The evaluation of the activity of medicinal remedies of plant and animal origin on the regeneration of the earthworms' tail segments

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ABSTRACT

Now, in the global community there is enough hard recommendation to replace the vertebrate test animals into simpler organisms at the development, testing, and evaluation of the quality pharmaceuticals. The feature of planarian to regenerate in new individual planarian from a piece, which is only 1/7 of the original animal, allowed to create the alternative methods of testing of drugs, dietary supplements, water quality, influence of electromagnetic fields, and other radiations. The tests on planarian can replace the ones that are held today on mammals. However, the lacks of the bioassays based on the planarian regeneration are the need for complex and expensive video equipment for recording the regrowth of worms' body, the difficulties of culturing of flatworms and fairly long period of response. These difficulties can be avoided by using another group of the worms of type *Annelida*. The new individual can be fully recovered only from the front half of the body in many species of earthworms. Thus, the influence of the pharmaceuticals from earthworms, mummy, and *Orthilia secunda* on the ability of earthworms to regenerate lost tail segments has been investigated. The relations of the activity of preparations tested with doses and the time of the storage have been revealed. The principal possibility of applicability of the test reaction studied as a way to evaluate the effects and quality of remedies based on medicinal plants and earthworms has been shown.

Key words: Earthworms, medicinal remedies, mummy, *Orthilia secunda*, regeneration, test-reaction, vermipreparation

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INTRODUCTION

Every year millions of animals are killed in the course of scientific experiments.[1] The necessity of such experiments is understandable, but their ethics is in serious doubt. Issues of humane treatment to animals used are given the most attention in the world.[2] Now in the global community there is enough hard recommendation to replace the vertebrate test animals into simpler organisms at the development, testing, and evaluation of the quality pharmaceuticals.[3] Not only ethical considerations, but also the financial aspects initiate the development of alternative methods of bioassay using the invertebrates. The basic biochemical processes are quite close to higher animals and the lower ones so the special obstacles for the development of the alternative methods are absent and the advantages are obvious. Moreover, main among them is the reducing of number of the vertebrate animals used in scientific experiments. High regenerative abilities of the worms are well known. For example, a new individual planarian can be grown from a piece, which is only 1/7 of the original animal.[4] This feature of planarian allowed to create the alternative methods of testing of drugs, dietary supplements, water quality, the influence of electromagnetic fields, and other radiations.[5] The tests on planarian can replace the ones that are held today on mammals.[6]

However, lacks of the bioassays based on the planarian regeneration are the need for complex and expensive video equipment for recording the regrowth of worms' body, the difficulties of culturing of flatworms, and fairly long period of response. These difficulties can be avoided by using of another group of the worms of type *Annelida*. The new individual can be fully recovered only from the front half of the body in many species of earthworms.[7-9]

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In this context, the aim of this work was verification of the capability of use for evaluation of the regenerative activity of drugs with plant and animal origin by their influence on the processes of recovery of amputated earthworms' tail segments.

MATERIALS AND METHODS

The red Californian hybrid was used as an object of study. It refers to the phylum of *Annelida*, class of *Oligochaeta*, family of *Lumbricidae*, genus *Eisenia* Malm 1877 Emend Michaelsen, 1900, species *Eisenia fetida* andrei Bouche, 1972. 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.

Vermipreparations

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.[10] The vermipreparations were obtained of zooids of red Californian hybrid *Eisenia fetida* andrei Boche by the traditional method.[11] Worms were kept for 2 days in the acidified water without food for cleansing of the bowel and 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 vermipowder at 100°C for 30 min. 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 h. Then the suspension was centrifuged for 5 min at 3000 rpm. The protein concentration in the supernatant was estimated by the Lowry method.[12] The decoctions of the medicinal plants were prepared from 1 g of raw material ratio of 20 ml of boiling water. We used decoctions cooled to the room temperature in the experiments. The dechlorinated tap water served as the control.

Mummy

It is organic and mineral product of natural origin, which is pieces of different shapes and sizes of the inhomogeneous dense, solid mass with a rough or grainy, matte or glossy surface, fragile or low-plasticity consistency with inclusions of plant, mineral or animal origin, retinoid compound, brown, deep brown, black with pale gray spots of color, and peculiar smell.[13] Mummy is used in ayurvedic and other forms of alternative medicine. We used the "golden mummy" (JSC "Evalar", Russia) in experiments.

Orthilia secunda, L., 1753 (upland uterus) is a flowering plant of the genus *Orthilia* in the family *Ericaceae*. It is the only member of the genus.[14] The plant has a circumboreal distribution, growing throughout much of the Northern Hemisphere. *O. secunda* is recommended by herbalists as an herb for gynecological disorders and inflammations. *O. secunda* preparations are used for sterility, bleedings, infantilism, cervical erosion, for menstrual cycle derangements, toxicosis. In some countries, *O. secunda* is used as diuretics and antiseptics for kidney and urinary bladder inflammations.

The obtained results were statistically processed using the software package Microsoft Excel 2010. All experiments were performed in five independent experiments in three parallel replicates. Difference reliability was determined by Student's *t*-test. Conclusions are made at $P < 0.05$.[15]

The effects of different drugs on the rate of regeneration of earthworms we tested as following. The mature zooids of earthworms were kept for 1-day without food in the containers with sterile filter for cleansing of their intestine and covers from the soil. The paper in containers was moistened with the acidified sterile water with 6, 5 pH. The worms were washed repeatedly with sterile water. The paper mats were changed too. The five tail segments of worms were dissected with a sterile scalpel in the control group. We used a magnifying lens also for this purpose. The wounds were washed with 1 ml of physiological salt solution immediately after the cut and once a day for 3 days. In the experimental group, the wound was washed with a solutions or suspensions of medicines with required containing.

The earthworms were contained without food in a petri dish containing sterile filter paper moistened with sterile water after resection. Worms were not fed specifically but they ate litter. The intensity of regeneration of the tail segments was fixed and compared within 2 weeks on the number of segments regrown in the experimental and control groups of earthworms. The data processing was performed by calculating the rate of regeneration with formula 1:

$$V = \frac{n}{t} \quad (1)$$

Where n is the number of segments regrown and t is the time of exposure.

RESULTS AND DISCUSSION

The study of the influence of vermipreparations and other drugs on the regeneration of the earthworms showed the following. The vermipreparations (in a concentration of 0.01%) both as intact and with reduced activity accelerated the regeneration of the tail segments of earthworms

on the average 2.6 times (t_d = 9; $P \leq 0.05$) compared to controls [Figure 1a and 1b].

The increasing of the concentrations of intact vermipreparation to 0.1% led to a suppression of regeneration on average 56% (t_d = 3.1; $P \leq 0.05$) [Figure 1a], while animals were insensitive to the vermipreparation attenuated by heating in the same concentration [Figure 1b]. A further increase in the concentration of both types of vermipreparations to 0.5% and 1% did not significantly alter the rate of the segment's regeneration. The preparation "golden mummy" inhibited regeneration of earthworms at 0.1% and 1% on average by 32% and 50% (t_d = 2.6, $P \leq 0.05$; t_d = 4.2, $P \leq 0.05$), respectively and stimulated on 112% (t_d = 6.2; $P \leq 0.05$) with content of the mummy 0.01% compared to the control [Figures 2 and 3].

The decoctions (1:20) of *O. secunda* of 1-year storage of raw materials inhibited the rate of regeneration of tail segments of earthworms on average by 52% (t_d = 7.7; $P < 0.05$) compared with the decoction of *O. secunda* 10 years ago [Figure 4].

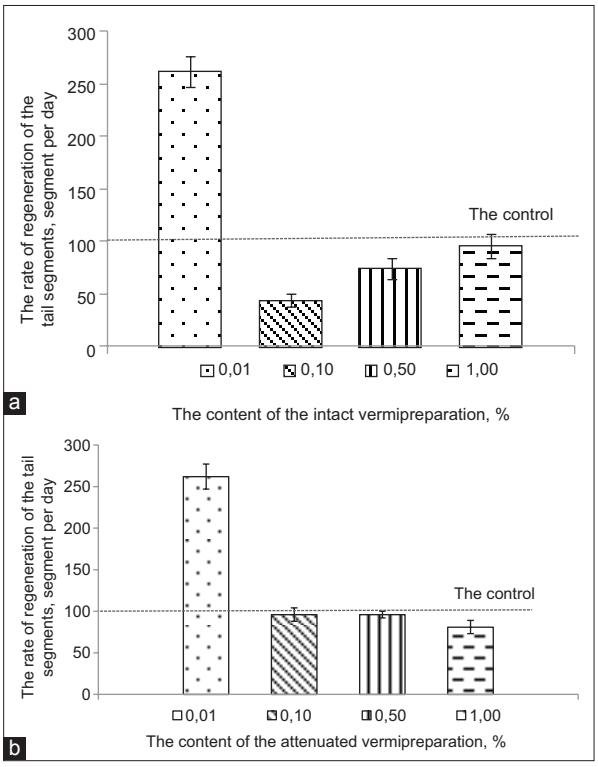

Figure 1: The regeneration of the tail segments of earthworms under the influence of: (a) Intact vermipreparation (b) vermipreparation attenuated by heating

Figure 3: The regeneration of the tail segments of *Eisenia fetida* on the 22 days of exposure of oligochaetes after treatment by: (a) 0.01% solution of the preparation "golden mummy", (b) 0.1% solution of the preparation "Golden mummy", (c) 1% solution of the preparation "golden mummies", (d) physiological saline (control)

CONCLUSION

Thus, vermipreparations and mummy stimulate at low and inhibit in higher concentrations of the regeneration of the tail segments of earthworms. The decoctions of fresh raw

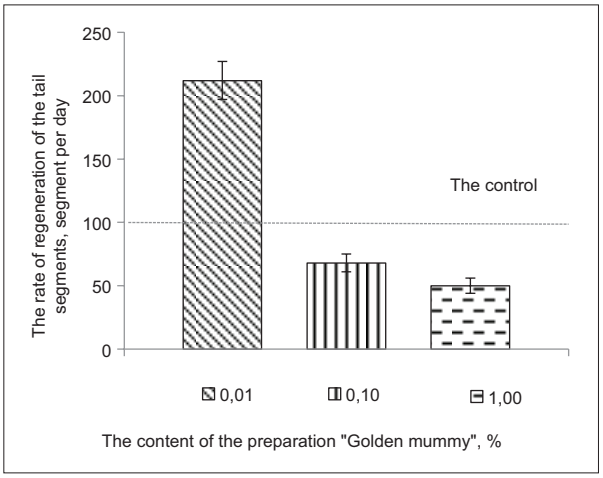

Figure 2: The regeneration of the tail segments of earthworms exposed with the preparation "Golden mummy"

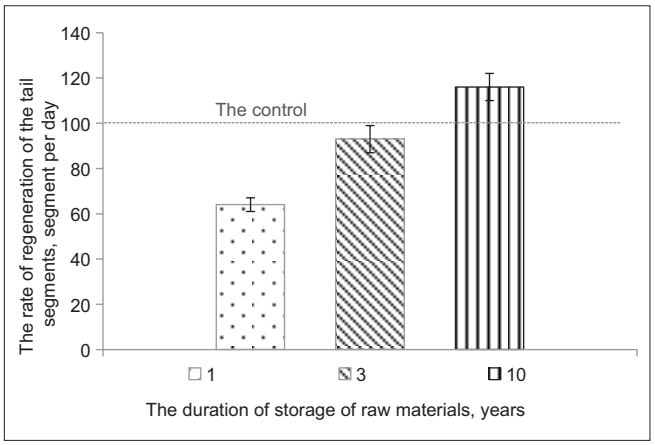

Figure 4: The effect of decoctions of *Orthilia secunda* of different periods of storage (1, 3, and 10 years old) on the regeneration of the tail segments of earthworms

O. secunda is strongly suppressed the regeneration of the earthworm's tail segments than older ones. This reaction is a promising bioassay, as it allows to select effective doses and evaluate the biological activity of vermipreparation and other drugs. The advantages of studied bioassay are the ease of implementation, technical simplicity, low cost, the ability to assess the quality of medicines on the field conditions. It gives an integral assessment of the activity of drugs in contrast to the known methods of analytical chemistry.

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