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The Cytoprotective Properties Assessment of 6-Substituted Uracil Derivatives Under the Influence of Deltamethrin on Rat Gasser's Ganglion Neurinoma Cell (RGGN-1).

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ABSTRACT

The authors took a cytoprotective properties assessment of various connections under the pesticide influence of the synthetic pyrethroidsdeltamethrin group on rat Gasser's ganglion neurinoma cells. The synthetic pyrethroids, deltamethrin, was used in the experiment. The deltamethrin purity was evaluated by using HPLC/MS (High Performance Liquid Chromatography Mass Spectrometry). The immortalized rat Gasser's ganglion neurinoma cell culture (RGGN-1) was used as a test object. The cells were cultivated in DMEM medium with addition of 10% fetal calf serum at 37^{O}C and 5% CO_2. The specimens were dissolved in DMSO and 96% alcohol mixes at the ratio 1:1. The test substances and deltamethrin were added to the cell culture medium. After 24 hours of cultivation, the cell layer was estimated using an inverted microscope by the following parameters: the percentage of surface coverage, the shape of cells, the number of cellular aggregates, the number of floating cells. The cells were counted in the hemocytometer. The number of live and dead cells was estimated by Trypan blue (0.1% solution). The effect of the test compounds on the cultural and morphological properties of cells was determined taking into account the following parameters: viability factor is the ratio of living cells to their total number in percentage terms; proliferation index is the ratio of the number of grown cells to the number of sown ones; cytotoxity index is the ratio of living cells remaining after exposure with a compound to the number of living cells in the control. According to the results of studies it was found that 6-isopropyl-2-thiouracil and 6-*tert*-butyl-2-thiouracil showed the best cytoprotective properties on rat Gasser's ganglion neurinoma cells under the influence of deltamethrin. These compounds increase the survival and proliferative activity of RGGN cells under the influence of deltamethrin. It indicates the advisability of further research on the use of these compounds as potential antidotes.

Keywords: pesticide, synthetic pyrethroids, deltamethrin, treatment, cytotoxity.

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INTRODUCTION

The rapid development of industry, the intensification of agriculture, the chemicalization of animal husbandry to accelerate the growth and fattening of animals leads to environmental pollution [1; 2]. The pesticides circulation along food chains with accumulation of residues in animal feed and food products of plant and animal origin is the most dangerous for animals and humans [3-5]. According to results of the European researches of phytogenic food, about 30% of samples have residues of two or more pesticides. In recent years, insecticides belonging to the group of synthetic pyrethroids are increasingly used in agriculture. Deltamethrin takes the first place in the scale of use. It has at least 30% of the market volume of pyrethroids [6; 7]. These compounds are sensitive. However, when they are used in agriculture and veterinary medicine, they can get into environmental objects and cause poisoning of humans and animals [8; 9]. In general, the study of biological activity of substances at the first stage assumes assessment of their toxicity, regardless of the subsequent purpose of their use. The toxicity assessment methods alternative to the classical tests on experimental animals, namely, models using cell cultures, are increasingly used in biochemical-toxicological studies [10-12]. Besides the solution of the ethical problems connected with mass use and death of experimental animals, such methods make it possible to significantly reduce the time and cost of preliminary research of new chemical preparations, especially at the stage of their preclinical testing. In addition, the use of cell cultures allows to establish the nature of biological activity of the studied compounds directly at the cellular level [13; 14].

MATERIALS AND METHODS

The synthetic pyrethroids, deltamethrin was used in the experiment. The deltamethrin purity was evaluated by using HPLC/MS (High Performance Liquid Chromatography Mass Spectrometry). The researches were conducted on HPLC MS/MS system of Bruker HPLC-DionexUltiMate 3000 RSLC; MS / MS-Bruker ESI- (Qq) TOF maxis II System of high resolution (Figure 1).

The immortalized rat Gasser's ganglion neurinoma cell culture (RGGN-1) was used as a test object. The cells were cultivated in DMEM medium with addition of 10% fetal calf serum at $37^{O}C$ and 5% CO_2. The specimens were dissolved in DMSO and 96% alcohol mixes at the ratio 1:1. The test substances and deltamethrin were added to the cell culture medium.

After 24 hours of cultivation, the cell layer was estimated using an inverted microscope by the following parameters: the percentage of surface coverage, the shape of cells, the number of cellular aggregates, the number of floating cells. The cells were counted in the hemocytometer. The number of live and dead cells was estimated by Trypan blue (0.1% solution) [15]. The effect of the test compounds on the cultural and morphological properties of cells was determined taking into account the following parameters: viability factor is the ratio of living cells to their total number in percentage terms; proliferation index is the ratio of the number of grown cells to the number of sown ones; cytotoxity index is the ratio of living cells remaining after exposure with a compound to the number of living cells in the control [16].

10 groups were formed in order to select potential therapeutic agents under the influence of deltamethrin on a eukaryotic cell. The first group served as a control, the second group was with the addition of deltamethrin. The test compounds were added to the remaining groups together with deltamethrin. The third group was with the addition of ionol, the fourth one - with vitamin E, the fifth one - with bisphenol-5, the sixth one - with 6-isopropyl-2-thiouracil, the seventh one - with 6-*tert*-butyl-2-thiouracil, the eight one - with 3-methyl- 6-cyclopropyluracil, the ninth one - with 6-ethyluracil and the tenth one - with 3-methyl-6-ethyluracil, all compounds were added at a concentration of 0.02 mM. In the experiment, deltamethrin was used in concentrations: 0,01 and 0,07 mM.

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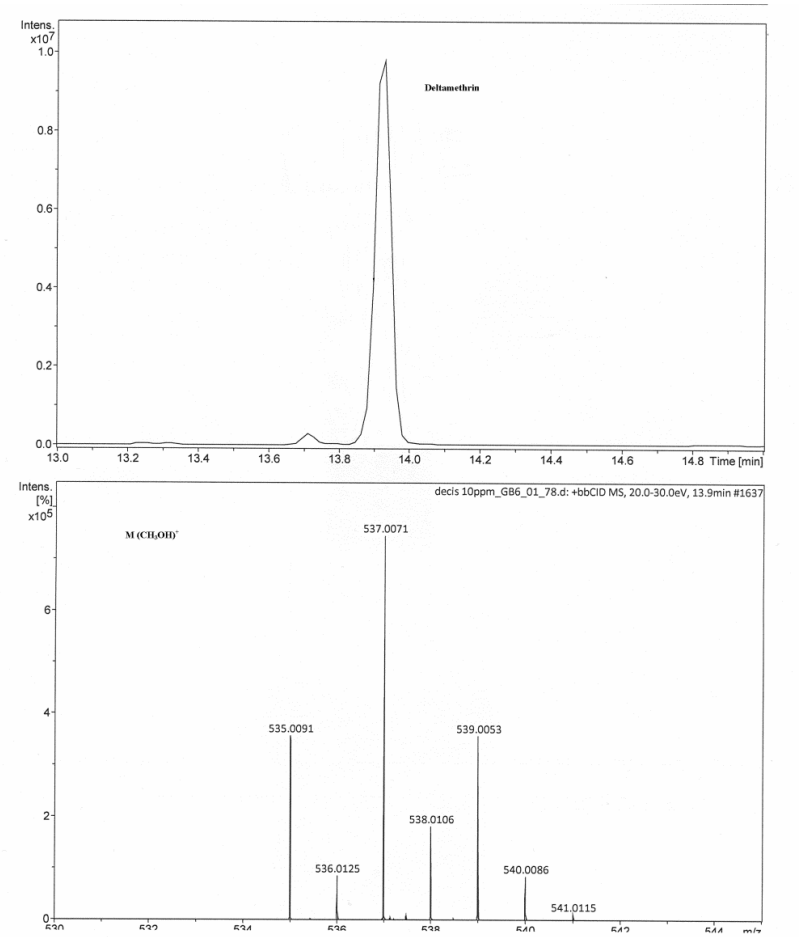

Figure 1: Results of HPLC and mass spectrometry of deltamethrin

RESULTS OF RESEARCH

Comparative studies on the effect of deltamethrin on the viability of rat Gasser's ganglion neurinoma cells on the back of the use of potential therapeutic agents are presented in Figure 2.

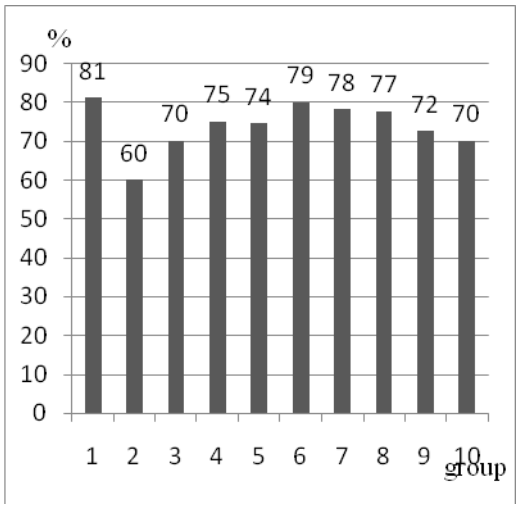

Figure 2 (a) – Viability of RGGN-1 cells under the influence of deltamethrin in a dose of 0.01 mM on the back of the use of potential therapeutic agent

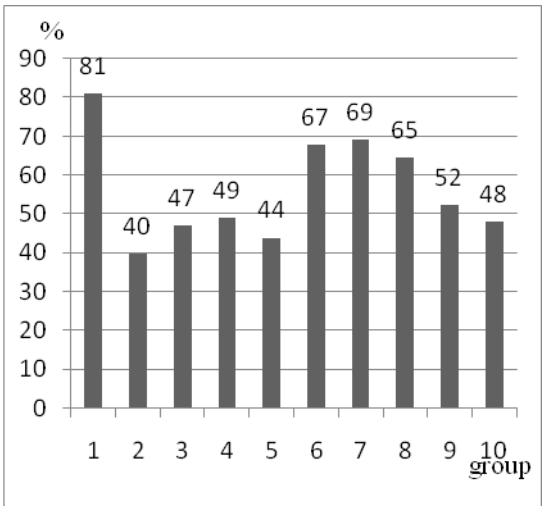

Figure 2 (b) – Viability of RGGN-1 cells under the influence of deltamethrin in a dose of 0.07 mM on the back of the use of potential therapeutic agent

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Figure 2 (a) shows that in the second group under the influence of deltamethrin in the amount of 0.01 mM there was a decrease in the coefficient of viability by 26.0%, in the third group there was a decrease in the coefficient of viability of the RGGN-1 cells line by 12.3%, in the fourth and fifth ones - by 7.5 and 8.2% relative to the control. In the sixth and seventh groups, the decrease in this coefficient relative to control was 1.4 and 4.0% respectively. In the eighth, ninth and tenth groups, the reduction in the cell line viability was 4.2, 11.0 and 14.0% compared to the control.

Figure 2 (b) shows that in the second group under the influence of deltamethrin in the amount of 0.07 mM there was a decrease in the coefficient of viability by 51.0%, in the third one there was a decrease in the coefficient of viability of the RGGN-1 cells line by 42.1%, in the fourth and fifth groups - by 39.0 and 46.0% relative to control. In the sixth and seventh groups, the decrease in this coefficient relative to control was 17.0 and 15.0% respectively. In the eighth, ninth and tenth groups, the reduction in the cell line viability was 20.5, 45.1 and 40.0% compared to the control.

Comparative studies on the effect of deltamethrin on the proliferative activity of rat Gasser's ganglion neurinoma cells in the application of potential therapeutic agents are presented in Figure 3.

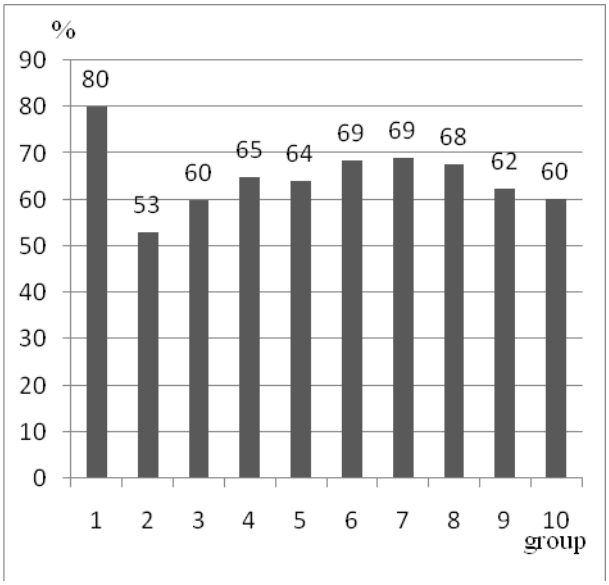

Figure 3 (a) – Proliferative activity of the RGGN-1 cells under the influence of deltamethrin in a dose of 0.01 mM on the background of the use of potential therapeutic compounds

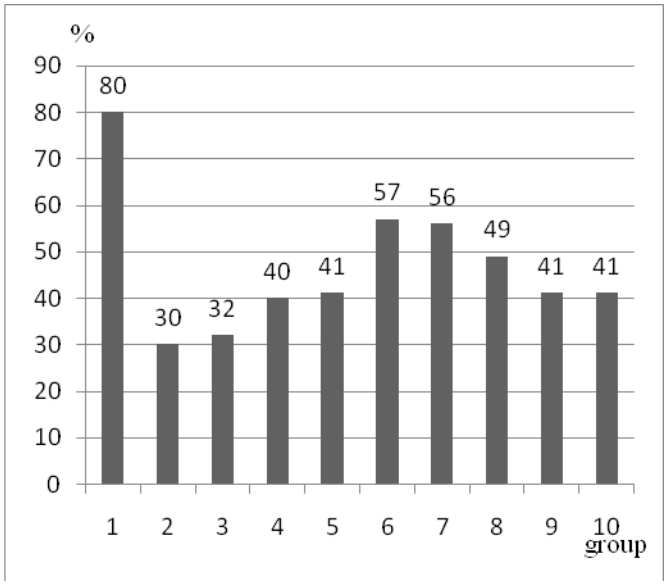

Figure 3 (b) – Proliferative activity of the RGGN-1 cells under the influence of deltamethrin in a dose of 0.07 mM on the background of the use of potential therapeutic compounds

Figure 3 (a) shows that in the second group under the influence of deltamethrin in the amount of 0.01 mM there was a decrease of proliferative activity by 34.3%, in the third one there was a decrease of cell proliferation by 25.0%, in the fourth and fifth groups - by 19.2 and 20.0% relative to control. In the sixth and seventh groups, the decrease in this coefficient relative to control was 14.3 and 13.7% respectively. In the eighth, ninth and tenth groups, the reduction in the coefficient of cell line proliferation was 16.0, 12.0 and 15.0% compared to the control.

Figure 3 (b) shows that in the second group under the influence of deltamethrin in the amount of 0.07 mM there was a decrease of proliferative activity by 73.5%, in the third one there was a decrease of cell proliferation by 60.3%, in the fourth and fifth groups - by 50.1 and 51.2% relative to control. In the sixth and seventh groups, the decrease in this coefficient relative to control was 29.0 and 30.0% respectively. In the eighth, ninth and tenth groups, the reduction in the coefficient of cell line proliferation was 39.0, 48.0 and 49.0% compared to the control.

In a cellular monolayer with the use of medicines there is a more pronounced cellular aggregation, a single presence of large cells with dark cytoplasm, a slight presence of debris compared to the group without the use of therapeutic compounds.

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Comparative studies on the effect of deltamethrin on the cytotoxic efficiency of RGGN-1 cells in the application of potential therapeutic compounds are presented in Figure 4.

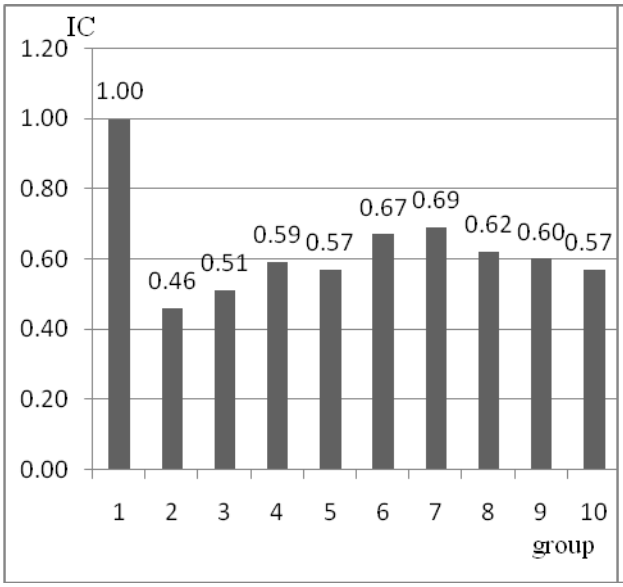

Figure 4 (a) – Cytotoxic efficiency of RGGN-1 cells under the influence of deltamethrin in a dose of 0.01 mM on the background of the use of potential therapeutic compounds

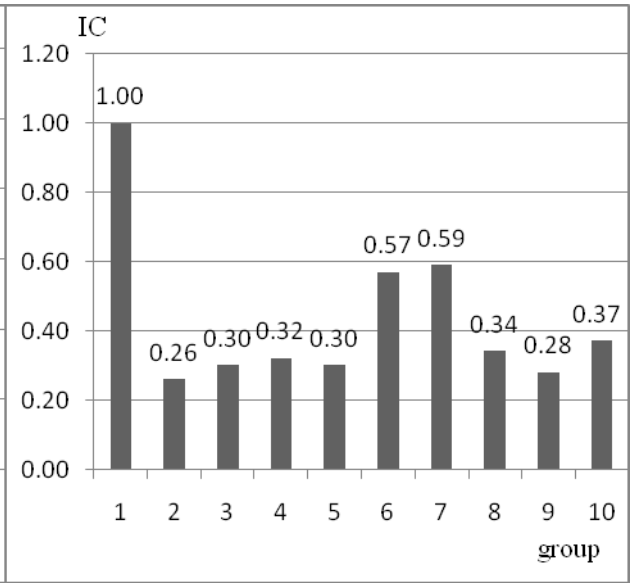

Figure 4 (b) – Cytotoxic efficiency of RGGN-1 cells under the influence of deltamethrin in a dose of 0.07 mM on the background of the use of potential therapeutic compounds

Figure 4 (a) shows that in the second group under the influence of deltamethrin in the amount of 0.01 mM there was a decrease of the cytotoxic efficiency by 54.3%, in the third one there was a decrease of the cytotoxic efficiency by 49.0%, in the fourth and fifth groups - by 41.0 and 43.0% relative to control. In the sixth and seventh groups, the decrease in this coefficient relative to control was 33.0 and 31.0% respectively. In the eighth, ninth and tenth groups, the reduction in the coefficient of cytotoxic efficiency was 38.0, 40.0 and 43.0% compared to the control.

Figure 4 (b) shows that in the second group under the influence of deltamethrin in the amount of 0.07 mM there was a decrease of the cytotoxic efficiency by 74.0%, in the third one there was a decrease of the cytotoxic efficiency by 70.0%, in the fourth and fifth groups - by 68.0 and 70.0% relative to control. In the sixth and seventh groups, the decrease in this coefficient relative to control was 43.0 and 41.0% respectively. In the eighth, ninth and tenth groups, the reduction in the coefficient of cytotoxic efficiency was 66.0, 72.0 and 63.0% compared to the control.

CONCLUSION

According to the results of studies it was found that among the test compounds, the best cytoprotective properties on rat Gasser's ganglion neurinoma cell under the influence of deltamethrin was presented by 6-isopropyl-2-thiouracil and 6-*tert*-butyl-2-thiouracil. These compounds increase the survival and proliferative activity of RGGN cells under the influence of deltamethrin. It indicates the advisability of further research on the use of these compounds as potential antidotes.

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