. 25. New York: CRC Press Taylor and Francis group; 2007. p. 83-99.
6. EUCAST Disk diffusion method for antimicrobial susceptibility testing-Version 2.1. Available from: http://www.eucast.org [Last accessed on Feb 2012].
7. Zhmur NS, Orlova TL. Methods of determining the toxicity of water, aqueous extracts from soils, sewage sludge and waste by changing the level of chlorophyll fluorescence and the number of algal cells. FR 1.39.2007.03223. Moscow: Akvaros; 2007. p. 48.
8. Stom DI, Ivanova GG, Bashkatova GV, Trubina TP, Kozhova OM. About the Role of Quinones in the Action of Some Polyphenols on the Streaming of Protoplasm in Nitella sp. Cells. Acta Hydrochimica et Hydrobiologica; 1974;2:407-12.
9. Dmitrieva AG. Bioassay method to determine the living and the dead algae cells using fluorescence microscopy. Methods of bioassay water: Festschrift. Chernogolovka: Union of Soviet Socialist Republics (USSR); 1988. p. 85-9.
10. Stom DI. Effect of polyphenols on shoot and root growth and on seed germination/D.I. Stom. Biologia Plantarum; 1982;6:1-6.
11. Pellissier F. Improved germination bioassays for allelopathy research. Acta Physiol Plant 2013;35:23-30.
12. Balayan AE, Stom DI, Kazarionova TF. Test-Reactions of the baikal sponges for estimating their physiological state. Abstracts of the third International Symposium of the series Speciation In Ancient Lakes (SIAL-3); Ancient lakes: Speciation, development in time and space, natural history. Novosibirsk: Nauka Publisher; 2002. p. 18.
13. Rakhleeva AA, Terekhova VA. Methodology for determining the toxicity of wastes, soils, sewage sludge, and surface and ground waters with the use of paramecium caudatum ehrenberg Infusoria (FR 1.39.2006.02506). Moscow: Edition of Moscow State University; 2006.
14. FR.1.39.2007.03222. Method for determining toxicity of water and aqueous extracts from soil, sewage sludge, waste by mortality and fertility changes in Daphnia. Moscow: Akvaros; 2007. p. 52.
15. Stom DI, Balayan AE, Polekhina SV, Bybin VA. Bioassay method drugs derived from earthworms. Patent RF No 2377561 from 11.02.2008. Official Bulletin "Inventions. Utility Models". Moscow: FIPS; 2009; p. 100.
16. Mikhailova LV. Provisional guidance on the valuation levels of chemicals in sediments of surface water bodies (for example, oil), Russian ecological federal information agency, Independent chemical information agency., Moscow: Nature; 2002.
17. Hardle W. Applied multivariate statistical analysis. Heidelberg: Springer-Verlag; 2007. p. 458.

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