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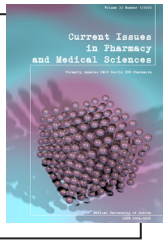

The evaluation of lipid peroxidation and oxidative modification of proteins in blood serum under obesity development and the consumption of aqueous kidney beans *Phaseolus vulgaris* pods extract

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ABSTRACT

Our interest has focused on the investigation of the anti-obese potential of kidney beans (*P. vulgaris*) pods extract. In the course of the study, obesity development in rats was induced with high-calorie diet. Control and obese rats then have consumed with aqueous kidney beans (*P. vulgaris*) pods extract during 6 weeks (200 mg/kg). Results show that the long-term consumption of P. vulgaris pods extract can lead to the reduction of hyperglycemia and insulin resistance development. Furthermore, we saw a normalization of lipid peroxidation parameters and oxidative modification of protein due to the consumption of the kidney beans (*P. vulgaris*) pods extract. Our experimental data demonstrate the ability of the kidney beans (*P. vulgaris*) pod extracts to mitigate obesity development but the details of this mechanism remains to be not fully understood.

INTRODUCTION

Obesity is a condition wherein over-accumulation of adipose fat tissue causes abnormal increase of weight [1,2]. This disease increases the risk for a variety of chronic disorders. For example, excessive Body Mass Index (BMI) is thought to account for 60% of the risks of type 2 diabetes development, and over 20% of that for hypertension and coronary-heart disease and between 10 and 30% for various cancers [3-5]. Approximately half of the individuals who were diagnosed with type 2 diabetes mellitus (T2DM) are obese [6]. Other co-morbidities include gall-bladder disease, fatty liver, sleep apnoea and osteoarthritis.

It is known that insulin resistance and the reduction in insulin production are the major characteristics of T2DM pathogenesis [7,8], but a modern lifestyle, physical inactivity, abdominal obesity and the excess of adipokines can also cause insulin resistance [9]. Insulin secretion in the fasting state results in the linear increase of glucose load with increased BMI [10]. Insulin resistance is accompanied by a disruption of the reactive oxygen species (ROS) metabolism that leads to the development of oxidative stress [11,12]. Oxidative stress, hence, is a trigger of obesity and also can be a consequence of it.

Oxidative stress activates stress-signaling kinases. Herein, c-jun N terminal kinase 1 (JNK1) playsa key role in the etiology of insulin resistance. JNK1 prevents recruitment of protein to the activated insulin receptor and disrupts the downstream events of the insulin signaling pathway by phosphorylation of IRS-1 at the inhibitory sites. Insulin resistance may also be caused be the chronic inflammation promoted by oxidative stress. The pro-inflammatory cytokines TNFα and IL-6 have been shown to trigger phosphorylation of IRS-1, thus exerting an inhibitory action on insulin signaling. Accordingly, genetic ablation either of TNFα or of its receptor can improve insulin resistance caused by obesity in rodent models [13]. Beyond the aforementioned, the oxidative damage of proteins in insulin-sensitive tissues could potentially affect their function and, thus, influence the propagation of insulin-stimulated signals [14].

Some studies based on rat models have demonstrated that obesity is associated with increased lipid peroxidation [15]. Lipid peroxidation is an oxidative damage that affects cellular membranes, lipoproteins, and other molecules that contain lipids in conditions of oxidative stress. This process is advanced by a free radical chain reaction mechanism.

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The reaction consists of three major steps: initiation, propagation and termination. In the initiation step, pro-oxidants detach the allylic hydrogen. As a result, a carbon-centered lipid radical is formed. In the propagation phase, the lipid radical rapidly reacts with oxygen to form a lipid peroxyl radical. It then detaches hydrogen from another lipid molecule and generates lipid hydroperoxide (LOOH). In the termination reaction, antioxidants such as vitamin E donate a hydrogen atom to the species and form a corresponding vitamin E radical that reacts with another radical to create nonradical products [16]. The end products of lipid peroxidation are malondialdehyde (MDA), which is known as the most mutagenic product of lipid peroxidation, and 4-hydroxynonenal (HNE) – the most toxic one [17].

In the traditional medicine of many countries, plants are often used to control diabetes mellitus [18-20]. Some of these whileused as antidiabetic remedies, are edible [21]. The biologically active molecules derived from plants are generally recognized to be safer, less toxic, more accessible and affordable than synthetic drug derivatives. Their huge advantage is that they can be ingested in everyday diet.

The study of *Phaseolus vulgaris* pods dry extract composition by chromatographic methods allowed the identification of such phenolic compounds as rutin, isoquercitrin, ferulic acid and kaempferol-3-O-glucoside [22]. A number of clinical and research studies have suggested that flavonoids have positive effects in the treatment, prevention and alleviation of various diseases such as cancers [23], diabetes [24], obesity, cardiovascular diseases and other age-related diseases [25]. Flavonoids can function as antioxidants to prevent diseases by modulation of the oxidative stresses in the body [26]. Flavonoids can also regulate carbohydrate digestion, adipose deposition, insulin release and glucose uptake in insulin-responsive tissues. There are a variety of studies that confirm the antioxidant properties of rutin and quercetin [27]. The antioxidant properties of bioflavonoids are due to the neutralization of free radicals (-OH and -O_2) – ROS.

Despite the well-known mechanisms of oxidative stress formation during obesity development, currently there are no complex studies that will help to find effective preventive or therapeutic agents. Hence, in this study, we wanted to test the influence of kidney bean *Phaseolus vulgaris* pod extract on the lipid peroxidation and oxidative modification of proteins in blood serum under obesity development.

MATERIALS AND METHODS

In vivo experimental procedures

All work was in compliance with the standards of the Convention of Bioethics of the Council of Europe in 1997, European Convention for the protection of vertebrate animals that are used for experimental and other scientific purposes, the general ethical principles of animal experiments approved by first National Congress of Bioethics of Ukraine and other international agreements and national legislation in this field.

Experiments were carried out for 10 weeks duration on white nonlinear male rats of the Wistar line with initial weight of 195-205 g. The experiments followed 7 days of acclimation in the animal facility of Taras Shevchenko National University of Kyiv, under constant conditions of temperature (22±3°C), humidity (60±5%) and light (12 h light/12 h dark cycle). Standard *rodent* food and water were provided *ad libitum*.

After one week, the animals were randomly placed within 3 groups (10 rats per group). The first group ("Control")was fed with standard food during all ten weeks and water *ad libitum*. The second group ("HCD") was on a high-calorie diet (38.8% fat, 15. 5% protein and 45.7% of carbohydrate, 28.71 kJ·g^{-1}) that consisted of a standard meal (60%), pork fat (10%), eggs (10%), sugar (9%), peanuts (5%), dry milk (5%) and sunflower oil (1%) [28],and drank water *ad libitum* for the whole experimental period. Animals of the third group ("HCD+Ex") were also on a high-calorie diet and (initially) water *ad libitum*. After 4 weeks of the experiment, they started to receive a water extract of *P. vulgaris* (200 mg/kg): one day all theratsreceived the water extract (free access) and another day they drank water*ad libitum*.

Body weights were recorded once a week and the food intake were recorded daily in all animal groups. The body mass index (BMI) (body weight (g)/nose-to-anus length^2 (cm^2)) and Lee index (cube root of body weight (g)/nose-to-anus length (cm)) were calculated at the end of the experiment [29].

At the end of the 10th week, the animals were fasted overnight and sacrificed**.** The blood was collected and used for the estimation of the blood glucose and glycosylated hemoglobin levels. The serum used for the determination of other biochemical parameters was prepared by centrifugation at 1.000 g for 30 min of blood samples previously incubated at 37°C for at least 30 min and stored at -20°C until it was used.

Preparation of plant extract

The aqueous kidney beans *P. vulgaris* pods extract were prepared according to the method [30]. The 132 g of grinded up dry kidney bean pods was diluted with 1 liter of boiling distilled water and brewed on a boiling water bath for 15 minutes. The extract was then cooled to room temperature at 25°C, filtered through several layers of gauze and centrifuged at 1,000 g for 10 minutes to remove the plant debris. The supernatant was lyophilized by incubation at -20°C in a deep freezer for 8 h, followed by drying in a freeze-dryer (The Telstar LyoQuest, Spain) at -56°C for 24 h under pressure of 0.05 mbar. In the studies, we used freshly prepared aqueous solutions of dry extractin the concentration of 200 mg/kg.

Biochemical analysis

Determination of the glucose concentration in blood was carried out by way of the glucosidase method,using glucometer Gluco Dr. auto (AGM-4000) (Allmedicus Co., Ltd, Korea). All procedures were carried out in accordance with the manufacturer's instructions.

The content of the glycated hemoglobin in the rat blood was determined spectrophotometrically according to the method [31]. Standard sets of reagents were used (Lachema, Czech Republic).

The determination of the insulin concentration in the blood serum of rats was carried out by following the method of immunoassay analysis, according to the general method for soluble proteins. This was performed in 96-well microplates with sorption capacity for soluble proteins [32]. The serum samples were previously diluted (1:10) in Tris-buffered saline (TBS, 50 mM Tris, 137 mM NaCl, pH 7.4) and added in the volumes of 100 µl/well to 96-well ELISA plates (Thermo FisherScientific, USA) and left to adsorb overnight at 4°C. After incubation, the plates were washed 3 times with TBS containing 0.05% Tween 20 and blocked with 5% nonfat dry milk for 1 h at 37°C. After washing, the corresponding primary antibodies (Insulin Antibody (2D11-H5): sc-8033, Santa Cruz Biotechnology, Inc., USA) were added to the plates and incubated for an hour at 37°C. The plates were then washed and incubated for an hour at 37°C with corresponding secondary antibodies conjugated to horseradish peroxidase (Sigma-Aldrich, USA). After washing, the substrate (OPD and hydrogen peroxide) was added. The reaction was stopped by the addition of 2.5 N H_2SO_4. Plates were read at 492 nm,using a µQuant™microplate reader (BioTek Instruments, Inc., USA).

Assessment of the level of malondialdehyde in the rat blood serum was carried out by reaction with 2-thiobarbituric acid and the formation of a coloured trymethine complex with a maximum absorption at a 532 nm [33,34]. The content of diene conjugates was determined in heptane-isopropanol extracts by means of the spectrophotometric method, while the content of the schiff bases was assessed by applying the fluorometric method [35,36].

Moreover, the levels of total, protein-bound and non-protein sulfhydryl (SH) groups were measured by means of the Elman method [37], and the content of the oxidative modification of proteins (OMB) was determined by measuring the level of carbonyl derivatives that were detected in reaction with 2, 4-dinitrophenylhydrazine[34,38]. Finally, the amount of protein was made known by applying the Bradford method based on the ability of the proteins to bind to kumasi diamond blue G-250 [39].

Statistical analysis

The data of biochemical estimations were reported as mean ± SEM for each group (n=10). Statistical analyses were performed using analysis of variance (ANOVA). The difference between the parameters was considered statistically significant at $p \leq 0.05$.

RESULTS

The body weight, body mass index and some nutritional parameters of the experimental rats during 10 weeks of a high-calorie diet with or without aqueous kidney beans (*P. vulgaris*) pods extract

The first step of our experiment was to identify the key obesity indicators in rats that were on a high-calorie diet, to compare these parameters with the same in rats from the control group, and to study the effect of the kidney beans (*P. vulgaris*) pods extract on obesity development. After 10 weeks of the experiment, an increase of BMI by 0.2 points of the "HCD" animals was observed when compared to the same of the rats from the "Control" group. However, no statistically significant changes in BMI were noted in the rats from "HCD+Ex" group when compared to the same control animal parameter (Table 1).

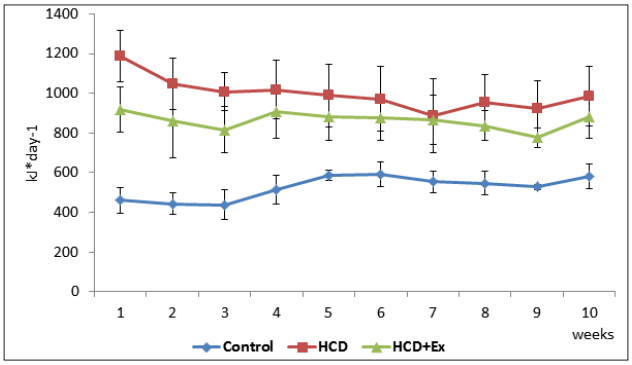
Table 1. General characteristics of the control animals("Control"), rats that were on a high-calorie diet("HCD") and rats that received the kidney bean *P. vulgaris* pod extract in combination with high-calorie diet fed (M±m, n=10)

	Food intake (g/day)	Liquid intake (ml/day)	Initial body weight (g)	Final body weight (g)	BMI (g/cm²)
Control	34.4 ±2.86	30 ±2.17	200 ±13.9	296 ±14.3	054 ±0.017
HCD	35.6 ±2.31	31. 7 ±2. 27	198 ±16.9	404 ±22. 1*	0.74 ±0.029*
HCD+Ex	30.6 ±1.28#	33.1 ±2.86	202 ±9.27	336 ±19.1*#	0.56 ±0.025#

*p≤0.05 significantly different from the control group
#p≤0.05 significantly different from the "HCD" group

The data present in Figure 1 demonstrate the amount of energy received by all groups of animals. The amount of energy consumed by animals from "HCD" and "HCD+Ex" groups was almost double that of the control rats, even though the animals of the "HCD+Ex" group had lower rates of body weight gain and BMI.

Figure 1. Effects of consumption of kidney beans (*P. vulgaris)* podextract on food intake and body weight in male rats pre-exposed to a high-calorie diet (M±m, n=10)

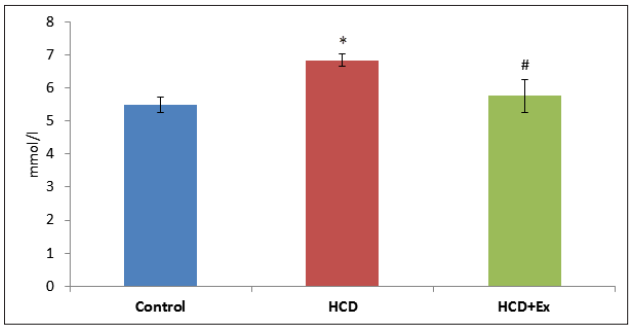


*p≤0.05 significantly different from the control group
#p≤0.05 significantly different from the "HCD" group

Experimental high-fat diet rat glucose homeostasis parameters at the end of the 10th week with or without aqueous kidney beans (*P. vulgaris*) pod extract

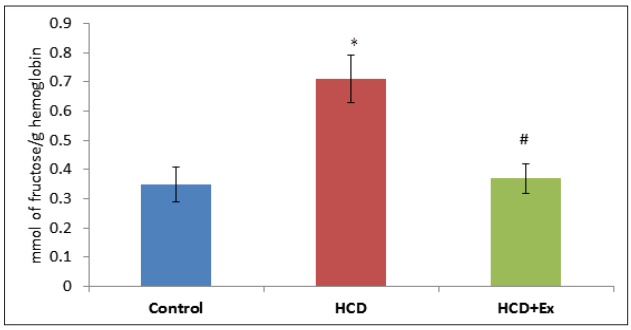
In the next step of our research, we measured blood glucose concentration. This parameter in the control group of animals was 5.4±0.23 mmol/l, and, according to the previous research data, is within the normal ranges of 3.5-5.5 mmol/l (Fig. 2). In contrast, consumption of a high-calorie diet led to a 1.3 times increase of the blood glucose level, compared to the same in the control rats. However, the six-week intake of a kidney beans (*P. vulgaris*) pod extract in combination with a high-calorie diet led to a 1.2 times decrease of blood glucose level, compared to this parameter in the "HCD" group of animals.

The data in Figure 3 show glycated hemoglobin concentration. This is used as an indicator of the long-term average level of glucose in the blood. In the "HCD" group of rats, this parameter increased 2 times, compared to the same of the "Control" group, whereas in the rats of "HCD+Ex" group, this decreased 1.9 times, compared to that in the group with obesity development.



*p≤0.05 significantly different from the control group
#p≤0.05 significantly different from the "HCD" group

Figure 2. The concentration of glucose in the blood of "Control", "HCD" and "HCD+Ex" groups (M±m, n=10)



*p≤0.05 significantly different from the control group
#p≤0.05 significantly different from the "HCD" group

Figure 3. The concentration of glycated hemoglobin in the blood of "Control", "HCD" and "HCD+Ex" groups (M±m, n=10)

The blood insulin concentration in the "HCD" rats also indicated a 2.6 times decrease, compared to this parameter in the "Control" animals. At the same time, the results shown that the level of insulin of the rats from "HCD+Ex" group increased 1.4 times, compared to the same of the animals from the "HCD" group.

Lipid peroxidation and oxidative modification parameters of proteins in the blood serum of experimental rats at the end of the 10th week of a high-fat diet –with or without aqueous kidney beans (*P. vulgaris*) pods extract

As shown in Table 2, the spontaneous and ascorbate-dependent MDA contents in the "HCD" group were 4.4 and 12.5 times higher, respectively, compared to those of the Control group. In the rats from the "HCD+Ex" group, these parameters showed a tendency to normalize (Table 2).

Table 2. The content of lipid peroxidation products in the blood serum of rats of "Control", "HCD" and "HCD+Ex" groups. (M±m, n=10)

Group of rats	Indicator			
	Spontaneous MDA nmol×mg protein^{-1}	Ascorbate-dependent MDA nmol×mg protein^{-1}	Schiff bases conv. unit × mg of protein^{-1}	Conjugated dienes nmol × mg of protein^{-1}
Control	0.0068 ±0.003	0.020 ±0.009	40.3 ±12. 86	0.0197 ±0.004
HCD	0.0299 ±0.009*	0.250 ±0.080*	163.7 ±32.58*	0.0365 ±0.009*
HCD+Ex	0.0081 ±0.005#	0.097 ±0.035#	51.3 ±18.44#	0.021 ±0.005#

*p≤0.05 significantly different from the control group
#p≤0.05 significantly different from the "HCD" group

Moreover, the content of conjugated dienes in "HCD" group was 1.8 times higher, compared to the same in "Control" group. In the animals from the "HCD+Ex" group, this parameter was 1.7 times lower, compared to that in "HCD" rats. Furthermore, the consumption of a high-calorie diet led to a 4 times increase of the schiff bases content, compared to the same in "Control" rats, while in the "HCD+Ex" rats, this indicator evidenced a 3.2 times decrease compared to that in the "HCD" group of animals (Table 2).

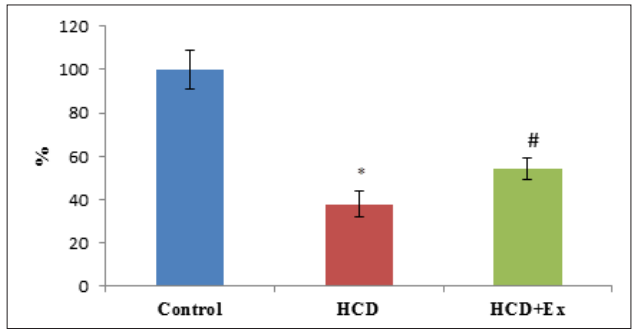
We also saw that under obesity development, the content of the total sulfhydryl groups in the blood serum of "HCD" rats was 1.25 times lower compared to the same in the "Control" rats group. In contrast, there was no significant differences in this parameter within the "HCD+Ex" group of rats, in comparison to the same of the control animals (Table 3). In addition, a decrease of non-protein SH-groups in rats that consumed the high-calorie meal was observed, but this parameter was not significantly different from that in the control rats. According to the obtained data, the results of the rats that consumed the kidney beans (*P. vulgaris*) pods extract in addition to the high-calorie diet and those of the rats that were only on a high-calorie diet were the same.

Table 3. The content of total, non-protein and protein sulfhydryl (SH) groups in the blood serum of rats of "Control", "HCD" and "HCD+Ex" groups (M±m, n=10)

Group of rats	SH groups		
	Total μmol×mg protein^{-1}	Non-protein μmol×mg protein^{-1}	Protein μmol×mg protein^{-1}
Control	0.70 ±0.066	0.102 ±0.008	0.598 ±0.081
HCD	0. 56 ±0.048*	0.093 ±0.003	0. 467 ±0.032*
HCD+Ex	0.66 ±0.035#	0.098 ±0.006	0.562 ±0.055#

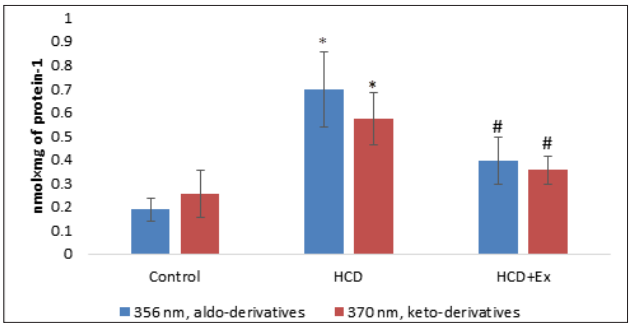
*p≤0.05 significantly different from the control group
#p≤0.05 significantly different from the "HCD" group

Finally, the results indicated in Figure 4 demonstrate that the content of OMP products with the absorption peaks of 356 nm and 370 nm in the "HCD" rats blood serum saw a 3.73 and 2.24 times increase, respectively, when compared to those in the "Control" rats. In contrast, the rats from the "HCD+Ex" group indicated a 1.78 and 1.53 times reduction of these parameters, respectively, as compared to the same in the "HCD" group (Fig. 5).



*p≤0.05 significantly different from the control group
#p≤0.05 significantly different from the "HCD" group

Figure 4. The insulin concentration in the blood serum of rats of "Control", "HCD" and "HCD+Ex" groups (M±m, n=10)



*p≤0.05 significantly different from the control group
#p≤0.05 significantly different from the "HCD" group

Figure 5. The content of products of oxidative modification of proteins in the blood serum of rats of "Control", "HCD" and "HCD+Ex" groups (M±m, n=10)

DISCUSSIONS

The results of this research suggest that a high-calorie diet brought about obesity, however, a decrease of body weight was indicated in those groups of rats who received the *P. vulgaris* extract when compared to this parameter of the "HCD" group of animals. Herein, previous studies demonstrate that the mechanism that lay behind the weight loss while consuming the *P. vulgaris* extract relies on its alpha-amylase-inhibiting activity [40-42]. Still, there is another possible mechanism of the effect of *P. vulgaris.* This is associated with phytohaemagglutinin activity. The last can bind to the cells of the gastric epithelium and intestinal mucosa thus stimulating the release of cholecystokinin and glucagon-like peptide – the hormones that play a key role in the digestion and eating behavior [43].

The excessive increase of the body weight of the animals from the "HCD" group is probably due to the accumulation of fatty tissue. These changes could evolve into disbalance between the amount of consumed and expended energy. Moreover, the amount of energy that the animals have received increased as a result of the higher caloric intake of the feed. Our results demonstrate that the use of kidney beans *P. vulgaris* pods extract not only decreases the energy consumption (Figure 1), but also affects the food intake (Table 1), and thus contributes to the decrease of the body weight in the rats of the "HCD+Ex" group.

From previous studies, it is known that the administration of the aqueous extract of *P. vulgaris* pods, along with glibenclamide to diabetic rats almost restored to control levels, changes in the parameters of the body weight and the levels of total and glycosylated hemoglobin. In control rats, the levels of total and glycosylated hemoglobin remained unchanged [44].

The hypoglycemic action of the *P. vulgaris* pods extract might also increase insulin sensitivity in peripheral tissues and act insulin-like and hence catalyze glycolysis in the peripheral tissues, thus preventing glucose absorption or inhibiting gluconeogenesis [45].

Obesity is a complex, polyethiologic disease that can lead to the deterioration of sensitivity to insulin in glucose utilization sites such as skeletal muscle, liver and adipose tissue. This condition is known as insulin resistance. The development of resistance to insulin activity is characterized by the reduction in the glucose consumption of the skeletal muscle and the increase to the blood stream of glucose excretion from the liver, as well as lipids from the adipose tissue. All these events result in glucose intolerance and chronic hyperglycemia [46].

The important parameter for the diagnosis of insulin resistance development is the determination of the serum insulin level. The serum insulin level results from the excessive production and secretion of insulin by pancreatic β-cells through the compensatory mechanism, and is a response to the decrease of the sensitivity of peripheral tissues to its action because of insulin resistance development [47].

The mechanism by which the kidney beans (*P. vulgaris*) pods extract exerts its hypoglycemic action in obese rats may be due to the potentiation of the insulin effect by either the increase of the pancreatic secretion of insulin from the existing β-cells or its release from the bound form. This was demonstrated by the significant enhancement of the insulin level in rats that consumed the kidney beans (*P. vulgaris*) pod extract, along with a high-calorie diet. The *P. vulgaris* pods are reported to be rich in flavonoids [22]. These flavonoids also might be responsible for the stimulation of the insulin secretion from the existing β-cells of the obese rats and thereby mediate its antihyperglycaemic effect. Furthermore, flavonoids are reported to regenerate the damaged pancreatic β-cells in diabetic animals [48].

Due to disease, the antioxidant defense system reduces its own activity and thus this leads to oxidative modification of DNA, carbohydrates, proteins and lipids. Table 2 shows the concentration of MDA, this being one of the parameters that characterize the intensity of free radical production. We observed that the spontaneous and ascorbate-dependent MDA contents in the "HCD" group were higher compared to the control levels. Such results are aligned with the previous reports of the increased MDA contents in patients with metabolic syndrome [49].

According to the data from various experimental models, obesity promotes increased plasma lipid peroxidation [50]. While lipid peroxide oxidation (LPO) generates a wide range of oxidation products, the conjugated dienes are the main primary products of lipid peroxidation. The final substances of LPO are fluorescent compounds of oxide copolymerization of lipids and proteins – other wise known as schiff bases, gaseous products, nitrates and nitrites [51]. Based on our results, there was a significant increase of the conjugated dienes and Schiff bases in the obese rats when compared to those parameters in the control group of animals. In contrast, obese rats who had consumed the aqueous extract of *P. vulgaris* pods were characterized by lower levels of conjugated dienes and Schiff bases when compared to those in the group of obese rats.

The active forms of oxygen and the products of lipid peroxidation induce the oxidative modification of proteins (OMP) and are determined by the specificities of the amino acid composition of the proteins. The acceptor groups that intercept electrons may include disulphide, sulfhydryl, carbonyl, carboxyl and amino groups of proteins. Accumulation of such modified proteins breaks the normal functioning of the cell and promotes the development of various pathological conditions. The important criterion of protein

modification is an oxidation of sulfhydryl groups. This process takes place both directly and enzymatically with the participation of glutathione peroxidase [52,53].

Flavonoids detected in *P. vulgaris* pods dry extract act as antioxidants by neutralizing the effects of the reactive oxygen and nitrogen species [22]. It has been reported that flavonoids, particularly flavonols such as quercetin, exhibit a wide range of biological functions [54]. One of these is the ability to inhibit lipid peroxidation. It is also known that kaempferol protects pancreatic β-cells from hyperglycaemia. Hence, it can prevent development of obesity [55,56]. The *in vitro* results of the studies confirm that the treatment with kaempferol (10 μM) promoted cell sustainability and suppressed cellular apoptosis. Moreover, it continually reduced the activity of the caspase 3 in β cells and human islets vulnerable to the hyperglycaemic conditions [57].

Thus, the quercetin and its glycoside derivatives that are present in *P. vulgaris* pod dry extract may promote glucose-stimulated insulin secretion and repress oxidative stress and nitric oxide accumulation. Furthermore, the reducing oxidation of protein molecules in the blood serum of the "HCD+Ex" group of rats may be associated with the antioxidant properties of flavonoids of *Phaseolus vulgaris* pod dry extract. Therefore, we can state the beneficial effects of the *P. vulgaris* pods dry extract on glucose homeostasis, as well as its antihyperglycaemic and antioxidant properties under the development of obesity.

CONCLUSION

In this work, the anti-obese potential of kidney beans (*P. vulgaris*) pods extract was investigated. Our experimental data align with the results of the previous studies performed on animals [58,59] and confirm that the long-term consumption of *P. vulgaris* pods extract can lead to the reduction of hyperglycemia and insulin resistance development. Furthermore, we saw a normalization of lipid peroxidation parameters and oxidative modification of protein due to the consumption of the kidney beans (*P. vulgaris*) pods extract.

Considering the rapid increase of the morbidity and mortality rates associated with the development of obesity, there is an urgent need for safe and effective remedies with anti-obesity activities. Therefore, the determining of the mechanisms that are involved in the kidney beans (*P. vulgaris*) pod extract action under the development of obesity would provide valuable insights in the field of drug development.

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