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MODELING OF CONTENT OF BIOLOGICALLY ACTIVE HEPATOPROTECTIVE SUBSTANCES IN THE COMPOSITION OF NATURAL HONEY

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ABSTRACT. Aim. *The search for ways to create hepatoprotective food additives based on biologically active substances of plant origin and bee honey is an important area of research in the field of biology and medicine.*
Materials and Methods. *The material for the research was bees of Ukrainian breed (Apis mellifera sossimai). The process of enriching the natural properties of natural honey was carried out in a natural way through the bee family by feeding colonies of bees with a special composition of natural origin components based on Avéna satíva and Silibum marianum. Sieve honey with monofloral honey from white acacia (Robinia pseudoacacia) 55.0-55.0% was used for feeding of the bee families. The first research group was exclusively fed honey water (control). For the second and third experimental groups, Avena Sativa and Milk Thistle Dry Extract were introduced into the main feed, respectively. Preparation of honey water was carried out on distilled water. Each group of bee families was evaluated according to the complex of biological and economic-useful features during the whole study period. The concentrations of active substances in the obtained batches of honey were determined. Ferulic acid was chosen as the active substance for oat, sum of Silibinin A and Silibinin B for milk thistle. The mass concentration of ferulic acid was measured according to European Standards EN 15662:2008 using Gas Chromatography-Tandem Mass Spectrometry following acetonitrile extraction/partitioning and clean-up by dispersive solid phase extraction (Quechers) method. The limits of quantitation of ferulic acid and sum of Silibinin A and Silibinin B were 0.02 mg/kg. The high content (83.3±0.4 mg/kg) of Silibinin flavonoids (A and B), Isosilibin (A and B), Silichristin A, etc. in honey persists. Instead, ferulic acid precipitated in fairly low concentrations of honey (1.13±0.02 mg/kg).*
Conclusions. *The obtained results indicate that the extract of milk thistle is promising for the creation of enriched natural honey with hepatoprotective properties, as the biologically active components of this plant have characteristics that allow to obtain the final product with a high content of active substances.*
Key Words: *honeybee, biological active substances, hepatoprotectors.*

Introduction. This material is a fragment of the research work on topic: "Toxicological support of creation of innovative dietary supplements (adaptogens), capable to increase the protective capabilities of the organism in case of harmful influence of the vital environment" (fragment of researcher 0112U001133), deadline 2018-2020. The studies were carried out by the L.I. Medved's Scientific Center of Preventive Toxicology, Food and Chemical Safety of the Ministry of Health of Ukraine together with the National University of Life and Environmental Sciences of Ukraine (Department of beekeeping and horse breeding), in 2019 and reflect the results of thefirst stage of research aimed at the development of honey with targeted properties.

From the 1970s to the present, flavonoids and flavonoligands (lat. *Silibinin, Isosilibinin, Silicristin, Silidianin and others.)* of Milk thistle has been the subject of scientific research, and it is proved that the biologically active substances (BAS) of milk thistle are endowed with various beneficial properties. Recent years' research is mainly focused on the in-depth study of molecular structure, participation in chemical processes, bioavailability, metabolism in the BAS, and the search for new forms of their introduction [1, 2].

Science has looked into the depths of the neuro-, nephro-, hepato-, cardioprotective and antioxidant effects of flavonoids [3], which allowed the use of drugs based on the BAS of milk thistle in the treatment of acute toxic lesions

of the liver (including ammonitic hepatitis) [4], biliary cirrhosis, viral hepatitis [5, 6]. Today, the spectrum of research includes antioxidant, chelating, antiapoptotic properties of the BAS of milk thistle and their role in the regulation of inflammatory processes [7], treatment of liver fibrosis [8], formation of oncoprotective effects [9]. Methods of modern chemical toxicological analysis (high performance liquid chromatography), in silico methods using QSAR models are applied [10]. For example, the use of alternative models of toxicological studies of the BAS of milk thistle gives the opportunity to simulate and study the synergistic effects of the interaction of various flavonoids in compositions with natural honey.

The need to develop a science-based technology to create bee honey with targeted hepatoprotective properties in Ukraine has led to this study.

Aim. Honey bee from the standpoint of the content of high-quality components of natural origin, the BAS — hepatoprotectors.

The subject of the study is the hepatoprotective properties of enriched natural honey made by feeding bees specially selected qualitative and quantitative composition of components of natural origin.

The purpose — scientific substantiation of technology of creation of enriched natural honey containing biologically active substances of hepatoprotective action.

Objectives of the research: scientific substantiation of technology of enrichment of natural honey, which is realized through the bee family by feeding them optimally selected qualitative and quantitative composition of high-quality components of natural origin.

Material and Methods. The bee of the Ukrainian breed (Lat. *Apis mellifera sossimai)* was the material of the research. Of these, 12 families were formed, which comprised 3 study groups (n = 3), the first group was the control group, the second and the third groups were experimental. Each group contained 4 colonies. For the formation of study groups, the method of balanced analog groups was used, so that after their distribution, the difference between the groups at the beginning of the experiment was not greater than 5.0 % in terms of: family strength, number of brood stock, egg production of bee queens, feed stocks and the rate of their development.

The technique of natural honey enrichment. The process of enriching the healing and prophylactic properties of natural honey is carried out in a natural way through the bee family by feeding the colonies of bees optimally selected qualitative and quantitative composition of high-quality components of natural origin, which have a high level of specific activity and provide balanced activation. Common oat (Lat. Avйna Satнva) and milk thistle (Lat. Silibum marianum) were selected as natural components, and pharmaceutical preparations based on them were used. Transformation of biologically active substances of pharmaceuticals Avena Sativa 10:1 (manufacturer by “Naturex”) and Milk Thistle Dry Extract (manufacturer by “Naturex”) into natural honey was carried out with the help of working bees for the purpose of controlled increase of the biological activity of the latter.

The experimental part was carried out in the active apiary period of 2018 on the apiary of the breeding bee breeding farm of PE “Prybuzky medobory” of Khmelnitsky region. Using the conditioned reflex of bees, we have trained them to process cooked feed. It was used sieve honey with monofloral honey from white acacia (Lat. Robinia pseudoacacia) 55.0-55.0% Brix for feeding of the bee families. The honey water was prepared on distilled water. The first research group was exclusively fed honey water (control) developed under the scheme contained in the Table 1.

For the second and third experimental groups, Avena Sativa 10:1 and Milk Thistle Dry Extract were introduced into the main feed, respectively. Preparation of honey water was carried out on distilled water.

For feeding the feed was prepared in the following sequence:

— 150.0 g Milk Thistle Dry Extract was dissolved in 1.0 liter of prepared warm water; this solution was added to 5.0 kg of acacia honey + 4.0 liters of H_2O;
— 50.0 g of Avena Sativa 10:1 was dissolved in 0.5 liter of prepared warm water;
— this solution was added to the 5.0 kg of acacia honey + 4.5 liters of H_2O.

Each group of bee families was evaluated according to the complex of biological and economic-useful features during the whole study period. Prepared bee families were fed cooked compositions with natural honey in a carefree period. Each bee family was fed only one type of solution (feed) in accordance with the scheme above.

Bee colonies were given as much feed as they could digest per day according to the scheme. Selected honey was ripened in bee families,the

Table 1

Study groups and feeding scheme for bee colonies

1 group (n=3)			2 group (n=3)			3 group (n=3)		
period, day	**amount of feed, kg (morning/ evening)**	**feeding**	**period, day**	**amount of feed, kg (morning/ evening)**	**feeding**	**period, day**	**amount of feed, kg (morning/ evening)**	**feeding**
1	0,3/0,7	honey water	1	0,3/0,7	honey water + *Avena Sativa* PE 10:1	1	0,3/0,7	honey water + Milk Thistle Dry Extract
2	0,3/0,7		2	0,3/0,7		2	0,3/0,7	
3	0,3/0,7		3	0,3/0,7		3	0,3/0,7	
4	0,3/0,7		4	0,3/0,7		4	0,3/0,7	

whole process was controlled by a refractometer. Humidity was maintained within no more than 20.0%.

For the production of enriched honey, medium-strength bee families (8-9 streets) and those held in beehives of the vertical type were used as they are ideally suited for the production of enriched (multivitaminized) honey by the express method. Thanks to this design, hives are easy to use and accessible. Most importantly, it is possible to control the physiological processes of bee growth and to bring and store forage compared to other types.

Frames with honey were selected and then centrifuged. Then honey was cleaned with the help of special filters. Purified honey was packaged in clean, sterile containers. Glass or enamelware was used to store the enriched honey. Enriched honey was stored in clean, dry rooms, without direct sunlight, with humidity up to 60.0 %, while avoiding placing near highly odorous substances. The complete production process took about 5-12 days (depending on the quantity and composition of the preparation).

In the course of the study, the methods of toxicological research were used: chemical-analytical (chromatography, mass spectrometry) and biomedical statistics.

Results and Discussion

In the first stage of the study, the selection of medicinal products based on vegetable raw materials., Extracts of common oats and milk thistle have been selected to enrich the natural honey referring to many years of clinical experience in the successful use of certain natural BAS. The process of natural honey enrichment was carried out in a natural way through the bee family by feeding the colonies of bees components of natural origin Avena Sativa 10:1 (manufactured by “Naturex”) and Milk Thistle Dry Extract (manufacturer by “Naturex”) were introduced into the main feed, respectively.

According to the results of the first stage of the study, bees with the same intensity processed (absorbed) feed, but biologically active additives from common oat always remained in the feeders in the form of insoluble dry residues, which was not observed in the feeder with the addition of milk thistle.

The second stage of the study was the determination of the concentrations of active substances in the obtainedbatches of honey. Ferulic acid was chosen as the active substancefor Avena Sativa, sum of Silibinin A and Silibinin B for Milk Thistle.

The mass concentration of ferulic acid was measured according to EN 15662:2008 (Foods of plant origin – Determination of pesticide residues using GC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – Quechers method). The limits of quantitation (LOQ) of ferulic acid and sum of Silibinin A and Silibinin B were 0.02 mg/kg. The studies were performed on a liquid chromatograph Shimadzu LC-30 A coupled with Shimadzu LCMS-8050 mass detector No. 1083547010 US, the chromatographic column was Kinetex 2.6 mkm Biphlenil, 100* 2.1 mm.

The results of the study found that the content of ferulic acid in the obtained honey, enriched with the drug Avena Sativa 10:1 (manufacturer by “Naturex”) was in modest concentrations, which made it impossible to continue further studies with this substance. Instead, the content of the

sum of Silibinin A and Silibinin B in the resulting media that enriched drug Milk Thistle Dry Extract detected in large quantities, allowing us to consider milk thistle as a promising material for the enrichment of natural honey (Fig.1,2).

Tables 2, 3 show the concentration of biologically active substances (BAS) in the mixture for feeding bees and in finished honey.

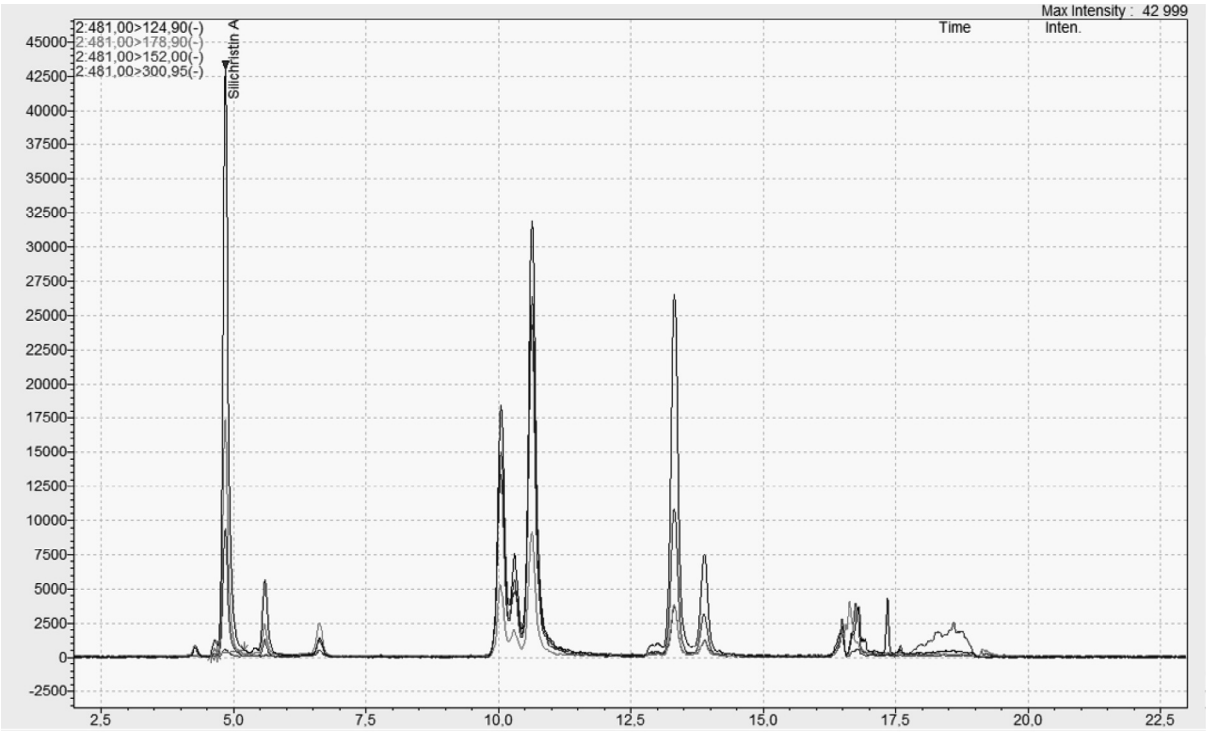

Fig.1. Chromatogram of a control sample of honey.

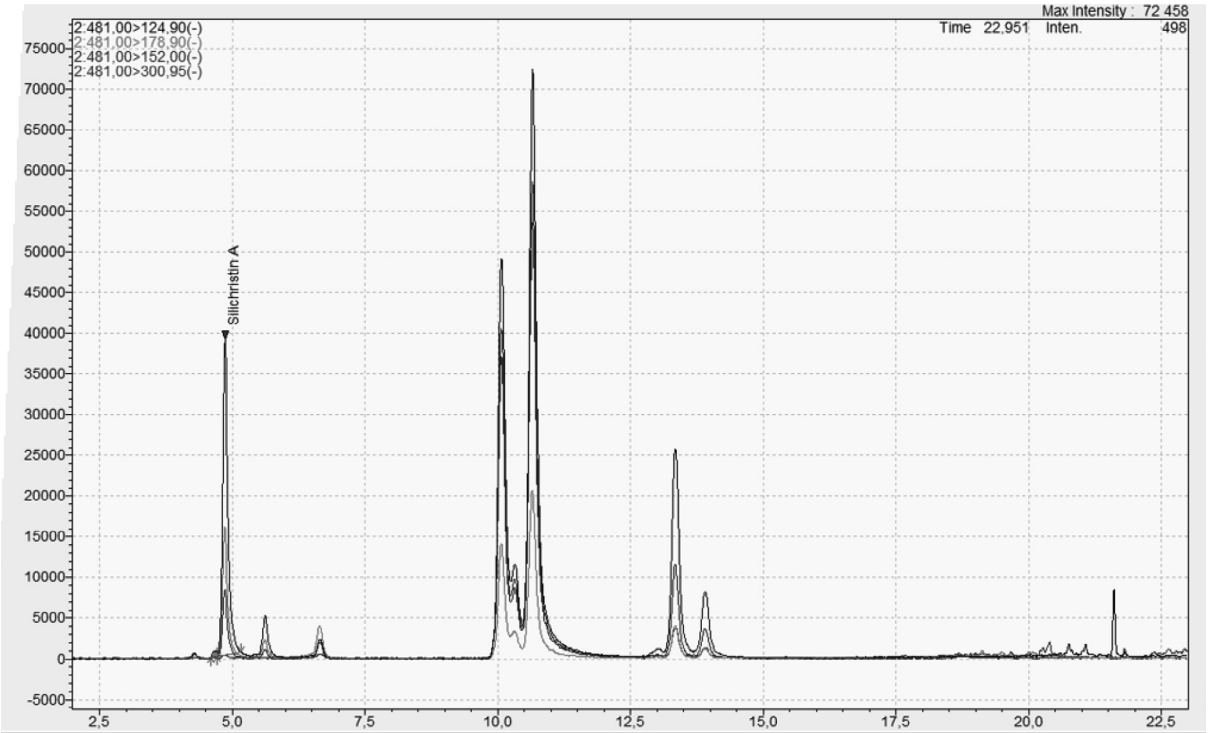

Fig. 2. Chromatogram of a sample of honey enriched with Milk Thistle Dry Extract (sample №2, standard - the amount of Silibinin A and B).

Table 2

The results of the study of the content of BAS *Avena Sativa* in the composition of the control sample of honey and sample No. 1, standard – ferulic acid

The object of analysis	The object of analysis
Honey, control	0,22±0,01
Honey, sample No.1	1,13±0,02
Honey, control with the introduction of 0.5 mg/kg ferulic acid	0,50-0,22=0,28 (56 %)

Table 3

The results of the study of the content of BAS *Silibum marianum* in the control sample of honey and sample No. 2, standard – the amount of Silibinin flavonoids (Silybin A+B mixture)

The object of analysis	Measurement result, mg/kg
Honey, control	0,72±0,01
Honey, sample No.2	83,63±4,0
Honey, control with the introduction of 0.95 mg/kg amount of Silibinin A+Bmixture	1,64-0,72=0,92 (97 %)

It is noted that the high content of Silibinin flavonoids (Silybin (A+B mixture), Isosilibin(A+B mixture), Silichristin A, etc.)in honey persists (83.3±0.4 mg/kg). Instead, ferulic acid precipitated in fairly low concentrations of honey (1.13±0.01 mg/kg).

At the final stage of the study, it is planned to determine the optimal quantitative composition of the BAS, adaptogens in the composition of enriched honey, which can increase the adaptation of the organism to harmful factors in the environment. Upon completion of the study of the finished product is planned to design specifications and other regulatory documents for the production and sale of innovative product and dietary supplements made on its basis.

The results of the first stage of the research confirm the prospect of developing innovative products based on natural honey and biologically active substances of vegetable origin, which will allow to obtain new useful varieties of honey and significantly improve the economic performance of enterprises producing these products.

Conclusions

For the creation of enriched natural honey, milk thistle extracts (lat. Silybum marianum) are promising, since the biologically active components of this plant have characteristics that allow to obtain the final product with a high content of active substances.

The emergence of new forms of enriched honey based on natural hepatoprotectors will open up new opportunities for treatment of acute and chronic toxic processes occurring in the body under the influence of environmental factors.

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МОДЕЛЮВАННЯ ВМІСТУ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН ГЕПАТОПРОТЕКТОРНОЇ ДІЇ У СКЛАДІ НАТУРАЛЬНОГО МЕДУ

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РЕЗЮМЕ. Мета. *Пошук харчових добавок гепатопротекторної дії на основі біологічно активних речовин рослинного походження і бджолиного меду є актуальним напрямком досліджень в галузі біології та медицини.*

Матеріали та методи. *Матеріалом для досліджень були бджоли української породи (Apis mellifera sossimai). Процес збагачення природних властивостей натурального меду здійснювався природним способом через бджолину сім'ю шляхом згодовування колоніям бджіл спеціальної суміші на основі вівса посівного (Avéna satíva) і розторопші плямистої (Silybum marianum). Для підгодівлі бджолиних сімей використовували ситу монофлорного меду білої акації (Robinia pseudoacacia) 50-55%. Першу групу бджіл підгодовували виключно медовою ситою (контроль). Для другої та третьої груп в основний корм вводили препарати вівса посівного (Avena Sativa) і препарати розторопші плямистої (Milk Thistle Dry Extract) відповідно. Приготування медової ситі здійснювали на основі дистильованої воді. Кожну групу бджолиних сімей оцінювали за комплексом біологічних і господарсько-корисних ознак протягом усього періоду дослідження.*

Результати та обговорення. *Визначали концентрації біологічно активних речовин в отриманих партіях меду. Ферулова кислота була обрана в якості активної речовини для вівса, а для розторопші – сума силібінінів А і В. Масову концентрацію ферулової кислоти вимірювали відповідно до Європейського стандарту EN15662:2008 за допомогою газової хроматографії-тандемної мас-спектрометрії з подальшою екстракцією/поділом і очищенням ацетонітрилом методом дисперсійної твердофазної екстракції (Quechers). Межі кількісного визначення ферулової кислоти і суми силібінінів А і В становили 0,02 мг/кг. У складі меду зберігався високий (83,3±0,4 мг/кг) вміст флавоноїдів: силібіну А і В, ізосилібіну А і В, силіхристину А та інших флавоноїдів. Феруловая кислота визначалася в значно нижчих (1,13±0,02 мг/кг) концентраціях.*

Висновки. *Отримані результати свідчать про те, що для створення збагаченого натурального меду з гепатопротекторними властивостями перспективним є екстракт розторопші плямистої, оскільки біологічно активні компоненти цієї рослини мають характеристики, що дозволяють отримати кінцевий продукт з високим вмістом діючих речовин.*

Ключові слова: *мед бджолиний, біологічно активні речовини, гепатопротектори.*

МОДЕЛИРОВАНИЕ СОДЕРЖАНИЯ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ ГЕПАТОПРОТЕКТОРНОГО ДЕЙСТВИЯ В СОСТАВЕ НАТУРАЛЬНОГО МЕДА

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РЕЗЮМЕ. Цель. *Поиск пищевых добавок гепатопротекторного действия на основе биологически активных веществ растительного происхождения и пчелиного меда является актуальным направлением исследований в области биологии и медицины.*

Материалы и методы. *Материалом для исследований являлись пчелы украинской породы (Apis mellifera sossimai). Процесс обогащения природных свойств натурального меда осуществлялся естественным способом через пчелиную семью путем скармливания колониям пчел специального состава на основе овса посевного (Avéna satíva) и расторопши пятнистой (Silybum marianum). Для подкормки пчелиных семей использовали сыту монофлорного меда белой акации (Robinia pseudoacacia) 50-55%. Первую группу пчел подкармливали исключительно медовой сытой (контроль). Для второй и третьей групп в основной корм вводили препараты Avena Sativa (производитель Naturex) и препараты расторопши пятнистой (Milk Thistle Dry Extract) соответственно. Приготовление медовой сыты осуществляли на дистиллированной воде. Каждую группу пчелиных семей оценивали по комплексу биологических и хозяйственно-полезных признаков в течение всего периода исследования.*

Результаты и обсуждение. *Определяли концентрации активных веществ в полученных партиях мёда. В качестве активного вещества для овса была выбрана феруловая кислота, для расторопши – сумма силибининов А и В. Массовую концентрацию феруловой кислоты измеряли в соответствии с Европейским стандартом EN15662:2008 с помощью газовой хроматографии-тандемной масс-спектрометрии с последующей экстракцией/разделением и очисткой ацетонитрилом методом дисперсионной твердофазной экстракции (Quechers). Пределы количественного определения феруловой кислоты и суммы силибининов А и В составляли 0,02 мг/кг. В меде сохранялось высокое (83,3±0,4 мг/кг) содержание флавоноидов: силибина А и В, изосилибина А и В, силикристина А и других флавоноидов. Феруловая кислота определялась в более низких (1,13±0,02 мг/кг) концентрациях.*

Выводы. *Полученные результаты свидетельствуют о том, что для создания обогащенного натурального меда с гепатопротекторными свойствами перспективным является экстракт расторопши пятнистой (Silibum marianum), поскольку биологически активные компоненты этого растения имеют характеристики, позволяющие получить конечный продукт с высоким содержанием действующих веществ.*

Ключевые слова: *пчелиный мед, биологически активные вещества, гепатопротекторы.*

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