

Research Article

Study of safety and influence on the wound defect tissues regenerative activity of the new product for oral care

Lyudmila Kravchenko^{1,*}, Natalya Ivchenko¹, Natalya Bas¹, Elena Zagradskaya¹, Yana Ivanova¹

¹*Department of Therapeutic Stomatology of the Odessa National Medical University, Odessa, Ukraine*

Abstract Difficulties of prevention and treatment of diseases of periodontal tissues, oral mucosa with metabolic syndrome make it an important problem of dentistry. The development of the new oral hygienic drug for improving the treatment and prevention of periodontal tissue diseases, oral mucosa is considered well timed.

Purpose of research: To investigate safety of the new product for oral cavity and determine the effect on regenerative processes under conditions of the experimental trauma.

Materials and methods: The study of acute toxicity of the new hygienic preparation in the form of elixir was performed. The elixir was administered intragastrally to white nonlinear mice. Timeframes of intoxication development and death of animals registration was obligatory. Acute toxicity was investigated by a single application on the skin mice. Subacute (chronic) toxicity was studied when elixir was applied to the skin of mice for one month. The study of subacute toxicity was performed on white rats of Wistar line with intragastric administration for 60 days. The condition of the animals was evaluated by their appearance and behavior, as well as by a number of objective indicators (live weight, analysis of cellular blood composition, hemoglobin level, blood serum biochemical parameters).

The study of reparative activity of the new elixir was carried out on an experimental model of the wound process—a round aseptic plane wound in rats.

Results: Oral administration of the new product in different concentrations to mice didn't result in death of animals in any case. According to obtained results the investigated agent isn't toxic. All the tested biochemical parameters of blood serum were not significantly different from the control, indicating that there were no metabolic disorders. Study of subacute toxicity did not reveal any abnormalities as compared with the control animals.

Positive dynamics of wound healing process was revealed visually in animals that received topical application of hygienic products based on propolis, which was confirmed by a quantitative calculation by the planimetry method. The difference at the area of wound defect of the animals, which were made application with the new product, and in rats of the control and comparative groups was determined on the 3rd, 7th, 14th days of the experiment. The difference in changes at the wound area in rats of the main and comparative group was on the 3rd day—25.7%, on the 7th day—37.5% and on the 14th day—45.2%.

Conclusions: a complete safety of the new hygienic product for the oral cavity—a tooth elixir on the basis of biologically active substances of bee products and compounds of plant origin—during studies of acute and subacute toxicity was determined. Reparative activity of the new product for oral cavity was determined on the model of wound process, which is better in drug of comparison “Phytpropolis”.

Keywords: elixir, acute toxicity, subacute toxicity, reparative activity, model aseptic plane wound.

How to cite: Lyudmila Kravchenko et al., Study of safety and influence on the wound defect tissues regenerative activity of the new product for oral care. J Med Discov (2020); 5(4):jmd20045; DOI:10.24262/jmd.5.4.20045; Received July 10th, 2020, Revised August 28th, 2020, Accepted September 15th 2020, Published October 1st, 2020.

Introduction

In recent years, most researchers have noted the prevalence of inflammatory diseases of the periodontal complex

* Correspondence: Lyudmila Kravchenko, Candidate of biological sciences, Odessa National Medical University, Odessa, 65082. Ukraine. Email: lyudmila.kravchenko.52@mail.ru

among patients with metabolic disorders, taking into account their interrelation with each other. So, the presence of a metabolic syndrome (MS) in a patient leads to a hyperinflammatory effect on the pathogenic microbiota of the periodontium [1]. Periodontitis, in turn, negatively affects the glycemic level, contributes to the development of metabolic complications. An increase in systemic pro-inflammatory mediators enhances insulin resistance [2]. However, the mechanisms underlying the relationship between these pathological conditions are not fully defined. The main feature of periodontal diseases, oral mucosa (OM) against the background of MS is an earlier generalization of the pathological process with more pronounced signs of inflammation, which are accompanied by discharge of pus from periodontal pockets. The course of periodontitis is continuous and recurrent, there is resistance to traditional therapy. Remission of the disease is unstable.

The known methods of prevention and treatment do not always provide the proper therapeutic effect, so the adequate treatment requires a reasonable use of agents that directly affect the injured areas in the oral cavity, removing stomatogenic risk factors, or correcting changes that have occurred against the background of the concomitant pathology.

Based on the analysis of available literature data, often contradictory, the development of a new oral hygienic drug for improving the treatment and prevention of periodontal tissue diseases, OM against the background of MS is considered well-timed and economically justified.

In the course of implementation of the state financed research project of the Ministry of Health of Ukraine №0120U002197 “Development of new therapeutic and prophylactic agents and pathogenetic substantiation of their use with inflammatory periodontal diseases against the

background of metabolic syndrome” on the basis of the Odessa National Medical University, the formula of a new hygienic preparation for oral cavity on the basis of biologically active substances of bee products and natural compounds was developed, which requires detailed study [3].

The aim of the work

To investigate safety of the new product for oral cavity and determine the effect on regenerative processes under conditions of the experimental trauma.

Materials and methods of research

The study of acute toxicity of the new hygienic preparation in the form of elixir was performed according to the methods [4].

The elixir was single administered, intragastrally to white nonlinear mice weighing 22 ± 2 g at doses at 50 mg/kg, 500 mg/kg, 5000 mg/kg. For intragastric administration of the elixir, the animals were provided with a gastric injection syringe with a cut and polished needle with a plum-shaped welding on the end. The elixir solution of 0.9% sodium chloride solution in a volume of 0.5 ml was administered on an empty stomach after a 12 hour fasting of the animal. The mice were accessed for food two hours after the manipulation. Each dose was studied for 10 animals, the control group (10 mice) were administered intragastrically saline solution. The duration of the mouse observation was 14 days. At the first day the animals were kept under continuous observation. The following parameters were used to fix the severity of the toxic effect: general somatic state of animals, intensity and nature of motile activity,

features of behavior, skeletal muscle tone, presence and nature of seizure, rate and depth of breathing, movement coordination, condition of hair and skin, mucous color, shells, feed and water intake, changes in body weight. Timeframes of intoxication development and death of animals registration was obligatory.

Acute toxicity of the new hygienic product was investigated by a single application at the rate of 1000 and 5000 mg/kg animal weight on the skin of 20 mice for 14 days.

Subacute (chronic) toxicity was studied when elixir was applied to the skin of 10 mice at a single daily dose of 500 mg/kg for one month, with the determination of skin sensitivity to a long-term action of the agent, behavioral abnormalities. The mice of the control group were rubbed saline into the skin. The study of subacute toxicity was performed on mature white rats of the Wistar line aged 2.5–3 months, weighing 180–230 g with intragastric administration through a probe in the form of a 2% aqueous solution at a dose of 500 mg/kg in the volume of 0.5 ml for 60 days, the control animals were injected saline. All the animals received a standard complete animal facility diet. The condition of the animals was evaluated by their appearance and behavior, as well as by a number of objective indicators (live weight, analysis of cellular blood composition [5], hemoglobin level [6], blood serum biochemical parameters—protein concentration [7], alkaline phosphatase [8], glucose [9]).

The analysis of the functional state of the skin of 10 rats after rubbing of the new preparation diluted with water (1:10) into the exposed areas for 30 days was done according to the severity of inflammatory reaction.

A local irritant effect of the elixir on the OM was examined in 14 white rats daily after application for 3–5 min,

monitoring the state of OM for 7 days.

The sensitizing effect of the new agent was studied in applications to 14 shaved lateral trunk surfaces.

The study of reparative activity of the new hygienic product was carried out on an experimental model of the wound process — a rounded aseptic plane wound [10] in rats kept under conditions of the animal facility of the Odessa National Medical University, with access to water and feed and 12-hour illumination. 28 Wistar rats of 2 months of age, weighing 220 ± 10 g after anesthesia with ether in advance dehaired skin body, after the surgical area processing by 70% alcohol, a rounded plane wound of diameter 1 cm was made by a sterile scalpel. In order to create the equal wound surfaces, a stencil with a hole of 1 cm in diameter made of a 2 mm thick organic glass was used. Rats were expelled from the experiment under anesthesia with thiopental 20 mg/kg by total bleeding from the heart at the 3rd, 7th and 14th day. All experimental rats were divided into 4 groups: group 1 — a model of a plate wound with an untreated surface, group 2 (the control) — the wound treated with sterile 0.9% sodium chloride, group 3 (the main) — the wound treated with the new elixir, group 4 (the comparative) — the wound surface treated with comparator agent — elixir “Phytpropolis” (trade mark “Medok”, Kharkiv, Ukraine).

The assessment of changes at the area of the simulated wound process was determined by macroscopic (examination) and planimetric methods [10, 11].

In order to study the plane wound healing, the wound surface reduction rate in time was calculated, on which the planimetric method was based [10]. At the 3rd, 7th and 14th day cellophane was applied to the wound surface, on which the contours of the wound defect were transferred, then using a trammel, the smallest and the largest diameter of

the oval was calculated by the formula $S = \pi Rr$, where R — the largest radius of the circle, r — the smallest radius of the circle. The wound process model applied by the stencil had a standard plane and was calculated by the formula according to the circle square which was 78.5398 mm^2 . The percentage of wound area healing was calculated and the wound healing rate at the area of the wound defect was evaluated.

The research and care of laboratory animals was carried out in accordance to the scientific and practical recommendations set out in the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes” [12] and according to the provisions of the General Ethical Principles for Experiments on Animals, approved on II National Congress of Bioethics (Kyiv, 2004) [13].

All the research results were processed statistically by using the program STATISTICA 6.1 for estimation of errors, their integrity [14].

Results of the study and their discussion

The formula of the new dental elixir includes biologically active components of bee products (propolis, comb capping wax), plant origin compounds, which were determined experimentally by composition. It is known that propolis contains a large number of active compounds, including many classes of polyphenolic compounds, flavones, flavonols, phenolic acid, vegetable oils, esters [15]. It has an effect on the normalization of processes in the human body with simultaneous manifestation of antibiotic, anti-inflammatory, anesthetic, antimicrobial, fungicidal, antioxidant, antitumor, immunostimulating, angioprotective effects. Propolis effects on the regeneration of tissues,

promotes rapid epithelialization and wound surfaces healing. Comb capping wax is highly immunostimulating and has antibacterial properties. By their properties, the plant components have an antioxidant effect, inhibiting the occurrence of excessive amount of peroxide compounds that violate the OM cells membrane. Besides, the natural constituents exhibit anti-inflammatory, capillaries strengthening, desensitizing effects, taking part in the regulation of lipid, mineral and water metabolism, the processes of periodontal tissues regeneration.

As a result of choosing optimal concentrations of extracts of phytocomponents and bee products, the new dental elixir was developed, the prospect of which can be determined first of all by toxicological and hygienic studies.

Oral administration of the new product in different concentrations to mice did not result in death of animals in any case. There were no changes in behavior and in the weight of animals, general somatic condition of the animals was satisfactory, there was a moderate motion activity (Table. 1).

According to obtained results the investigated agent is not toxic, on this occasion LD 50 is impossible to calculate.

When studying acute toxicity with a single application of the new product at a rate of 5000 mg/kg of animal weight on the skin in 5 mice did not reveal signs of intoxication and reliable difference from the animals of the control group, which were rubbed saline solution, no animals died during the observation (10 days).

When studying acute toxicity, the animals of the research groups who received the new product did not differ from the animals of the control group by appearance and

behavior. The increase in weight of rats was greater than in the corresponding control group.

Table 1. The results of the study of acute toxicity of a new hygienic product with intragastric administration in mice ($M \pm m$)

| Parameters | Concentration of the used agent | | |
|---------------------------|---------------------------------|-----------------|------------------|
| | 50 mg/kg, n=10 | 500 mg/kg, n=10 | 5000 mg/kg, n=10 |
| General somatic condition | Satisfactory | Satisfactory | Satisfactory |
| Motion activity | Moderate | Moderate | Moderate |
| Seizures | Absent | Absent | Absent |
| Color of mucosa | Pale pink | Pale pink | Pale pink |
| Body weight | 23±4.0 | 23±3.0 | 22±2.0 |
| Death of animals | 0 | 0 | 0 |

Analysis of the cellular composition of blood is presented in table 2 [5]. The data evidence that the cellular composition of the blood of rats, which received the new elixir, did not significantly differ from the corresponding

parameters of control.

Table 3 presents the results of biochemical studies of blood serum and blood hemoglobin concentration of rats that received the new hygienic product intragastrically.

Table 2. Effect of long-term intragastric administration of the new elixir on increase of weight, parameters of blood cell composition of rats ($M \pm m$)

| Parameters | Group of animals | |
|-----------------------|------------------|---------------------|
| | control (n=10) | experimental (n=10) |
| Increase of weight, g | 134.6±10.2 | 141.0±8.4 |
| P | | >0.05 |
| Erythrocytes, T/l | 5.10±0.24 | 5.88±0.32 |
| P | | >0.05 |
| Leukocytes, g/l | 10.80±0.54 | 11.96±0.70 |
| P | | >0.05 |
| Leukogram, % | | |
| Eosinophils, P | 3.50±0.30 | 2.34±0.24 |
| | | >0.05 |
| Rod-like | 4.30±0.32 | 2.66±0.22 |
| P | | >0.05 |
| Segmental | 30.20±0.80 | 31.00±1.40 |
| P | | >0.05 |
| Lymphocytes | 58.40±1.60 | 62.00±2.00 |
| P | | >0.05 |
| Monocytes | 3.00±0.40 | 2.00±0.20 |
| P | | >0.05 |

Note: P — is the probability index calculated in relation to the control group.

Table 3. Biochemical parameters of blood serum and hemoglobin concentration of rats with intragastric administration of the agent ($M \pm m$)

| Parameters | Groups of animals | |
|--|-------------------|---------------------|
| | control (n=10) | experimental (n=10) |
| Blood hemoglobin, mmol/l P | 8.82±0.14 | 9.38±0.18 >0.05 |
| Protein level, g/l P | 1.32±0.11 | 1.10±0.10 >0.05 |
| Alkaline phosphatase, μ kat/l P | 0.64±0.04 | 0.70±0.05 >0.05 |
| Glucose, mmol/l P | 5.8±0.2 | 5.8±0.2 >0.05 |

Note: P — is the probability index calculated in relation to the control group.

All the tested biochemical parameters of blood serum were not significantly different from the control, indicating that there were no metabolic disorders.

The presented results of biochemical studies indicate safety of the new hygienic agent, as evidenced by the lack of significant differences in the parameters of the experimental and control groups.

Study of subacute toxicity when applied to the skin of 10 rats at a single daily dose of 500 mg/kg of elixir during the month did not reveal any abnormalities in the behavior or physiological condition as compared with the control animals.

According to the results of toxicological studies and microscopic examination of the internal organs at autopsy of animals after euthanasia on the 3rd, 7th, 14th, 30th, 40th, 60th day of the experiment did not reveal any deviations from the norm.

The histomorphological picture of the vital parenchymatous organs of rats: liver, kidney, spleen, heart, lungs, stomach did not reveal the presence of toxic effect of the new hygienic agent on organ tissues with repeated administration, which is safe.

At present, no data on absorption in the gastrointestinal tract of constituents of the new elixir have been identified in the scientific literature, which eliminates their resorptive action and confirms non-toxicity.

Analysis of the functional state of the skin by severity of inflammatory reaction in 10 white rats after rubbing-in of the diluted elixir (1:10) into the exposed area for 30 days determined that the index of skin irritation was 0 points, so irritation was absent.

Local irritation of the new agent on OM was examined in 14 white rats daily after application for 2–3 min, monitoring the state of OM for 7 days. The irritation ratio was determined to be less than 0.1, indicating no irritation.

The results determine sensitizing action of the elixir with application to the shaved lateral areas of the trunk showed no sensitization when the index is less than 1 ($S = 0.36 \pm 0.04$).

Positive dynamics of wound healing process was revealed macroscopically (visually) in animals that received topical application of hygiene products based on propolis, which was confirmed by a quantitative calculation by the planimetry method.

The difference at the area of wound defect of the animals, which were made application with the new product, and in rats of the control and comparative groups was determined on the 3rd day of the experiment. On the 7th day, it was determined that the wound area in the group of rats treated with the new elixir was significantly reduced as compared to the control and comparison groups. On the 14th day, the

reduction of the wound area in the animals after the application of the new hygiene product was significantly more pronounced as compared to other experimental animals and almost complete healing was observed. The difference in changes at the wound area in rats of the main and comparative group was on the 3rd day — 25.7 %, on the 7th day — 37.5% and on the 14th day — 45.2%.

Table 4. Changes of wound damage area in rats under the influence of the hygienic means (M±m, mm²)

| Groups of animals | Term of experiment | | | |
|--|--------------------|-------------------------------------|-------------------------------------|------------------------------------|
| | before treatment | 3 rd day | 7 th day | 14 th day |
| Model of wound n=8 | 78.5±0.1 | 72.4±0.2 | 46.2±0.2 | 5.2±0.1 |
| Control n=8 P | 78.5±0.1 | 69.6±0.2 <0.05 | 34.4±0.3 <0.05 | 4.9±0.1 <0.05 |
| Main n=8 P P ₁ P ₂ | 78.5±0.1 | 42.8±0.3 <0.05 <0.05 <0.05 | 17.6±0.2 <0.05 <0.05 <0.05 | 2.3±0.1 <0.05 <0.05 <0.05 |
| Comparison n=8 P P ₁ | 78.5±0.1 | 57.6±0.2 <0.05 <0.05 | 28.2±0.2 <0.05 <0.05 | 4.2±0.1 <0.05 <0.05 |

Notes: P — significant difference for the group of the wound model; P₁ — significant difference for the control group; P₂ — significant difference for the group of comparison.

Table 5. Results of planimetric study

| Animal groups | Healing of wound area at the 14 th day (%) | Rate of wound healing (mm ² /d) |
|-----------------------|---|--|
| Model of wound, n=8 | 93.4 | 5.20 |
| Control group, n=8 | 93.8 | 5.25 |
| Main group, n=8 | 97.0 | 5.44 |
| Comparison group, n=8 | 94.6 | 5.30 |

Conclusions

1. A complete safety of the new hygienic product for the oral cavity — a tooth elixir on the basis of biologically active substances of bee products and compounds of plant origin — during studies of acute and subacute toxicity was determined.
2. Reparative activity of the new product for oral cavity was determined on the model of wound process (a rounded aseptic planar skin-fascial wound), which is better in drug of comparison “Phytpropolis”.
3. The research results indicate the prospect of further study of the new elixir properties in order to create a greater variety of home produced dental means for dental institutions practice.

Conflicts Of Interest

None

Acknowledgments

None

References

1. Denga O.V., Pindus T.A., Verbitskaya T.G. Molekulyarno-geneticheskaya otsenka markerov zhirovogo obmena i vospaleniya u patsientov s parodontitom na fone metabolicheskogo sindroma [Molecular-genetic evaluation of markers of fat metabolism and inflammation in patients with periodontitis on the background of metabolic syndrome]. Vestnik morskoy meditsiny 2017;4:149-154. [In Russian]
2. Han D.H. The association of metabolic syndrome with periodontal disease is confounded by age and smoking in a Korean population /D.H. Han, S.Y. Kim, B.C. Sun, D. Pack, H.D. Kim. J. Clin. Periodontol 2010;37:609-616.
3. Kravchenko L.S., Appelkhans O.L., Ivanova Ya.I., Goncharenko O.V. Zayavka na vynakhid Ukrainy a202002339 vid 10.04.2020. Zubnyi eliksir dlya mistsevoyi profilaktyky i likuvannya zapalnykh protsesiv slyzovoyi obolonky porozhnyny rota ta tkanyin parodonta [Dental elixir for local prevention and treatment of inflammatory processes of the oral mucosa and periodontal tissues]. [in Ukrainian]
4. Metodicheskie ukazaniya po eksperimentalnomu (farmakologicheskomu) i klinicheskomu ispytaniyu gigienicheskikh i lechbeno-profilakticheskikh sredstv dlya ukhoda za polostyu rta. Farmkomitet MZ Ukrainy [Methodological instructions for experimental (pharmacological) and clinical testing of hygienic and therapeutic prophylactic agents in oral care], 1994:43. [in Russian]
5. Goryachkovskiy A.M. Klinicheskaya biokhimiya v laboratornoy diagnostike [Clinical biochemistry in laboratory diagnostics] 3rd. ed, Odessa; Ekologiya, 2005:616. [in Russian]
6. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.I. Protein measurement with Folin phenol reagent. J. Biol. Chem 1951;193:265-275.
7. Levitskiy A.P., Marchenko A.I., Rybak T.L. Sravnitel'naya kharakteristika trekh metodov opredeleniya fosfataz slyuny cheloveka [Comparative characterization of three methods for the determination of phosphatase of human saliva]. Laboratornoe delo 1973;10:624-625. [in Russian]
8. Kuzina M.I., Kostyuchenok B.M. (eds.) Rany i ranevaya infektsiya [Wounds and wound infection]. A manual for doctors. Moscow, Meditsina, 1990:591. [in Russian]
9. Popov V.A. (ed) Ranevoy protsess: nanobiotehnologii optimizatsii [The wound process: nanobiotechnology optimization]. SPb, Spets. Lit 2013:199. [in Russian]
10. Khabraev R.Ts. Rukovodstvo po eksperimentalnomu (doklinicheskomu) izucheniyu novykh farmakologicheskikh veschestv [A guide for experimental (preclinical) study of new pharmacological substances]. 2nd revised edition, Moscow, OAO Meditsina 2012:832. [in Russian]
11. Kozhemyakin Yu.M., Khromov O.S., Filonenko M.A., Saifetdinova G.A. Naukovo-praktychni rekomendatsii z utrymannya laboratornykh tvaryn ta roboti z nymy [Scientific and practical recommendations for keeping and working with laboratory animals], Kyiv Avitsenna, 2002:156. [in Ukrainian]
12. Kosenko K.M., Sukmanskyy O.I. Eksperymentalne modelyuvannya khvorob, za ta proty: bioetychni aspekty [Experimental modeling of diseases — pros and cons: bioethical aspects]. Materialy Natsionalnogo kongresu z bioetyky z mizhnarodnoyu uchastyu, Kyiv, 2004:163-164. [in Ukrainian]

13. Glants S. Mediko-biologicheskaya statistika [Biomedical statistics]. Moscow, Praktika, 1998:459. [in Russian]
14. De Groot A.C. Propolis: a review of properties, applications, chemical composition, contact allergy and other adverse effects. *Dermatitis*, 2013;24(6):263-282.



This work is licensed under a Creative Commons

Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

© 2020. This article is published under <https://creativecommons.org/licenses/by/4.0/>(the “License”). Notwithstanding the ProQuest Terms and Conditions, you may use this content in accordance with the terms of the License.