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The Influence of the Xymedon Preparation on the Regeneration of *Girardia tigrina* Planarians

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Abstract Synthesis of new medicines and analysis of their effects have become increasingly important lately. Hepatoprotectors is a general name of medicinal preparations for liver treatment and recovery. The Xymedon preparation (Hydroxyethyldimethyldihydropyrimidine), an immunostimulant, can be presumably used as a hepatoprotector. To date, the influence of this preparation has been insufficiently studied. The purpose of this paper is to investigate the effect of the pyrimidine derivative Xymedon on the regeneration of *Girardia tigrina* (Girard, 1850) planarians, one of the perspective models for analysis of the influence produced by various substances and preparations on proliferation rates of the regeneration bud (blastema). The study revealed that the Xymedon preparation is not toxic for *G. tigrina* planarians and does not cause their death even when its concentration is gradually increased in the solution. The selected working concentrations of the Xymedon preparation produced different effects on planarians during the experiment. The influence of small concentrations (0.0004 and 0.004 mg) was little different from that in the control group, i.e., the regeneration criterion was low. Higher concentrations (0.1 mg) stimulated regeneration processes, i.e., the regeneration criterion was high. The largest concentration of the Xymedon preparation (0.4 mg) led to a slight decrease of the regeneration criterion, being, however, characterized by the high regeneration coefficient.

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1 Introduction

In today's world, searching for medicinal preparations with potential hepatoprotective efficacy is an important task. This is determined by various factors, such as unhealthy feeding, infections, and environmental pollution, all contributing to liver dysfunction and disorders [1]. The Xymedon preparation (1-(ß-oxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxypyrimidine) synthesized in Russia stimulates tissue regeneration and wound healing, as well as has anti-inflammatory and immunostimulating effects [2]. Its hepatoprotective action has been proved by the recent research [3]. The Xymedon preparation is characterized by low toxicity (LD50 = 7000 mg/kg) and has never been tested on any living objects other than mammals. Testing medicinal preparations on invertebrate hydrobionts is a promising model to characterize their action. In this study, *Girardia tigrina* planarians that are characterized by the high regenerative ability [4] were used as a test object to analyze the influence of the Xymedon preparation on regeneration process. Planarians are a convenient subject of basic research in the field of regeneration, morphogenesis, and stem cell biology [4].

2 Material and Methods

The laboratory culture of *G. tigrina* (Plathelminthes, Tricladida) asexual planarians was used as the object of study. It was precultured under laboratory conditions at 26 °C. The planarians were fed with larval dipterans. A total of 450 planarian specimens were involved in the experiments. They

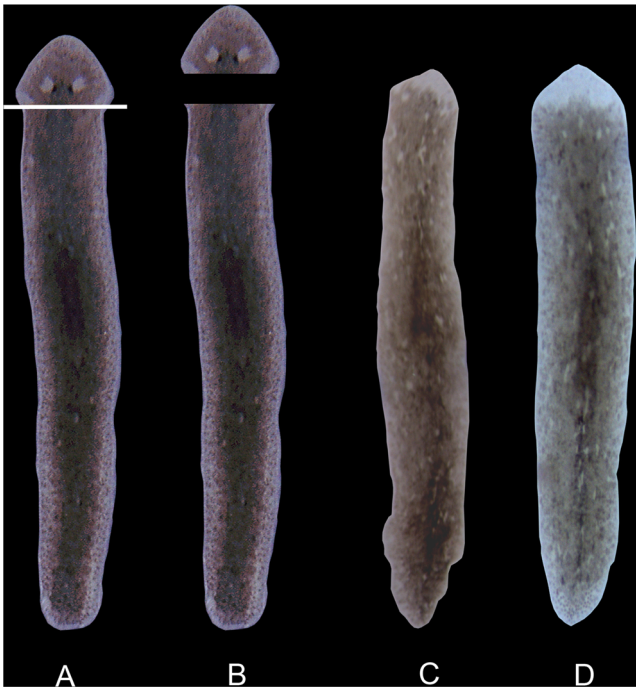

Fig. 1 Scheme of the operation. Animals were operated behind the eyes (**a**, **b**). Animals in control group (**c**). Animals after concentration of the Xymedon preparation 0.1 mg/100 mL (**d**)

were kept in starvation for 7 days and had the body length of 9–10 mm. Regeneration in the planarians was initiated by amputation of the head end in the area of eyes. At the first stage, the lethal (CL50) and no observed effect (NOEC) concentrations of the Xymedon preparation for *G. tigrina* planarians were determined. Based on the data on CL50 and NOEC values, the optimal working concentrations were selected to study the influence of the preparation on the regeneration of *G. tigrina* planarians. Growth of the head end was investigated in the decapitated planarians using the method of vital computer morphometry [5–7] based on the registration of photocontrast between old (pigmented) and novel (without pigment) body parts (Fig. 1). Within the framework of the method of vital computer morphometry, the regeneration criterion (R) was calculated as a relation between the regenerating body part (blastema) area (s) and the area of the whole body (S), which is an indirect indicator of cell proliferation. The effect was estimated as a percent difference in the regeneration criterion (R) between the control and experimental groups. Each of the determined values, the result is averaged over 30 animals in the experiment ($n = 30$) and in the control ($n = 30$). The experiments for each concentration were carried out in at least three replicates. The blastema areas were examined under a Carl Zeiss.V12 stereo microscope. Differences among the control group and four groups of concentrations were assessed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) multiple comparison. The diagram was created using the program Past 3.11.

3 Results and Discussion

The obtained CL50 values show that extremely high concentrations of the preparation (0.3 g/100 mL, 0.9 g/100 mL, and 2 g/100 mL) are not toxic for *G. tigrina* planarians, i.e., they neither inhibit motion nor cause tissue lysis. According to the previous research [8, 9], the critical concentration of any substance for hydrobionts is estimated as 2 g/1 L. Concentrations higher than that do not occur under natural conditions.

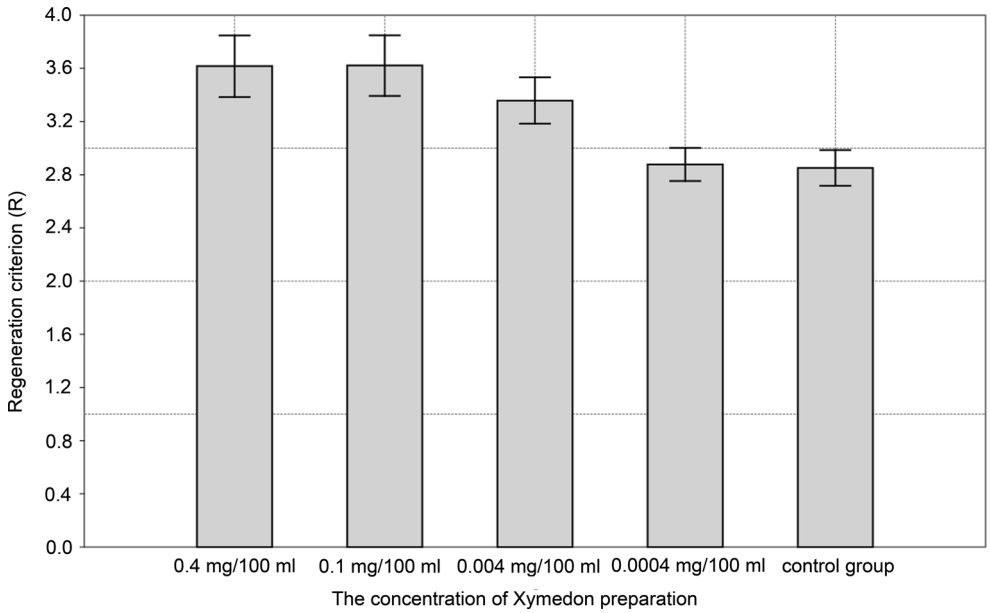

Fig. 2 The regeneration criterion level in *D. tigrina* planarians at different concentrations of Xymedon (medium value ± standard error)

The working concentrations selected were as follows: 0.4 mg/100 mL, 0.1 mg/100 mL, 0.004 mg/100 mL, 0.0004 mg/100 mL, and control group. The one-way ANOVA test showed differences between the compared sets ($F = 4.915$; $df = 4$; $p < 0.001$). The subsequent multiple pairwise comparisons (Tukey's HSD test) allowed concluding that the observed differences from the control group were statistically significant (Fig. 2) at the highest concentrations of the preparation (0.4 mg/100 mL and 0.1 mg/100 mL).

The concentration of 0.0004 mg/100 mL is close to ultra-low, but it does not have any noticeable effect (the average value of the regeneration criterion is 2.87 compared to 2.85 in the control group). The concentration of 0.0004 mg/100 mL does not show significant differences from the control group. The regeneration criterion is positive (3.36 on average) at the concentration of 0.004 mg/100 mL. At the highest concentrations selected, the regeneration criterion is positive: 3.61 at 0.4 mg/100 mL and 3.62 at 0.1 mg/100 mL.

4 Conclusions

It was revealed that the Xymedon preparation is most effective for the regeneration of *G. tigrina* planarians at the concentration of 0.1 mg/100 mL. When the concentration of this preparation is higher (0.4 mg/100 mL), the regeneration criterion slightly decreases. On the contrary, the lower concentrations (0.004 and 0.0004 mg/100 mL) of the preparation have a minor positive effect on cell proliferation as compared to the control group.

Xymedon is known to effect biochemical processes at the cellular and subcellular levels by activating adenylate cyclase, thereby leading to a rapid accumulation of cAMP in the cell; the drug stimulates metabolism and protein synthesis [10]. We assume that Xymedon has a similar influence on the blastema regeneration in *D. tigrina* planarians, as confirmed in some studies [11].

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