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Low abundant bovine colostrum proteins in combination with amaranth oil reveal topical analgesic activity

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Abstract

Colostrum is an arsenal of proteins and peptides required at the earliest stage of a newborn development. Proteins involved in all biological processes of an infant development have been found in its composition. It is expected as cost-effective source of biologically active proteins and peptides. In this work, we separated water-alcohol-soluble low abundant proteins from bovine colostrum in preparative amounts that were further utilized in combination with amaranth oil for topical cream composition. The ratio of the obtained proteins' fraction made a thousandth of the colostrum dry mass. The partial sequences of 37 identified proteins were established by mass-spectrometer and using BLAST search in NCBI database. In our previous work, we established the chemical composition of amaranth seed oil with ~6 % squalene by mass. The physical mixtures of these natural resources were fabricated into cream using hyaluronic acid as moisturizing agent and their analgesic activities were established. The optimal ratio of proteins and oil was determined in terms of their effects as analgesic means by experiments carried out on mice. Several proteins could possibly be responsible for the revealed biological efficacy. Among them, G-protein coupled receptor and synaptotagmin were previously linked with analgesic activity. Establishing an optimum ratio of ingredients proved also the contribution of higher quantity of amaranth oil, a rich source of squalene and unsaturated fatty acids.

Introduction

Colostrum is a rich source of proteins, fats, carbohydrates, and other ingredients. As a product secreted after birth, it has a higher content of proteins than milk. Immunoglobulins, lactoferrin, lysozyme, and different growth factors compile the main content of colostrum (Silva *et al*. 2019). Albumin, heavy chains of immunoglobulins M and G, antitestosterone antibody, lactotransferrin, and polymeric immunoglobulin receptors were demonstrated as the main content of the colostrum whey (Zhang *et al*. 2011). By two-dimensional analysis, IGHG1 (immunoglobulin heavy constant gamma 1), C1 with uncharacterized function and VI1a protein were found as low abundant proteins in bovine colostrum whey (Golinelli *et al*. 2011). Recent research analysis revealed that exosomes of colostrum are enriched with proteins regulating the immune response and growth. The biological roles

of the proteins from exosomes were found to belong to immune regulation, growth and infant development (Samuel *et al*. 2017). A number of colostrum-based products have reached the market as cosmetics, bioadditives, and other means. In combination with other ingredients, colostrum has been patented as a pharmaceutical and cosmetic composition (Gobbi 2007). In another document, different ratios of horse colostrum and milk were earlier patented with other ingredients as pain-relieving agents against sun burns and fire burns (Gobbi 1993).

In-depth analysis of bovine colostrum with 2D-LC-MS/MS analysis identified 403 proteins in the nonfractionated colostrum (Nissen *et al*. 2012). In the fluid phase, 69 additional proteins were found. Among all identified proteins, 38 % of them belonged to cellular processes, biological regulations, response to stimulus, and regulations of biological processes. Studying the proteomic changes in bovine colostrum and milk revealed that the highest number of proteins belonged to immunity, transport and cell processes both in colostrum and milk (Zhang *et al*. 2015). Number of proteins in colostrum involved in cellular process, response to stimulus, metabolic processes, and regulation of biological processes were found as the highest both in bovine colostrum and milk; but all of them made lower number in the composition of milk (Nissen *et al*. 2017).

A study on the effects of heat treatment on bovine colostrum protein profile demonstrated that low abundant proteins, involved in cellular processes and immune response, are affected by heat (Tacoma *et al*. 2017). Besides, higher number of low abundant proteins was found to belong to metabolic processes, biological regulation, multicellular organismal processes and response to stimulus. Recent advances in research allowing find low and very low abundant proteins from colostrum and milk. Using so-called combinatorial peptide library technology (CPLL) treatments made it possible to detect the signal of proteins masked by major proteins that constitute >95 % of total bovine colostrum protein mass (Altomarea *et al*. 2016). Another tandem with LC-MS/MS in this approach used isobaric tag for relative and absolute quantification (iTRAQ) (Yang *et al*. 2017). Thus, the method allowed to compare differentially expressed proteins in whey of human and bovine colostrum and milk. In this work, we identified the low-abundant proteins in bovine colostrum after enrichment and by eluting with water-alcohol solution. The enrichment process was carried by trapping the proteins in polytetrafluoroethylene sorbent that was further eluted with water-alcohol solution. The idea came from the isolation of the ones of more hydrophobic nature. The proteins of hydrophobic interaction could have higher membrane positioning and thus cross the membrane more easily (Lomize *et al*. 2017). Sequences of the isolated proteins and peptides were established by mass-spectrometric analysis. Further analgesic activity of the fraction was studied in combination with amaranth oil and hyaluronic acid; optimum mass ratio of the ingredients were established. In our previous work, we demonstrated high hyperlipidemic efficiency of amaranth oil that could be resulted from high concentration of unsaturated fatty acids and squalene (Bozorov *et al*. 2018). Oil samples obtained from local amaranth varieties (Olimjonov *et al*. 2020) can contribute to economic competitiveness. Skin surface lipids contain up to 13 % squalene (Passi *et al*. 2002) that can contribute to higher interaction with squalene that ease the penetration of active compounds.

Experimental

Separation of proteins from bovine colostrum

Bovine colostrum samples obtained from cattle (*Bos taurus*) on the 1st, 2nd, and 3rd days of the birth were gathered and the mixture was further used for analysis. To precipitate high-molecular-weight proteins, 60 mL of 54 % (w/v) perchloric acid ($HClO_4$) solution was added to 945 mL of colostrum and kept in the refrigerator for 24 h capped. The precipitate was centrifuged and supernatant was collected, the volume was 600 mL. The excess amount of $HClO_4$ in the obtained supernatant was neutralized with potassium alkali until pH reached 7.5 and kept in refrigerator for 10 h. The resulted mixture was centrifuged to remove excess of $KClO_4$. Supernatant volume compiled was 550 mL. It was left in the refrigerator overnight and used for column chromatography.

Column chromatography

Hydrophobic chromatography with a sorbent polytetrafluoroethylene (polychrome-1) filled in a 400 mL column connected to LKB Bromma pump chromatography system was used to separate the fractions in a 550 mL mixture and desalinate the solution. For that, the obtained solution was first passed through a column. The column was washed out with water; the elution of the trapped proteins from the sorbent was carried out using an aqueous solution of 60 % ethyl alcohol (120 mL solution was used once with a flow rate 60 $mL.h^{-1}$). The eluents were collected and the excess alcohol was removed using a rotory evaporator (RE 2000A). Further, the solutions were gathered and lyophilized (The Virtis Automatic Freeze-Dryer, 10-010).

Mass spectroscopy analysis of low-abundant fraction of proteins

For mass-spectral analysis, 2 – 5 µL of digested protein samples were subjected to CHIP (150 µm × 43 mm) Zorbax SB C18 column in Agilent Technologies 1260 Cap Pump with a flow rate of 4 µL per min, elution rate made 0.6 µL per min. B solution was managed as following: 5 %, 3 min; 80 %, 25 – 30 min. The solution was degassed in Agilent Technologies 1260 µ-degasser. Mass detection was performed in CHIP-Q-TOF LC-MS Agilent Technologies 6520B Series mass spectrometer. Mass data was recorded in positive ionization mode (ionization source – CHIP interface, gas flow rate – 4 $L.min^{-1}$ at 350 ^{o}C, skimmer current power – 65 V, MS/MS – 50 – 2400 m/z, mobile phase: A – 0.1 % formic acid, B – acetonitrile + 0.1 % formic acid) and partial sequences were identified with Spectrum Mill database. For functional annotations, obtained fragments were further searched by BLAST analysis in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi): taxid: 9903 (belonging to *Bos taurus*) identity was chosen as organism. For functional annotations and reliability, NCBI search results of the identified peptides with percentage identity higher than 85 % were checked with higher identities in the proteome of other mammals.

Establishment of amaranth oil composition

The chemical composition of amaranth oil was established as described in our previous work (Bozorov *et al*. 2019). The seeds of Helios variety were ground in a porcelain mortar using quartz sand. The oil was extracted from the resulting powder with acetone by stirring for 30 min. The squalene content in the oil sample was 5.7 %.

Fabrication of the cream

The basic components of the cream included 5 g of T-1 emulsifier and 0.2 g of sorbic acid as antibacterial means in a total mass of 100 g cream. In order to establish optimum ratio of putative biologically active ingredients, we suggested 10 compositions using various ratios of bovine colostrum proteins, amaranth oil (Lacatusu *et al*. 2018) and hyaluronic acid (Juncan *et al.* 2021) (Table 1). The composition variants were added to the cream basis respectively, and filled up with water to a final mass of 100 g and further emulsified using a mixer. The obtained cream was of a dairy colour that is stable for more than a year when stored at 4 ^{o}C.

Analgesic activity

The efficiencies of the compositions were determined by the analgesic activity during contact-thermal irritation in the hot plate test following a commonly used method as described by Deuis *et al*. (2017). The thermal irritation model reveals a central component in the mechanism of the analgesic action of a substance. The analgesic activity was established by the change in the threshold of pain sensitivity in animals during contact-thermal stimulation under the influence of the cream. The initial reaction of the white outbred mice (the time to start licking the hind limbs) was determined when they were placed on a standard plate with a temperature of 55 °C. Ethical norms of evaluating pain behaviors, described by Deuis *et al.* (2017), were followed when analgesic activity of cream samples was determined. Then the rate of the same reaction was taken into account after certain time of applying the cream compositions to the animals on the hind and fore paws (15, 30 and

45 min before the study), and its measurements were expressed as a percentage relative to the initial value.

Animal experiments were carried out according to requirements used for scientific purposes (European Directive 2010/63/EU).

Table 1. Establishment of optimum ratio of ingredients to fabricate analgesic means.

Compositions	Amaranth oil [g]	Bovine colostrum proteins [mg]	Hyaluronic acid [g]
1	5	0	0
2	0	10	0
3	0	0	2
4	6	8	1
5	**5**	**10**	**2**
6	4	7	3
7	3	10	1
8	2	10	2
9	**5**	**8**	**3**
10	1	9	4

Samples that revealed highest analgesic activity are indicated in bold.

General and acute toxicity

The study of the acute toxicity and general action of the investigated cream was carried out on white outbred mice weighing 20 ± 1.5 g. Five mice were included in each group. The tested substance was orally administered at doses up to 5,000 $mg.kg^{-1}$; cream samples were dermally used at doses up to 2500 $mg.kg^{-1}$. The observation was carried out 3 times a day for 2 – 3 days in a laboratory condition and further for 10 days in a vivarium.

Results

Proteins were isolated from bovine colostrum as above described. Obtained fraction of the water-alcohol-soluble fraction of low-abundant proteins made by dry mass approximately a thousandth of the colostrum dry mass. Proteins, highly abundant in colostrum such as casein, immunoglobulins, and lactoglobulins, are mainly transport proteins and/or ones involved in immune processes (Nissen *et al.* 2017). Our hypothesis originated from isolating the ones that interact with hydrophobic sorbent polytetrafluoroethylene – polychrome (despite fluorine is electronegative, the polymerized sorbent is of hydrophobic nature). In total, ~1.4 g of protein fraction of low abundant ones were obtained and subjected to sequence analysis. In Table 2, were provided the sequence of the identified proteins, NCBI annotation as well as accession number, query cover, and percentage identity. Query cover only higher than 90 % were selected.

Our results by analgesic activity showed the initial reaction of mice in the control group occurred after 9.3 ± 0.7 seconds. Some of the cream samples that did not contain either amaranth oil alone or amaranth oil and bovine colostrum fractions (samples 2 and 3) revealed lowest effect that could be attributed to their presence. They enhanced analgesic activity in 45 min by 175.3 and 207.5 %, respectively. The effect of hyaluronic acid in pain relief can be noticed in samples 6 and 10 that contain 3 and 4 g of hyaluronic acid, respectively. Samples 7, 8, and 10 turned out to be moderately active despite the highest quantity of protein fraction included. When these samples were applied, analgesic activity in 45 min increased by 296.8 % (36.9 ± 3.6 s), 267.7 % (34.2 ± 3.2 s) and 284.9 % (35.8 ± 4.3 s), respectively, compared to the initial latent period. The most active ones were samples 5 and 9, the analgesic activity of which enhanced almost 3.84 % (45.0 ± 3.8 s) and 3.6 times (42.6 ± 4.1 s), respectively (Table 3). The observed time lag in pain relief can be concluded with highest quantity of amaranth oil, bovine colostrum protein fraction, and hyaluronic acid. Based on the obtained results of the studied samples, the most optimal choice in terms of the ratio of components was sample 5 that contained 5 g of amaranth oil, 10 mg of bovine colostrum protein fraction and 2 g of hyaluronic acid (Table 1). In relation to the initial indicators, this composition increased the analgesic activity by 131.2 %, 230.1 % and 383.9 % after 15, 30, 45 min of topical application, respectively (Table 3).

Based on the data obtained, we developed cosmetic cream that was named "Zumara". The cream consists of T1 emulsifier, hyaluronic acid, bovine colostrum proteins, amaranth oil, ethyl alcohol, sorbic acid and purified water.

The highest biological activity among samples belonged to samples 5 and 9 that could be attributed to higher content of bovine colostrum protein fraction, amaranth oil that contains

Table 2. Identified sequences of low-abundant proteins in bovine colostrum and their accession numbers, query covers and percentage identities in NCBI database.

	Sequences identified by MASS analysis	Accession number	Query cover	Per. Identity	Proteins name/NCBI annotation
1.	RLVRFYPRADRVMSVCLRVELYGCLWKAGLXSTQTG	XP_019841431.1	100 %	100 %	Discoidin domain receptor family, member 1
2.	VLLGWGSLLIMLKNEGFYSSMCPAENSTNSTQDEQRR	XP_027418094.1	91 %	85.29 %	Neutral amino acids transporter small subunit 3
3.	NISYGGCMAQMFFLSWSVAAEVLLFTAMAYDRYVA	XP_027385372.1	100 %	77.14 %	Olfactory receptor 13A1-like
4.	AIYHCREQPASDWPEAVCLLIGRQDLSKQA	XP_005891645.1	100 %	100 %	Anaphase-promoting complex subunit 1 isoform X1
5.	VLSVELPGLLAQLARSFALLLPVYALGYLGLSFSWVLLALALLAW	XP_027394252.1	97 %	88.64 %	Extended synaptotagmin-2
6.	SIGGTPIRGALQI	XP_027405056.1	100 %	100 %	Tyrosine phosphatase, receptor type, δ isoform 1 precursor
7.	SIPHLLLPLA	NP_001192519.1	90 %	87.50 %	Integrin alpha-10
8.	QLLSQWSAHAMEVYSWKLVHPTD	ELR61599.1	100 %	95.95 %	Cytoplasmic FMR-1 interacting protein
9.	VLVVASFDELQRRASEARGKIIVYNQP	ELR52717.1	100 %	77.78 %	Plasma glutamate carboxypeptidase
10.	QLEKSWSQYLACPEDISGLLHS	XP_005892933.1	100 %	95.45 %	Huntingtin interacting protein 1
11.	SSGPYLTSVMGKAAGEREF	ABG67017.1	94 %	77.78 %	Transcobalamin II precursor
12.	GLLCVCWSPDGKYIVTGGEDDLVTV	XP_024837551.1	100 %	96 %	WD repeat containing protein 20
13.	PKETPFLVSFIIAQIVTTCSGILSYPF	AIC38345.1	100 %	92.59 %	ADP/ATP translocase 4
14.	GEPGKTGPPGLPGAGGSGAISTATYTTVPRVAFYAGLKNPH	ELR50847.1	100 %	100 %	CRF, C1q-related factor
15.	NPVACELKAFV	DAA15478.1	90 %	80 %	DNA-directed RNA polymerase III
16.	SFQTGSWLLDHCQESYCEPTVCQ	NP_001074209.1	100 %	86.96 %	Keratin associated protein 11-1
17.	LYRWQGIFVPFEDLSIE	XP_005907599.1	100 %	100 %	Very large G protein-coupled receptor
18.	SLPPPPLQP	XP_027384342.1	100 %	100 %	The peroxisome proliferator-activated receptor γ coactivator-1

Table 2. Identified sequences of low-abundant proteins in bovine colostrum and their accession numbers, query covers and percentage identities in NCBI database – continued.

Sequences identified by MASS analysis	Accession number	Query cover	Per. Identity	Proteins name/NCBI annotation
19. MLENYSNLVFLGLAVSKPQLVTFLEQR	ELR60893.1	96 %	80.77%	Zink finger protein ZNF720
20. IQVTDDDINEIIQIDGTGDNSSAEEG	XP_027411025.1	100 %	72 %	Transcription factor IIA α/β
21. TSSIHSKEIFHSLT	XP_027422939.1	100 %	92.7%	DNA polymerase epsilon (ε) catalytic subunit A
22. IQGAYYPRRGSSEIAFHTIPLIQR	XP_027411356.1	96 %	86.4 %	Retinol saturase
23. EFRRRDQFPLTRGRAIQECRSPVPPPA	XP_027369964.1	96 %	95.8 %	Striated Muscle Preferentially Expressed Protein Kinase (SPEG)
24. QGVEYAKAVPLGTPIQS	NP_991380.2	100 %	94.45 %	γ-Protocadherin-C3 isoform
25. TILLGPMSTLVHTDQISTPETP	NP_991380.2	100 %	95.45 %	Hepatocyte nuclear factor 4 γ
26. MISQLVLEQFLLIGHCKD	XP_024835263.1	94 %	64.7 %	Zinc finger and SCAN domain containing protein 4D
27. QKLEQQLKVVPRFQPISEHQT	XP_024846738.1	100 %	95.24 %	A kinase anchor protein 9
28. DSTFANISKDDSDLIYSTYGEDPDLPSDFSIH	XP_005218672.1	100 %	100 %	Bromodomain containing protein 7
29. HIVIYSSIEEKTTLKDKNALHLFSINGKYLGSQI	XP_027420629.1	100 %	85.3 %	Neurobeachin-like protein 1
30. PRGPKGHMGDSVIGQKGER	NP_001160001.2	100 %	84.21 %	Collagen 4 alpha 3
31. VHGEPLGYGV	XP_027413406.1	100 %	80 %	Serine/Threonine-protein kinase LATS2
32. NLLDLLLNHQVLVPGCLDPFPLLSAYV	DAA13671.1	100 %	100 %	Glutathione S-transferase P
33. SIQSQSSLTITVSSTP	NP_776434.1	93 %	73.33 %	Hematopoietic progenitor cell antigen CD34
34. APNVQSRELNYD	NP_001179782.1	100 %	83.33 %	Parathyroid Hormone B1
35. GHRNTVLLTWKPPD	XP_027370093.1	100 %	100 %	Obscurin-like 1
36. SDKIHFPS	E1BPQ3.1	100 %	87.5 %	G-protein coupled receptor family C gr. 6 member A
37. SVNSEVLGATRVKRHKNLLAERWEAHIYA	XP_005209316.1	96 %	75 %	Mitotic interactor and substrate of PLK1

Table 3. Effects of the fabricated creams on the latency period and analgesic activity in the thermal nociceptive hot plate test (M ± m; n = 6).

Fabricated cream compositions	Time with the moment of cream application [min]					
	15		30		45	
	Latent period (seconds)	Analgesic activity (percentage increased by)	Latent period (seconds)	Analgesic activity (percentage increased by)	Latent period (seconds)	Analgesic activity (percentage increased by)
Control (initial)	9.3 ± 0.7		9.3 ± 0.7		9.3 ± 0.7	
1	18.2 ± 1.8	95.7	20.0 ± 2.0	115.1	22.5 ± 3.4	141.9
2	10.5 ± 2	12.9	12.2 ± 0.7	31.2	25.6 ± 2.8	175.3
3	15.3 ± 1.5	64.5	17.5 ± 1.8	88.2	28.6 ± 3.2	207.5
4	20.3 ± 1.5	118.3	25.6 ± 1.5	175.3	40.2 ± 3.5	332.3
5	**21.5 ± 1.8**	**131.2**	**30.7 ± 2.2**	**230.1**	**45.0 ± 3.8**	**383.9**
6	20.3 ± 2.0	118.3	25.7 ± 0.7	176.3	38.7 ± 2.8	316.1
7	19.6 ± 1.8	110.8	24.5 ± 2.0	163.4	36.9 ± 3.6	296.8
8	18.4 ± 1.5	97.8	23.6 ± 1.5	153.8	34.2 ± 3.2	267.7
9	**22.5 ± 1.8**	**141.9**	**31.2 ± 2.2**	**235.5**	**42.6 ± 4.1**	**358.1**
10	19.6 ± 2.0	110.8	24.6 ± 0.7	164.5	35.8 ± 4.3	284.9

$P < 0.01$ – reliability in relation to the mean value of initial control. Samples revealing highest activity and their effects, respectively, are shown in bold letters. ± bars indicate standard deviation value of a data set.

squalene up to 5.7 % in the composition and hyaluronic acid. Further analysis will prove our hypothesis.

Discussion

Previous researchers demonstrated different mechanisms of analgesic activities of peptides. Dooley *et al*. (1994) reported RFWINK peptide that do not resemble to any known opioid peptide. Reported six amino acid-long peptide was demonstrated to induce long-lasting analgesia in mice. The peptide was suggested to cross blood brain barrier. In another work, GYCAEKGIRCDDIHCCTGLKCKCNASGYNCV CRKK peptide, isolated from *Alopecosa marikovskyi* spider venom, was found to possess analgesic activity by influencing purinergic P2X3 receptors. The maximum bioactivity of the peptide, studied by thermal hypersensitivity test, was 0.5 $mg.kg^{-1}$when administered intravenously (Palikova *et al*. 2019).

ZRCCNGGCSSRWCRDHSRCC peptide (SIIIA) and similar structures are expected to be potent blocking agents of TTX-R and TTX-S sodium channels (Bulaj *et al*. 2005; Green *et al*. 2007). μ-conotoxins were established to block voltage-gated sodium channels (VGSC) and more than twenty sequences belonging to conotoxins were already identified that interact with VGSC subtypes (Green *et al*. 2014). Another mechanism of analgesic effect of peptides was shown to be connected to a linkage with clathrin heavy chain. Synthetic peptide, obtained by modifying salmon calcitonin sCT_{16-21} fragment retained the activity of a full molecule (Kotin *et al*. 2019).

Some peptides, identified in this work, were reported to be associated with analgesic mechanisms. Very large G-protein coupled receptor 1 (VLGR1) is one of these peptides included in these analgesic processes. The large ectodomain of VLGRl contains calcium exchanger repeats that possess resemblance to sodium-calcium exchanger proteins. Very large G protein-coupled receptor 1 (VLGR1), having a large ectodomain, contains multiple calcium exchanger repeats resembling regulatory domains of sodium-calcium exchanger proteins. (McMillan *et al*. 2002). G protein-coupled receptors mediate (GPCR) human sense of vision, smell, taste and pain, and are involved in

cell recognition processes. They structurally are classified as GPCR possessing short N-terminal ectodomain (50 – 80 residues) and long ectodomain (80 – 600 residues). The long N-terminus can be involved in ligand recognition; the bound ligand can possibly move to the transmembrane region to activate G protein (Vaidehi *et al.* 2002). VLGR1 is a core component for the development of ear inner cells. Mutations in VLGR1 genes were defined to cause Usher syndrome with symptoms congenital hearing loss and progressive retinitis pigmentosa (Hu *et al.* 2014).

We suggested synaptotagmin-2 (Syt-2) protein as the next one involved in analgesic processes. Syt-2 was reported to be involved in neuronal processes (Bouhours *et al*. 2017). Extended synaptotagmin-like proteins, are a family of proteins that are involved in Ca^{2+}-regulated secretion and characterized by an N-terminal transmembrane sequence and two C-terminal C2 domains (C2A and C2B) (Rizo and Rosenmund 2008). E-Syts consist of a short, nonconserved N-terminal transmembrane region and C2 domains. Their C2 domains generally act as Ca^{2+}- and phospholipid-binding domains and/or as protein–protein interaction domain (Min *et al*. 2007). The E-Syt2 C2A domain binds up to four Ca^{2+} ions, whereas the C2B domain does not bind Ca^{2+} (Xu *et al.* 2014).

Other proteins that could be involved in analgesic activity among the ones, identified in this work, are receptor protein tyrosine phosphatase (RPTP) delta isoform, corticotropin releasing factor, and γ-protocadherin C3. Since analgesic effects of these proteins were not conducted separately, our suggestions are speculative only. However, obtained fraction of water-alcohol soluble proteins, possibly of more hydrophobic nature, has been demonstrated as proper natural resources that can be obtained in preparative amounts. Further researches will clarify deeper fundamental basis of the work.

Amaranth oil is another factor contributing to the efficiency of the cream. Beneficial effects of squalene in the composition of creams and other means of cosmetic dermatology provide advantages to amaranth oil over other sources (Huang *et al.* 2009). Up to 13 % concentration among the components of the skin surface lipids (Passi *et al.* 2002) causes higher interaction of the cream composition with a high ratio of squalene that contributes to the penetration of active compounds.

The study of the general and acute toxicity of the substances and the cosmetic cream showed that they belong to practically harmless substances. LD_{50} level of the product reached more than 25,000 $mg.kg^{-1}$ with cutaneous and oral administration in mice and rats. The study of the specific toxicology of the substance and the cream showed that they do not have a local irritating effect on the skin of rats and the conjunctiva of the eyes of rabbits and do not have an allergenic effect.

The study of the chronic toxicity of the substance and the developed cream showed that the cream revealed prolonged action and did not lead to any changes in the biochemical parameters of the peripheral blood and internal organs of the experimental animals (results are not shown).

The study of specific activity showed that the cream smoothes the skin by retaining moisture resulted from hyaluronic acid (Sundaram *et al.* 2018; Lubart *et al.* 2019). Hyaluronic acid, another significant component in the composition of the product, is found in many connective tissues (Gupta *et al.* 2019; Chang *et al.* 2021). It is mostly used moisturizing agent used in cosmetology. Hyaluronic acid is responsible for the regeneration process of the skin: it helps the synthesis of collagen (Silva *et al.* 2017) and elastin (Joddar and Ramamurthi 2006), thus contributing to their correct positioning. Thanks to this, the turgor and elasticity of the skin are improved. In addition, the unique ability of hyaluronic acid to retain water provides stability to the product. In the upper layers of the skin, it works as a conductor of nutrients and facilitates their diffusion (Dovedytis *et al*. 2020).

Conclusion

In this work, we identified 37 low abundant proteins in bovine colostrum that are soluble in 60 % ethanol. The identified peptides contribute to the extension of proteomic data of bovine colostrum. The used method can serve to identify low-abundant proteins from the colostrum and milk of other mammals. The sum of these peptides was found likely to possess analgesic effects when used as topical cream. We hypothesized the observed

analgesic activity might be attributed to at least two proteins: VLGR1 and synaptotagmin-2. Amaranth oil containing high amount of squalene was concluded as efficient additive that likely contributes to biological efficiency.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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