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Research Article

ANTI-OBESITY PROPERTIES OF KIDNEY BEAN (PHASEOLUS VULGARIS) HUSK PEPTIDES IN DIET-INDUCED OBESE RATS: A FUNCTIONAL AND METABOLIC STUDY

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Abstract

Obesity is a global problem that is spreading at an incredible rate. Bioactive peptides of plant origin, e.g., extracts of *Phaseolus vulgaris* beans, are currently considered as one of the possible ways to treat this disease. In this work, we shed a light on the peptides from *P. vulgaris* husks, evaluating their anti-obesity properties for the first time. By acetic acid treatment of the kidney bean husk extract, we obtained hydrolysis-derived peptides and fed them to rats with diet-induced obesity. During the experiment, we measured rats' weight, and compared the weight of main organs right after euthanasia. Biochemical blood parameters were measured using specialized biochemical analyzers. Serum and brain serotonin levels were determined spectrofluorometrically. It was determined that group which consumed kidney bean peptides had improved visceral, brown and subcutaneous adipose tissue weights. In addition, this group showed improvements in total protein, total and indirect bilirubin, creatinine, aspartate aminotransferase, alpha-amylase, glucose, alanine aminotransferase, gamma-glutamyl transferase levels, and had improved serotonin levels. We believe that the anti-obesity properties of our peptides are directly related to their hypoglycemic activties. It may also be related to the already discovered antioxidant activity, or anti-inflammatory properties that our peptides may have.

1. Introduction

Obesity and overweight are serious health complications that are rapidly spreading around the world. In 2022, 43% of people 18 y.o. or above were overweight (body mass index (BMI) >25 kg/m2), and 16% were obese (BMI >30 kg/m2), according to the World Health Organization (WHO). The situation for children is more serious: over 390 million children between the ages of 5 and 19 were overweight, with 160 million of them being obese (Obesity and overweight, 2025). The World Obesity

Federation estimates that there will be 1.53 billion obese adults in 2035. Furthermore, the rate at which obesity is spreading is independent of a nation's level of prosperity and is a natural occurrence in high-, middle-, and low-income nations (World Obesity Atlas 2024: No area of the world is unaffected by the consequences of obesity, 2024).

Obesity can be characterized as a complex disease, which can affect practically every organ system in the body. Moreover, the level of impairment of a particular body organ may vary across the population, since the complexity of this disease revolves around individual trait of each organism. The most common obesity-related health complications are chronic kidney disease, acute kidney injury, heart diseases, difficult breathing, loss of mobility, increased blood pressure, development of type 2 diabetes mellitus, fatty liver disease and many-many other (Anderson and Shashaty, 2021). As of 2022, there were 7 officially approved drugs for treatment of obesity: phentermine, phentermine-topiramate, naltrexone-bupropion, orlistat, liraglutide, semaglutide and tirzepatide, however, these medications have many negative adverse effects and limitations, for example their prescription is not recommended for pregnant or lactating women (Gudzune and Kushner, 2024). There is a popular opinion, that glucagon-like peptide receptor agonists (GLP-1 RA), such as abovementioned liraglutide, semaglutide and tirzepatide, is a very promising type of anti-obesity drugs, research of which may be the key to the ideal anti-obesity medication development (Bailey *et al*., 2023). In addition to that, anti-obesity and anti-diabetes activities were found in food-derived peptides, which may act as inhibitors or agonists of various receptors in human body. Plant-derived bioactive peptides, for example, are very promising candidates in this aspect, because plant material is cheap, widely available, and, in contrast to animal-derived peptides, it doesn't rise any ethical or religious concerns (Suryaningtyas and Je, 2023).

Among a wide variety of plants, kidney bean (*Phaseolus vulgaris*) is known from folk medicine for its antiglycemic, antidiabetic and anti-inflammatory activities (Helmstädter, 2010). Moreover, modern studies on mice and rats prove the presence of such effects in *P. vulgaris* beans and bean husk aqueous extracts (Bhide *et al*., 2022; Kyznetsova *et al*., 2015; Yurchenko *et al*., 2021). Recently, Shchypanskyi *et al.* have discovered prominent anti-oxidant activities of common bean husk-derived peptides in in vitro studies (Shchypanskyi *et al*., 2025). Our study was aimed to develop this topic further, investigating the effect of peptides from bean husk extracts on the obesity progression in rats, emphasizing the effect on body weight, possible morphometric changes of organs, alterations of main biochemical parameters in serum and shifts in serotonin levels.

2. Materials and methods

2.1. Bean Husk Extract Preparation

With a few minor modifications, *P. vulgaris* bean husk extracts were produced following the technique outlined by Kyznetsova *et al*. (Kyznetsova *et al*., 2015) The husks of kidney beans that we utilized came from a local field in Kyiv region, Ukraine. One litre of boiling distilled water and around 132 grams of dried, powdered bean husks were mixed, and this mixture was then placed in a covered container for twenty minutes. After that, we left it to cool overnight. After cooling and filtering, the mixture was centrifuged for 10 minutes at 1,000 g. A laboratory freeze-drier LyoQuest (Telstar, Barcelona, Spain) was used to lyophilize the resultant supernatant, producing around 8 grams of freeze-dried material.

2.2. Peptide Extraction and Purification

In our study we aimed to obtain non-native peptides by acidic hydrolysis of proteins derived from an extract. To obtain these peptides, 8 grams of lyophilized bean husk extract were mixed with 250 mL of 1 M acetic acid (Product code 502000, CARLO ERBA Reagents SAS, Val de Reuil, France). The mixture was hydrolyzed for 1 hour under constant agitation. Following this, the solution was heated to 100 °C, boiled for 1 hour, and then cooled to room temperature. The resulting suspension was

centrifuged at 2,800 g for 45 minutes. After that, the collected supernatant was dialyzed in order to remove impurities of non-protein nature, and then the purified fraction was lyophilized.

2.3. Animal Housing and Ethical Compliance

For the experiment we have chosen 30 non-linear albino male rats. Throughout the study, all animals were housed in the accredited vivarium of Taras Shevchenko National University of Kyiv. All procedures involving animals were conducted in compliance with ethical guidelines and regulations for the use of laboratory animals, as outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Additionally, the procedures adhered to Ukrainian legislation, specifically the law enacted on February 21, 2006 (No. 3447-IV), titled "On the Protection of Animals from Cruel Treatment."

The animals were maintained under the following conditions: temperature 20–24 °C, humidity 30–70%, natural light/dark cycle. Initially, the rats were randomly divided into three groups of 10 animals each. Each group was placed in separate cages, with five animals per cage, and provided with free access to water and standard rodent chow in pellet form.

2.4. Obesity Induction in Rats

After three days of acclimatization and initial weighing of the animals, we initiated the process of obesity induction using a high-calorie diet. The high-calorie chow was prepared by mixing the following ingredients in 2 liters of hot water to soften them: standard rodent chow pellets (60%), chicken eggs (10%), lard (10%), crushed peanuts (5%), white sugar (9%), dry milk powder (5%), and sunflower oil (1%) (Yurchenko *et al*., 2021). From this mixture, solid spheres approximately 4 cm in diameter were formed. After drying, these spheres were used as high-calorie food for the experimental groups.

The obesity modelling experiment lasted 12 weeks and was divided into two stages. In the first stage, all rats were weighed, randomly divided into three groups of 10 animals each, and housed in separate cages with five animals per cage. The control group was provided with filtered water and standard rodent chow pellets *ad libitum*. The other two groups were given filtered water and the custom high-calorie food described above, also *ad libitum*. Throughout the experiment, the rats' weights were measured once every two weeks. The second stage began at the 6th week. One of the experimental groups receiving the high-calorie diet were additionally provided with aqueous solutions of *P. vulgaris* peptides (200 mg/kg of body mass) in place of drinking water, *ad libitum*, yet the feeding regimen for the remaining animal groups remained unchanged. According to the weight of animals, weekly rate of body mass gain compared to the initial weight was calculated using the formula:

$$\text{Body mass gain (\%)} = 100 * (\text{Weight}_{\text{week n}} - \text{Weight}_{\text{week 0}}) / \text{Weight}_{\text{week 0}} \quad (1)$$

where $\text{Weight}_{\text{week n}}$ – animals' weight on corresponding number of week (n), $\text{Weight}_{\text{week 0}}$ - animals' initial weight at the beginning of experiment (week 0)

2.5. Euthanasia and Sample Collection

Euthanasia of the experimental animals was performed on 12th week after the start of the experiment. To collect an adequate volume of blood samples and avoid the potential influence of anesthetics on biochemical parameters in blood and tissues, the decapitation method of euthanasia was employed. Immediately after decapitation, blood samples (approximately 10 mL per animal) were collected for serum preparation. Subcutaneous fat, visceral fat, brown fat, spleen, and heart were isolated and weighed.

For the preparation of brain homogenates, 1 g of brain samples were mixed with 50 mM Tris-HCl buffer (pH 7.4) (Product code C4706, Sigma-Aldrich, Saint Louis, USA) containing 140 mM NaCl (Product code A57006, ThermoFisher Scientific, Waltham, USA) in a mass ratio of 1:9 (organ to buffer). The tissues were homogenized at +4 °C using hand-held homogenizers (Product code 11799, Reichelt

Chemietechnik GmbH+Co., Heideilberg, Germany). The resulting homogenate was centrifuged at 600 g for 15 minutes, and the supernatant was centrifuged again at 10,000 g for another 15 minutes. Rat serum samples were prepared by centrifuging freshly drawn blood at +4 °C and 1,300 g for 20 minutes. The obtained homogenates and serum samples were aliquoted and stored frozen at −20 °C.

2.6. Biochemical analysis

Determination of the main biochemical parameters, namely total protein (TP), glucose, total cholesterol (TC), direct, indirect and total bilirubin, urea, creatinine, alanine aminotransferase (ALT) aspartate aminotransferase (AST), α-amylase, alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) in rat serum were performed in a private laboratory using cobas c311 analyzer (Roche Diagnostics, Basel, Switzerland).

2.7. Serotonin content analysis

Serum and brain samples were thawed at 37 °C, after which 0.4 M perchloric acid (Product code 1005191001, Merck, Darmstadt, Germany) was added in a 1:5 ratio. The resulting mixture was incubated at 4 °C for 60 min. After that, the solution was centrifuged for 5 min at 800 g in a cooled rotor. After centrifugation, the supernatant was removed and the pH was adjusted to 5-6 with 2 N KOH (Product code P4494, Sigma-Aldrich, Saint Louis, USA). The samples were centrifuged again for 5 min at 800 g in a cooled rotor. The resulting supernatant was applied to a pre-equilibrated with 0.01 M Na-phosphate buffer solution (pH 6.2) (Product code 76847, Sigma-Aldrich, Saint Louis, USA) column with CM-Sepharose (Product code 17127703, Cytiva Life Sciences, Marlborough, USA). The elution was carried out at room temperature with buffer solution (0.03 M Na-phosphate buffer solution, pH 6.2) (Product code 76847, Sigma-Aldrich, Saint Louis, USA) which eluted serotonin. To 1 ml of the eluted fraction with serotonin was added 0.3 ml of 11.6 M HCl (Product code 528525 CARLO ERBA Reagents SAS, Val de Reuil, France). The measurements were performed using a spectrofluorophotometer RF-6000 (Shimadzu Europa GmbH, Duisburg, Germany) at an excitation wavelength of 295 nm and an absorption wavelength of 550 nm against a blank containing distilled water instead of the sample (Yurchenko *et al*., 2021).

2.8. Statistical analysis

Statistical analysis of experimental data was achieved using Statistics Kingdom software Statistics Kingdom, 2025). In order to access the normality of distribution of obtained data, the Kolmogorov-Smirnov Test of Normality was conducted. Significance of differences between the groups was assessed via one-way analysis of variances (ANOVA) with a Tukey's post hoc test at $p<0.05$. Data in figures and table is presented as mean ± standard error of mean (M ± m).

3. Results and discussions

Rapid and constant weight gain is a direct major indicator of overweight and obesity development. In this study, we examined weight gain rates in rats that consumed standard chow (Control), rats with diet-induced obesity (DIO) that ate high-calorie custom-made chow, and DIO group that also consumed aqueous solutions of peptides (DIO+P). As shown in Figure 1a, all groups exhibited continuous weight gain throughout the experiment, but at different rates. Between weeks 2 and 12, the control group gained weight at an average rate of 11.2 ± 0.7 % of initial body weight every two weeks, with a maximum increase of 16.2 ± 1.0 % at week 6 and a minimum increase of 8.6 ± 0.4 % at week 12. By the end of the experiment, the final body weight in this group was 81.4 ± 7.3 % higher than the baseline.

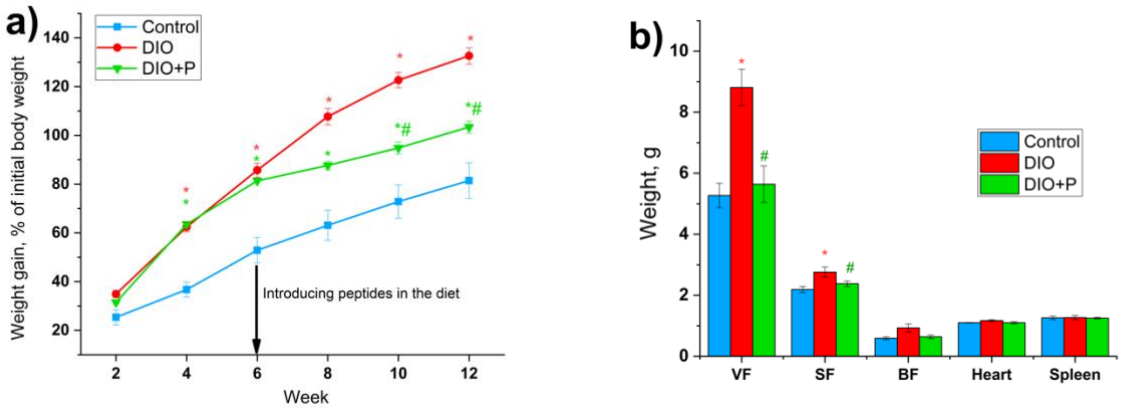

Figure 1. Weight gain rates (a) and final weights of internal organs (b). VF – visceral fat, SF – subcutaneous fat, BF- brown fat. * - p< 0.05 (compared to control group), # - p< 0.05 (compared to DIO group), n=10

In the DIO group, weight gain averaged 19.5 ± 1.0 % every two weeks, with a peak increase of 27.6 ± 0.5% at week 4 and a minimum gain of 10.0 ± 0.3 % at week 12. The final weight in this group was 132.6 ± 3.4 % above baseline. In contrast, the DIO+P group had a lower average weight gain of 14.4 ± 0.7 %, with a peak increase of 32.0 ± 0.3 % at week 4 and a minimum of 6.3 ± 1.0 % at week 8. By the conclusion of the study, the final body weight in the DIO+P group was 103.4 ± 2.5 % of the baseline values.

These findings suggest that peptide supplementation reduced the rate of weight gain, resulting in much lower total weight gain compared to DIO group. These results are supported by previous papers, that demonstrate the weight-reducing effects of *P. vulgaris* dry extracts in both animal models (Carai *et al*., 2011; Fantini *et al*., 2009) and human subjects (S. Wang *et al*., 2020). Moreover, our results prove the potential of kidney bean husk-derived peptides to be considered as bioactive anti-obesity compounds along with whole kidney bean extracts, yet the main advantage of our peptides is their cost-effectiveness, because bean husks aren't as valuable as whole beans.

Changes in the weight of internal organs during obesity progression serve as one of major indicators of its development, with adipose tissue mass being most informative. In this study, visceral fat weight in the control group was approximately 67% lower than in the DIO group (5.3 ± 0.4 g and. 8.8 ± 0.6 g, respectively) (Fig. 1b). In contrast, visceral fat weight in peptide-supplemented group was comparable to that of the control group, being 5.6 ± 0.6 g. A similar trend was observed for subcutaneous fat. In the control group, subcutaneous fat weight was 2.2 ± 0.1 g which is approximately 26% lower than in the DIO group (2.8 ± 0.2 g). In the DIO+P group subcutaneous fat weight was approximately 2.4 ± 0.1 g, respectively, which is close to the control values. Brown adipose tissue weight in the control group averaged 0.6 ± 0.1 g, which was 58% lower than in the DIO group (0.9 ± 0.1 g), whereas in the DIO+P group (0.6 ± 0.1 g), it did not differ from the control group. These findings are similar to previous researches, in which the fat-reducing effects of *P. vulgaris* extracts were proved. In a study by Wang *et al.* (S. Wang *et al*., 2020), individuals who consumed *P. vulgaris* extract for 35 days had an 8% less adipose tissue mass compared to control values. Similarly, Neil *et al.* (Neil *et al*., 2019) reported that consumption of *P. vulgaris* beans led to a 12% reduction in visceral fat and a 28% reduction in subcutaneous fat in obese C57BL/6 mice. Organ weights such as the heart and spleen are also related to obesity progression, because obesity induces structural cardiac changes, namely left ventricular enlargement (Stencel *et al*., 2023), and hypertrophy of spleen, due to its role in low-density lipoprotein clearance (He *et al*., 2022).

However, we did not observe statistically significant differences in heart or spleen weights under our experimental conditions.

As shown in Table 1, the biochemical analysis of serum revealed the most pronounced difference in ALP activity between the control and DIO groups, with the latter exhibiting a 321% increase. In the DIO+P group, ALP activity was 231% higher than in the control group, suggesting a positive effect consumption of peptides in regulation of ALP activity. ALP is one of the key liver enzymes, alterations in levels of which serve as biomarkers for liver dysfunction. Recent studies have reported an association between elevated ALP activity and obesity (Jalili *et al*., 2022). Although the precise mechanism is still unclear, it is possible that obesity may lead to excessive release of this enzyme in adipose tissue (Khan *et al*., 2015). The effect of *P. vulgaris* consumption on ALP levels was also demonstrated in a study by Forster (M. Forster, 2012), in which a diet with kidney beans significantly reduced ALP levels in obese dogs.

Table 1. Rat serum biochemical parameters

	Control	DIO	DIO+P
ALP, U/L	126.8 ± 22.5	(407.6 ± 17.3)*	(293.0 ± 9.8)*#
TC, mmol/L	2.0 ± 0.1	(3.5 ± 0.2)*	(3.0 ± 0.1)*#
Glucose, mmol/L	4.3 ± 0.1	(6.5 ± 0.2)*	(4.4 ± 0.1)#
GGT, U/L	23.0 ± 1.4	(11.8 ± 0.7)*	(19.0 ± 0.6)*#
Total protein, g/L	76.2 ± 2.0	(88.8 ± 1.2)*	(79.6 ± 1.1)#
Total bilirubin, μmol/L	10.3 ± 0.2	(8.8 ± 0.3)*	(10.2 ± 0.1)#
Direct bilirubin, μmol/L	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
Indirect bilirubin, μmol/L	9.1 ± 0.2	(7.4 ± 0.3)*	(8.5 ± 0.1)#
Creatinine, μmol/L	50.6 ± 0.9	(59.2 ± 0.9)*	(53.4 ± 1.5)#
Urea, mmol/L	6.3 ± 0.1	5.9 ± 0.3	6.3 ± 0.2
ALT, nmol/L	79.6 ± 6.5	(69.1 ± 2.6)*	(78.8 ± 1.6)#
AST, nmol/L	260.8 ± 4.9	(218.8 ± 12.8)*	(253.4 ± 2.2)#
α-amylase, U/L	636.0 ± 14.6	(758.4 ± 27.0)*	(688.6 ± 3.9)#

* - $p < 0.05$ (compared to control group), # - $p < 0.05$ (compared to DIO group), n=5

Visible differences between the control and DIO groups were also observed in serum TC and glucose concentrations, as well as GGT activity. TC is an important indicator of lipid metabolism and it is closely associated with obesity and metabolic syndrome. In the DIO group, total cholesterol levels were 77% higher than in the control group and in the DIO+P group this increase was 54% (Table 1). These findings can be supported by previous research by Nchanji and Ageyo (Nchanji & Ageyo, 2021), who reported a 19% reduction in total cholesterol levels in volunteers consuming *P. vulgaris* extract. However, clinical trials conducted by Singh *et al.* (Singh *et al*., 2024) on the commercial kidney bean extract Phaseolean® did not report significant alterations in cholesterol levels.

As shown in Table 1, glucose levels in the DIO group were 50% higher than in the control group, indicating a direct correlation between blood glucose concentration and obesity progression in our study. At the same time in the DIO+P group glucose concentration was just 3% higher – almost at the same level as in non-obese control rats. High blood glucose concentration is a major marker of carbohydrate metabolism disorders, including type 2 diabetes, which is closely related to obesity. *P. vulgaris* is widely recognized as a source of α-amylase inhibitors, which contribute not only to their hypoglycemic effects but also to their anti-obesity properties in general (Peddio *et al*., 2022). In a recent study, *P. vulgaris* extract demonstrated dose-dependent glucose-lowering effects, but, unlike our findings, glucose levels in extract-fed rats

did not return to control values (Almuaigel *et al*., 2017).

In our study, GGT activity in the DIO group was 48% lower than in the control group (Table 1). In the DIO+P group, GGT activity was reduced by 17%, compared to control values. GGT is one of the most obesity-associated liver enzymes. Elevated levels of this enzyme are often observed in overweight individuals (Jalili *et al*., 2022). A decrease in GGT activity in obese rats may be a result of dysfunctional antioxidant system, as this enzyme plays a critical role in the glutathione regeneration cycle (Bai *et al*., 2022). Similar findings were reported in a study, where four-month consumption of *P. vulgaris* extract by individuals with type 2 diabetes led to an increase in their GGT levels (Feng *et al*., 2022). However, the mechanism responsible for the reduction in GGT activity observed in obese rats still remains unclear.

We observed less pronounced changes in TP, total and indirect bilirubin, creatinine, ALT, AST, and α-amylase levels. In the DIO group, total protein levels were 16% higher than in the control group. At the same time, in the DIO+P group this increase was only 4%(Table 1). The elevation in TP levels observed in obese rats may be result of increased levels of circulating acute-phase proteins, inflammatory cytokines, and tissue-derived proteolytic products, because these molecules are usually associated with obesity-induced inflammation. The ability of *P. vulgaris* extracts to lower total protein levels was demonstrated in a rat model of induced diabetes (Khatija & Marikkar, 2022). Our findings support this evidence by confirming a similar effect in our obesity model.

Serum total bilirubin levels in the DIO group were nearly 14% lower than in the control group, while DIO+P group didn't show statistically significant differences from control values, with reductions of only 1% (Table 1). Similarly, concentration of indirect bilirubin was 18% lower in the DIO group compared to controls, while in the DIO+P group, this reduction was approximately 5% (Table 1). However, we did not observe any significant changes in direct bilirubin levels among our experimental groups. These findings are supported by previous research by Siddiq *et al*. (Siddiq *et al*., 2018), who reported no effect of kidney bean extract on total and direct bilirubin levels in healthy rabbits. Similarly, Patel *et al.* (Patel *et al*., 2024) found that anti-obesity pellets containing kidney beans had no significant effect on total bilirubin levels in Sprague Dawley rats.

Obesity is usually associated with impaired kidney function, which can be assessed through various biomarkers, including serum levels of creatinine and urea, both of which are known as indicators of renal dysregulation (Abdelfattah *et al*., 2024). As shown in Table 1, creatinine concentrations in the DIO group were 17% higher compared to the control group. In the DIO+P group, creatinine level was similar to the control group, showing 6% increase. In contrast, urea levels of DIO and DIO+P groups had no significant difference, compared to the control group (Table 1). These findings are consistent with the results of Wang *et al*. (S. Wang *et al*., 2020), who observed no significant changes in creatinine or urea levels after bean extract consumption by obese human subjects. Nonetheless, Abdelfattah *et al*. (Abdelfattah *et al*., 2024) reported elevated creatinine and urea levels in obese rats, but supplementation with a *P. vulgaris* extract-containing mixture reduced these markers to baseline levels.

ALT and AST are commonly used biomarkers of liver function, and their blood concentrations are closely associated with obesity progression. There is a direct correlation between elevated ALT and AST levels and the severity of obesity, mostly due to obesity-related liver dysfunction (Jalili *et al*., 2022). Although most studies report increased ALT and AST levels in obesity, our findings showed lower concentrations of these enzymes in obese rats compared to controls. As shown in Table 1, ALT levels in the DIO group were 13% lower than in the control group. Meanwhile, the DIO+P group exhibited reduction of 1%, compared to the control. Similarly, AST levels in the DIO group were 16% lower than in the control group, but in the DIO+P group AST concentration was reduced only by 3%, with no significant difference from control values (Table 1). The reduction in liver enzyme concentrations could

be related to metabolic adaptations in the liver without its' significant damage. The possible explanation of this phenomenon is a downregulation in the synthesis of these enzymes or alterations in their release into circulation, which may occur in the early stages of metabolic dysfunction without significant liver injury (Zheng *et al*., 2023).

In our study, α-amylase activity in the DIO group was increased by 19% compared to control values, while in the DIO+P group this indicator was increased by 8% (Table 1). Alpha-amylase is a key enzyme involved in carbohydrate metabolism. Recent studies indicate that obesity leads to increased circulating α-amylase activity, mainly due to overexpression of the Amy2 gene, which regulates pancreatic amylase production (Azzout-Marniche *et al*., 2019). Our results do not differ from previous studies that showed that *P. vulgaris* extract can significantly reduce α-amylase activity in the blood - by about 28% in an obesity model - suggesting a potential regulatory effect of bean compounds on amylase activity (Micheli *et al*., 2019). This inhibitory effect is likely due to biologically active peptides with α-amylase-inhibitory effects. As we mentioned above, P. *vulgaris* is a rich source of α-amylase inhibitors, such as phasolamine, which can reduce postprandial glucose levels (Peddio *et al*., 2022).

Summarizing the results of the biochemical analysis DIO+P group had a significant effect on most of the assessed parameters. The mechanisms of peptide effects on these biochemical markers may be complex. As we have already mentioned, proteins with α-amylase-inhibitory properties from *P. vulgaris* are able to regulate postprandial glucose levels by reducing carbohydrate digestion and absorption (Almuaigel *et al*., 2017; Peddio *et al*., 2022). Such regulation of glucose homeostasis may, in turn, reduce metabolic stress on key organs, including the liver and kidneys, contributing to improved lipid metabolism, enzymatic function, and systemic inflammation. In addition, the bioactive peptides present in legumes have been shown to have antioxidant and anti-inflammatory effects, which may also contribute to the results obtained (Matemu *et al*., 2021; Shchypanskyi *et al*., 2025). Although the exact molecular pathways underlying these effects remain unclear, it is possible that the bioactive peptides exert their effects through a combination of direct enzyme inhibition, modulation of inflammatory signalling, and enhancement of antioxidant defence systems.

Serotonin, or 5-hydroxytryptamine (5-HT) is very important neurotransmitter, which regulate many processes in our bodies, but in context of obesity its' key role lies in energy balance and appetite regulation (van Galen *et al*., 2021). Up to 10 % of total body serotonin is synthetized in the brain, mostly in raphe nuclei. However, these quantities are enough to regulate food intake and control appetite – there is an inverse correlation between brain serotonin levels and food consumption (Conde *et al*., 2023). In contrast, nearly 90 % of total serotonin, also known as peripheral serotonin is synthetized by enterochromaffin cells in the gut, and its effect on the energy regulation is the opposite to central (brain) serotonin. There are reports, suggesting that high peripheral serotonin levels in blood are related to gain weight, probably by inducing gluconeogenesis in liver, and inducing adipogenesis in adipocytes (Namkung *et al*., 2015). In our study, the brain serotonin levels in the DIO group were significantly reduced, measuring 105.6 ± 3.2 ng/mg of protein, which is approximately 2.5 times lower than in the control group (259.6 ± 12.9 ng/mg of protein) (Fig. 2A). In contrast, DIO+P group exhibited serotonin levels of 334.7 ± 13.4 ng/mg of protein, which is nearly 1.3 times greater compared to the control group. These results support the generally supported opinion about the positive impact of brain serotonin levels on appetite and weight gain reduction. In one of previous studies, serotonin levels in obese rats' brains were decreased by 1.42 times, compared to the control, but rats that consumed *P. vulgaris* beans had the same values as control rats (Yurchenko *et al*., 2021).

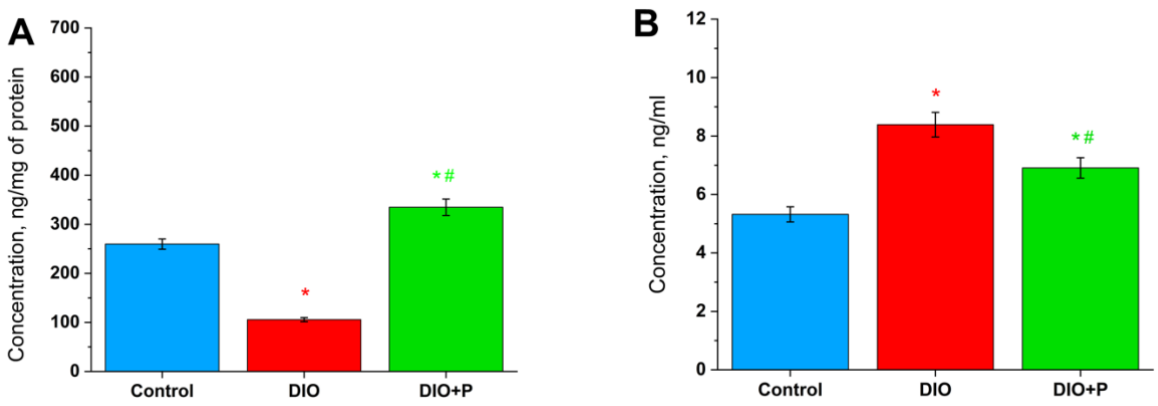

Figure 2. Concentrations of serotonin in rats' brain (a) and sera (b). * - p< 0.05 (compared to control group), # - p< 0.05 (compared to DIO group), n=10

In the DIO group serum serotonin was 8.4 ± 0.4 ng/ml, which is a 1.6-fold increase relative to the control group (5.3 ± 0.2 ng/ml). Meanwhile, the DIO+P group showed a relatively slight increase, with serotonin levels reaching 6.9 ± 0.2 ng/ml, approximately 1.3 times higher than in the control group (Figure 2B). Our results are in line with previous studies and common thought about obesity stimulating effect of increased peripheral serotonin levels (Nonogaki, 2022). However, in one of the recent studies there was a 1.8 time decrease in obese rats' serum serotonin levels, compared to the control, which differs from results of our and other studies (Yurchenko *et al*., 2021).

Our findings indicate that obesity is associated with a significant reduction of brain serotonin and simultaneous increasing of peripheral serotonin concentrations, and aligns with results of other studies on this topic (Namkung *et al*., 2015; Young *et al*., 2018). Serotonin levels greatly depend on the body inflammation status, microbiome composition, stress and many other factors (Cîmpeanu *et al*., 2025). The potential mechanism behind these effects remains unclear, yet there are a few possible explanations. For example, Buey et. al. (Buey *et al*., 2023) highlighted the possible mechanisms of milk bioactive peptide influence on serotonergic system, among which the ability of peptides to regulate tryptophan (which is 5-HT precursor) intake by gut cells, as well as to influence the activity of key 5-HT synthesis enzymes. Moreover, these peptides can possibly form complexes with 5-HT, regulating its potential, and influence gut microbiota, role of which in obesity development shouldn't be underestimated. Another potential mechanism may lie in the upregulation of 5-HT receptor synthesis by bioactive peptides, and facilitation of 5-HT binding to its receptor in their presence (J. Wang *et al*., 2022). Soy-derived peptide deprestatin was found to influence serotonin release and subsequent 5-HT_{1A} receptor activation, most likely by transmitting signal from intestinal tract to brain via vagus nerve (Mizushige, 2021). However, in order to describe precise mechanism of *P. vulgaris* peptides' effects further researches need to be conducted in the future.

4. Conclusions

In this study, we analyzed the effect of novel peptide group obtained from *P. vulgaris* husks on the organometric and biochemical parameters of rats with induced obesity. Summarizing the results of our work, we can say that these peptides have anti-obesity properties to a certain extent: we found a decrease in the rate of weight gain, and weight of adipose tissue, alignment of most biochemical parameters to control values and improved levels of serotonin in serum and brain. The exact mechanisms of the peptides' effect on all studied parameters are not yet known, but in our opinion, the decrease in weight gain and improved biochemical

parameters may be mainly a result of hypoglycemic effect inherent to *P. vulgaris* extracts. In addition, antioxidant, and potential anti-inflammatory properties of our peptides may be related to this as well.

The results of our work may also indicate that peptides from bean husks, having an advantage from an economic point of view, exhibit the same activities as bean extracts at almost the same level. These data can be taken into account in the development of new drugs based on bioactive peptides, since the cheapness of bean husks makes them an attractive type of raw material.

5. References

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Bio-Ethical certificate

MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE, THE NATIONAL UNIVERSITY NAMED AFTER TARAS SHEVCHENKO, 01033, Kyiv, Volodymyrska St., 60, **Protocol No. 7** of November 21, 2024, Committee on Bioethics of Scientific Research, **date 21. 11.2024.** *The Committee on Bioethics in Scientific Research at Taras Shevchenko National University of Kyiv (hereinafter referred to as the Committee) at its meeting (Protocol No. 7 of November 21, 2024) reviewed the materials of the dissertation research "The effect of exogenous peptides of various origins on the development of experimental obesity in rats" by graduate student Serhii Andriiovych Shchypanskyi. According to the materials submitted to the Committee for consideration, the research will be conducted in 2025, with Oleksii Mykolaiovych Savchuk as the scientific supervisor. The materials contain all the documents necessary for making a decision: an abstract, an application for permission to use laboratory animals with a detailed description of the work protocol. The research will be carried out taking into account existing bioethical and scientific standards for conducting dissertation research involving laboratory animals.*