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STUDY OF A POSSIBLE ALLERGIC EFFECT OF UBIVAKS OINTMENT

Akopian K.A.[1*], Poghosyan S.B.[2], Movsisyan M.R.[3], Poghosyan Y.M.[1], Matinyan S.V.[2]., Ter-Zakaryan S.O.[2], Muradyan S.A.[2], Janjapanyan A.N.[2]

[1] Department of Maxillofacial Surgery, Yerevan State Medical University, Yerevan, Armenia
[2] Scientific Research Center, Yerevan State Medical University, Yerevan, Armenia
[3] Department of Clinical Allergology, Yerevan State Medical University, Yerevan, Armenia

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Abstract

One of the topical issues of modern therapy and surgery is research of new effective ways for treatment of burn wounds.

Poghosyan Y. and Papyan A. created a complex ointment, named Yubivaks, with the following ingredients: sea-buckthorn oil (86-90%), bees wax (7-10%), propolis (1.5-2%), clove oil (1.75-2.6%). Each of the ingredients has multipurpose biological activity.

The most essential product in sea-buckthorn processing is sea-buckthorn oil, which has gained increased interest in the recent years not only in pharmacology, but also in food industry and cosmetology. The therapeutic effect of sea-buckthorn oil is usually explained to be due to vitamins, carotenoids, tocopherols and other biological active substances. Propolis is a resinous substance with nectar impurity, collected by the bees from surfaces of leaves. It includes 65% different chemical substances such as, resins, balms, essential oils and aromatic compounds. In addition, propolis also contains wax, vitamins, mineral salts, microelements, natural antibiotics, and amino acids. The antibacterial and immunomodulation properties of propolis are well known. One to three percent liniment of propolis has an anti-inflammatory effect. To date, propolis is proven to have many biological effects, such as: anti-inflammatory, anesthetizing and antioxidant. Side effects of propolis and its products are manifested in some allergic reactions. Clove oil is used in medicinal products, especially when preparing remedies for gums and teeth. Also, tinctures, extracts and essential oils are used. Clove oil has antibacterial, antifungal and antioxidant properties. High levels of eugenol content in clove essential oil are responsible for antimicrobial activity. There are data on sensitizing effect of eugenol as well.

The ointment is subject to preclinical testing for its anti-burn activity. Taking into consideration that even a highly efficient product cannot be introduced to medicine without studying its side effects, we have assessed the possibility of an allergic effect of the ointment by testing it on experimental animals in therapeutic and toxic doses.

Studies have demonstrated that the ointment needs to be used only in therapeutic doses, because toxic doses cause regional lesions, such as local hyperemia, skin hypostasis, and foci of petechial hemorrhage, as well as significantly intensified frequency of specific leukocytes lysis processes.

Keywords: *ointment Yubivaks, sensitization, allergic effects.*

Introduction

One of the topical problems of modern therapy and surgery is research of new effective ways of treatment of burn wounds [*Paramonov BA, 2000*].

In recent years, in the treatment of burn wounds, clinicians have been more interested in testing new remedies of plant and animal origin, which include different biologically active substances of antibacterial, anti-inflammatory actions, as well as having the capacity to mobilize regional reparative-proliferative processes [*Jarvis D, 1981*].

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Address for Correspondence:
Department of Maxillofacial Surgery, Yerevan State Medical University after M.Heratsi, 2 Koryun Street, Yerevan 0025, Armenia
Tel. (+374 077) 823 012
Email: akopian karen@gmail.com

gredients: sea-buckthorn oil (86-90%), bees wax (7-10%), propolis (1.5-2%), clove oil (1.75-2.6%). Each of the ingredients has multipurpose biological activity [*Poghosyan YM, Papyan AA, 2011; Poghosyan YM 2012*].

The most essential product in sea-buckthorn processing is sea-buckthorn oil, which has gained increased interest in the recent years not only in pharmacology, but also in food industry and cosmetology. The therapeutic effect of sea-buckthorn oil is usually explained to be due to vitamins, carotenoids, tocopherols and other biological active substances. Propolis is a resinous substance with nectar impurity, collected by the bees from surfaces of leaves. It includes 65% different chemical substances such as, resins, balms, essential oils and aromatic compounds. In addition, propolis also contains wax, vitamins, mineral salts, microelements, natural antibiotics, and amino acids. The antibacterial and immunomodulation properties of propolis are well known. One to three percent [*Jarvis D, 1981; Khlghatyan SV et al., 2008; Konoplyova MM, 2011*] liniments of propolis have an anti-inflammatory effect. To date, propolis is proven to have many biological effects, such as anti-inflammatory, anesthetizing, and antioxidant [*Pessolato AG et al., 2011*]. Side effects of propolis and its products are manifested in some allergic reactions. Clove oil is used in medicinal products, especially when preparing remedies for gums and teeth. Also, tinctures, extracts and essential oils are used [*Atal CK, Kapur BM, 1982*]. Clove oil has antibacterial, antifungal and antioxidant properties [*Lee KG, Shibamoto T, 2001*]. High levels of eugenol content in clove essential oil are responsible for antimicrobial activity. There are data on sensitizing effect of eugenol as well.

The ointment Yubivaks is subject to preclinical testing for its anti-burn activity. Taking into consideration that even a highly efficient product cannot be introduced to medicine without studying its side effects, we have assessed the possibility of an allergic effect of the ointment by testing it on experimental animals in therapeutic and toxic doses [*Egorov UL et al., 1980; Golikov SN et al., 1986*].

The objective of this study was to investigate possible allergenic effects of ointment Yubivaks on the body of warm-blooded animals.

Materials and Methods

The material of the study was ointment Yubivaks designed for treatment of local 2nd and 3rd degree thermal burns of the skin of neck and face.

The study to identify the ointment allergic effect was conducted in accordance with the standard methods of preclinical evaluation of the safety of the pharmacological products [*Burov YV et al., 1992*] suggested by Alekseeva O.G. [*Alekseeva OG et al., 1992*] and conventional methods [*Marchenko NE et al., 1980; Ministry of Health of the Latvian SSR, 1980*].

Medical ethanol was used as a solvent. Three groups of white rats were involved in the study. Toxic doses of the ointment were used in the first group: 20 *g* per day, exceeding the therapeutic dosage more than 7 times, (toxic ratio). The second group was administered 3 *g* per day (therapeutic dosage), and the 3rd – control group – received 3 *ml* of ethanol.

Primary assessment of the Yubivaks was performed via single intradermal sensitization, which allows, in a short period of time, to obtain findings on the presence of sensitizing properties. Doses of the solutions were calculated in a phased manner [*Alekseeva OG, Dueva LA, 1992*].

In the first phase of the study, the rats of the first group were injected with a single dose of 0.02 *ml* solution with 1400 *μg* of experimental substance on the external surface of the ear intradermally with a tuberculin syringe, taking into account the toxic ratio. The second group received 0.02 *ml* of the solution with 200 *μg* of the substance. The control group received 0.02 *ml* ethanol. Effects on the skin treated with the solution was checked after 30 minutes, 2, 24, 48, and 72 hours for the presence of erythema, infiltrates, hypostases, and necrosis in the areas under study.

After 72 hours (2nd phase of the study) the rats of the 1st and 2nd groups were exposed to 10-fold epicutaneous applications of the ointment (5 times per week) of 20 *g* and 3 *g*, respectively, in the lateral sites of the trunk, equal to 2x2 *cm*. In the con-

trol group 3 *ml* ethanol was used. Exposure to the effects took 4 hours. The animals were placed in special individual compartment in order to prevent them from licking it and smearing the ointment on the cage walls. Effects on the skin after application were checked after 4, 24 hours and further on till the end of the experiment, that is during 30 days.

All the 3 groups of animals were inflicted with scars using 0.1% hydrochloride histamine solution (3rd part of the study) after finishing the epicutaneous applications.

The testing by way of reaction of the specific lysis of the leukocytes (RSLL) was performed by calculating the quantity of leucocytes in Goryayev camera, with subsequent calculation of a RSLL indicator. Reaction is positive if the indicator is 10% and more.

Reaction of the specific agglomeration of leukocytes (RSAL) was assessed, which is based on the strengthening of adhesion (agglomeration) of leukocytes by adding in vitro a sensitizing allergen in the blood of a sensitized individual. The phenomenon of agglomeration is the first stage of the allergic reaction in the cells. Evaluation of the reaction was performed by comparing the percentage of agglomeration leukocytes in the allergen test sample with that in the control sample, that is, with the level of spontaneous leukergy according to Fleck at the 500 leukocytes count. Ointment ethanol emulsion was used as an antigen in the presence of RSLL and RSAL.

Sensitizing effect was also estimated by the number of the sensitized animals, taking into account the results of all the conducted research of the techniques we have applied.

Results of the study were statistically analyzed for quantitative evaluation of the pharmacological effect of the preparation under study. Evaluation of mean group indicators was performed using Student's *t*-test, via SPSS version 13 software, ANOVA.

Results and Discussion

As the study results show, single intradermal injection of the solutions along the external surface of the rats ears after 30 minutes and 4 hours did not cause visible signs of skin irritation in both the two test groups and the control group. After 24 hours, the rats of both groups were gathering in the corner of the cages with their eyes half closed. The sites of injection with effective solution doses and rat pinnae got erythematous and edematous with the presence of wounds. The experimental animals responded very poorly to painful and tactile irritants, meal and water. After 24 hours, in the areas of injection of the rat group subjected to toxic doses, a number of regional disorders, such as local hyperemia and skin hypostasis, as well as dot foci of hemorrhages. That picture was intact until the end of the observed period. The second group of rats, as compared with the control group, had some minor changes, manifested in dot hemorrhages. After 72 hours, rats in the control and test groups showed the same signs.

The results of inflicted scar testing of the rats treated with the ointment were not significantly different from those in the control group. At the same time, no presence of erythema, hypostasis, necrosis or other pathological changes of the skin was observed.

The results of RSLL in the study of immunological tolerance and allergic effect of Yuvibaks ointment are showed in table 1. As table 1 shows, the use of toxic doses caused the number of the structurally intact leukocytes to decrease, whose content was 2.5 times lower than the control level. The effect of therapeutic dose administration demonstrated that the content of structurally intact leukocytes was virtually no different from that of the control group animals (Table 1).

As it is known, the reaction of specific agglomeration of leukocytes can be evaluated in each group as the ratio of the test sample indicators to the control one. In our study, the result was negative for both test groups (percentage of the agglomerated leukocytes was less than the value of 1.5) (Table 2).

Thus, the application of Yubivaks ointment at a toxic dose led to undesirable effects, manifested by a number of regional changes as focal hyperemia and hypostasis of the skin, dot hemorrhages, and significant intensification of the processes of

Table 1.

Evaluation of possible allergic effect of ointment Yubivaks.

Study groups	Leukocytes	*p*-value
control	140.5 ± 12.2	
1st test	56.0 ± 2.3	<0.0005
2nd test	131.3 ± 3.2	>0.25

Note: *p – 1st and 2nd group indicators as compared with those of the control group*

specific lysis of leukocytes. At the same time, the use of therapeutic dosage did not lead to any of the above mentioned symptom complex, observed in the animals of the 1st group.

Table 2.

Evaluation of possible allergic effect of Yubivaks ointment according to the results of the reaction of specific agglomeration of leukocytes

Study groups	Number of agglomerations		
	Test sample	control sample	Test to Control Ratio
control	0.6	1.7	0.35
1st test	0.83	0.67	1.23
2nd test	0.5	0.67	0.75

The changes found in the animals of the 1st and the 2nd groups allowed us to rank Yubivaks ointment as a product with a weak allergenic effect.

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