

**THE BIOLOGICAL AND CLINICAL ANALYSIS OF PROSTATE-PROTECTIVE
EFFECTS OF THE PEPTIDE PRO IPH**

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Abstract. The article describes the result of research of biological and clinical prostate-protective effects of peptide IPH PRO. The peptide IPH PRO has a high biological activity in to the controlling of the normal formation of the reproductive system in humans at the genetic level according to the expression of genes for the formation of male genitals and hormones on cell culture. The article also describes that the peptide IPH PRO has a regulatory effect on the functional activity of male genital cells, which is manifested by cytostatic and anti-inflammatory action against prostate cells according to experimental studies. The article describes the clinical parameters: the application of the peptide IPH PRO has a regulatory effect on the functional activity of prostate cells, contributes to the normalization of urination, reduces the number of daily urinations and improves the quality of life. Therefore, the application of the peptide PRO IPH is recommended like a biologically active supplement with therapeutic and preventive effects.

Key words: peptide, prostate, protective effects, biological effects, chronic prostatitis, adenoma of prostate gland.

Introduction.

The study of the effects of peptides has a great interest today. Peptides have the same structure as proteins, but the size of these molecules is smaller. It is also important that short peptides are a natural metabolic product in the body and they can't be detected in blood or urine. That's why, it is interesting to study the effects of peptides on cell cultures [1,2,3].

The peptide IPH PRO contains a complex of low molecular weight peptides and has a normalizing effect on prostate function [4,5].

The experimental studies have shown that the peptide IPH PRO regulates metabolic processes in prostate cells, increases its reserve capacity. These facts suggest that the peptide IPH PRO has an effect for the normalization of prostate function in men of different ages by different genesis [6-8].

The diseases of prostate occupy a significant place among urological pathology today [7,8]. According to some authors, more than 70% of men over the age of 50 have prostate diseases, including adenoma of prostate gland and cancer. Thus, the research of new effective methods for the prevention and treatment of prostate diseases is actual [1,6,8].

The development of chronic prostatitis is no less dangerous for the social life of men. More than 50% of the male population have chronic prostatitis. The men aged 20 to 40 years have chronic prostatitis is more often. This man are in the period of greatest sexual, reproductive and labor activity. This fact proves the medical, socially significant aspects of this disease. It's important to search new therapeutic and preventive techniques for man [1,6].

The aim of this study was to identify biological and clinical effects of prostate-protective effects of the peptide IPH PRO.

Material and methods.

We conducted 3 areas of research to identify the biological and clinical effects of the peptide IPH PRO:

1. The study of the effects of the peptide IPH PRO on the cell.
2. The study of the effects of the peptide IPH PRO in the experiment.
3. The clinical study of the effects of the peptide IPH PRO.

In a cell-based study we selected embryonic stem cells (SC5, origin: human, embryonic stem cells (ESC), blastocyst (5-6 days of development), collection: Institute of Cytology of the Russian Academy of Sciences, Federal state institution of science «Institute of Cytology of the Russian Academy of Sciences», http://www.cytspb.rssi.ru/eotk/infbull_ru.htm, M. S. Bogdanova, G. G. Polyanskaya, A. M. Koltsov "Cell cultures" Information Bulletin. Vol. 34 (2018), http://www.cytspb.rssi.ru/rkkk/katalog1n_2017_with_figs.pdf), which are of pluripotent type.

We carried out the expression of the main genes involved in the formation of organs and glands of the urinary-reproductive system: the genes involved in the differentiation of gonads and ontogenesis of the genitals, and the genes responsible for the synthesis of sex hormones and receptors to these hormones.

We also assessed the biological active markers. We used immunofluorescence technique using primary antibodies to SSEA-4 (1:150, Abcam) and p53 protein (1:50, Abcam). We have created the following groups for the study: 1 group – the study of molecular expression before the study; 2 group-control (we added the culture medium, incubation with serum albumin); 3 group – we added the control dipeptide Glu-Trp at the concentration of 100 micrograms (mcg); 4 group – we added the peptide IPH PRO at a concentration of 100 micrograms (mcg). We selected the peptide Glu-Trp with the immune properties and well described in the literature as a control.

The PCR method was used to measure the level of gene expression using Novocasta's reagents and sets of monoclonal antibodies produced by Biosource (Belgium). We used confocal microscope Olympus FluoView FV1000 with indicator of 200, 400, 600. We conducted the measurement of the expression in %.

We have chosen the most commonly used species of laboratory animals for the study for the experiment recommended by the Ministry of Health of the Russian Federation in the Manual for preclinical studies of drugs - rats. We created a model of hypokinetic stress, in which prostatitis developed in experimental rats. Was studied 40 rats at the age of $14,2 \pm 1,2$ months and weighing $409,6 \pm 9,5$ g.

All procedures of animal keeping and testing were carried out in accordance with standards ISO 10993-1-2003 and GOST RISO 10993.2-2006. The rats were divided into 2 groups – the control (n=20) and the main group (n=20). The rats of the main group were given orally through a pipette-dispenser a solution consisting of water for injection in a dosage of 1 ml, in which the lyophilized powder of IPH PRO peptides was dissolved in a concentration of 0.59 micrograms (mcg) per rat body weight per day for 14 days. A pipette-dispenser allowed to control the volume and the fact of liquid consumption.

The rats were killed after 26 days. Then the prostate gland was removed, fixed by immersion in a solution of 4% paraformaldehyde in phosphate buffer (PBS pH = 7.3) for 24 hours at a temperature of 4 °C. We produced slices with a thickness of 20 μm using cryotome of Leica CM 1510S model (Germany). Then the sections were mounted on a slide and stained with hematoxilin and eosin. We used the Olympus IX81 microscope for the study. The Danet criterion was used to assess the reliability of the difference in the results obtained in the groups before the use of the peptide, compared with the groups after the application of the peptide IPH PRO.

The clinical studies of the peptide IPH PRO were conducted in 57 patients with chronic prostatitis and adenoma of prostate gland. We conducted studies the effectiveness of peptides in the dosage of 50 µg (n= 55 people) and 150 µg (n=56 people) to assess the effectiveness of the dose of 100 µg (n=57 people) for the peptide IPH PRO. All patients were exposed to diagnoses and appropriate treatment carried out in accordance with the Russian clinical guidelines in urology 2013 (<http://www.minzdravrb.ru/minzdrav/docs/urol.pdf>).

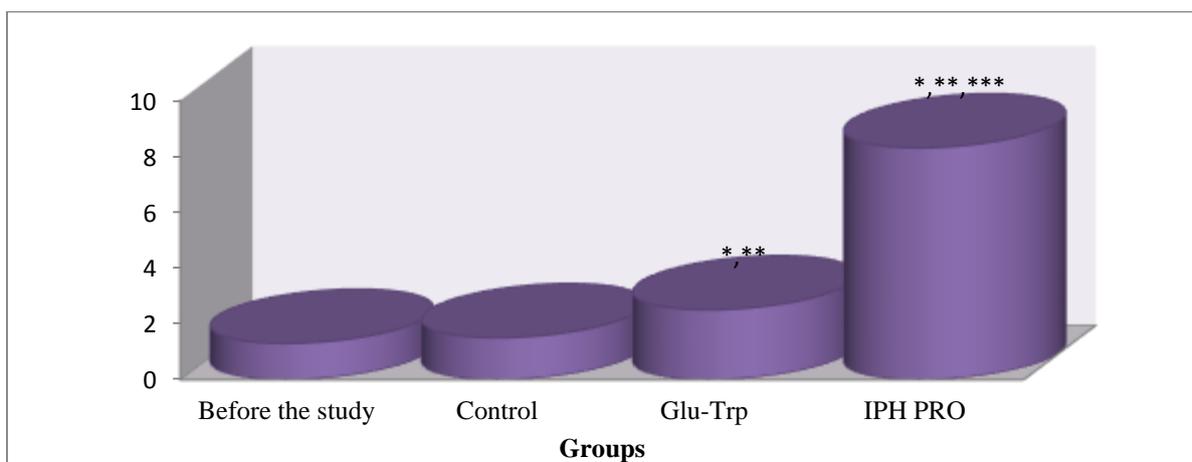
The peptide IPH PRO was administered orally: 1 capsule (100 µg peptide) 1 time per day for 30 days, then 30 days a break in the medication. And repeat the same course for another 30 days, again 30 days a break in the medication - and the third course for 30 days. The total course was 6 months (3 courses of 30 days and 3 a break in the medication of 30 days). We studied the effectiveness of the improved management scheme of such patients using the peptide IPH PRO after 3 and 6 months. The control values was selected the results before the study. The efficacy of the peptide IPH PRO was evaluated of the dynamics of patients' complaints, the degree of abdominal pressure during urination, fluorometric index and the quality of life.

We used standard methods of medical and biological research.

Results and discussion.

The biological analysis of prostate-protective effects of the peptide PRO IPH on cell culture

The effect of the IPH PRO peptide on the expression of genes responsible for the human reproductive system is shown in figure 1 and 2. It was found that the peptide IPH PRO significantly increases the expression of genes responsible for the normal formation of both male genitals and factors that form the hormonal system.

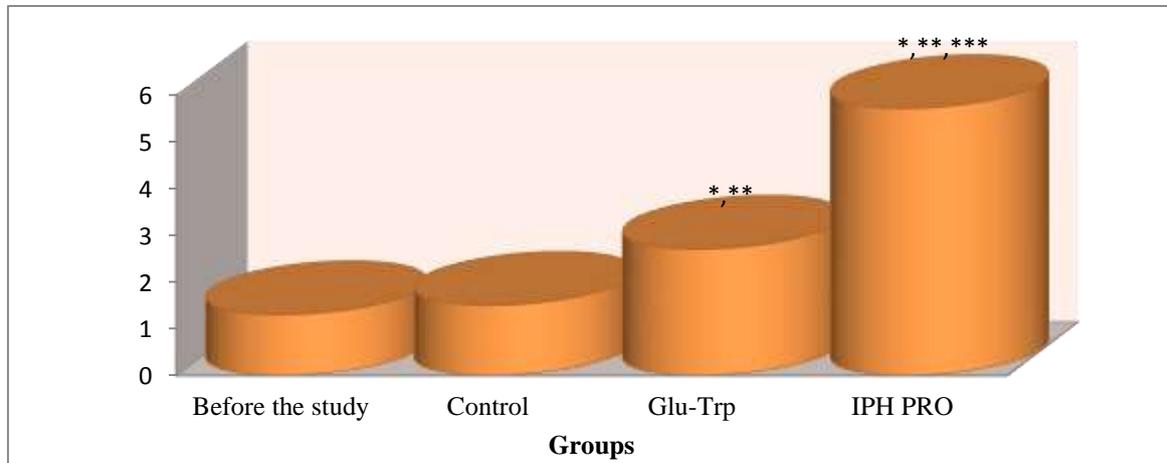


* $p < 0.05$ compared to baseline data;

** $p < 0.05$ compared to control;

*** $p < 0.05$ between the indicators of the level of expression used Glu-Trp and the peptide PRO IPH.

Figure 1. Total expression of genes involved in the differentiation of the gonads and the ontogeny of male genital organs: SRY, SOX9, WT1, a gene that encodes steroidogenic factor SF1.



* $p < 0.05$ compared to baseline data;

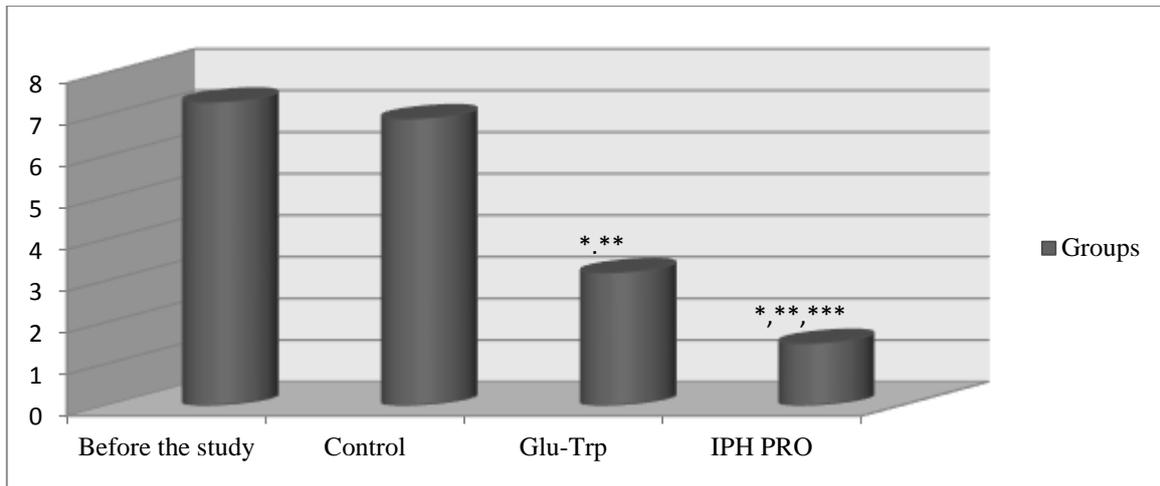
** $p < 0.05$ compared to control;

*** $p < 0.05$ between the indicators of the level of expression used Glu-Trp and the peptide PRO IPH.

Figure 2. Total expression of genes responsible of synthesis of sex –hormones and receptors to these hormones: FSH (gene follicle stimulating hormone), LH (luteinizing hormone gene), AMN (gene antimullerian hormone), AMHR (gene receptor AMHR), TAR (the gene for the androgen receptor), GnRH (gene gonadotropin-releasing hormone), GnRH-R (GnRH receptor gene), LH-R (receptor gene and LH - luteinizing hormone), FSH-R (the receptor gene FSH - follicle stimulating hormone).

It is proved that under the influence of the peptide PRO IPH in human cell culture there is a statistically significant increase the expression of genes responsible for the ontogenesis of male genital organs and hormonal background. These studies showed that the peptide IPH PRO significantly increases in the culture of human cells "cascade" of signal molecules, which is necessary for the activation of proliferation and differentiation of stem cells of the male genital organs, in particular, prostate cells.

The effect of the peptide IPH PRO on the expression of SSEA-4 in human cell cultures is shown in figure 3. It was found that the use of the peptide IPH PRO reduces the expression of SSEA-4 in 4 times from the baseline. These types of cells are detected in prostate cancer and other tumors in the human body.



* $p < 0.05$ compared to baseline data;

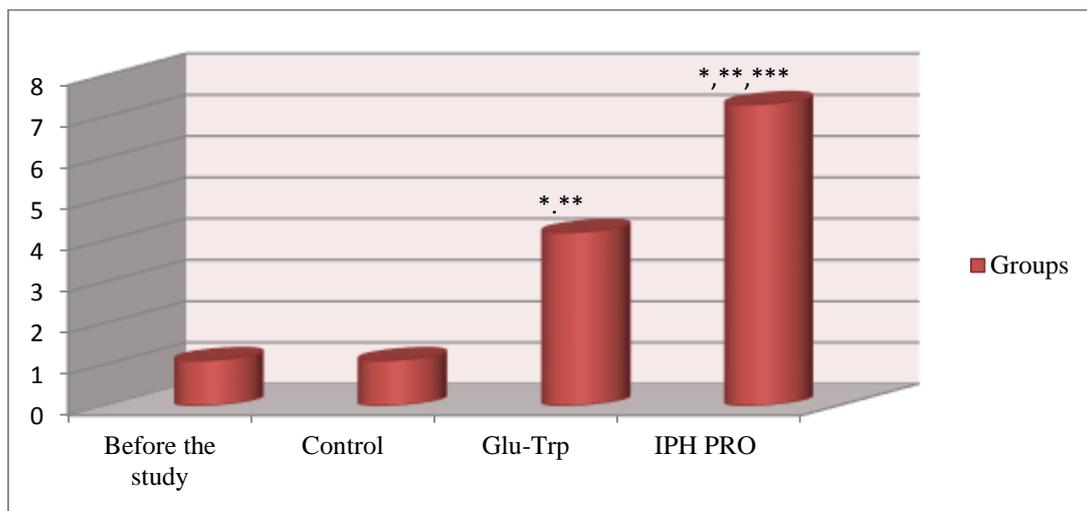
** $p < 0.05$ compared to control;

*** $p < 0.05$ between the indicators of the level of expression used Glu-Trp and the peptide PRO IPH.

Figure 3. The effect of the peptide IPH PRO on the expression of the SSEA-4 in the cell culture.

Consequently, the application of the peptide IPH PRO is protected against cancer, in particular, against malignant tumors of the prostate gland according to the level of expression of the marker SSEA-4 on cell culture.

The effect of the peptide IPH PRO on the expression of p53 protein in cell cultures is presented in figure 4. The use of the peptide IPH PRO increases the production of protein p53, which is a transcriptional factor and acts as a suppressor of malignant tumor formation by the way of activating apoptosis in the tissues. This results lead to the conclusion about the antitumor properties of the peptide IPH PRO.



* $p < 0.05$ compared to baseline data;

** $p < 0.05$ compared to control;

*** $p < 0.05$ between the indicators of the level of expression used Glu-Trp and the peptide PRO IPH.

Figure 4. The effect of the peptide IPH PRO on the expression of p53 in the cell culture.

P53-dependent apoptosis also avoids the accumulation of mutations. In the case when mutations have already arisen, p53-dependent apoptosis allows to eliminate this potentially dangerous cells. On this fact we can make a conclusion about the cytoprotective effect of the peptide IPH PRO.

The peptide IPH PRO had a high onco-protective activity in relation to the cells of the reproductive system of men according to the expression of biological molecules in cell culture.

Biological analysis of prostate-protective effects of the peptide IPH PRO in an experimental model

We found significant areas of leukocyte infiltration were noticeable in the prostate stroma in the control group in an experimental model ($76,8 \pm 1,2\%$). But in rats were given orally the peptide IPH PRO the area of leukocyte infiltration was $41,4 \pm 0,4\%$ (figure 5).

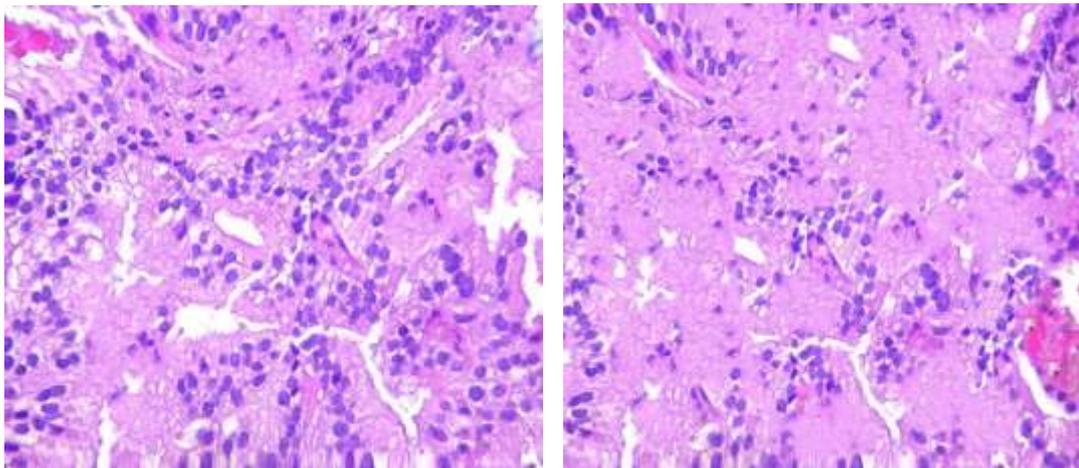


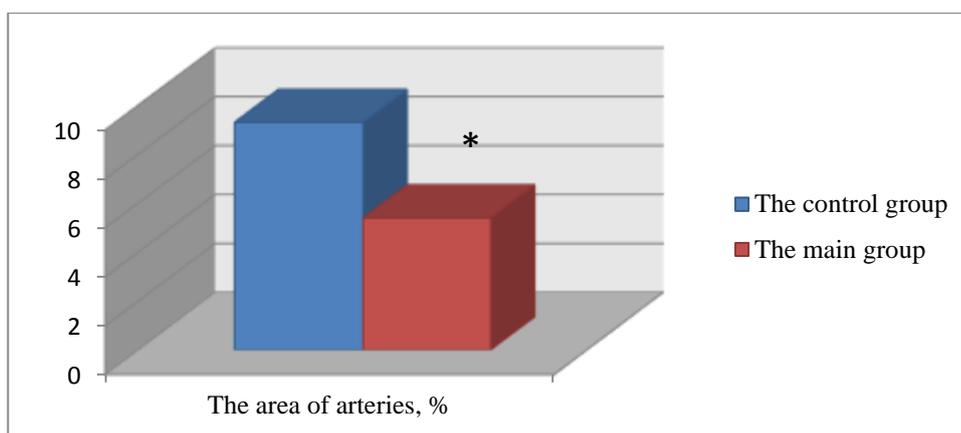
Figure 5. The infiltration of leukocytes in the prostate stroma (hematoxylin and eosin, x 400).

A - without the application of the peptide IPH PRO.

B - with the application of the peptide IPH PRO.

Consequently, the peptide IPH PRO reduces the degree of inflammation in the development of prostatitis in the experimental model.

We found the developed thickening and edema of tissues and there were areas of necrotic endotheliocytes in capillary and the accumulation of amyloid plaques. All these morphological parameters indicate a decrease in reparative capabilities into cells.



* $p < 0.05$ compared to the control group.

Figure 2. The area of arteries (%).

We found that in rats were given orally the peptide IPH PRO the area of arteries was in 1,7 times less than in rats with untreated prostatitis (figure 6). We can make a conclusion that such a decrease in the area of the arteries indicates a lower need for compensatory reactions.

Therefore, the peptide IGF PRO has a cytostatic effect and increases the adaptive capacity of cells in rats with prostatitis according to experimental model.

Clinical analysis of prostate-protective effects of the peptide PRO IPH

The results of a clinical study have shown, that pollakiuria (frequent urination) had completely stopped at 73.2% of chronic prostatitis patients, 88.1% of patients disappeared the need for nighttime urination. Stranguria (difficult urination) had stopped at 67.1% of patients, 21.5% of patients noted an increase in urine flow and improved urination (table 1).

Table 1

The dynamics of fluorometry after the application of the peptide PRO IPH in patients with chronic prostatitis

Indicator	Before the study	In 3 months			In 6 months		
		The dosage of the peptide PRO IPH			The dosage of the peptide PRO IPH		
		50 µg	100 µg	150 µg	50 µg	100 µg	150 µg
The average urine flow rate, ml/s	16,9±1,2	16,8±1,2	24,3±1,7*, #	24,2±1,7*	16,6±1,2	28,1±1,6*, #	28,3±1,6*
Maximum speed urination, ml/s	19,6±1,1	19,3±1,1	25,2±1,7	25,1±1,7	19,9±1,1	28,4±1,7*, #	28,1±1,7*
The time to achieve peak flow rate, sec.	16,1±1,2	15,9±1,2	10,2±0,8	10,8±0,8	16,6±1,2	5,3±0,1*, **, #	5,2±0,1*, * *

* $p < 0.05$ compared to the control group (before the study).

** p<0.05 between 3 months and 6 months.

p<0.05 between 50 µg and 100 µg.

It should be noted that the most positive results were found about the time to achieve the maximum urination rate, which after 6 months of the study decreased to $5,3 \pm 0,1$ seconds. It's refers to the norm.

The dynamics of the results in patients with adenoma of prostate gland after 3 and 6 months the application of the peptide PRO IPH is shown in table 2.

Table 2.

The dynamics of fluorometry after the application of the peptide PRO IPH in patients with adenoma of prostate gland

Indicator	Before the study	In 3 months			In 6 months		
		The dosage of the peptide PRO IPH			The dosage of the peptide PRO IPH		
		50 µg	100 µg	150 µg	50 µg	100 µg	150 µg
Urinary delay time, sec.	4,4±0,5	4,1±0,5	2,7±0,3*,#	2,8±0,3*	4,7±0,5	2,3±0,3*	2,2±0,3*
The number of urinations:	9,1±0,7	9,2±0,7	6,1±0,4*,#	6,0±0,4*	9,6±0,7	5,1±0,4*,#	5,2±0,4*
- during the day	4,1±0,6	4,2±0,6	2,7±0,1	2,9±0,1	4,5±0,6	1,8±0,1	1,9±0,1
- at night							
The degree of abdominal pressure, points	3,1	3,2	2,5	2,6	3,0	2,1*,#	2,0*
The character of the urine stream, scores	3,6	3,3	2,3*,#	2,3*	3,3	2,0*,#	2,0*
The average urine flow rate, ml/s	13,5±1,3	13,4±1,3	15,0±1,6	15,3±1,6	13,6±1,3	19,4±1,4*,#	19,3±1,4*
Maximum speed urination, ml / s	16,9±1,3	16,7±1,3	21,3±1,7	21,4±1,7	16,8±1,3	22,2±1,7	22,1±1,7
The time to achieve peak flow rate, sec.	15,1±1,2	15,8±1,2	9,0±0,3	9,2±0,3	15,3±1,2	6,2±0,1*,* *,#	6,1±0,1*,* *

* p<0.05 compared to the control group (before the study).

** p<0.05 between 3 months and 6 months.

p<0.05 between 50 µg and 100 µg.

We found the fact of a significant decrease in the number of urinations in the daytime after the application of the peptide IPH PRO in 1,5 times in 3 months and in 1,8 times in 6 months compared to the baseline, p<0.05 compared to the control group (before the study), p<0.05 between 3 months and 6 months. Probably, this fact also influenced on the quality of life of patients with adenoma of prostate gland.

It should be noted that the results of uroflowmetry in patients with adenoma of prostate gland stage I and stage II showed the reconstruction of the main parameters of urination to normal values. The results of uroflowmetry in patients with adenoma of prostate gland stage III showed increase in urine flow.

The state of health of the patients with adenoma of prostate gland after application of the peptide IPH PRO was characterized by improvement of subjective and objective characteristics of urodynamics.

We assessed the quality of life using the questionnaire EuroQol EQ-5D (table 3).

Table 3.

The assessment of quality of life using the questionnaire EuroQol EQ-5D (M±m, points)

Indicator	Before the study	In 3 months			In 6 months		
		The dosage of the peptide PRO IPH			The dosage of the peptide PRO IPH		
		50 µg	100 µg	150 µg	50 µg	100 µg	150 µg
Mobility	3	3	4	4	3	4	4
Self service	4	4	4	4	4	4	4
Habitual daily activities	2	2	3	3	2	3	3
Pain/ Discomfort	2	1	4	4	2	4	4
Anxiety/ Depression	2	2	4	4	2	4	4
Visual analog scale, mm	40	40	70	70	40	70	70

Thus, the quality of life of patients has improved for each parameter, as much as possible in terms of pain and the development of anxiety and depression. It should be noted that these parameters have increased in 2 times. The subjective assessment of the quality of life on the visual-analog scale increased in 1,8 times compared to the initial data after 3 months of the application of the peptide IPH PRO and remained positive throughout the study.

We haven't found significant differences between the results in the application of 100 mcg and 150 mcg in all the studied parameters like in 3 months as in 6 months. Also, we have not found significant differences between the indicators in the application of 50 mcg and before the study on all parameters. This fact proves that the effective optimal dosage for the peptide IPH PRO is 100 mcg.

Conclusion.

These studies confirm the high biological activity of the peptide IPH PRO in relation to the control of the normal formation of the reproductive system in humans at the genetic level according to the expression of genes responsible for the formation of male genitals and hormones in cell

culture. The results indicate a high onco-protective activity of the peptide PRO IPH of prostate cells according to the expression of biological molecules in cell culture.

It was also proved that the peptide IPH PRO has a regulatory effect on the functional activity of male genital cells, which is manifested by cytostatic and anti-inflammatory action against prostate cells according to experimental studies.

We have found that the use of the peptide IPH PRO has a regulatory effect on the functional activity of prostate cells, contributes to the normalization of urination, reduces the number of daily urinations and leads to a subjective improvement in the quality of life according to clinical studies.

The application of the peptide IPH PRO is recommended as a supplement with a therapeutic and preventive purpose for patients with chronic prostatitis and adenoma of prostate gland in the optimal effective dosage of 100 µg as a supplement to normalize the functions of the prostate gland.

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