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PHARMACOLOGICAL RESEARCH OF THE DENTAL GEL WITH CARBON DIOXIDE HUMULUS LUPULUS EXTRACT

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As a result of the study of acute and subacute toxicity of the gel with carbon dioxide Humulus lupulus extract found, that it belongs to low-toxic substances (class IV toxicity) and has good tolerability and safety of long-term use.

Keywords: gel with carbon dioxide Humulus lupulus extract, acute and subacute toxicity, laboratory animals, clinical observations, reference drug - Kamistad-Gel H

Despite advances in modern dentistry, the problem of treatment of diseases oral cavity is very acute. The effectiveness of most drugs and treatments is not at a high level [1]. The emergence of bacterial resistance to the drug, colonization unusual forms of microorganisms on the surface of the oral mucosa, followed by their penetration and their decay products deep into the epithelium interfere with effective therapy. Widespread use of chemotherapy drugs often leads to unwanted side effects of the human organism, while drugs based on plant material is low-toxic and well tolerated by patients of all ages. Herbal preparations distinguishes from other drugs presence in them of microelements, vitamins, essential oils, nutrients. So clear interest in herbal medicines polyvalent action for topical application in the treatment of inflammatory periodontal disease and oral mucosa. One promising direction in this area is the use of Humulus lupulus [2-4].

Among drugs that used in dentistry in modern pharmaceutical practice most effective is gel, which easily applied to mucosa and fairly long hold on the gums, forming a protective film and prolonging therapeutic effect of the drug. In this regard, has developed a new drug of hop extract carbon dioxide in the form of a gel for the treatment of oral cavity [5].

Humulus lupulus and preparations based refer to non-toxic substances. The literature describes no cases of poisoning by means of Humulus lupulus. However, an overdose of herbal medicines hop cones possible side effects.

The aim of our study was to investigate the acute and subacute toxicity the gel containing 0.5% carbon dioxide Humulus lupulus extract. The toxicity of the gel studied at the intragastric route of administration, due to the scope of drug - dentistry.

Materials and Methods

The object of the research was gel containing 0.5 % carbon dioxide Humulus lupulus extract.

Studies of acute toxicity gel with carbon dioxide Humulus lupulus extract was performed on white inbred laboratory mice of both sexes, weighing 19-21 gram. Animals received gel single intragastric at maximum tolerated this route of administration dose – 2.0 g/kg [6]. The experiment used by 5 mice of both sexes. The criteria of judgment about the toxicity was the clinical picture of intoxication, animal survival, dynamics of body weight of mice (raw data, 3, 7, 14 days). Observation of animals were carried out within two weeks.

Subacute toxicity studied on white inbred laboratory rats of both sexes, weighing 220- 250 g. Animals were divided into 3 groups, each experimental group were 5 male and 5 female rats. Total experiment used 30 rats.

Rats was injected gel with carbon dioxide Humulus lupulus extract once a day for 14 days at doses of 0.2 g/kg and 1.0 g/kg (1/10 and 1/2 of the maximum dose in acute experiment). Control animals were injected comparison drug Kamistad-H gel at a dose of 1.0 g/kg.

Evaluation of the toxic effects of investigational gel and reference medicine on the body of the experimental animals were carried out on the following parameters: clinical observations, the survival of animals, food and water consumption, the dynamics of body weight, hematological and biochemical parameters of blood.

Clinical observations of the animals was performed daily during the experiment, registering changes in their general condition, behavior, food and water consumption.

Clinical and biochemical parameters studied using a reagent kit of "Felicity Diagnostics" (Ukraine) and conventional methods [7, 8].

Body weight of animals recorded in dynamics. The animals were weighed before the experiment, then 7 and 14 days.

Experimental data acute and subacute toxicity were processed by methods of variational statistics.

The results and discussion

Results of the study parameters acute toxicity gel with carbon dioxide Humulus lupulus extract showed that intragastric administration to mice at a dose of 2.0 g/kg caused no clinical symptoms of intoxication. Animals calmly reacted to the introduction of the sample gel, their general condition and behavior remained unchanged. Animal deaths and abnormalities in their general condition and behavior during the observation period were noted.

Impact analysis of the sample of gel on the dynamics of body mass animal experiments showed that a single injection of a gel to mice at a dose of 2.0 g / kg did not significantly affect the dynamics of body weight (Table. 1).

Table 1. Dynamics of body weight of mice with acute action gel containing 0.5% carbon dioxide Humulus lupulus extract

The period of observation	Body weight, g	
	Males	Females
Gel containing 0.5% carbon dioxide Humulus lupulus extract		
Baseline data	19,8 ± 0,583	20,2 ± 0,374
3 days	20,00 ± 0,775	20,4 ± 0,51
7 days	21,4 ± 0,872	22,2 ± 0,374
14 days	23,2 ± 0,735 ¹⁾	23,8 ± 0,49 ¹⁾

Note: ¹⁾ – statistical significance of differences compared to the baseline data (p ≤ 0,05)

14 days after a single intragastric introduction gel at a dose of 2.0 g/kg was conducted pathomorphologic studies. According to section abnormalities were not found. The relative weight of internal organs of mice treated with the investigational gel remained within the physiological norm.

In experiments on rats were studied subacute toxicity gel with carbon dioxide Humulus lupulus extract. The results of 14 days intragastric introduction gel and reference drug studies at subacute toxicity showed that daily administration of no effect on the general condition, appearance (state of the skin and mucous membranes)

and the behavior of rats. Consumption of food and water in animal experimental and control groups had no differences from each other. During the experiment animal deaths were not reported.

Data characterizing the dynamics of increase in body weight of rats are presented in table 2. As can be seen, the animals of experimental groups and the control group by the end of the experiment had equivalent, statistically significant weight gain compared with the output data: males - $p \leq 0.0001$ females - from $p \leq 0.0001$ to $p \leq 0.0004$.

Table 2. Body weight (g) rats with subacute exposure gel containing 0.5 % carbon dioxide Humulus lupulus extract and drug-Gel N Kamistad

The period of observation	Gel Kamistad H dose 1.0 g / kg	Investigated gel at a dose of 0.2 g / kg	Investigated gel at a dose of 0.1 g / kg
Males			
Baseline data	246,50 ± 1,12	246,00 ± 1,45	244,50 ± 1,32
1 week	252,00 ± 1,46	250,00 ± 1,68	247,50 ± 2,53
2 weeks	258,00 ± 1,32 ¹⁾	257,50 ± 2,39	253,50 ± 2,53
Females			
Baseline data	216,50 ± 4,31	218,00 ± 4,22	217,50 ± 3,58
1 week	225,50 ± 4,03	225,00 ± 4,35	221,00 ± 2,92
2 weeks	233,00 ± 3,96 ¹⁾	232,50 ± 3,68 ¹⁾	226,50 ± 2,20 ¹⁾

Note: ¹⁾- $p \leq 0,05$ on baseline data

During the experiment, the animals of all experimental groups were observed physiological fluctuations that characterize the pattern of peripheral blood.

Results of biochemical studies (table. 3 and 4) showed that overall the tested parameters: the concentration of total protein, albumin, thymol test, the

activity of alanine and aspartate aminotransferase, the concentration of glucose and cholesterol in the blood serum of rats of all experimental groups answered normal values. There have been fluctuations in biochemical parameters in animals of all experimental groups compared with baseline data did not go beyond the physiological norm [9].

Table 3. Biochemical parameters of blood serum male rats with chronic steps gel containing 0.5 % carbon dioxide Humulus lupulus extract and drug-Gel N Kamistad

Indicator	Gel Kamistad H dose 1.0 g / kg	Investigated gel at a dose of 0.2 g / kg	Investigated gel at a dose of 0.1 g / kg
Baseline data			

Total protein, g/l	66,94 ± 0,95	66,94 ± 0,95	66,94 ± 0,95
Albumin , g/l	32,07 ± 0,92	32,07 ± 0,92	32,07 ± 0,92
Thymol test, U	1,66 ± 0,08	1,66 ± 0,08	1,66 ± 0,08
AlAT, mkkat/l	0,50 ± 0,01	0,50 ± 0,01	0,50 ± 0,01
AsAT, mkkat/l	0,87 ± 0,03	0,87 ± 0,03	0,87 ± 0,03
Cholesterol, mmol/l	1,41 ± 0,19	1,41 ± 0,19	1,41 ± 0,19
Glucose, mmol/l	4,66 ± 0,35	4,66 ± 0,35	4,66 ± 0,35
Urea, mmol / l	5,28 ± 0,22	5,28 ± 0,22	5,28 ± 0,22
14 days			
Total protein, g/l	68,78 ± 2,55	71,87 ± 1,52	71,09 ± 2,74
Albumin , g/l	31,09 ± 1,12	32,91 ± 1,72	30,37 ± 2,56
Thymol test, U	0,72 ± 0,19 ¹⁾	0,68 ± 0,21 ¹⁾	0,65 ± 0,18 ¹⁾
AlAT, mkkat/l	0,43 ± 0,05	0,38 ± 0,02 ¹⁾	0,47 ± 0,04
AsAT, mkkat/l	0,75 ± 0,05	0,80 ± 0,07	0,80 ± 0,06
Cholesterol, mmol/l	2,08 ± 0,23	2,92 ± 0,22 ¹⁾	2,43 ± 0,12 ¹⁾
Glucose, mmol/l	5,23 ± 0,57	4,72 ± 0,35	4,87 ± 0,38
Urea, mmol / l	3,97 ± 0,26 ¹⁾	4,21 ± 0,24 ¹⁾	4,14 ± 0,41 ¹⁾

Note: ¹⁾- p ≤ 0,05 on baseline data

Table 4. Biochemical parameters of blood serum of female rats with chronic steps gel containing 0.5% carbon dioxide Humulus lupulus extract and drug-Gel N Kamistad

Indicator	Gel Kamistad H dose 1.0 g / kg	Investigated gel at a dose of 0.2 g / kg	Investigated gel at a dose of 0.1 g / kg
Baseline data			
Total protein, g/l	71,31 ± 0,57	71,31 ± 0,57	71,31 ± 0,57
Albumin , g/l	36,36 ± 0,62	36,36 ± 0,62	36,36 ± 0,62
Thymol test, U	1,52 ± 0,45	1,52 ± 0,45	1,52 ± 0,45
AlAT, mkkat/l	0,45 ± 0,01	0,45 ± 0,01	0,45 ± 0,01
AsAT, mkkat/l	0,91 ± 0,05	0,91 ± 0,05	0,91 ± 0,05
Cholesterol, mmol/l	1,49 ± 0,20	1,49 ± 0,20	1,49 ± 0,20
Glucose, mmol/l	6,55 ± 0,19	6,55 ± 0,19	6,55 ± 0,19
Urea, mmol / l	4,56 ± 0,31	4,56 ± 0,31	4,56 ± 0,31
14 days			
Total protein, g/l	73,93 ± 2,29	74,29 ± 1,06	74,00 ± 0,70
Albumin , g/l	31,96 ± 1,50	34,82 ± 3,03	35,26 ± 1,04
Thymol test, U	1,13 ± 0,31	1,14 ± 0,22	0,82 ± 0,16
AlAT, mkkat/l	0,40 ± 0,03	0,35 ± 0,06	0,42 ± 0,03
AsAT, mkkat/l	0,71 ± 0,02 ¹⁾	0,74 ± 0,06	0,63 ± 0,04 ¹⁾
Cholesterol, mmol/l	2,27 ± 0,20 ¹⁾	2,43 ± 0,16	1,95 ± 0,15
Glucose, mmol/l	4,94 ± 0,46 ¹⁾	6,05 ± 0,31	5,96 ± 0,44
Urea, mmol / l	3,56 ± 0,12	3,42 ± 0,14 ¹⁾	4,18 ± 0,26

Note: ¹⁾- p ≤ 0,05 on baseline data

Conclusions

1. Study of acute toxicity after a single intragastric administration to mice gel with 0.5% carbon dioxide Humulus lupulus extract have shown that gel does not cause death of animals; no toxic effects on the general state, behavior, food consumption and water weight animals; does not affect the absolute and relative weight of internal organs; does not cause visible changes of internal organs.
2. According to the standard classification gel with 0.5% carbon dioxide Humulus lupulus extract refers to practically non-toxic substances [6].

3. Subacute toxicity study results showed that the gel with 0.5% carbon dioxide Humulus lupulus extract with 14 daily intragastric administration to rats at doses of 0.5 g / kg and 1.0 g / kg, does not cause death of animals; has no toxic effects on the general state, behavior and dynamics of body weight of the experimental animals; no negative impact on the performance of blood of animals; does not change the biochemical parameters characterizing the functional state of the liver of animals.
4. Pharmacological studies of dental gel with 0.5% carbon dioxide Humulus lupulus extract, such as the results of the study of acute and subacute toxicity found that designed gel to include low-toxic substances (IV

class of toxicity), has good tolerability and safety of long-term use.

References

1. Mazur I. P. The impact of drugs on oral health / I. P. Mazur, K. N. Kosenko // *Modern dentistry*. – 2008. – № 3. – P. 179 – 187.
2. Medicinal properties of the Humulus Lupulus L. / M. Lyashenko, M. Mikhailov, G. Galak, T. Khomenko // *Food and processing industries*. – 2002 – № 12. – P. 19–20.
3. Patent for useful model № 113906 UA, MPK A61K 31/00, A61K 8/97, C12C 3/08, A61Q 11/00, A61P 31/04. An antimicrobial method for the treatment of diseases of the oral cavity / Kazmirchuk V.V., Melnik A. L., Hristyan G. Je. et al.; State Institution "I. Mechnikov Institute of Microbiology and Immunology National Academy of Medical Sciences of Ukraine" – applicants 13.06.2016; published 27.02.2017, Bulletin № 4.
4. Melnik A.L. Antimicrobial properties and prospects of application in dentistry a new drug with a carbon dioxide extract of Humulus Lupulus L. / A. L. Melnik // *Experimental and Clinical Medicine*. – 2016 – № 3(72). – P. 39–43.
5. New antimicrobial agent on the basis of biologically active substances for the treatment of periodontitis Humulus Lupulus L. / V. V. Kazmirchuk, A. L. Melnik, O. O. Radchenko et al. // *Technological and biopharmaceutical aspects of drugs of different directions of action: Materials I of the international scientific-practical conference*. – Kharkov, 2014. – P. 81 – 82.
6. Experimental study of the toxic effect of potential drugs: methodological recommendations / V. M. Kovalenko, O. V. Stefanov, Y. M. Maksimov, I. M. Trachtenberg – Kiev, 2001. – P. 74 – 98.
7. Laboratory Methods in clinic. Directory / V. V. Menshikov, L. N. Delektorskaya, G. P. Zolotnitskaya et al. // Edited in. V. V. Menshikov – M.: Medicine, 1987. – 386 p.
8. Kamyshnikov V. S. Clinical and biochemical laboratory diagnostics: Directory: In 2 vols. V 1. – 2 ed. – Mn.: Interpresservis, 2003. – 495 p.
9. Problem of norm in toxicology. (Current concepts and methodological approaches, the basic parameters and constants) / I. M. Trachtenberg, R. E. Sowa, V. O. Sheftel et al. // Edited in. I. M. Trachtenberg. – M.: Medicine, 1991. – 204 p.

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Introduction. In contemporary pharmaceutical practice among drugs used in dental gels are most effective, that are easily applied to the mucous and long held on the gums to form a protective film and prolonging therapeutic effect. Therefore it developed a new drug with a carbon dioxide Humulus Lupulus extract antimicrobial action in gel form for treatment of the oral cavity. The aim of our study was to investigate the acute

and subacute toxicity the gel containing 0.5 % carbon dioxide Humulus lupulus extract. The toxicity of the gel studied at the intragastric route of administration, due to the scope of drug – dentistry. **Materials and methods.** The object of the research was gel containing 0.5% carbon dioxide Humulus lupulus extract. Studies of acute toxicity gel with carbon dioxide Humulus lupulus extract was performed on white inbred laboratory mice of both sexes, weighing 19–21 gram. Animals received gel single intragastric at maximum tolerated this route of administration dose – 2.0 g/kg [6]. The experiment used by 5 mice of both sexes. The criteria of judgment about the toxicity was the clinical picture of intoxication, animal survival, dynamics of body weight of mice (raw data, 3, 7, 14 days). Observation of animals were carried out within two weeks. Subacute toxicity studied on white inbred laboratory rats of both sexes, weighing 220 – 250 g. Animals were divided into 3 groups, each experimental group were 5 male and 5 female rats. Total experiment used 30 rats. Rats was injected gel with carbon dioxide Humulus lupulus extract once a day for 14 days at doses of 0.2 g/kg and 1.0 g/kg (1/10 and 1/2 of the maximum dose in acute experiment). Control animals were injected comparison drug Kamistad–H gel at a dose of 1.0 g/kg. Evaluation of the toxic effects of investigational gel and reference medicine on the body of the experimental animals were carried out on the following parameters: clinical observations, the survival of animals, food and water consumption, the dynamics of body weight, hematological and biochemical parameters of blood. Clinical observations of the animals was performed daily during the experiment, registering changes in their general condition, behavior, food and water consumption. Clinical and biochemical parameters studied using a reagent kit of "Felicity Diagnostics" (Ukraine) and conventional methods. Body weight of animals recorded in dynamics. The animals were weighed before the experiment, then 7 and 14 days.

Results and discussion. Results of the study parameters acute toxicity gel with carbon dioxide Humulus lupulus extract showed that intragastric administration to mice at a dose of 2.0 g/kg caused no clinical symptoms of intoxication. Animals calmly reacted to the introduction of the sample gel, their general condition and behavior remained unchanged. Animal deaths and abnormalities in their general condition and behavior during the observation period were noted. Impact analysis of the sample of gel on the dynamics of body mass animal experiments showed that a single injection of a gel to mice at a dose of 2.0 g/kg did not significantly affect the dynamics of body weight. In experiments on rats were studied subacute toxicity gel with carbon dioxide Humulus lupulus extract. The results of 14 days intragastric introduction gel and reference drug studies at subacute toxicity showed that daily administration of no effect on the general condition, appearance (state of the skin and mucous membranes) and the behavior of rats. Consumption of food and water in animal experimental and control groups had no differences from each other. During the experiment animal deaths were not reported. Data characterizing the dynamics of

increase in body weight of rats showed that the animals of experimental groups and the control group by the end of the experiment had equivalent, statistically significant weight gain compared with the output data: males – $p \leq 0.0001$ females – from $p \leq 0.0001$ to $p \leq 0.0004$. During the experiment, the animals of all experimental groups were observed physiological fluctuations that characterize the pattern of peripheral blood. Results of biochemical studies showed that overall the tested parameters: the concentration of total protein, albumin, thymol test, the activity of alanine and aspartate aminotransferase, the concentration of glucose and cholesterol in the blood serum of rats of all experimental groups answered normal values. There have been fluctuations in biochemical parameters in animals of all experimental groups compared with baseline data did not go beyond the physiological norm.

Conclusions. 1. Study of acute toxicity after a single intragastric administration to mice gel with 0.5 % carbon dioxide Humulus lupulus extract have shown that gel does not cause death of animals; no toxic effects on the general state, behavior, food consumption and water weight animals; does not affect the absolute and relative weight of internal organs; does not cause visible changes of internal organs. 2. According to the standard classification gel with 0.5 % carbon dioxide Humulus lupulus extract refers to practically non-toxic substances. 3. Subacute toxicity study results showed that the gel with 0.5 % carbon dioxide Humulus lupulus extract with 14 daily intragastric administration to rats at doses of 0.5 g/kg and 1.0 g/kg, does not cause death of animals; has no toxic effects on the general state, behavior and dynamics of body weight of the experimental animals; no negative impact on the performance of blood of animals; does not change the biochemical parameters characterizing the functional state of the liver of animals. 4. Pharmacological studies of dental gel with 0.5 % carbon dioxide Humulus lupulus extract, such as the results of the study of acute and subacute toxicity found that designed gel to include low-toxic substances (IV class of toxicity), has good tolerability and safety of long-term use.

Keywords: gel with carbon dioxide Humulus lupulus extract, acute and subacute toxicity, laboratory animals, clinical observations, reference drug – Kamistad-Gel H