

EXPERIMENTAL SUBSTANTIATION OF A SAFETY OF A NEW SEDATIVE APIFITODRUG "APISED"

Studied the safety of a new apiphytodrug capsules "Apised" by determining the LD₅₀ in the study of acute toxicity. Found that after a single intrastomach administration to rats of both sexes capsules "Apised" in a dose of 5000 mg / kg, signs of intoxication and death of rats not have been reported. It is not possible to establish the LD50 dose, and shows that its value is far beyond the 5000 mg / kg. According to the toxicological classification capsules "Apised" with intrastomach administration are V toxicity class - practically non-toxic substances. Set new standards in the use apiphytodrug dose of 5000 mg / kg, which is almost 100 times the therapeutic (60 mg / kg), the capsules "Apised" have a high safety profile and wide range of use in medicine and are promising for the implementation of the medical and sports practice and production.

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INTRODUCTION

The modern pace of life is a strong physical, emotional and psychological stress. This causes a disturbance of the central nervous system (CNS). Such violations need in the prevention and treatment with sedative drugs of natural origin. Such drugs are apiphytodrugs [2, 10, 16, 20, 22, 23].

Absence or at least side-effects of natural and herbal drugs is their advantage over synthetic drugs and contributes to their widespread use in clinical practice [1, 14, 17, 21]. But the range of natural and herbal sedative drugs of Ukrainian market of pharmacy with compliance dosage forms is insufficient or limited. This stimulates the search and the development and introduction of new drugs in this group with appropriate dosage form.

In order to expand the range of domestic natural and plant sedative drugs scientists from the department of pharmaceutical drug technology D.P.Salo behalf of the National University of Pharmacy was justified by a rational structure and the technology of the new combined apiphytodrug capsules "Apised." The composition of capsules includes the standardizing substance of natural honey and medicinal plants: grass balm (*Herbae Melissa officinalis L.*), hop cones normal (*Flos Humuli Lupuli L.*), narrow-leaved lavender blossoms (*Flores Lavandulae angustifolia Mill.*). Was established sedative, hypnotic and weak anxiolytic effect of capsules "Apised" in previous studies [12]. But for the final evaluation of the therapeutic effect of latitude of a new drug requires data on the safety capsules "Apised", because the more the effectiveness and safety of the drug, the greater its potential use in medical practice.

Therefore, the aim of this work is investigate the safety of capsules "Apised" through the definition of the indicator in the study of the LD₅₀ acute toxicity. This will assess the breadth of therapeutic action, and the ratio of "risk/safety" in the application of the drug in doses that are hundreds of times greater than the therapeutic dose. [4]

MATERIALS AND METHODS

Determination of the acute toxicity of capsules "Apised" held at the Central Scientific-Research Laboratory of NUPh under the supervision of Professor L. Yakovleva. In order to determine the LD₅₀ dose and reproduction of acute poisoning clinic the acute toxicity capsules "Apised" studied in accordance with the guidelines Ukraine DEC of MH [4] in male and female rats in a once intragastric administration. The route of administration was selected in accordance with the proposed dosage form - capsules. According to the guidelines of the Ukraine DEC of MH [4] when doses of intragastric administration of the limiting measure in determining the LD₅₀ is the maximum dose of fourth-grade toxicity (low-toxic substances) - 5000 mg/kg. If the dose administered to animals is not their destruction, the introduction of high-dose is impractical. Thus, for studies we chose dose capsules "Apised" – 5000 mg / kg, which was administered once intragastric to rats males and females weighing

180-200±20 g. Experimental animals were randomized into groups, which are presented in Table 1.

Table 1

Randomization of rats in an experiment in study the acute toxicity of capsules "Apised"

Conditions of the experiment	Dose, mg/kg	Number of rats	
		males	females
Intact control (IC)	–	6	6
Capsules "Apised"	5000,0	6	6

After drug administration the animals were monitored for 14 days and was evaluated their general condition, mortality and the dynamics of body weight. At the end of experiment the animals were taken out of the experiment after euthanasia under ether anesthesia. Further makes the macroscopic examination of internal organs and were determined their mass, which allowed the calculation of an integral index - mass coefficient of internal organs [4, 13].

During the experiment the animals were kept in a vivarium with natural light regime of "day-night" temperature conditions of 18-24⁰C and the relative humidity of 50-60%. The rats were kept in plastic cages on a balanced diet in accordance with applicable regulations. Studies were performed in accordance with National guidelines, "The General Ethical Principles of Animal Experimentation" (Ukraine, 2001), which are consistent with the provisions of " The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [3, 4, 7, 15].

The experimental data were analyzed by variation statistics using a standard statistical software package "Statistica 6.0" [11]. When applying the method of mathematical statistics was adopted significance level of $p < 0.05$. To obtain reliable statistical conclusions when comparing statistical samples relative variables, after univariate analysis of variance (ANOVA, or to experimental data elements that are repeated "ANOVA" / "MANOVA") revealed differences between experimental groups, to assess the intra-variance dispersion was used analysis with the use

parametric test Newman-Keuls [6, 8, 9, 11]. The results of the investigation are given in Table. 2-6.

RESULTS AND DISCUSSION

Established that the after a single intragastric administration of male and female rats of IC - drinking water, and the experimental group - capsules "Apised" in a dose of 5000 mg / kg (Table 2) of signs of intoxication on the day of administration and for 14 days after is not revealed: animals were neat, active, responsive to auditory and visual stimuli, the process of urination and defecation were normal, respiratory failure and convulsions were not observed, reflex excitability has been saved. The rats of both experimental groups consumed food and water normally. Not registered the death of animals during the entire observation period (Table 2).

Table 2

The acute toxicity studies capsules "Apised" after the single intragastric administration to white rats of both genders

Conditions of the experiment	Dose, mg/kg	The observed effect, the number of dead animals / total number of animals in the group	
		Males	Females
Intact control (IC)	–	0/6	0/6
Capsules "Apised"	5000,0	0/6	0/6

Conducted a study of the dynamics of body weight of the experimental animals to assess the toxicity of capsules "Apised" on the organism (Table 3) [4]. Established physiological authentic to baseline data regarding the increase in body weight of male and female rats after a single intragastric administration of capsules "Apised" and in the groups of intact animals during the study period (Table 3).

Table 3

Dynamics of the body weight of male and female rats after a single intragastric administration of capsules "Apised" in the study of acute toxicity

Conditions of the experiment	Imprint	3 days, g	7 days, g	14 days, g
Males				
Intact control (IC)	202,5± 3,82	205,0± 2,89	211,7± 1,67	221,7± 1,67*
Capsules "Apised"	205,0± 4,47	208,3± 3,57	217,5± 2,50	227,5± 2,50*
Females				
Intact control (IC)	200,0± 5,33	200,8± 4,55	206,7± 3,33	217,5± 3,10*
Capsules "Apised"	210,0± 4,65	210,8± 3,96	215,8± 3,52	218,3± 3,57**

Note: * – significant deviation indicator on the initial data, $p \leq 0,05$;

** – Rejection rate tends to probable on the initial data, $P > 0.05$.

Then performed the autopsy of animals and observed a neat coat and unaltered mucosal natural holes of all experimental male and female rats. During macroscopic examination of internal organs and organism systems of animals established absence of differences from the physiological norm. The analysis of the data of mass coefficients of the internal organs of male and female rats after a single intragastric administration of capsules "Apised" and in the groups of intact animals, which are shown in Tables 4 and 5. Established the absence of pathological changes in the functional state of the experimental animals in comparison with the intact rats (Tables 4 and 5).

Table 4.

The mass coefficients of the internal organs of male rats after a single intragastric administration of capsules "Apised" in the study of acute toxicity

Conditions of the experiment		Intact control (IC)	Capsules "Apised"
Body			
Liver		3,63±0,01	3,57±0,02
Kidneys	right	0,31±0,01	0,31±0,01
	left	0,31±0,01	0,31±0,01
Heart		0,36±0,01	0,36±0,01
Lights		0,75±0,01	0,75±0,02
Spleen		0,37±0,02	0,37±0,02
Adrenals		0,029±0,001	0,030±0,001
Thymus		0,132±0,009	0,112±0,010
Testes	right	0,54±0,01	0,56±0,01
	left	0,55±0,01	0,57±0,01

Table 5

The mass coefficients of the internal organs of female rats after a single intragastric administration of capsules "Apised" in the study of acute toxicity

Conditions of the experiment		Intact control (IC)	Capsules "Apised"
Body			
	Liver	2,96±0,06	2,86±0,14
Kidneys	right	0,28±0,01	0,28±0,01
	left	0,27±0,01	0,27±0,01
	Heart	0,30±0,01	0,30±0,01
	Lights	0,69±0,03	0,73±0,05
	Spleen	0,39±0,03	0,39±0,02
	Adrenals	0,032±0,002	0,028±0,001
	Thymus	0,109±0,009	0,114±0,016

Thus intragastric administration to rats maximum dose of capsules "Apised" 5000 mg/kg did not cause death of the animals and pathological changes of the functional state of the rats. Therefore the determination of the LD₅₀ dose capsules "Apised" is impossible (Table 2-5).

Thus, in the acute toxicity study established the absence of toxicity of the new apiphytodrug capsules "Apised" after a single intragastric administration into male and female rats in a dose 5000 mg / kg. This shows convincingly that the LD₅₀ dose is located administering to an animal outside of the dose - 5000 mg / kg. This allows us to substantiated conclusion that the by toxicological classification of substances according to К.К.Сидоров [4] capsules "Apised" after intragastric administration are included in V toxicity class – practically harmless substances (Table 6).

Table 6.

Options of acute toxicity of the capsules "Apised" after a single intragastric administration to rats of both genders

Drug	LD ₅₀ , mg/kg	Toxicity Class
Capsules "Apised"	> 5000	V Practically harmless substances (LD ₅₀ >5000 mg/kg)

CONCLUSIONS

1. Conducted a study on the acute toxicity and done experimental substantiation of a safety of the new sedative apiphytodrug capsules "Apised".
2. Established that after a single intragastric administration into male and female rats of capsules "Apised" in a dose of 5000 mg/kg signs of intoxication and death of animals is not registered in the period of observation. Therefore establish the dose of study drug LD₅₀ is impossible.
3. Capsules "Apised" (LD₅₀> 5000 mg / kg) after intragastric administration belong to V toxicity class - practically harmless substances according to the toxicological classification of substances according to К.К.Сидоров.
4. Established when using of the new sedative apiphytodrug capsules "Apised" in a dose of 5000 mg/kg, which is nearly one hundred times the therapeutic dose (60 mg/kg), that the capsules "Apised" have a high safety profile and extensive opportunities for using in medical practice, including and sports medicine, and are perspective for introduction in manufacture.

LIST OF SOURCES USED INFORMATION

1. Волошин О.І. Ліки рослинного походження: сучасні тенденції у вітчизняній та світовій клінічній медицині і фармації / О.І. Волошин, О.В. Пішок, Л.О. Волошина // Фітотерапія. – 2003. – № 3. – С. 3-7.
2. Воробьева О.В., Акарачкова Е.С. Применение комбинированных растительных препаратов при тревожных расстройствах / О. В. Воробьева, Е.С. Акарачкова // Фарматека. – 2007. – № 7 (142). – С. 47–50.
3. Западнюк И.П. Лабораторные животные: разведение, содержание, использование в эксперименте / И.П. Западнюк, В.И. Западнюк, С.А. Захария. – К.: Вища школа, 1983. – 552 с.
4. Коваленко В.М. Експериментальне вивчення токсичної дії потенційних лікарських засобів / Доклінічні дослідження лікарських засобів: [метод. рек.] / В.М. Коваленко, О.В. Стефанов, Ю.М. Максимов, І.М. Трахтенберг [за ред.: член-кор. АМН України О. В. Стефанова] – К.: Авіцена, 2001. – С. 74–97.

5. Костюченко С.И. Эпидемиология психического здоровья в Украине / С.И. Костюченко // *Нейронews*. – 2008. – №2. – С. 9-13.
6. Лапач С.Н. Статистические методы в медико-биологических исследованиях с использованием Excel. / С.Н. Лапач, А.В. Чубенко, П.Н. Бабич. – 2-е изд., перераб. и доп. – К.: МОРИОН, 2001. – 408 с.
7. Науково-практичні рекомендації з утримання лабораторних тварин та роботи з ними / [Кожем'якін Ю.М., Хромов О.С., Філоненко М.А., Сайфетдінова Г. А.]. – К.: Авіцена, 2002.– 156 с.
8. Основные методы статистической обработки результатов фармакологических экспериментов.: [Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ]. / Под общей редакцией члена-корреспондента РАМН, профессора Р.У. Хабриева – 2-е изд., перераб. и доп. – М.: ОАО «Издательство «Медицина», 2005. – С. 763-826.
9. Реброва О.Ю. Статистический анализ медицинских данных. Применение пакета прикладных программ STATISTICA / О.Ю. Реброва. – М.: МедиаСфера, 2006 – 312 с.
10. Фролов В.М., Пересадин Н.А. Продукты пчеловодства при синдроме хронической усталости // *Пчеловодство*. – 2006. – № 8. – С. 57-58.
11. Халафян А.А. STATISTICA 6. Статистический анализ данных: учебник. – 3-е изд. – М.: ООО «Бином-Пресс», 2007. – 512 с.
12. Шпичак О.С., Яковлева Л.В., Шаповал О.М. Експериментальне обґрунтування використання капсул «Апісед» як седативного лікарського засобу в період відновлення працездатності спортсменів // *Український біофармацевтичний журнал*. – 2012. – № 5-6(22-23). – С. 78-83.
13. Эвтаназия экспериментальных животных: [метод. реком. по выведению животных из эксперимента]. – М.: Медицина, 1985. – 13 с.
14. Braun L., Cohen M. Herbs and natural supplements: An Evidence-based Guide / 2nd Edition. Marrickville NSW: Elsevier, 2007. – 567 p.

15. European convention for the protection of vertebrate animals used for experimental and other scientific purposes // Council of European. – Strasbourg, 1986. – № 123. – 51 p.
16. Fang X.Sh. Pharmacological studies on the sedative-hypnotic effect of Semen Ziziphi spinosae (Suanzaoren) and Radix et Rhizoma Salviae miltiorrhizae (Danshen) extracts and the synergistic effect of their combinations / X.Sh.Fang, J.F.Hao, H.Y.Zhou [et al.] // *Phytomedicine*. – 2010. – Vol. 17. – P. 75-80.
17. Hanson B.A. Understanding medicinal plants their chemistry and therapeutic action. – New York, London, Oxford: The Haworth Press. – 2005. – 307 p.
18. Klein D. Therapeutic alliance in depression treatment: controlling for prior change and patient characteristics / D. Klein, J. Schwartz, N. Santiago // *J. Consult. Clin. Psychol.* – 2003. – Vol. 71(6). – P. 997-1006.
19. Konareva I.N. Influence of sedative vegetable preparations on emotional sphere of the person // *Uchenye zapiski Tavricheskogo Natsionalnogo Universiteta im. V.I. Vernadskogo. Series «Biology, chemistry»*. – 2009. – V.22 (61). – № 2. – P. 67-71.
20. Kurkin V.A. Phenylpropanoids from, medicinal plants: distribution, classification, structural analysis, and biological activity / V.A. Kurkin // *Chemistry of Natural Compounds*. – 2003. – Vol. 39, № 2. – P. 123-153.
21. Philips G. Standardization Techniques for Herbal Medicines. Enough techniques (too) many different plants. A joint symposium of the sections for Medicinal and Aromatic Plants, and Laboratories and Medicines Control Services / G. Philips // *Int. Pharmacy Journ.* – 2002. – Vol. 16, №2. – P. 18-19.
22. Spinella M. The importance of pharmacological synergy in psychoactive herbal medicines // *Altern. Med. Rev.* – 2002. – № 7(2). – P. 130-137.
23. Wake G., Court J., Pickering A., Lewis R., Wilkins R., Perry E. CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory // *J. Ethnopharmacol.* – 2000. – Vol. 64, № 2. – P. 105-114.

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ЭКСПЕРИМЕНТАЛЬНОЕ ОБОСНОВАНИЕ БЕЗОПАСНОСТИ НОВОГО СЕДАТИВНОГО АПИФИТОПРЕПАРАТА "АПИСЕД"

Проведено исследование по изучению безопасности нового апифитопрепарата капсул "Аписед" путем определения LD_{50} в процессе изучения острой токсичности. Установлено, что после однократного внутрижелудочного введения крысам обоих полов капсул «Аписед» в дозе 5000 мг/кг признаков интоксикации и гибели крыс не зарегистрировано, что не позволяет установить среднелетальную дозу LD_{50} и свидетельствует о том, что ее значение лежит далеко за пределами 5000 мг/кг. Согласно токсикологической классификации капсулы «Аписед» при внутрижелудочном введении принадлежат к V классу токсичности – практически нетоксичным веществам. В условиях применения нового апифитопрепарата капсул «Аписед» в дозе 5000 мг/кг, которая почти в 100 раз превышает терапевтическую (60 мг/кг), установлено, что они имеют высокий профиль безопасности и широкие возможности применения в медицинской практике и являются перспективными для внедрения в медицинскую, в том числе и спортивную практику и производство.

Ключевые слова: безопасность, растительные лекарственные средства, спортивная медицина, продукты пчеловодства, капсулы "Аписед", острая токсичность, седативное действие.

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