PharmacologyOnLine

Archives • 2021 • vol.3 • 140-145

STUDY OF THE PHARMACOLOGY OF SAFETY AND TOXICITY OF THE MEDICAL SPONGES WITH CHLORHEXIDINE DIGLUCONATE IN THE EXPERIMENT

Stechyshyn, Iryna; Chubka, Mariana; Pavliuk, Bohdana*; Hroshovyi, Taras
I.Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

*bohdana.vons@gmail.com

Abstract

Hemostatic sponges become a valuable addition to the arsenal of tools for external control of bleeding and for closing burn surfaces, especially in cases where the tourniquet cannot be applied (for example, in case of neck injury). The aim of the work is to study the acute and sub-acute toxicity of a hemostatic sponge based on water extract from the xenoderm with chlorhexidine digluconate (1 %). Materials and methods. Toxicity studies were performed on white Wistar rats weighing 250–260 g, which were injected intragastrically with the developed hemostatic sponge using a metal probe at a dose of 10,000 mg/kg. Results and discussion. The results of the study of the toxicity of the hemostatic sponge indicate the absence of any toxic manifestations when administered intragastrically in white rats, which allows them to be classified as class V toxicity (practically non-toxic substances).

Keywords: *acute toxicity, sub-acute toxicity, sponge, xenoderma, wounds*

Introduction

Bleeding is present in any injury, which is one of the leading causes of death. Therefore, research on the use of absorbent hemostatic material is important for both civilians and the military [1]. Therefore, it is important to develop new drugs and medical devices based on the material of natural origin, where the active pharmaceutical ingredients are biologically active substances of plant`s or animal`s origin [2-4].

Medical sponges are used in surgery, neurosurgery, dentistry, otolaryngology, and gynecology to stop blood loss or to close wound surfaces (burns, trophic ulcers) [5].

Medical sponge – a tool that has absorbent and antiseptic properties, as well as stimulates tissue regeneration. It should also be noted that depending on the composition of the active pharmaceutical ingredients, sponges exhibit bactericidal, antiseptic, antimicrobial, regenerating, tonic, and absorbent properties and purposefully act on the focus of pathology [6, 7, 8].

Hemostatic sponges become a valuable addition to the arsenal of tools for external control of bleeding and for closing burn surfaces, especially in cases where the tourniquet cannot be applied (for example, in case of neck injury) [9].

The Food and Drug Administration has published a retrospective review and identified resorbable collagen-based hemostatic agents as potentially suitable and effective.

Promising today are medical sponges developed on the basis of material of natural origin, namely cryolyophilized xenoderm. Xenoderma is developed by a special lyophilization technology (patent of Ukraine 55636 A, 2003). Cryolyophilized xenoderm is a material of natural origin, which contains a large number of amino acids that improve regeneration processes [1, 10, 11].

The study pharmacology of safety makes it possible to analyze the harmful pharmacodynamic effects of the drug on the physiological functions of a living organism, which is an important step for modern pharmacy [12-14]. The single-dose toxicity study provides information on the relationship between dose and systemic or local toxicity [15, 16, 17]. Moreover, toxicity studies play a significant role in drug development procedure providing information on toxic doses and therapeutic indices of potential drugs or medical devices. In this study, we report the acute and subacute toxicity studies of medical sponges on the basic of water extract from xenoderm powder with chlorhexidine digluconate in female Wistar rats.

Methods

Technology of medical sponges

Medical sponge was prepared based on a material of biological origin, namely xenoderm, which is enriched with macro-and micronutrients, amino acids and with chlorhexidine digluconate (1%) as the active pharmaceutical ingredient (APhI) [1].

Experimental animals

The experiments was performed on white Wistar rats weighing 250-260 g from I.Horbachevsky Ternopil National Medical University vivarium. The rats were fed orally with rat pelleted feed, with access to the drinking water and also they were housed in cages, in compliance with the Good Laboratorial practice (GLP) guidelines for the care and use of laboratory animals. All animals were identified by color markings on their body. The rats were kept under standard conditions at room temperature in isolated cages with a 12-hour day/night. Rats were acclimatized to above-mentioned conditions for one week prior to the toxicity studies. The studies were carried out in accordance with national and international recommendations for the protection of animals used for experimental and other scientific purposes (Strasbourg, 1986; Law of Ukraine № 3447-IV, 2006). In accordance with the requirements of the Bioethics Commission of I.Horbachevsky Ternopil National Medical University (Protocol, No. 56 of January 8, 2020).

Acute toxicity

In the study of acute toxicity of drugs, an integral indicator is the survival/mortality of animals, which allows you to calculate the average lethal dose (LD50) of the drug. The State Expert Center of the Ministry of Health of Ukraine recommends the use of the maximum dose of class IV toxicity in accordance with the route of administration for the study of acute toxicity. Due to bioethical principles, the route of

administration to be used in clinical practice has not been studied. However, it was decided to conduct a study of the drug by oral administration, according to the recommendations of safety pharmacology. The sponge was administered orally on an empty stomach as an aqueous suspension at a dose of 10,000 mg/kg in two doses, which allows for systemic action. A total of 12 female rats were used for the study of acute toxicity. Their weights were recorded and randomly divided into 2 groups (Control group of animal (group A) and Acute toxicity (group B)), each containing 6 rats. The group A were given tap water (10 ml/kg/body weight) [18]. For medicinal products and medical devices of biological origin, which in structure and pharmacological properties can be equated to drugs for which there is extensive experience in clinical practice, toxicological studies may not be conducted in full, but only in females that are more sensitive to toxic effect than males [19]. Toxicity was monitored 1, 2 and 4 hours after treatment and periodically for the first 24 hours, then daily for two weeks after treatment twice daily (morning and evening). Clinical observations included control of motor activity, skin condition, changes in respiration, food and water intake, changes in weight, and any changes in animal behavior. To control body weight, the individual weighing was performed at 0, 3, 7 and 14 days (Table 1). At the end of the observation, the number of animals remained unchanged (n = 6), both in the control group (group A) and in the study group (B).

Sub-acute toxicity

The subacute toxicity study was conducted for 28 days to examine the toxicity of the medical sponges. 12 rats were randomly distributed into two groups (Control group of animal (group C) and sub-acute toxicity (group D)) each consisting of six rats per group. The control group of animals were given tap water (10 ml/kg/body weight), whereas group D daily by oral administration were given of an aqueous suspension at a dose of 10,000 mg / kg in two doses for 28 days [20].

Clinical follow-up was performed for 28 days, weight was measured weekly during four weeks. On day 28, the final weight of the rats was measured and then anesthetized. The signs of toxicity were observed in the same way as in the case of the study of acute toxicity. To control body weight, the individual weighing was performed every week (Table 2). At the end of the observation, the number of animals remained unchanged (n = 6), both in the control group and in the study group.

Relative organ and body weights study

The changes in body weights were recorded on a weekly basis, while the organs were weighed using standard weighing balance to calculate relative organ weight for the different sets on the sacrifice day [21].

Relative organ weight (%) = (Absolute weight of organ (g) / weight of rat on sacrifice day (g)) x100

Statistical analysis

The obtained results were subjected to statistical analysis by methods of variation statistics. For all studies, the arithmetic means, as well as the standard error, were calculated. For statistical analysis of the obtained results, a one-way analysis of variance ANOVA was performed using the Student's t-test. Statistical analyzes were performed using GraphPad Prism, version 5.0 (GraphPad Software, Inc.). To assess the probability of the obtained results, the significance level $p \leq 0.05$ was taken.

Results and Discussion

Establishing the effect of the test sample on vital functions and body weight

Observations of the animals' behavior, appearance, coat condition, activity and appetite revealed no signs of clinical intoxication or death. Reflex excitability, urination, defecation were within the physiological norm. All animals showed weight gain without significant differences between groups. The body weight alterations of rats given medical sponges are indicated in Table 3. Daily administration of medical sponges at doses 10000 mg/kg did not result in significant changes in the body weight of rats when compared with the control [22].

Acute toxicity

No visible pathological changes in the appearance and behavior of experimental animals

on the 1st, 7th and 14th day after the start of application of the developed tool was registered.

In the process of studying the acute toxicity of the sponge, it was found that the introduction of intragastric suspension of the sponge at a dose of 10,000 mg/kg death of animals was not observed.

Therefore, the lethal dose (LD50) of medical sponges was higher than 10000 mg/kg

Sub-acute toxicity study

Administration of medical sponges for 28 days continuously did not induce morphological changes or general behavioural changes in treated rats compared to the control group. No deaths were observed during the period.

Relative organ and body weights study

At the end of the observation period, namely 14 and 28 days, the animals were euthanized under anesthesia. During the experiment, there was also no difference in the values of the relative mass of animal organs; the results of the studies are shown in table 3. All internal organs are located anatomically correct. The surface of the organs is smooth, shape, color and size are characteristic and unchanged. The liver is represented by lobes of normal size. The spleen is full-blooded, elastic, normal size. The kidneys of all animals are symmetrical with a characteristic structure. The lungs are airy; the leaves of the pleura are unchanged. Thymus without changes. The ovaries are normal.

According to the results of in vivo toxicity studies of the developed medical sponge based from xenoderm water extract with chlorhexidine digluconate at a dose of 10000 mg/kg, no signs of pathological changes or inflammatory reactions were found in the internal organs. After removing the animals from the experiment, as a result of macroscopic examination, it was noted that vital organs and systems whose functions are acutely critical for life were unchanged and were identical to control group of animals (group A and C). Therefore, according to the parameters of toxicity, the developed pharmaceuticals belong to the V class of toxicity (practically non-toxic substances) according to the classification of O. V. Stefanov and according to the classification of H. C. Hodge та L. H. Sterner [23].

Conclusions

1. Acute and subacute toxicity of medical sponges based on water extract from xenoderm with chlorhexidine digluconate was studied.

2. After completion of the observation, no mass metric or visual changes were detected.

3. It is established that according to the indicators of acute and sub-acute toxicity, medical sponges when administered intragastrically are practically non-toxic substances (toxicity class V).

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. Pavliuk, B., Chubka, M., Hroshovyi, T. The development of composition and characterization of biodegradable hemostatic and absorbable sponges for medical application. Farmacia, 2020; 68(3):419–425
2. Savych, A., Milian, I. Total flavonoid content in the herbal mixture with antidiabetic activity. PharmacologyOnLine, 2021; 2:68-75.
3. Slobodianiuk, L., Budniak, L., Marchyshyn, S., et al.: Experimental studies on expectorant effect of extract from Pimpinella saxifraga L. Pharmacologyonline, 2021; 1: 404–410.
4. Savych, A., Basaraba, R. Ascorbic acid content in the herbal mixture with antidiabetic activity. PharmacologyOnLine, 2021; 2: 76-83.
5. Kusuda K, Yamashita K, Tanaka S, Tanaka K, Ohta Y. Development of a Surgical Sponge Counting System Using Radiographic Images. Surgical Innovation, 2020; 27(6): 647-652.
6. Savych, A., Polonets, O. Study of hypoglycemic activity of antidiabetic herbal mixture on streptozotocin-nicotinamide-induced rat model of type 2 diabetes. PharmacologyOnLine, 2021, 2, pp. 62-67.
7. S. Okley, C. Mauprivez, P. Prada, M. Dubus, F. Velard, R. Rahouadj, H. Kerdjoudj & A. Baldit. Haemostatic sponges as an alternative to granular bone substitute for sinus lifts, Computer Methods in Biomechanics and Biomedical Engineering, 2020; 23:sup1, 206-208.
8. Darzuli, N., Budniak, L., Slobodianiuk, L. Investigation of the antibacterial and antifungal

activity of the Pyrola rotundifolia L. leaves dry extract. Pharmacologyonline, 2021; 1: 395–403.
9. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Demydiak, O. Investigation of the anti-inflammatory effect of the dry extract from the herb of stachys sieboldii MIQ. Pharmacologyonline, 2021, 2, pp. 590–597.
10. Bennett, B. L., Littlejohn, L. Review of new topical hemostatic dressings for combat casualty care. Military medicine, 2014; 179(5): 497-514.
11. Pavliuk, B., Stechyshyn, I., Kramar, S., et al.: Therapeutic efficacy of the developed gel "Xeliogel" on a burn wound model in rats. Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego. 2020; 48(287): 331-334.
12. Zuo, Y., Lu, S.: Dermis, acellular dermal matrix, and fibroblasts from different layers of pig skin exhibit different profibrotic characteristics: evidence from in vivo study. Oncotarget, 2017; 8(14): 23613-27.
13. N. A. Al-Afifi, A. M. Alabsi, M. M. Bakri, and A. Ramanathan, "Acute and sub-acute oral toxicity of *Dracaena cinnabari* resin methanol extract in rats," BMC Complementary and Alternative Medicine, 2018; 18(1): 50 pages.
14. Kanakia, S., Toussaint, J.D., Mullick Chowdhury, S., Tembulkar, T., Lee, S., Jiang, Y.P., Lin, R.Z., Shroyer, K.R., Moore, W., Sitharaman, B. Dose ranging, expanded acute toxicity and safety pharmacology studies for intravenously administered functionalized graphene nanoparticle formulations. Biomaterials, 2014; 35(25): 7022-31.
15. Prior, H., Haworth, R., Labram, B., Roberts, R., Wolfreys, A., Sewell, F. Justification for species selection for pharmaceutical toxicity studies. Toxicol Res (Camb), 2020; 9(6): 758-770.
16. Berezovskaya, I.V. Classification of chemicals according to the parameters of acute toxicity with parenteral administration methods. Pharm Chem J. 2003;37(3):32-4
17. Erhirhie, E.O., Ihekwereme, C.P., Ilodigwe, E.E. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol, 2018; 11(1): 5-12.
18. Ministry of Health of Ukraine. On Approval of the Recommendations of the Research Methods of Biological Effects of Natural Medical Resources and Preformed Medicines. Kyiv: Ministry of Health of Ukraine; 2009. Available from: http://www.old.moz.gov.ua/ua/portal/dn_20090928_692.
19. Chapman, K.L., Holzgrefe, H., Black, L.E., et al.: Pharmaceutical toxicology: designing studies to reduce animal use, while maximizing human translation. Regul Toxicol Pharmacol, 2013; 66(1): 88-103.
20. Zhou, X., Rong, Q., Xu, M. *et al.* Safety pharmacology and subchronic toxicity of jinqing granules in rats. *BMC Vet Res* 2017; **13,** 179. https://doi.org/10.1186/s12917-017-1095-3
21. Stechyshyn, I., Pavliuk, B., Demchuk, M., Chubka, M. Changes in mass measurement indices, cardiointervalogram parameters and duration of swimming in animals with experimental type 2 diabetes mellitus treated with drugs exerting antioxidant properties. Romanian Journal of Diabetes, Nutrition and Metabolic Diseases, 2020; 27(2): 146-152
22. Chinedu, E., Arome, D., Ameh, F.S. A new method for determining acute toxicity in animal models. Toxicol Int. 2013; 20(3): 224-226.
23. Ekanayake, C.P., Thammitiyagodage, M.G., Padumadasa, S., Seneviratne B., Padumadasa C., Abeysekera A.M. Acute and Subacute Toxicity Studies of the Ethyl Acetate Soluble Proanthocyanidins of the Immature Inflorescence of Cocos nucifera L. in Female Wistar Rats, BioMed Research International, 2019; Article ID 8428304. https://doi.org/10.1155/2019/8428304

Table 1. Dynamics of body weight of animals in the study of the acute toxicity (M±m)

Group of animals	White rats	Weight of animals (M ± m), g			
		beginning of the experiment	3rd day	7th day	14th day
Control (group A)	Female	247.17±0.70	249.00±0.68	254.17±0.65	260.0±0.37
Medical sponge (group B)	Female	254.72±1.68	257.67±1.17	261.83±1.01	265.83±1.14 $p_1 \geq 0.05$

Table 2. Dynamics of body weight of animals in the study of the subacute toxicity (M±m)

Group of animals	White rats	Weight of animals (M ± m), g			
		beginning of the experiment	7th day	14th day	28th day
Control (group C)	Female	220.53±1.01	224.62±0.95	228.0±1.07	232.03±0.68
Medical sponge (group D)	Female	235.12±0.52	240.32±0.81	241.83±1.25 $p_1 \geq 0.05$	248.16±0.98 $p_1 \geq 0.05$

Table 3. Mass coefficients of the internal organs of female rats in the study of acute and sub-acute toxicity (M±m)

Internal organs		Group of animals (M ± m), g			
		Control (group A)	Acute toxicity (group B)	Control (group C)	Sub-acute toxicity (group D)
White rats-female					
Liver		2.99±0.03	3.00±0.05 $p_1 \geq 0.05$	3.01±0.1	2.89±0.08 $P_2 \geq 0.05$
Kidneys	Right	0.34±0.01	0.34±0.01 $p_1 \geq 0.05$	0.31±0.05	0.34±0.02 $P_2 \geq 0.05$
	Left	0,33±0,01	0.32±0.02 $p_1 \geq 0.05$	0.32±0.04	0.30±0.01 $P_2 \geq 0.05$
Heart		0.41±0.01	0.43±0.01 $p_1 \geq 0.05$	0.40±0.01	0.42±0.01 $P_2 \geq 0.05$
Lungs		0.77±0.02	0.80±0.01 $p_1 \geq 0.05$	0.79±0.06	0.73±0.04 $P_2 \geq 0.05$
Spleen		0.46±0.02	0.48±0.01 $p_1 \geq 0.05$	0.47±0.01	0.47±0.03 $P_2 \geq 0.05$
Adrenal glands		0.041±0.001	0.040±0.001 $p_1 \geq 0.05$	0.042±0.009	0.040±0.007 $P_2 \geq 0.05$
Thymus		0.15±0.009	0.16±0.01 $p_1 \geq 0.05$	0.15±0.01	0.13±0.02 $P_2 \geq 0.05$

Note: p1 is the reliability of group A, p2 is the reliability of group C.