

Bovine Lung Peptides After Trypsinolysis Reveal Anti-Exudative Activity

Research paper

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Abstract Enzymatic hydrolysates of proteins isolated from cow lungs are used as anti-inflammatory agents for the growth and regeneration of new tissues. Hydrolysates were observed to inhibit the production of free radicals in cells and have a high anti-exudative activity even in low doses. In this study, we established the anti-exudative activity of peptides of protein hydrolysates isolated from bovine lungs. The anti-exudative activity of the peptides sum was compared with acetylsalicylic acid (ASA) and prednisolone, and the dynamics of inflammation were studied by tumour formation using carrageenan and formalin. Between 1 h and 24 h after administration of the drug, the effect of the peptides sum showed a significantly better indicator than the rest of the means. In general, the anti-exudative activity of the peptides sum was 2–3 times higher than the rest of the preparations. Biochemical indicators of prednisolone and peptides sum in two doses of 10^{-6} µg/kg and 10^{-4} µg/kg were studied. As a result of the study, it was found that the peptides sum at 10^{-6} µg/kg dose enhanced alanine aminotransferase (ALT) activity by 17.1% compared to those resulting from prednisolone treatment. In C-reactive protein (CRP) and antistreptolysin O (ASO) indicators, 10^{-4} µg/kg dose showed high activity. The sum of isolated peptides reduced ALT and ASO levels. This study provides additional support for preparing anti-inflammatory means from the peptides sum isolated from bovine lungs.

Keywords anti-exudative – carrageenan – inflammation – L-arginine – peptides

INTRODUCTION

Bovine lung protein hydrolysates have several potential benefits (O'Sullivan et al., 2016). Protein hydrolysates increase blood flow to the wound site, promote new tissue growth and regeneration (Rivera-Jiménez et al., 2022), and reduce inflammation (Ndiaye et al., 2012). In addition, it was observed that the production of inflammatory cytokines was inhibited by hydrolysates (Bamdad et al., 2017). Collagen hydrolysates from animals are also important in wound healing and tissue repair (Ao and Li, 2012). Bovine lung protein hydrolysates have been shown to inhibit the cellular production of free radicals and reduce cellular oxidative stress (Yuan et al., 2021). These hydrolysates can modulate the immune system (van Dijk et al., 2016) and activate T and B cells which are essential immune system components (Kiewiet et al., 2015). Protein hydrolysates are produced by breaking down proteins into smaller peptides and amino acids using enzymes. This

can be done using several methods, including enzymatic hydrolysis, chemical hydrolysis, and microbial fermentation (Cruz-Casas et al., 2021). Enzymatic hydrolysis of proteins and peptides extracted from the lungs of cattle and sheep can produce hydrolysates capable of reducing oxidation or inflammation. Hydrolysates, obtained by alcalase, were used as anti-inflammatory agents (O'Sullivan et al., 2017). Endogenous dipeptide L-tyrosine–L-arginine (kyotorphin) has been observed to have a high antinociceptive effect, even in low doses, with no negative effect on vision, memory, recognition, and behaviour parameters (Angelova et al., 2021). There are possibilities to treat ischemic stroke, haemorrhagic stroke, and Alzheimer's disease with the help of arginine-rich neuroprotective peptides. In addition, these peptides are also used as pain relievers (Edwards et al., 2020). Until now, biologically active peptides isolated from animal lungs have been extracted mainly from hydrolysates of proteins, and natural biologically active peptides have hardly

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been studied. In this study, the protein extract isolated from cow lungs was hydrolysed using trypsin. It was found that the resulting hydrolysate peptides have high analgesic and anti-inflammatory properties.

MATERIALS AND METHODS

Animal experiments

The experiments were carried out on 6-month-old white albino Wistar male and female rats weighing 160–180 g, provided by a company 'YTT ALSU' registered in Tashkent, Uzbekistan. The animals were fed on a polysaccharide-rich diet during the treatment. They were fed on special granules made up of wheat flour, corn flour, and oil, produced by the same company. The humidity of the room was 50%–55% and the temperature was $22 \pm 2^\circ\text{C}$. The light–darkness cycle took 14–10 h. The experimental animals were treated before noon and kept in special experimental cages. The studied drugs and saline solution were given to the animals orally an hour before formalin or carrageenan administration for 7 days in a volume of 0.1 mL. The behaviour and appearance of treated animals did not differ from control animals. No differences were observed in the weight of the animal as well.

Chemicals and reagents

Sephadex G-25 and CM-Sephadex C-25 were purchased from Pharmacia (Sweden). Sodium phosphate, ammonium sulphate, acetonitrile (I3ACN; $\geq 98\%$), formic acid 99%, ammonium phosphate, disodium hydrogen orthophosphate, and potassium dihydrogen orthophosphate were purchased from Sigma-Chemical (USA). All other chemicals or solvents were of analytical grade.

Peptide extraction

The lungs of the freshly slaughtered bovine (1.5 kg) were ground in a grinder. The ground mass was then boiled in 0.9% NaCl solution at 80°C for 20–25 min, cooled, and filtered through a hard grey cloth. Total proteins–peptides were precipitated by adding acetone to the supernatant in a ratio of 1:5. The obtained substance was extracted in 0.01 M sodium phosphate buffer at a ratio of 1:10, and high molecular proteins were precipitated by adding 67 mL of a saturated solution of ammonium sulphate to it. The pH of the remaining supernatant was reduced to 4 with 10% acetic acid, and peptides were precipitated by adding 45 g of ammonium sulphate powder to it. The study was continued with gel filtration.

Gel filtration

To separate the obtained proteins according to the difference in molecular mass, they were dissolved in 0.05 M

ammonium bicarbonate buffer at a ratio of 1:10, loaded onto a Sephadex G-25 column, and eluted in this buffer at a flow rate of 1 mL/min. We used a UV detector at a wavelength of 280 nm. The eluate was dried in a lyophilic freeze-drier. The separation of the peptides was continued by ion exchange chromatography.

Ion exchange chromatography

An amount of 1.5 g of the biologically active fraction separated from the gel filtration was dissolved in 20 mL of 0.02 M ammonium acetate buffer at pH 5 and analysed by ion exchange chromatography using the CM-Sephadex C-25 column. The flow rate is 0.5 mL/min. Solutions: A: 0.02 M ammonium acetate buffer, pH 5; B: 0.5 M NaCl dissolved in 0.02 M ammonium acetate buffer, pH 5. Gradient: B: 0% 10 min, B: 100% 11–80 min, B: 0% 81–90 min. The substances obtained were purified by desalting them with Sephadex G-25. The sum of separated peptides was lyophilized. The amount of peptides in the powder prepared was 30 mg.

HPLC analysis

An amount of 50 μg of the isolated peptide is added to 1 mL of distilled water and passed through a $0.45 \mu\text{m}$ filter. Then, 50 μL of the filtrate was analysed in a high-pressure chromatograph with a Zorbax XDB C₁₈ $4.6 \times 250 \text{ mm}$ column. The flow rate was 1 mL/min. Solutions: A: 0.1% TFA, B: acetonitrile. Acetonitrile gradient: 0%–40% 15 min. Absorbance 280 nm.

Process of trypsinolysis

Peptide bond breakage was carried out by enzymatic cleavage using trypsin. In this process, 1 mg of peptide was dissolved in 200 μL of 50 mmol/L ammonium bicarbonate solution. Before performing the reaction, the peptide solution was heated at 60°C for 50–60 min. Then, 30–60 μL of initial buffer and trypsin solution in 1 mmol/L HCl were added to the peptide of final ratio 1:50 (by mass). The reaction was incubated at 37°C for 18 h. The reaction was terminated by reducing the pH to 4 by adding formic acid.

Identification of peptides using LC-MS

The molecular mass of peptides with a column retention time of 12.8 min was determined by Agilent Technologies 6530B Chip-HPLC-Q-TOF mass spectrometer.

Blood sample collection and analysis

Blood samples were taken from rat veins on the first and seventh days of the experiment for biochemical and haematological analyses. Blood sera were collected after centrifugation (10 min at 3000 rpm) for biochemical parameters.

Cotton pellet implantation-induced granuloma formation

Experiments were conducted on white rats weighing 160–180 g. Rats were anesthetized with diethyl ether, the abdominal wool was carefully trimmed, and a 10 mg sterile cotton pellet was inserted under the skin and subcutaneous tissue through a skin incision with scissors under aseptic conditions. On the eighth day of the experiment, the implanted cotton pellet with granulation tissue was taken, weighed, and then dried to constant weight at 55°C–60°C. The parameters were determined based on the differences between initial and implanted cotton weight (Kumar et al., 2016).

Blood biochemical parameters

The Cypess protocol was used to determine the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and to quantify soluble proteins, glucose, and urea.

Exudative inflammation

An acute inflammatory reaction (edema) was reproduced by subplantar administration of phlogogens. The severity of the inflammatory reaction was assessed 1, 2, 3, 4, 5, and 24 h after the induction of inflammation, as well as on days 1–7 (in the formaldehyde edema model) by changes in paw volume (oncometrically). The swelling was expressed as a percentage relative to the initial volume of the paws before the introduction of phlogogens. The activity of the studied drugs was determined by their ability to reduce the development of edema compared with the control and was expressed as a percentage, which reflected the degree of suppression of the development of edema by a given substance in relation to the control, where the amount of edema was taken as 100%. Anti-exudative activity (A, %) was calculated using the following Eq. (1):

$$A = 100 - (V_1 - V_2) \times 100 / (V_3 - V_4) \quad (1)$$

where $(V_1 - V_2)$ is the increase in paw volume relative to the initial one in drug-administered animals, and $(V_3 - V_4)$ is the increase in the paw volume relative to the initial one in control animals.

Model of carrageenan edema

The experiments were carried out on 36 outbred rats of both sexes weighing 160–180 g, which were divided into groups of six animals in each group. Inflammation was caused by subplantar injection of 0.1 mL of a 1% carrageenan solution into the hind paws of rats. The drugs were orally administered to the animals in a volume of 0.1 mL: peptides sum at a dose

of 10–4 µg/kg, acetylsalicylic acid (ASA) at a dose of 100 mg/kg, and prednisolone at a dose of 7 mg/kg. Control animals received 0.1 mL saline solution.

Model of formalin edema

The experiments were carried out on 24 outbred rats of both sexes weighing 160–180 g, which were divided into groups of six animals in each group. Formalin inflammation was induced by subplantar injection of 0.1 mL of a 2% formalin solution into the hind paw of rats. The studied drugs were administered to the animals orally an hour before formalin administration for 7 days in a volume of 0.1 mL: peptides sum at a dose of 10⁻⁴ µg/kg, ASA at a dose of 100 mg/kg, and prednisolone at a dose of 7 mg/kg. Control animals received 0.1 mL saline solution.

Statistical analysis

Statistical analysis was performed in GraphPad Prism using the Tukey's test.

RESULTS AND DISCUSSION

Extraction and separation

Cow lungs were crushed in a meat grinder and extracted in 0.9% NaCl solution at 80°C for 20–25 min. At this temperature, bacteria like mycobacteria existing in lung tissues die (Doig et al., 2002). The isolation and purification of peptides sum from lung tissues passed several steps. At the final stage, their existence was proven by HPLC analysis, which was further researched using mass spectrometry.

Mass spectrum results

The sum of separated peptides was subjected to trypsinolysis and analysed in a mass spectrometer. As a result of the interpretation of the received mass spectrum data using the *Spectrum Mill* and *Mass Cot* databases, the fragmentary sequences of the peptides sum were determined. As a result, 10 peptide fragments were detected with a range of 932–2915 Da (see Table 1).

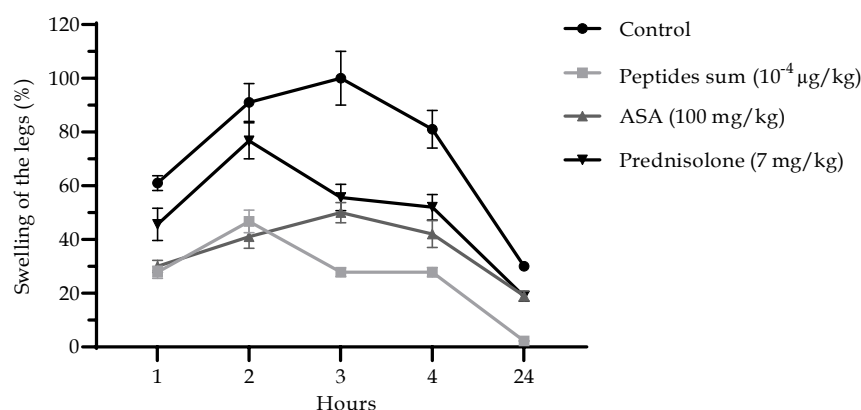
Study of the preventive effect peptides sum on a model carrageenan edema

The anti-exudative activity of the studied drugs was calculated using Eq. (1), and the results are presented in Fig. 1.

The peptides sum resulted in 73% activity. ASA and prednisolone led to 50% and 45% activity in relation to control. The highest effect of control was observed in 3 h. The ASA treatment revealed similar dynamics. But, compared with the control, ASA had twice the effect for the first 4 h. Prednisolone and the peptides sum showed similar dynamics;

Table 1. Molecular masses of peptides isolated from the substance peptides sum.

Sequence	MM	Accession	Protein name
RVGAGAPVYLAADVLEYLTAEILELAGNAARD	2915.588	28195394	Histone cluster 2, H2ab
KFFSAVSTVLTSKY	1286.7	122405	Hemoglobin alpha chain
RAGLQFPVGRI	944.532	4504255	H2A histone family, member Z
RLLVVPWTQRF	1274.726	4504351	Delta globin
KVNVDEVGGEALGRL	1314.665	4504349	Beta globin
KSAVTALWGKV	932.52	4504349	Beta globin
KSLVSGLWGKV	946.536	57113367	Similar to beta globin
KVFLENVIRD	989.578	817960	Histone H4 (55AA)
RLLLPGEAKH	953.604	1568557	Histone H2B
RISGLIYEETRG	1180.621	4504301	Histone cluster 1, H4a

Figure 1. Preventive effect of the peptides sum on the dynamics of inflammation in rats bearing carrageenan edema model (increase in the volume of the limbs, % relative to the initial one, $n = 6$).

however, it had a twofold greater impact than prednisolone. The peptides sum generally showed 2–3 times greater efficiency than the control. Three hours after the drug administration, the peptides sum effect was significantly better than ASA and prednisolone. The anti-exudative effect of the peptides sum returned to maximum efficiency within 24 h. These results prove the high anti-exudative effect of peptides sum.

The effects of prednisolone administration at a dose of 7 mg/kg were observed after 3 h, which was 1.8 times smaller volume than that of control. Thus, the peptides sum had high anti-exudative activity, exceeding the activity of ASA and prednisolone (Fig. 2).

Therapeutic effects of the peptides sum on a model of acute carrageenan inflammation

The peptides were administered orally once 2 h after the induction of inflammation to study the therapeutic effect of

the peptides sum on acute exudative inflammation caused by carrageenan. The therapeutic effect was studied over time 1, 2, 3, 4, and 24 h after administration of the peptides sum. Fig. 3 shows that peptides sum decreased the paw swelling by 42% in 1 h after administration compared with the control group of animals. After 2 h, it decreased by 30% or 2.9 times and in 3–4 h, it decreased by 22% or 2.9 times.

After 24 h, there was no swelling in the experimental group, but it was 39% in the control group. Thus, the peptides sum was effective in both preventive and therapeutic applications. L-Arginine is dose-dependent in the treatment of carrageenan-induced hyperalgesia. The antinociceptive effect of L-arginine is used based on the opioidergic mechanisms, not with carrageenan (Kawabata et al., 1992a). Studies with kyotorphin (L-tyrosyl-L-arginine) have been shown to inhibit carrageenan-induced hyperalgesia. Based on the results of these studies, it was determined that the antinociceptive effect of kyotorphin is 10-fold greater than that of L-arginine (Kawabata et al., 1992b). Electrostatic attraction between

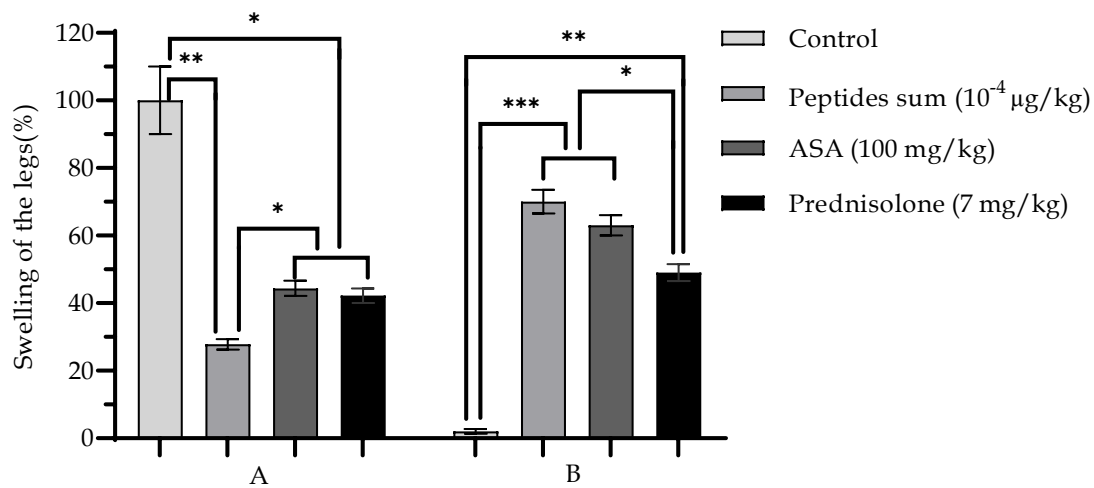


Figure 2. Effect of the peptides sum, ASA, and prednisolone on the dynamics of inflammation in rats on the model of exudative inflammation induced by carrageenan (increase in the volume of the limbs, % relative to the initial one, $n = 6$). (a) Increase in paw volume 3 h after induction, %. (b) Anti-exudative activity, %. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.005$. ASA, acetylsalicylic acid.

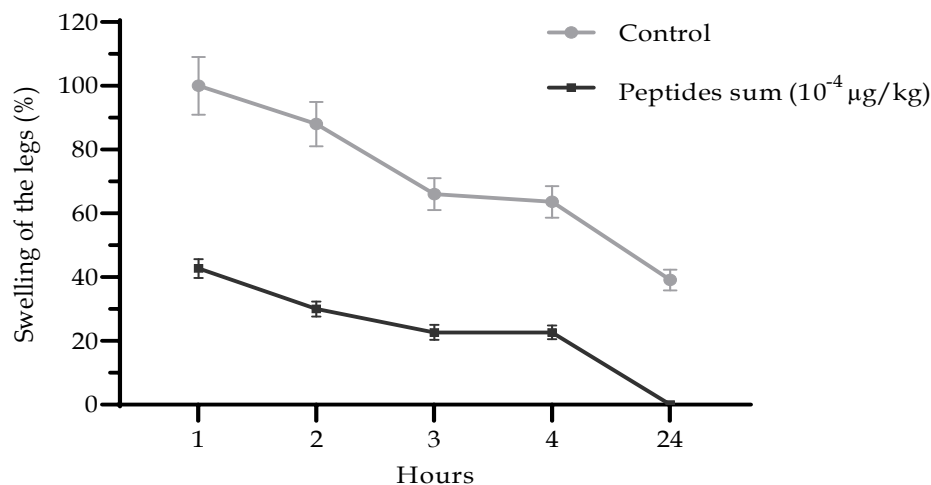


Figure 3. The therapeutic effect of the peptides sum on the dynamics of inflammation in rats bearing exudative inflammation induced by carrageenan (increase in the volume of the limbs, % relative to the initial one, $n = 6$).

carrageenan and amino acids such as arginine, lysine, and glutamine increases the mobility of water bound to carrageenan. These results show that the strength of the gel structure between carrageenan and water decreases under the influence of these amino acid groups (Dong et al., 2019). The role of nitric oxide (NO) is important in the development of hyperalgesia and swelling. NO is produced by the enzyme nitric oxide synthase (NOS). It is one of the main indicators of carrageenan-induced inflammation. L-arginine and its derivatives reduce its effect. NOSs are thought to contribute to tissue damage, inflammation-induced edema, and hyperalgesia by producing NO (Omote et al., 2001). It was determined that methyl-L-arginine acetate salt is one of the

inhibitors of NOS. Inhibition of this enzyme increases the antinociceptive effect (Ikarashi et al., 1998).

The antinociceptive and anti-inflammatory effects of isoflavone glabridin were investigated using various tests targeting L-arginine–NO interactions and Ca^{2+} and K^{+} channels. As a result, NO production decreased, Ca^{2+} and K^{+} channels were activated (Parlar et al., 2020). NO is a small cell-diffusible signalling molecule formed from arginine by NOS and from nitrate NO_3^- and nitrite NO_2^- ions. L-arginine and all nitrogen oxides are effective in diseases such as myocardial infarction, stroke, systemic and pulmonary hypertension, and stomach ulcers (Lundberg et al., 2008). During inflammation, NOS can produce high levels of NO. High-level production of

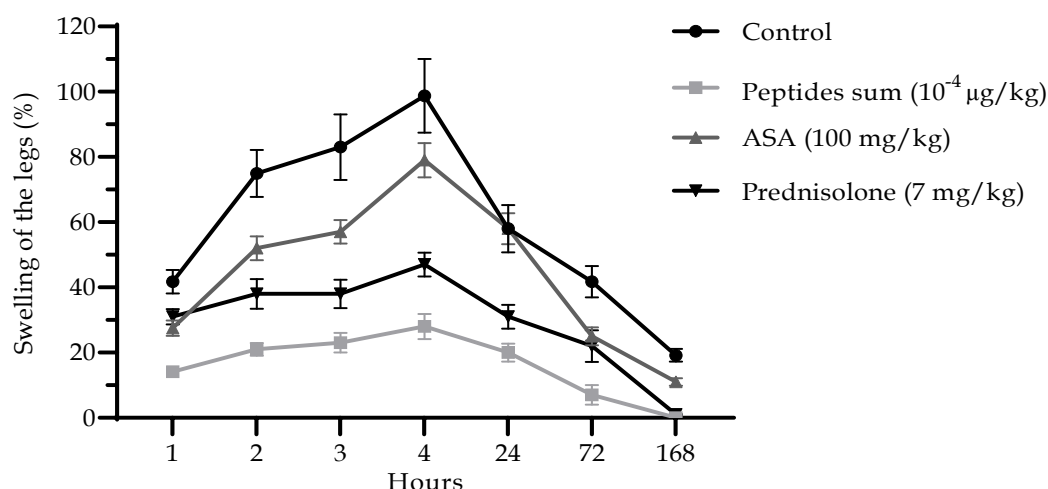


Figure 4. The effect of the sum peptides on the dynamics of inflammation in rats using a model of exudative inflammation induced by formalin (increase in the volume of the limbs, % relative to the initial one, $n = 6$).

NO can cause tissue damage (Yaren et al., 2007). L-arginine significantly reduces NO and, at the same time, reduces synovial inflammation and tissue damage in streptococci (Iwata et al., 2010). We suggest that L-arginine in the content of peptides isolated in this work could result in a similar effect due to arginine and lysine in their contents.

Formalin edema model

The trigger point in the development of exudative inflammation induced by formaldehyde is linked with membrane protein destruction. Moreover, its exudative effect lasts for 6–8 days. In 4 h, swelling of the paws of rats under the influence of formaldehyde in the control reached the maximum level and gradually decreased to 19.2% in 168 h (Fig. 4).

In the group of animals that were administered peptides sum at a dose of 10^{-4} μg/kg, after 4 h, the swelling of the paw was 28% of the control. It should be noted that already on the third day, the amount of peptide practically eliminated the swelling caused by formaldehyde: the increase in paw volume was only 7%. In the group of animals that were administered ASA at a dose of 100 mg/kg, after 4 h, the swelling of the paw was 79%. In rats administered prednisolone at a dose of 7 mg/kg, swelling of the paws after 4 h was 47%.

The anti-exudative activity of the drugs was determined in Eq. (1). As can be seen from Fig. 4, the anti-exudative activity of the peptides sum after 4 h was equal to 72%, ASA 21%, and prednisolone 34%. Fig. 4 shows swelling formed with formalin, which reached the highest level in 4 h in control animals. Similar dynamics were observed in ASA compared with the control, but the size of the leg swelling under the influence of ASA decreased by two times compared with the control. Prednisolone and the peptides sum showed a similar dynamics, and the peptides sum had twofold higher efficiency than prednisolone.

In general, animal experiments show 3–4 times greater efficiency of peptides sum than the control. Four hours after the drug administration, the effect of the peptides sum was found to be significantly higher than that of ASA and prednisolone. The anti-exudative effect of the peptides sum returned to its maximum value in 168 h. These results prove a high anti-exudative effect of the peptides sum.

L-Arginine also reduces inflammation caused by formalin and acetic acid. Its mechanism of action is associated with an increase in the amount of glutathione (Kaur et al., 2023).

Several amino acids, including arginine, are effective in inhibiting the action of several intestinal bacteria during formalin-induced inflammation using vancomycin. The improvement of the vancomycin effect is associated with higher concentrations of several amino acids, such as arginine, proline, and valine, in the intestine (Payne et al., 2021). It was found that L-arginine-containing agents also have a dose-dependent effect on reducing the antinociceptive effect of formalin-induced inflammation. In addition, aminoguanidine pre-administration enhanced the antinociceptive effect (Azevedo Ade et al., 2015).

Blood biochemical parameters

Taking the anti-inflammatory effect of bovine lung hydrolysates (10) into account, in this work, we studied the anti-exudative effect of the isolated peptides sum in comparison with prednisolone and ASA drugs. Therefore, our next experiments investigated their effects on some blood biochemical parameters such as ALT, AST, bilirubin, C-reactive protein (CRP), creatinine, antistreptolysin O (ASO), glucose, and urea in rats bearing cotton granuloma model. The obtained results are presented in Table 2.

Table 2. Effects of peptides sum and prednisolone on biochemical blood parameters in experimental cotton wool granuloma.

Biochemical parameters	Control	Prednisolone (10 mg/kg)	Peptides sum (10 ⁻⁶ mg/kg)	Peptides sum (10 ⁻⁴ mg/kg)
Glucose (mmol/L)	6.15 ± 0.13	5.97 ± 0.15	4.93 ± 0.59	4.83 ± 0.13
ALT (U/L)	72.3 ± 3.93	56.7 ± 4.18*	47.0 ± 6.57**	61.3 ± 2.97*
AST (U/L)	140.7 ± 3.18	154.7 ± 11.1	147.7 ± 11.7	150.3 ± 16.2
Total protein (g/L)	70.2 ± 1.59	64.8 ± 1.91	76.0 ± 1.16	72.3 ± 0.33
Bilirubin (μmol/L)	4.2 ± 0.66	4.17 ± 0.81	4.47 ± 0.97	4.2 ± 7.0
Creatinine (μmol/L)	42.9 ± 1.91	38.7 ± 1.35	45.0 ± 2.0	48.0 ± 3.8
Urea (mmol/L)	6.8 ± 0.89	6.33 ± 0.93	6.23 ± 0.45	7.87 ± 1.43
CRP (mg/L)	30.0 ± 0.4	29.0 ± 1.5	28.0 ± 0.7	26.0 ± 0.9
ASO (mmol/mL)	23.77 ± 7.2	10.9 ± 1.64*	10.0 ± 0.06*	9.47 ± 0.47*

ALT, alanine aminotransferase; ASO, antistreptolysin O; AST, aspartate aminotransferase; CRP, C-reactive protein.

* $P \leq 0.05$.

** $P \leq 0.001$.

Cotton pellet implantation-induced granuloma formation

The cotton pellet method is widely used to study the biochemical parameters in blood. It is one of the widely used methods for studying the effectiveness of anti-inflammatory drugs. A granuloma is formed by placing a cotton pellet under the animal's skin with an implant needle, and it is determined according to the weight of its wetness in blood.

ALT

ALT is an enzyme released into the bloodstream when the liver is damaged (Ozer et al., 2008) and is measured as part of a liver function test (Washington and Van Hoosier, 2012). The most common causes of elevated ALT levels are viral hepatitis, excessive alcohol consumption, and certain medications that can damage the liver and cause elevated ALT levels (Rafter et al., 2012).

AST

AST is one of the enzymes that are detected in cases of muscle damage caused by heavy exercise and liver tissue damage (McGovern et al., 2015). AST combines with amino acids in the blood and provides purine and pyrimidine nucleotides in the liver. It is also involved in the synthesis of L-arginine and circulating nucleotides in nerve tissues and muscles (Otto-Ślusarczyk et al., 2016).

CRP

CRP is a protein produced by the liver in response to inflammation. Inflammation is the body's normal response to injury or infection, but it can also be a sign of chronic disease (Sproston and Ashworth, 2018), as CRP levels rise rapidly and then fall again after the inflammation subsides (Ridker, 2003). The level of CRP in the blood plasma in rheumatoid arthritis and cancerous tissue damage increases very quickly (Ciubotaru et al., 2005). No changes were observed in CRP following the treatments with prednisolone and peptides.

Bilirubin

The breakage of haemoglobin, an oxygen-carrying protein in the blood, forms bilirubin. Haemoglobin is found in red blood cells that are constantly broken down and recycled in the body. It is conjugated in the liver (Ngashangva et al., 2019). Haemolytic anaemia, decreased liver function, and blockage of the bile ducts can cause high levels of bilirubin in the blood (Coucke et al., 2023). At both concentrations, the peptides sum did not cause significant changes in bilirubin content (Table 2).

Creatinine

Creatinine is produced when creatine phosphate, an energy-storing molecule, is broken down in the muscles. The level of creatinine in the blood evaluates the function of the kidneys. Creatinine levels can also be affected by other factors, such as muscle mass (Park et al., 2013), diet, and certain medications.

We observed no significant changes in the content of creatinine following the treatment with peptides (Table 2).

ASO

The ASO test measures the level of antibodies in the blood. Its rise may indicate a recent or chronic streptococcal infection (Hembrom et al., 2014). This test is often used to diagnose rheumatic fever, post-streptococcal glomerulonephritis, strep throat, and scarlet fever. All treatments, including prednisolone, resulted in more than twofold lower ASO levels, explaining their positive effect.

The results showed that ALT activity was reduced by 22% in prednisolone, 35% in the 10^{-6} dose of the peptide, and 15% in the 10^{-4} dose of the peptide. ASO levels were reduced by 54.1% in prednisolone treatment, 57.9% at a dose of 10^{-6} of the peptides sum, and 60.2% at a dose of 10^{-4} of the peptides sum compared with the control. Reductions were observed in ASO levels. All other biochemical parameters were similar to those in the control.

Overall, the research suggests that bovine lung protein hydrolysates may have several potential benefits, including antioxidant, anti-inflammatory, immunomodulatory, wound healing, and tissue repair activities. However, more research is needed to determine the optimal dosage and formation of protein hydrolysates. Thus, the peptides sum at the studied dose showed higher anti-exudative activity than ASA and prednisolone in the formaldehyde edema model at the studied dose.

CONCLUSION

The peptides sum isolated from bovine lung hydrolysates were evaluated for their anti-exudative and anti-inflammatory activities. The anti-inflammatory activity of the peptides sum

was compared with ASA and prednisolone in the carrageenan-induced tumour model in rats. The peptides showed significantly higher activity than ASA and prednisolone, even at a 1000-fold lower concentration. Significant improvements were also observed in the formalin-induced exudative inflammation model. No significant changes were found in blood biochemical parameters.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

ETHICAL APPROVAL

Animal experiments were carried out according to the *International Convention on Humane Treatment of Animals* (The European Communities Council Directives of 24 November 1986-806/609/EEC).

AUTHOR CONTRIBUTIONS

Concept – N.G., J.F.Z.; Design – N.G., N.V.; Supervision – N.G., J.F.Z.; Resources – Sh.A.Kh.; Materials – N.G., N.V.; Data Collection and/or Processing – U.J.I., A.M.A., N.M., S.Sh.A.; Analysis and/or Interpretation – U.J.I., Sh.S.O., A.M.A., S.Sh.A.; Literature Search – A.M.A., A.W., U.J.I.; Writing – U.J.I.; and Critical Reviews – A.M.A., N.G., U.J.I., J.F.Z.

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