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RESEARCH OF THE TOXICOLOGICAL PROFILE OF THE NEW GEL, WHICH CONTAINS AN EXTRACT OF THE OAK BARK AND ALOE EXTRACT

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На сьогоднішній день хвороби пародонту значно поширені, діагностуються більш ніж у 75 % населення всього світу, при цьому уражують всі вікові групи. Перспективними лікарськими препаратами слід вважати засоби на основі лікарської рослинної сировини, які поряд із вираженою терапевтичною дією мають мінімум негативних реакцій.

***Метою** роботи було дослідження гострої токсичності нового гелю, що містить екстракт кори дуба та екстракт алое.*

***Методи.** Дослідження гострої токсичності проводили відповідно до методичних рекомендацій на білих нелінійних щурах масою 180–220 г, у декілька етапів: дослідження гострої токсичності компонентів нового гелю, дослідження гострої токсичності нового гелю за умов внутришньошлункового ведення, внутрішньоочеревинного та при нашкірному нанесенні.*

***Результати.** Встановлено, що за умов внутришньошлункового введення екстракту кори дуба та екстракту алое неможливо встановити $ЛД_{50}$, тому що введення максимальної дози 15100 мг/кг не викликало летальності щурів. У зв'язку з цим у подальших дослідженнях, з метою підтвердження цього твердження була досліджена гостра токсичність екстрактів лише у дозі 15100 мг/кг. При внутришньоочеревинному введенні досліджувані $ЛД_{50}$ екстракту кори дубу становить 2580 (1930–3220) мг/кг, екстракту алое 2180 (1460–2900) мг/кг. На подальших етапах дослідження не було зареєстровано видимих ознак токсичного впливу нового гелю на функціональний стан тварин під час спостереження протягом 14 діб при введені у максимально рекомендованих дозах: внутрішньошлуноково 15100 мг/кг та нашкірно 22600 мг/кг.*

***Висновки.** Комплекс проведених досліджень дозволив встановити, що новий гель не чинить токсичного впливу на органи та системи дослідних тварин та не має летальної дії. Екстракт кори дуба та екстракт алое за умов внутрішньошлункового введення відносяться до VI класу токсичності «Відносно нешкідливі речовини». При внутришньоочеревинному введенні досліджувані екстракти відносяться до V класу токсичності «Практично нетоксичні речовини». За показником вивчення гострої токсичності новий гель за умов внутришньошлункового та нашкірного нанесення відносяться до VI класу токсичності «Відносно нешкідливі речовини»*

***Ключові слова:** гостра токсичність, екстракт кори дуба, екстракт алое, гель, токсичність, екстракт*

1. Introduction

Nowadays, dystrophic and inflammatory diseases of periodontal tissues are widespread and are diagnosed in more than 75 % of the world's population, and affect all age groups [1]. According to N. N. Savelieva (2017) [2] in recent decades in Ukraine there was marked tendency to increase the frequency of periodontal lesions that reaches 90 %.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Optimization of the treatment of periodontal disease is one of the important factors not only for restoring the health of the oral cavity, but also can improve the overall health of the patient and have no negative effect on the course of concomitant systemic pathologies.

Recent scientific studies on the research of the etiopathogenetic components of inflammatory diseases of the cavity of the mouth have allowed to establish that along with local lesions of the periodontal system, also develop systemic pathological reactions. So in the work Panezai J. et al. (2017) [3] proved that inflammatory periodontal disease is correlated with a change in the content of 92 serum cytokines, affecting the level of chemokines, growth factors and the activity of markers enzymes for the development of destructive processes. In a study by Wang Y. (2017) [4], dystrophic-inflammatory diseases of the periodontal disease are accompanied by excessive reactive forms of oxygen produced by hyperactive neutrophils. The endogenous antioxidant system of the body is not able to balance the excess reactive forms of oxygen, resulting in an increase in lipid peroxidation metabolites, damage to mitochondrial DNA. The study confirmed that the inflammatory reaction of the periodontal tissues is associated with local and systemic oxidative stress and a decrease in the activity of the antioxidant system.

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

Established links between inflammatory diseases of the oral cavity and systemic biochemical changes in the body, allows better understanding of the positive correlation with diseases that extend beyond the oral cavity. For example, the two-directional effect of periodontal disease and diabetes mellitus has been proved [5]. For decades, it was believed that diabetes contributes to the deterioration of the oral cavity and increases the development of periodontitis. It has now been established that perio-

dontal disease may worsen insulin resistance and affect glycemic control. Adequate periodont-protective therapy improves glycemic control in type 2 diabetics.

The study by Aoyama N. (2017) [6] found that the severity of inflammatory diseases of the periodontal system affects the development of systemic inflammation and complicates cardiovascular disease, in particular coronary heart disease.

There are also data on the negative effects of dystrophic-inflammatory diseases of the oral cavity on the course of atherosclerosis, rheumatoid arthritis, Alzheimer's disease, etc. [7, 8].

4. The field of research considering the general problem, which is described in the article

An urgent general problem of pharmacy and medicine is the optimization of treatment of destructive inflammatory diseases of the oral cavity and the development of new drugs. Prospective drugs for the treatment of degenerative and inflammatory diseases of the cavity of the mouth should be considered as the means developed on the basis of medicinal plant material, which, along with the expressed therapeutic effect, have a minimum of negative reactions. Confirmation of this is the direction of modern scientific research on the search for new drugs for the treatment of periodontal disease, namely from the effective plant substances, it is the herbal medicinal products with pronounced antimicrobial, antioxidant, antiseptic, anti-inflammatory, anticollagenase activity due to polymodal pharmacological action [9].

Taking into account that an integrated approach is important for the treatment of dystrophic-inflammatory diseases of the oral cavity, dental preparations should have an impact on the various pathogenesis of diseases. In particular, it is typical for complex medicinal products based on plant material. It should be noted that the amount of multi-component herbal preparations is considerably inferior to mono-component ones, in the pharmaceutical market of dental products, which proves the expediency of their development and implementation in order to improve the provision of dental care to the population.

According to the above-mentioned, the scientists of the NUPh, under the direction of prof. N. V. Hochlenkova a new gel containing an extract of oak bark and aloe extract was developed for the treatment of inflammatory diseases of the oral cavity [10], for which in previous studies a pronounced membrane-protective, antimicrobial, anti-inflammatory action and periodontal protective activity were established [11, 12].

In the study of new pharmacologically active agents, the most important characteristic along with high efficiency is the study of their toxicological profile. The particular features of the toxicological profile are usually the main obstacle to the next stage of the introduction of new drugs and significant constraints at the stage of clinical application of already registered drugs [13].

In this regard, in the study of any active pharmaceutical ingredient, the prerequisite for preclinical examination is the study of acute toxicity [14].

5. Formulation of goals (tasks) of article

The aim of this work was to study the acute toxicity of a new gel that contains extract of oak bark and aloe extract.

6. Presentation of the main research material (methods and objects) with the justification of the results

Materials and methods. The study of acute toxicity of a new gel and its components was conducted in accordance with the methodological recommendations of the Ministry of Health of Ukraine on preclinical drug research [15] on white nonlinear rats with the weight of 180–220 g. Studies were carried out on animals obtained from vivarium of CDC of NUPh, which were kept on the usual food and water ration. Experiments were carried out in accordance with the "Rules for carrying out experiments using experimental animals" (Strasbourg, March 18, 1986) [16].

The object of the study was a new gel of the following composition in %: a thick extract of oak bark – 5 %, dry aloe extract – 3 %, carbopol 934P – 3 %, trometamol – 2.5 %, EDTA – 0.05 %, glycerol – 10 %, nipagin – 0.1 %, fructose – 10 %, purified water to 100 %. [10].

The acute toxicity of the new gel was studied in 3 stages:

1) study of acute toxicity of components of a new gel: an extract of oak and aloe extract under intragastric and intraperitoneal administration;

2) study of the acute toxicity of a new gel under conditions of intragastric administration;

3) study of the acute toxicity of a new gel after the applying to the skin.

In the first stage, the acute toxicity of the extract of aloe and extract of oak was studied by the method of P. V. Pastushenko and co-authors. Experiments were conducted on white non-breeding rats in males with the weight 180–220 g in condition of single administration.

At the second and third stage, the study was conducted in males and females, taking into account the possible difference, depending on sex. Subcutaneous application was carried out in accordance with the guidelines. For 24 hours before the application to the skin of the test substance, the fur on the lateral surface of the body of the experimental animal was removed by a hair-style. Cutting area was at least 10 % of the total body surface.

At the end of the experiment, the animals were withdrawn from the experiment under anesthesia and organs were extracted from the bodies for macroscopic analysis.

Results and discussion. At the first stage of the study, the acute toxicity of the extract of oak and aloe extract was investigated. Studies were conducted on male rats. In accordance with the guidelines for the purpose of establishing a range of toxic doses and minimizing the participation of animals in a lethal experiment, a preliminary study was conducted in groups of two animals. The results are presented in table 1. It was found that it is impossible to establish LD_{50} for the oak and aloe bark extract under intragastric administration, since administration of a maximum dose of 15100 mg / kg did not cause

lethality of rats. In this connection, in further studies, to confirm this assertion, the acute toxicity of extracts was studied only at a dose of 15100 mg / kg in groups of 6 animals (Table 2)

Table 1

Preliminary stage of study of acute toxicity of components of a new gel: oak extract and aloe extract

Dose mg/kg	Number of animals	Mortality
Intragastric administration of an oak bark extract		
5000	2	0/2
10000	2	0/2
15100	2	0/2
Intraperitoneal administration of an oak bark extract		
1000	2	0/2
2000	2	1/2
3000	2	2/2
Intragastric administration of an aloe extract		
5000	2	0/2
10000	2	0/2
15100	2	0/2
Intraperitoneal administration of an aloe extract		
1000	2	0/2
2000	2	1/2
3000	2	2/2

Table 2

Study of acute toxicity of components of a new gel: oak and aloe extract in terms of intragastric administration to rats

Oak bark extract			Aloe extract		
Dose mg/kg	Number of animals	Mortality	Dose mg/kg	Number of animals	Mortality
15100	6	0/6	15100	6	0/6

The above data suggest that the average mortality dose of LD_{50} for oak and aloe extract in the intragastric introduction can not be established since the dose of more than 15100 mg / kg is the maximum recommended for study by this route of administration.

It is established that according to the classification by K. K. Sidorov [17] extract of oak and aloe extract under conditions of intragastric administration are classified as the VI class of toxicity "Relatively harmless substances".

Intraperitoneal administration of extracts in the previous study caused animal lethality in some groups, which resulted in a more complete study of "dose-lethality" in groups of 6 animals (Table 3).

The LD_{50} dose under the conditions of intraperitoneal administration to rats of extract of oak and aloe extract was calculated by probit analysis using mathematical calculations [17]. The results are shown in the Table. 4

The complex of conducted researches allowed to establish that under the conditions of intraperitoneal administration to rats LD_{50} with a confidence interval of extract of oak bark is 2580 (1930–3220) mg / kg, LD_{50} with a confidence interval of aloe extract is 2180 (1460–2900) mg / kg, thus in classification by K. K. Sidorov investigated extracts belong to the class V toxicity "Practically nontoxic substances".

Taking into account the low toxicity of the components of the new gel, further studies examined the acute toxicity of the new gel in the maximum doses recommended for study under conditions of intragastric and intraperitoneal administration. The results are presented in the Table. 5.

Table 3

Study of acute toxicity of components of a new gel: extract of oak and oak bark extract under intraperitoneal injection to rats

Oak bark extract			Aloe extract		
Dose mg/kg	Number of animals	Mortality	Dose mg/kg	Number of animals	Mortality
1260	6	0/6	1260	6	0/6
1580	6	1/6	1580	6	1/6
2000	6	2/6	2000	6	3/6
2500	6	4/6	2500	6	5/6
2820	6	6/6	2820	6	6/6

Table 4

Calculation of LD_{50} for extract of oak and aloe extract in conditions of intraperitoneal administration to rats

Dose mg/kg	% mortality	probit y	weight coef. B	xB	x^2B	yB	xyB
Oak bark extract							
1260	0	3.3	1.6	1.6	1.6	5.2	5.2
1580	16.7	4.0	3.7	7.4	14.8	14.9	29.9
2000	33.3	4.6	4.6	13.8	41.4	20.9	62.9
2500	66.7	5.4	4.6	18.4	73.6	25.0	100.1
2820	100	7.7	1.2	6.0	30.0	9.3	46.3
			45.7	47.2	161.4	75.5	244.58
Aloe extract							
1260	0	3.3	1.6	1.6	1.6	5.2	5.2
1580	16.7	4.0	3.7	7.4	14.8	14.9	29.9
2000	50	5.0	5.0	15.0	45.0	25.0	75.0
2500	83.3	5.9	3.2	14.0	56.0	20.8	83.3
2820	100	7.7	1.2	6.0	30.0	9.3	46.3
			15.0	44.0	147.4	75.3	239.8

Table 5

Investigation of acute toxicity of a new gel with intragastric and intraperitoneal administration

Males			Females	
Dose mg/kg	Number of animals	Mortality	Dose mg/kg	Number of animals
Intragastric administration				
15100	6	0/6	6	0/6
15100	6	0/6	6	0/6
Intraperitoneal administration				
22600	6	0/6	6	0/6
22600	6	0/6	6	0/6

Visible signs of toxic effects of this agent on the functional state of animals during the observation for 14 days was not recorded. There were no possible external manifestations of the toxic effect of the new gel on the condition and behavior of animals, such as drowsiness, changes in motor activity, elevated salivation, changes in the general condition of the skin, changes in the colour of the mucous membranes, edema, high fever, cramps and exposure to respiration.

At the end of the experiment, a macroscopic examination of the internal organs was performed to confirm the presence of pathological changes. According to the results of macroscopic examination, no pathological changes were noted.

This allows to classify a new gel by K. K. Sidorov as VI class toxicity "Relatively harmless substances" under conditions of intragastric and intraperitoneal administration to males and females rats.

7. Conclusions from the conducted research and prospects for further development of this field

1. It has been established that a new gel containing an extract of oak bark and aloe extract does not have a toxic effect on organs and systems of experimental animals.

2. Pharmacologically active gel components extract of oak bark and aloe extract under conditions of intragastric administration are related to the VI class toxicity "Relatively harmless substances", it is impossible to establish an average mortality dose of LD_{50} because it exceeds the maximum dose for administration of 15100 mg/kg.

3. In intraperitoneal administration, the investigated extracts belong to the class V toxicity "Practically non-toxic substances", LD_{50} for extract of oak bark is 2580 (1930–3220) mg / kg, LD_{50} for aloe extract – 2180 (1460–2900) mg / kg.

4. By the indicator of study of acute toxicity, a new gel, in conditions of intragastric administration and skin application, belongs to the VI class toxicity "Relatively harmless substances".

A new gel containing the extract of oak bark and aloe extract is promising for further pharmacological studies with the aim of developing a national periodontal protective agent and introducing it into medical practice, since, in addition to the establishment in previous studies, the expressed pharmacological activity does not exert a toxic effect on the body at extremely high doses.

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