ORIGINAL ARTICLE

Experimental Basis of the Bee Products Standard Substances Composition Safety for the Treatment of the Urogenital System

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Abstract

Introduction: The work presents the data of the literary sources review which suggests that nowadays, treatment of the urinary tract, including prostatitis and prostate adenoma, which are the most common and serious male disorders of and tend to increase their frequency, is the topical issue of the modern medicine. It is proved that the compounds of natural substances, standardized bee products in particular - honey powder (HP), propolis phenolic hydrophobic preparation (PPHP) and bee pollen (BP), are promising for the effective treatment of this disease. **Materials and Methods:** The results of studying the acute toxicity and safety of a new national medication of natural origin for treating the diseases of the genitourinary system, including prostatitis and prostate adenoma, which contains above named natural substances, are presented in the experimental part. Pharmacological, physiological, instrumental research methods and mathematical statistics methods have been used in the study. **Results and Discussion:** The analysis of morphological studies of testes and sperm functional state of rats demonstrates no change of morphological and functional indices in the experimental group compared with the control group. Basing on these studies, we can conclude that the mixture of active pharmaceutical ingredients (APIs) in the form of bee products standardized substances composition at a dose of 100 mg/kg demonstrates no toxic effects on spermatogenesis in male rats. The results of the study allergenic activity of the studied composition at the same dose indicate no hypersensitivity of immediate type reactions and no accumulation of homocytotrophic antibodies in the blood, and accordingly the no sensitizing activity, which was also confirmed by the "conjunctival test" on guinea pigs. The research of the local irritating effect of the mixture of the APIs on the mucous membrane demonstrates the lack of such effect after a single application to the eye of rabbits. **Conclusions:** The study of the specific toxicity of the APIs mix in the form of a bee products standardized substances composition - HP, PPHP, and BP at a dose of 100 mg/kg, demonstrated no capacity to gonadotoxic and allergenic effect. It has been found that the formula of APIs has no effect on the secretory function and the state of the gastric mucosa and no irritating action at contacting with mucous membranes of an eye. The results of the studies of pharmacological activity and specific toxicity of the bee products standard substances composition - HP, PPHP, and BP – allow to conclude that manufacturing such preparation is promising, and further preclinical researchers to obtain a permit to introduce a new preparation into clinical practice and industry are desirable.

Key words: Allergenic, bee pollen, genitourinary system disorders, gonadotoxic, honey powder, local irritating action, propolis phenolic hydrophobic preparation

INTRODUCTION

Nowadays, the problem of treating urogenital system is still an urgent problem for modern medicine.[1-4] Therein, the prostate disorders require considerable attention, including prostate and prostate adenoma, which are supposed to be the most common and serious male pathology and tend to increase their frequency.[5-7]

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Currently, different methods of treating patients with adenoma and chronic prostatitis are used widely, but there is no sufficient amount of reasonably grounded approaches to the treatment of these diseases yet. The basic principles of treating chronic prostatitis are affecting all links of the etiology and pathogenesis of the disease; category, activity, and extent of the inflammatory process accounting; and the use of complex pharmacological measures. However, the standard therapy of patients with chronic prostatitis is not always effective enough, and the disease recurrence often occurs after the treatment.[8]

Some scientists believe that in most cases the etiology, pathogenesis, and pathophysiology of chronic prostatitis are still considered uncertain.[2-4,9-14] However, among the etiopathogenic factors of this disease, the main one is considered to be the prostate infection in the course of the chronic urethritis. There is also the assumption that in almost 90% of cases the microorganisms causing inflammation in the prostate gland penetrate from the urethra through the glands ducts, and other ways of infection contamination are extremely rare.[15] In addition, there may be observed severe microcirculation complications and neurotrophic disorders developing.[9,15]

Allergy, self-aggression, hormonal and immunological imbalance, etc., can play an important role. Typically, in such cases, even massive and long-lasting antibiotic therapy is not sufficient for a clinical cure and for sanitation for the infection as well. In this regard, only using complex therapy, which will affect various factors of the chronic prostatitis pathogenesis, can provide relatively favorable results.

In our opinion, one-way to solve this problem is to create new highly efficient domestic preparations of complex action that have anti-inflammatory and reparative properties with minimal side effects. In this regard, promising compounds are medications based on bee products standardized substances that are increasingly used to create natural medicines of different directional effect at the pharmaceutical market of Ukraine.[16-19]

EXPERIMENTAL PART

According to the State Pharmacological Center of the Ministry of Health of Ukraine guidelines, while researching a new promising medication along with the study of pharmacological activity, a mandatory characteristic is appraising toxic level, which, in its turn, allows to appraise its safety level.[19] In this context, the aim of this work was to study the acute toxicity and safety of a new national preparation of natural origin for treating the genitourinary system diseases, including prostatitis and prostate adenoma.

Pharmacological, physiological, instrumental research methods and mathematical statistics techniques have been used in the work.

Studying specific toxicity of the preparation being developed was conducted in the Central Research Laboratory of the National University of Pharmacy.

Researching gonadotoxic effects of the composition of the standardized substances of honey powder (HP), propolis phenolic hydrophobic preparation (PPHP), and bee pollen (BP) were performed on male rats weighing 180-240 g according to the methodological recommendations.[20]

The research has been conducted according to the national "general ethical conduct of experiments on animals" (Ukraine, 2001), which correspond the statements of "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986).[21]

A mixture of active pharmaceutical ingredients was delivered at a dose of 100 mg/kg within one period of spermatogenesis (48 days). At the end of the experiment the animals were taken out by decapitation, their testes were taken out and their morphological characteristics - weight, length - were determined, and also their weight coefficient was calculated. Along with this, the sperm functional status parameter was researched, and testicular tissue was selected for histological research.

To investigate the sperm functional state, the suspension along the split cercus of a seminal gland in normal saline solution was used. The indicators of sperm function were: Their number, relative number of dead and abnormal sperm forms, their osmotic and acid resistance.[20]

The obtained experimental data were processed by the method of variation statistics (calculated the mean arithmetic and standard error).[22] For comparison of the normal distribution, one-factor ANOVA and Newman-Calex for multiple comparisons were used, for nonparametric data, the Kruskal–Wallis (ANOVA) and the Mann–Whitney criterion. The verification of the normality of the distribution of factual data was performed using Leven's test.[23] Differences between groups were considered statistically significant at $P < 0.05$.

When using the Mann–Whitney test, the significance level for multiple comparisons is listed with the Bonferroni correction according to the formula $P = p0/k$,[23] where $p0 = 0.05$, k is the number of pair comparisons, which in this study is equal to 2: "Intact control - negative control," "intact control - test sample," level of significance $P = 0.0250$. Statistical processing of the data was performed using the Statistica 6.0 software package.[22,23]

These morphological studies of the testes and functional parameters of spermatogenesis in rats are, respectively, presented in Tables 1 and 2.

While studying new upcoming medications preclinically, the exclusive requirement is to conduct experimental studies to identify allergenic properties.[24] In this regard, a possible of the API formula of HP, PPHP, and BP was carried out using indirect mast cells degranulation reaction, conjunctival test, and active cutaneous anaphylaxis.

Researching allergenic effect of the bee products standard substances composition was active cutaneous anaphylaxis model-based. Testing was performed on mature guinea pigs weighing 450-600 g. Sensitization of animals was realized orally for 14 days with the medication which was studied at a dose of 100 mg/kg. The control animals received the solvent - purified water.

During the experiment, on the 21st day after the sensitization, the animals of experimental and control groups got subdermally 40 ml of the medication suspension into shaven parts of skin on the back at concentrations that do not cause nonspecific inflammatory response. To control the solvent, 40 ml of saline were inserted subdermally into the area on the left flank of each experimental animal. Then, the animals were injected intravenously with 0.5 ml of 1% solution of Evans blue. 30 min later, the animals were taken out of the experiment with the overdose of ether and their skin was separated. The results of the experiment are presented in Table 3.

Studying allergenic effects of the bee products standard substances composition – HP, PPHP, and BP in a mast cell degranulation reaction were based on the ability of the API formula to cause appearance of homocytotrophic antibodies in the mast cell degranulation test.[25] In the experiment animals weighing 150-200 g were used.

Sensitization of rats was performed orally daily for 14 days. Control animals received orally solvent - purified water. During the experiment on the 21st day, the animals were sacrificed under ether anesthesia, and serum was obtained for setting the reaction.

Table 1: Some morphological parameters of the testes of the rats treated with the bee products standard substances composition – HP, PPHP, and BP at the dose of 100 mg/kg

Groups of animals	*n*	Weight	Testes weight				Testes length	
			Right		Left		Right, cm	Left, cm
			Weight, g	Mass coefficient	Weight, g	Mass coefficient		
Control group	8	216.43±8.22	1.65±0.13	0.79±0.08	1.66±0.12	0.77±0.05	2.10±0.09	2.08±0.11
API formula	8	223.75±5.69*	1.69±0.16*	0.81±0.07*	1.68±0.14*	0.80±0.06*	2.12±0.10*	2.13±0.13*

Values are mean of six individual observations in each group mean±SD. **P* denotes statistical significance of ANOVA to test the difference between the experimental groups. SD: Standard deviation, API: Active pharmaceutical ingredients, HP: Honey powder, PPHP: Propolis phenolic hydrophobic preparation, BP: Bee pollen

Table 2: The functions of spermatogenesis of the rats treated with the bee products standard substances composition – HP, PPHP, and BP at the dose of 100 mg/kg

Groups of animals	*n*	Number of sperm cells, mln.	Percentage of dead sperm cells, %	Percentage of pathologic forms of sperm cells, %	Time of sperm motility, min	Osmotic fragility of sperm cells, % in NaCl concentrated solution	Acid resistance
Control group	8	80.66±14.13	29.88±1.97	5.29±0.48	245.0±5.59	3.69±0.08	3.91±0.13
API formula	8	78.35±12.34*	30.25±1.25*	6.14±0.66*	295.5±12.43*	3.72±0.06*	3.80±0.21*

Values are mean of six individual observations in each group mean±SD. **P* denotes statistical significance of ANOVA to test the difference between the experimental groups. SD: Standard deviation, API: Active pharmaceutical ingredients, HP: Honey powder, PPHP: Propolis phenolic hydrophobic preparation, BP: Bee pollen

Table 3: Allergenic effect of the bee products standard substances composition – HP, PPHP, and BP, based on active cutaneous anaphylaxis model

Terms of the experiment	Dose, mg/kg	Sensitization	Number of animals in the group	Area of a colored spot, mm²
Control	–	–	6	6.18±1.04
API formula	100	Oral	6	6.84±0.85*

Values are mean of six individual observations in each group mean±SD. **P* denotes statistical significance of ANOVA to test the difference between the experimental groups. SD: Standard deviation, API: Active pharmaceutical ingredients, HP: Honey powder, PPHP: Propolis phenolic hydrophobic preparation, BP: Bee pollen

In the previous experiments, the substances concentration was selected, causing no more than 5% of non-specific degranulation. Preparations were prepared on subject glass plates, painted with 0.3% alcoholic solution of neutral red. 30 mql of serum from experimental or control animals and 30 mql of coarse dispersion of the preparation in question were added to 30 mql of mast cells coarse dispersion obtained from intact animals. When setting the reaction, to exclude spontaneous mast cells degranulation and degranulation under the influence of the solvent, the following kinds of control were considered:

1. The serum control: 30 mql of the mast cells coarse dispersion, 30 mql of the serain question, and 30 mql of saline solution
2. The allergen control: 30 mql of the mast cells coarse dispersion, 30 mql of saline solution, and 30 mql of allergen.

After the 15 min incubation at 37°C the preparations were microscope, and the mast cells degranulation rate (MCDR) was calculated using the formula:

$$MCDR = \frac{1a + 2b + 3A + 3d}{100},$$

Where, *a*, *b*, *c*, *d* – number (average of three repetitions) of degranulated cells according to the degree of degranulation.

In each interzone, 100 cells were counted. The reaction was considered positive if MCDR was more than 0.2.

The results of the experiment are presented in Table 4.

Studying the allergenic effect of the bee products standard substances composition – HP, PPHP, and BP was also carried out in the "conjunctival test," which was performed on 10 guinea pigs while the preparation was inserted orally for 14 days. During the experiment, on the 21st day after the start of sensitization 1 drop of the corresponding doses of the substance was instilled under the upper eyelid, and 15 min, 1 h, and 24 h later the reaction of the mucosa was observed. The other eye was used as a control; solvent - purified water was instilled in it.[26,27]

Because the API formula– HP, PPHP, and BP is intended for oral application, researching the effect of standard substances composition on the functional state of the gastrointestinal tract and determining their effect on gastric secretory activity and possible ulcerogenic effect was desirable.

The effect of the standard substances composition on the secretion of gastric acid was researched according to the appropriate method.[28] Albino rats weighing 200-220 g were kept on an absolute diet for 48 h without limiting drinking water. The preparation was inserted into animals orally at doses of 100 mg/kg. The control animals received the equivalent amount of solvent. 1 h after inserting the preparation the animals of treated and control groups were intraperitoneally injected with 1% solution of barbamil in the amount of 0.8 ml per 100 g of the animal. Then, the abdomen of the animals was cut, and the pyloric sphincter of the stomach was threaded. 4 h later the other sphincter of the stomach was threaded. The animals were taken out of the experiment by decapitation; their stomach was taken out and the volume of gastric acid was measured.

The intensity of gastric acid secretion was recounted per 100 g of an animal's body weight. Total and free acidity was determined by titrating gastric acid with NaOH 0.1 N solution over phenolphthalein and bromothymol blue indicators. Total and free acidity was determined by the number of milliliters of NaOH 0.1 N solution, necessary for neutralizing of 100 ml of gastric acid. Combined acidity was determined by the difference between the total and free acidity. The results are shown in Table 5.

Researching the effect of the bee products standard substances composition – HP, PPHP, and BP on the state of the gastric mucosa was carried out by the appropriate method.[28] The rats weighing 180-200 g were kept on an absolute diet for 48 h without limiting drinking water. Then, the animals of the experimental group were orally inserted the preparation in question, and the animals of the control group were inserted the equivalent amount of solvent (purified water). 3 h later the animals were taken out of the experiment, their stomachs were taken out, and the state of the gastric mucosa was examined with a magnifying glass. The results of the research are presented in Table 6.

Researching local irritating effect of the bee products standard substances composition – HP, PPHP, and BP on the mucous membrane was carried out according to the methodological guidelines.[29] The research was performed on rabbits weighing 2.0-2.4 kg by instilling 1 drop of the API formula

Table 4: The effect of the bee products standard substances composition – HP, PPHP, and BP, on the MCDR

Terms of the experiment	Dose, mg/kg	Sensitization	Number of animals in the group	MCDR
Control	–	–	7	0.11±0.01
API formula	100	Oral	7	0.12±0.01*

Values are mean of six individual observations in each group mean±SD. **P* denotes statistical significance of ANOVA to test the difference between the experimental groups. SD: Standard deviation, API: Active pharmaceutical ingredients, HP: Honey powder, PPHP: Propolis phenolic hydrophobic preparation, BP: Bee pollen, MCDR: Mast cells degranulation rate

Table 5: The effect of the bee products standard substances composition – HP, PPHP, and BP, on the gastric secretory function of rats (*n*=5)

Terms of the experiment	Gastric acid volume, ml/100 r of an animal's weight	Total number, ml of 0.1 N NaOH/100 ml of gastric acid	Free number, ml of 0.1 N NaOH/100 ml of gastric acid	Combined number, ml of 0.1 N NaOH/100 ml of gastric acid
Control	1.03±0.30	79.60±14.75	46.80±5.59	29.94±9.74
API formula, 100 mg/kg	1.12±0.22*	68.25±6.44*	41.56±6.44*	26.68±4.14*

Values are mean of six individual observations in each group mean±SD. **P* denotes statistical significance of ANOVA to test the difference between the experimental groups. SD: Standard deviation, API: Active pharmaceutical ingredients, HP: Honey powder, PPHP: Propolis phenolic hydrophobic preparation, BP: Bee pollen

Table 6: The effect of the bee products standard substances composition – HP, PPHP, and BP, on the state of the gastric mucosa of rats (*n*=5)

Terms of the experiment	Dose, mg/kg	Number of ulceration
Control	–	1.80±0.66
API formula	100	0.00±0.00*

Values are mean of six individual observations in each group mean±SD. **P* denotes statistical significance of ANOVA to test the difference between the experimental groups. SD: Standard deviation, API: Active pharmaceutical ingredients, HP: Honey powder, PPHP: Propolis phenolic hydrophobic preparation, BP: Bee pollen

aqueous suspension into the conjunctival sac of an animal's right eye once, and nasal lacrimal canal in the inner corner of the eye was pressed for 1 min. The left eye was used as a control. The possible irritating effect of 0.5%, 1%, and 5% substance suspension was researched. The mucous membrane of an animal's eye was examined in 5, 15 min, 1 h, and then daily for 5 days. The evaluation of the damaging effect was done according the rating scale is given in the guidance.[30,31]

RESULTS OF THE RESEARCH AND THEIR DISCUSSION

The analysis of the morphological research of the testes of the rats [Table 1] demonstrates no change of morphological parameters in the experimental group in comparison with the control group.[19,20,22-24] The results of the research of the functional state of spermatozoa of the rats [Table 2] which received the bee products standard substances composition – HP, PPHP, and BP at a dose of 100 mg/kg, also demonstrated no changes in the functional parameters, including such as sperm count, time of their motility and pathological form sperm count in comparison with the control group.[22,23]

On the basis of these studies, we can conclude that the API formula as the bee products standard substances composition – HP, PPHP, and BP at a dose of 100 mg/kg demonstrates no toxic effects on spermatogenesis in male rats.[19,24]

The results of studying allergenic activity of the bee products standard substances composition based on active cutaneous anaphylaxis model [Table 3] suggest that oral inserting of the API formula which was researched at a dose of 100 mg/kg does not cause an immediate hypersensitivity reaction.[22,23]

The results of studying allergenic properties of the composition of the bee products standard substances composition – HP, PPHP, and BP in the mast cells degranulation reaction [Table 4] demonstrates that the API formula does not cause the accumulation of homocytotrophic antibody in the blood and, consequently, does not demonstrate sensitizing activity. The data of studying the allergenic properties of the API formula the obtained from the "conjunctival test," which was performed on 10 guinea pigs after oral intake, also demonstrate that all experimental animals had a negative reaction of the conjunctival samples, the state of the mucous membrane of researched and control eyes remained consistently the same, indicating no sensitizing action of the preparation in question.[19,20,22-24]

The results of determining the combined acidity by the difference between the total and free acidity [Table 5] show that the API formula – HP, PPHP, and BP at a dose of 100 mg/kg did not affect the secretory activity of gastric glands.

The data on the impact of the standard substances composition – HP, PPHP, and BP on the state of gastric mucosa of the rats demonstrated that the API formula demonstrates no ulcerogenic effect on the gastric mucosa state.

Researching the local irritating effect of the API formula on the mucous membrane, which was performed on rabbits, demonstrated that at instilling 0.5%, 1%, and 5% the API formula (HP, PPHP, and BP) coarse dispersion in the quantity of 1 drop into the conjunctival, there was no visible reaction from the mucosa, indicating that there is no local irritating action of the bee products standard substances composition on the mucous membrane of the eye of rabbits after a single application.[22,23]

Thus, the results of researching pharmacological activity and specific toxicity of the bee products standard substances composition – HP, PPHP, and BP suggest long-term benefits of the development of this preparation and feasibility of a further preclinical research to obtain a permit to introduce a new preparation into clinical practice and industry.

CONCLUSIONS

1. Researching the specific toxicity of the API formula as the standard substances composition – HP, PPHP, and BP at a dose of 100 mg/kg revealed no capacity to gonadotoxic and allergenic action
2. No the API formula (HP, PPHP, and BP) impact on the secretory function and the state of the gastric mucosa or irritating action on the contact with the mucous membranes of an eye was found out
3. The results of researching the pharmacological activity and specific toxicity of the bee products standard substances composition – HP, PPHP, and BP allow to make a conclusion about long-term benefits of the development of this preparation and feasibility of a further preclinical research to obtain a permit to introduce a new preparation into clinical practice and industry.

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