

## ORIGINAL ARTICLES

# PASTES BASED ON ROYAL JELLY, AN ALTERNATIVE FOR THE MINIMALLY INVASIVE TREATMENT OF PULPITIS (HISTOPATHOLOGICAL EXPERIMENTAL DATA)

Aida METO<sup>1</sup>, Agron METO<sup>2</sup>

<sup>1</sup>Restorative Dentistry Department, Faculty of Dental Medicine, University of Medicine, Tirana, Albania

<sup>2</sup>Department of Dentistry, Faculty of Dental Sciences, Aldent University

(Corresponding author: [aida\\_meto@yahoo.com](mailto:aida_meto@yahoo.com))

## Abstract

**INTRODUCTION:** Royal jelly is the most interesting of trio product that comes from bees. The 10-hydroxydeconoic acid is a precious ingredient of royal jelly that exerts antibacterial and antitumoral activity. It looks like a white-yellow cream, with a pH around 4-4.5.

The purpose was to define through histopathological slides, the anti-inflammatory and regenerative actions of pastes based on royal jelly in vital amputations in the near future.

**MATERIAL AND METHODS:** In 16 patients, aged from 35-60 years, there were planned extractions of 16 teeth for orthodontics and prosthetics purposes. Patients were treated at the University Clinic of Aldent, Tirana. The teeth were divided into three groups, and were treated with pastes based on royal jelly. Pulpal disease diagnosis was acute partial pulpitis based on clinical data. the coronary vital amputation was used, removing the inflamed pulp and aiming at a well-preserved retention of the radicular pulp.

**RESULTS:** From clinical examination, patients did not express concerns (as pains, pulsations, reactions on percussion or changes to the surrounding tissues) in conjunction with treated teeth. **Conclusions:** After 30 days of treatment, by the histopathologic examination a normal pulp was observed, with well expressed odontoblasts proliferation, a cell proliferation and lack of inflammation at the 1<sup>st</sup> group.

**KEYWORDS:** anti-inflammatory, propolis, regeneration, royal jelly, vital amputation.

## Introduction

The propolis is a mixture of substances and other elements as: tannin, propolis resin, wax of bees, essence, pollen, different vitamins, microelements, etc.

(Ahn, et al. 2009; Li-Chang Lu et al, 2005). Being a secondary product of bees, it is known for his antitumoral, antioxidant, antimicrobial, anti-

inflammatory, and immunomodulatory effects (Al-Shaher, et al. 2004; **Scazzocchio**, et al. 2006).

A well-kept hive can produce during 5-6 months of spring-summer seasons about 500 grams of royal jelly. This is the most interesting of trio product that comes from bees.

Royal jelly is one of the most valuable products of the bee hive. It is excretion of hypopharynx glands and jaw of bees. The precious ingredient of royal jelly is 10-hydroxydeconoic acid which exerts antibacterial and antitumoral activities (Baker, et al. 1990; Hattori, et al. 2007).

It contains 70% water, 30% dry substance, from which 15% are proteins, 12% carbohydrates and 3% lipids, it contains enzymes, cholinergic factors, vitamin B1, B2, B6, PP, Biotin, B5, B9, B12, inositol. Minerals and 28 oligoelements. Antitoxic factors, antibiotic (roializina), a growth factor that is biopterin, and antitumoral cytostatic factor (neopterin), etc. (Bonomi 1983; Fernandes Júnior et al 2003; Xiao et al, 1995).

It is a stimulant that facilitates the cellular metabolism, strengthens the immune defense and resistance to stresses, operating in depression and fatigue (**Table.1**). It looks like a white - yellow cream, perfumed and with a pungent flavor. Its PH is 4-4.5.

Table 1. Properties of royal jelly.

General revitalizer	Stimulate the level of anti-anemic senile (aging)	Stimulant of appetite	Stimulant of humor	Equilibrator of neurovegetative and psychological systems
Immunomodulatory (incentive to anticorps)	Antibacterial	Anti-hypertensive	Antiviral	Antitoxic

## Aim

To define through histopathological slides, the anti-inflammatory and regenerative actions of pastes

based on royal jelly, in vital amputations in the near future.

## Materials And Methods

In 16 patients, aged from 35-60 years, including 7 women and 9 men, extractions of 16 teeth were planned for orthodontics and prosthetics purposes. For the interventions, a sub-agreement with the patients was done, on the method of treatment and dental extractions. Patients were treated at the University Clinic of Aldent, Tirana. The teeth were divided into three groups, where were treated with pastes based on royal jelly (**Table.2**). Pulpal disease diagnosis based on clinical data was acute partial pulpitis. As a technique, we used the coronary vital amputation removing the inflamed pulp and aiming at

the well-preserved retention of the radicular pulp. Anesthesia in all cases was infiltrative with 4% Articaine. Cavity was opened by traditional techniques.

Table 2. Therapeutic pastes taken in treatment based on royal jelly.

Group 1	Group 2	Group 3
Zinc oxide + royal jelly	Zinc oxide + royal jelly + 5% propolis dissolved in propylene glycol	Ca(OH) <sub>2</sub> + royal jelly + 5% propolis dissolved in propylene glycol

Pastes were prepared ex-tempore, thanks of royal jelly obtained from the pipe (Fig.1) in the mashed consistency, applicable in the cavity. Hemorrhage was banned in most of cases with saline. After drying the cavities under sterile conditions, application of paste was done without pressure on the radicular pulp in a thickness of 2 mm, and then the setting the layer of cement without pressure above, and in the end the definitive composite fillings.

Patients were observed for a period of 30 days. After 30 days the extractions of teeth were done and fixed in 10 % formalin. EDTA was used as a decalcicator, in the Histopathologic Laboratory of the QSUT "Hospital Center University of Mother Teresa" Tirana, whereas stains Hematoxylin - Eosin and Masson's Trichrome were used. Slides were performed longitudinally and transversally as well.



Fig. 1: Pipe with royal jelly, used for the preparation of pastes

## Results Of The Treatment

During clinical examination, patients did not complain of any concerns (as pains, pulses, reactions on percussion or changes to the surrounding tissues) in relation to treated teeth.

In 1<sup>st</sup> group, as the intention was to see the action of royal jelly mixed with an indifferent powder, but not radiotransparent such as zinc oxide, after 30 days of treatment and observation, by histopathologic examination, it was noted a normal pulp with well expressed odontoblasts proliferation, and in the pulp it was noticed a cell proliferation and lack of inflammation (Fig.2,3,4).

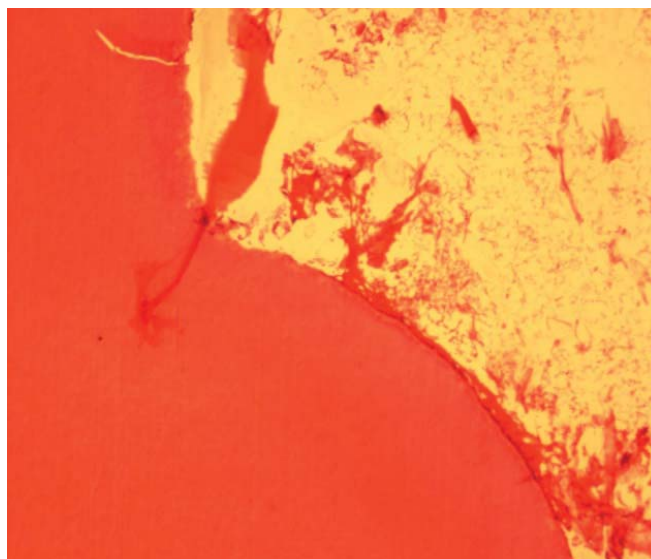


Fig.2: Longitudinal slide of pulp with Hematoxylin-Eosin stain, normal histological structure.

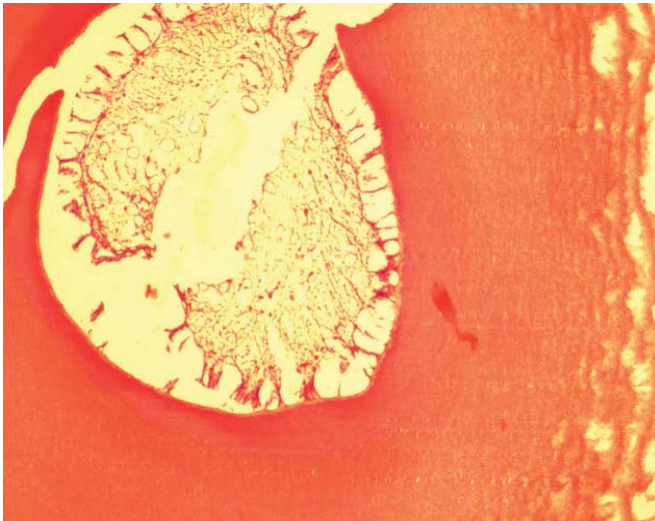


Fig.3: Transversal slide of pulp with Hematoxylin-Eosin stain after treatment with Zinc Oxide + royal jelly.

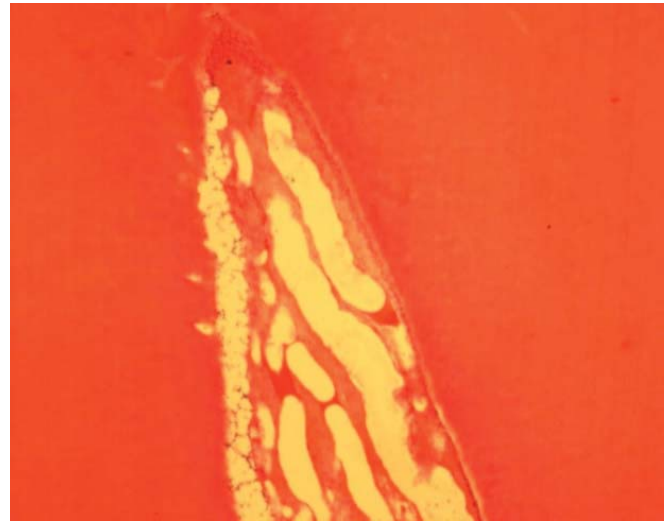


Fig.5: Normal pulp after 30 days of treatment of paste based on ZnO + royal jelly + 5% propolis dissolved in propylene glycol. Hematoxylin-Eosin stain.

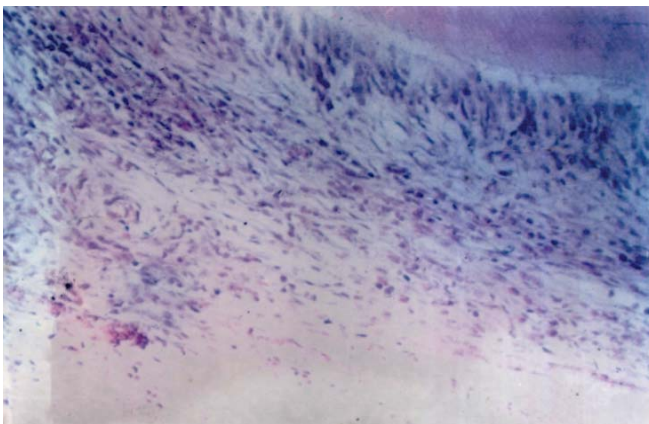


Fig.4: Apical pulp - odontoblasts borderless with a regular palisades placement, blood capillary and normal pulp in the apex. Hematoxylin-Eosin stain.

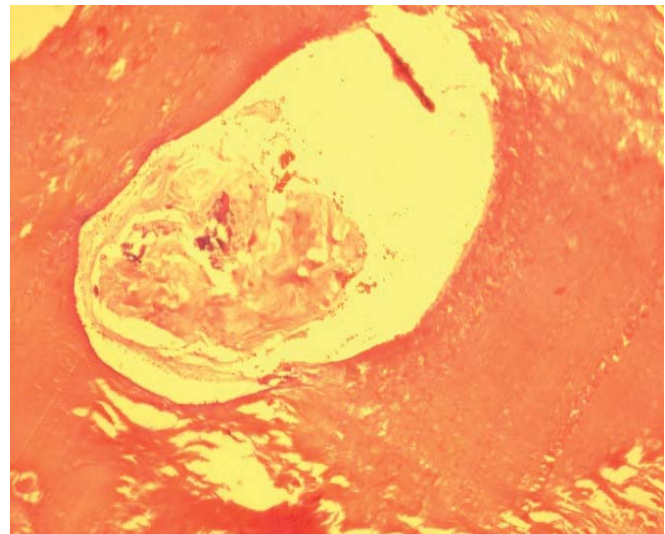


Fig.6: It was noted in this transverse slide, the presence of fibrosis and calcification of the pulp. Hematoxylin-Eosin stain.

In 2<sup>nd</sup> group, treated with the paste based on oxide zinc + royal jelly + 5% propolis dissolved in propylene glycol, normal pulp was observed after 30 days of treatment, odontoblasts borders in 50% of cases, while in the rest of cell structure fibrocytes and fibrotic tissue prevailed, with calcic clusters in spherical shapes (Fig.5,6,7).

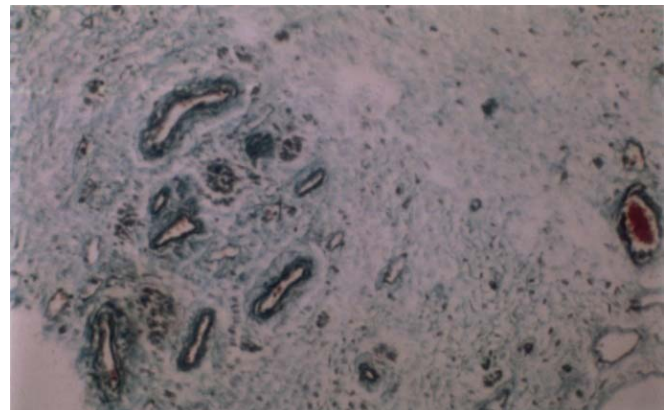


Fig.7: Neurovascular bundles of radicular pulp, after 30 days of treatment. Coloration with Masson's Trichrome.

In the 3<sup>rd</sup> group, treated with paste based on calcium hydroxide + royal jelly + 5% propolis dissolved in propylene glycol, in the longitudinal slide was noticed fibrotic pulp where prevailing fibrocytes after 30 days of treatment, while in the transversal slide was surveyed fibrosis and calcium agglomerations. In one of the cases, in the preparation stained with the Masson's Trichrome we discerned a superficial demineralization lesion and expressed tissue fibrosis. The presence of sclerotic pulp with calcic foci was noticed in this group (Fig.8,9,10,11).

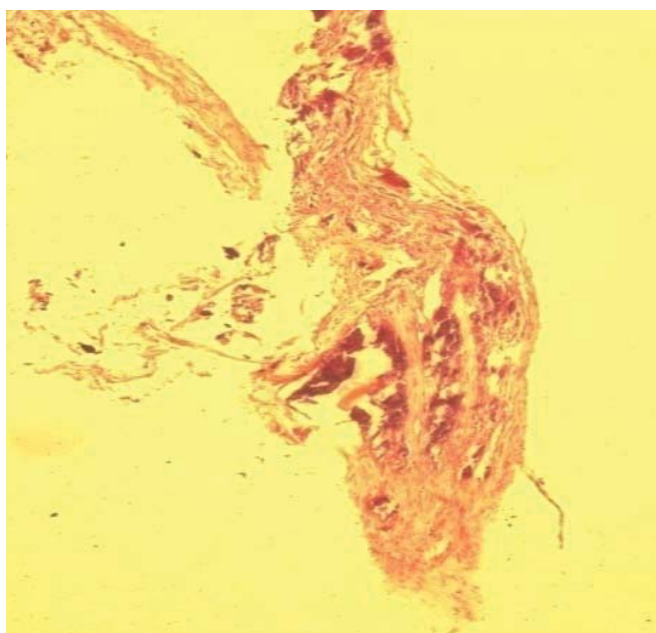


Fig.8: Fibroblastic proliferation, large fibrous and calcic clusters no condensed, after 30 days of treatment with Ca (OH)  $\square$  + royal jelly + 5% propolis dissolved in propylene glycol.

In 2<sup>nd</sup> group, treated with the paste based on zinc oxide + royal jelly + 5% propolis dissolved in propylene glycol results were better, while in 3<sup>rd</sup> group where calcium hydroxide was present the result did not change, but in the pulpal tissue were more present fibrosis, radicular pulpal calcifications and pulpal sclerosis.

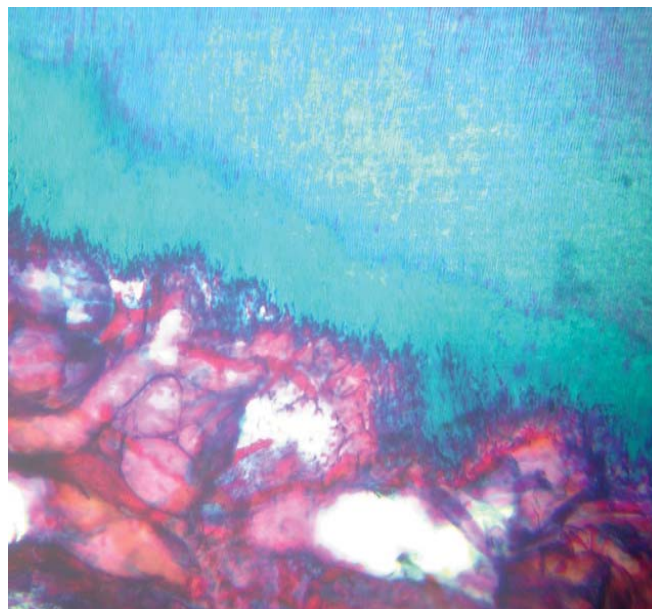


Fig.9: Fibrosis and micro calcic, with Masson's Trichrome stain.

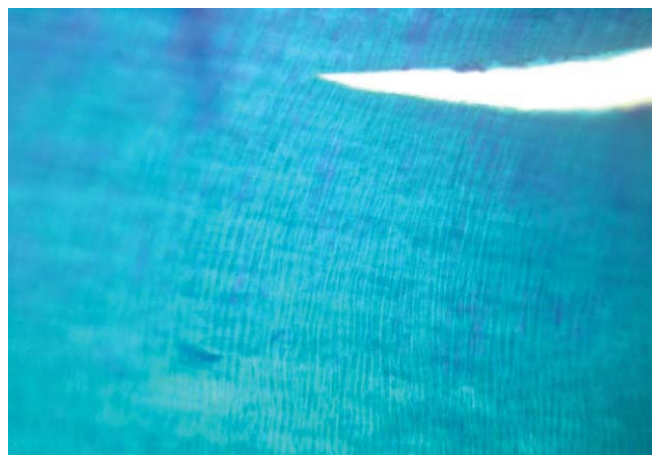


Fig.10: Fibrosis + canalar structure of dentin, after 30 days of treatment. Masson's Trichrome stain.

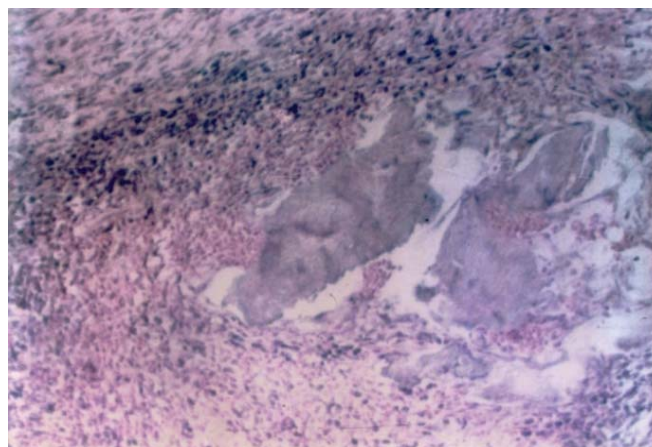


Fig.11: Chronic inflammation with pulpal calcification, after 30 days of treatment. With Hematoxylin - Eosin stain.

## Discussion Of Results

As we stated in the beginning, the purpose of this study was the determination of anti-inflammatory and regenerative actions of royal jelly in inflamed pulpal tissue after coronary amputation. This was confirmed from the analysis of the **first group**, where we implemented the composition of royal jelly only. It is well known in literature that the dentinal bridge stimulated by calcium hydroxide is incomplete, porous and does not provide long-term protection of pulp (Gomes., Brenda. 2002). Today, calcium hydroxide does not hold the position of glory as a single recovery pulp agent (Athanasiadis et al, 2007). Creating a suitable terrain in pulp through the curative material, which allows the healing, has opened the way for new alternative research efforts, where our modest work is also included.

Our histopathological results showed that we respected the conditions of radicular pulp to be healthy (Rroku C, Pavli E. 1979).

In various biological treatments, preparations based in antibiotics, sulfanilamide, glucocorticoids, antiseptic, proteolytic enzymes, and bio-substrates, etc. have been used

These preparations in a number of cases reduce inflammation, but do not stimulate the reparative dentin, and may even annihilate the dentine-genesis (such as glucocorticoids) (Gomes, Brenda. 2002).

Royal jelly is an albumin metabolic bio-stimulator and of cell regenerative processes (Rembold H. 1965; Xu Ming et al., 1993). Decenoic acid is the main factor

of antimicrobial action of royal jelly. It shows a high level of antimicrobial activity in relation to gram positive microorganisms, antiviral action was evident in water fractions of jelly, and among them, especially in albumin substance such as gamma globulin. So, the active factor of royal jelly, with antibacterial function, antiviral and antitoxic, are fatty acids, and in particular decenoic acid, but also the albumin substance (Hattori N. 2007).

Pastes with royal jelly positively influence on radicular pulpal tissue, haste the healing and stimulate the odontoblasts. It is important to note that, medicinal paste without the presence of calcium hydroxide stimulated the odontoblasts with relation to the presence of royal jelly, which is a biocatalyst of vital processes of the cell (Wu Cui-Wen., et al. 1993; Xu Ming et al 1993).

## Conclusion

In conclusion, we managed to, that royal jelly composed in the pastes combined with propolis, possessed an expressed action of:

- ✓ analgesic,
- ✓ anti-inflammatory,
- ✓ odontoblasts stimulant,
- ✓ regenerative.

## References

1. Ahn MR, Kunimasa K, Kumazawa S, Nakayama T, Kaji K, Uto Y, Hori H, Nagasawa H, Ohta T. Correlation between antiangiogenic activity and antioxidant activity of various components from propolis. *Mol Nutr Food Res.* 2009
2. Li-Chang Lu, Yue-Wen Chen, Cheng-Chun Chou; Antibacterial activity of propolis against *Staphylococcus aureus*. *International Journal of Food Microbiology*, Volume 102, Issue 2, 15 July 2005, 213-220.
3. Abdul Al-Shaher, James Wallace, Sudha Agarwal, Walter Bretz, Dean Baugh. Effect of Propolis on Human Fibroblasts from the Pulp and Periodontal Ligament *Journal of Endodontics*, Volume 30, Issue 5, May 2004.
4. F. Scazzocchio, F.D. D'Auria, D. Alessandrini, F. Pantanella. Multifactorial aspects of antimicrobial activity of propolis. *Microbiological Research*, Volume 161, Issue 4, 17 November 2006.
5. Baker S.A., Foster A.D., Lamb S.D., Hodson N. et al. Identification of 10-hydroxy-2-decenoic acilin royal jelly. *Nature.* –1990.–V.183, 996-997.
6. Hattori N, Nomoto H, Fukumitsu H, Mishima S, Furukawa S. Royal jelly and its unique fatty acid, 10-hydroxy-trans-2-decenoic acid, promote neurogenesis by neural stem/progenitor cells in vitro. *Biomed Res.* 2007 Oct; 28(5):261-6.
7. Bonomi, A. Acquisizioni in tema di composizione chimica e di attivita' biologica della pappa reale. *Apitalia.* 1983; 10 (15): 7-13.
8. Fernandes Júnior A, Balestrin ECC, Cunha MLRS. Anti-*Staphylococcus aureus* activity of bee propolis extracts prepared with different ethanol concentrations. *Rev Ciênc Farm* 2003; 24:147–52.
9. Xiao J., Wang R., Li J. Les Peptides actifs de la gelee royale inhgibent les Bacteries. XXXIV Congres international d'apiculture. – Bucarest: Apimondia, 1995, 441.
10. Gomes, Brenda (2002) In Vitro Antimicrobial Activity of Calcium Hydroxide Pastes and Their Vehicles Against Selected Microorganisms . *Brazilian Dental Journal* 13 (3).
11. Athanassiadis B., Abbott P.V., Walsh L.J. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. *Austr. Dent. J.* 2007. Mar; 52 (Suppl 1), S. 64-82.
12. Rroku C, Pavli E: "Teknika histopatologjike", Tirane, 1979; 96.
13. Rembold H. Biologically active substance in royal jelly. *Vitam. Horn.* –1965, Vol 23. 359-382.
14. Xu Ming, Yuan Ze-liang, Ling-yun. Extraction of 10-HAD from the filter residue of fresh royal jelly. XXXIII Congres international d'apiculture. – Bucarest: Apimondia, 1993. – P. 389
15. Wu Cui-wen, Zhang Fu-xing, Fang Bing-bing Glucose oxidase (GOD) in royal jelly. XXXIII Congres international d'apiculture. – Bucarest: Apimondia, 1993. – P. 377-381