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Effect of Aqueous Extract from *Phaseolus vulgaris* Pods on Lipid Peroxidation and Antioxidant Enzymes Activity in the Liver and Kidney of Diabetic Rats

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ABSTRACT

The social significance of diabetes mellitus lies in the fact that in addition to significant prevalence, this disease is associated with many complications. To facilitate the course of diabetes and its complications medicinal plants are widely used in traditional medicine. One of such plants is kidney bean (*Phaseolus vulgaris*). This plant is used in traditional medicine, especially for the secondary complications of diabetes. Since complications of diabetes are often associated with increased oxidative stress, the study of antioxidant properties of *P. vulgaris* is important to clarify the mechanism of its therapeutic effect. Present investigation shows that long-term oral administration of aqueous *P. vulgaris* pods extract in dose of 200mg/kg b.w. besides its pronounced hypoglycemic action also has a positive influence on the liver and kidney function markers in STZ-treated diabetic rats. The extract also inhibits free radical production and lipid peroxidation and activates antioxidant enzymes in liver and kidneys of rats with STZ-induced diabetes. Thus, our data reveal antioxidant properties of aqueous *P. vulgaris* pods extract that might have beneficial effect in treatment of diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by the elevated blood glucose level and subsequent pathological effects (Shafiee *et al.*, 2012). It is well known that chronic hyperglycemia is associated with increased oxidative stress. Production of free radicals caused by elevated blood glucose level may occur via four different routes as: 1) enhanced glycolysis, 2) activation of the polyol pathway (also known as sorbitol-aldose reductase pathway), 3) glucose autoxidation and 4) non-enzymatic protein glycation (Ceriello *et al.*, 1992; Williamson *et al.*, 1993; Ceriello, 2000). Activation of these mechanisms leads to a number of other biochemical disorders including formation of highly active lipid peroxides and reactive oxygen species (ROS) which can directly damage cells. For these reasons, oxidative stress can be considered at the same time as a cause and as consequence of micro and macrovascular complications of diabetes (Goycheva *et al.*, 2006; Yang *et al.*, 2011). Nowadays, development of new effective therapeutic strategies for diabetes is task of extremely high importance.

The use of antioxidants for dealing with diabetes, and particularly for treatment of its complications, is being intensively studied. Plants, including herbs and spices, contain many phytochemicals, which are a potential source of natural antioxidants such as phenolic diterpenes, flavonoids, alkaloids, tannins and phenolic acids. Multiple studies have been made in order to find out the antioxidant activities of various herbs, fruits, vegetables, spices and their role in the prevention and treatment of diabetes complications (Shukia *et al.*, 2000; Khan *et al.*, 2012; Patel *et al.*, 2012).

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Phaseolus vulgaris, also known as kidney bean, is a common vegetable that possess plenty of curative and therapeutic properties. Various parts of this plant are extensively used in traditional medicine for the treatment of DM. *P. vulgaris* contains bioactive components with antihyperglycemic activity (Roman-Ramos *et al.*, 1995; Pari and Venkateswaran, 2003). However, in order to understand more about the therapeutic values of this plant in prevention and treatment of DM further investigations are necessary. Purpose of the given study was to study the effect of the aqueous extract from *P. vulgaris* pods on the functional state of liver and kidney in rats under conditions of streptozotocin-induced diabetes.

MATERIAL AND METHODS

Preparation of plant extract

The aqueous extract was prepared by boiling 132 g of dried powdered *P. vulgaris* pods in 1 liter of distilled water for 20 min. After boiling, extract was left overnight to infuse (Venkateswaran and Pari, 2002). In order to remove plant debris obtained extract was filtered and centrifuged at 1000×g for 10 min. 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. Dry extract (8 g) was stored at -20˚C. Right before use, required doses were taken and resuspended in 2 ml of distilled water.

Experimental animals

White non-linear rats of both sexes, each in the weight range of 100-120g, were obtained from the Animal house of Taras Shevchenko National University of Kyiv, Ukraine. All experimental protocols were approved by the Ethical Committee for Conduction of Animal Studies at the Educational and Scientific Center 'Institute of Biology' of Taras Shevchenko National University of Kyiv, Ukraine. Experimental DM was induced by single intraperitoneal injection of 45 mg/kg b.w. streptozotocin (STZ; Sigma, USA) (Zafar and Naqvi, 2010) dissolved in 0.5 ml of 0.01 M citrate buffer, pH 4.5. Control group received 0.5 ml of buffer that was used for dissolving of STZ. Two days after STZ injection, fasting animals with glycemic values more than 25 mM were chosen for the experiment.

Experimental design

The rats were weighed, tagged and randomly divided into four groups of ten animals each as followed. "Control" and "Diabetes" (the untreated diabetic rats) were given by gavage de-ionized water (2 ml/day); "Control + Extract" and "Diabetes + Extract" were treated with *P. vulgaris* aqueous extract (200mg/kg b.w. per day) dissolved in 2 ml of deionized water and applied orally. The experiment was conducted for 28 days (Hernández-Saavedra *et al.*, 2013). During the experiment animals were kept under standard conditions (temperature, humidity, 12 hour dark-light cycle and were fed with standard commercial food and water available *ad libitum*).

Analytical methods

After 28 days, the animals were deprived from food overnight and killed by decapitation. Blood was collected and used for the estimation of blood glucose levels and glycosylated hemoglobin. Serum was separated by centrifugation at 2500×g for 25 min and stored at -20˚C until used for biochemical analysis. Blood glucose level was evaluated using glucometer «Hlyukofot II» (Norma, Ukraine) and level of glycosylated hemoglobin was measured spectrophotometrically using commercial kit (ERBA-Lachema, Czech Republic).

Liver and kidney function markers as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), creatinine, uric acid and urea were estimated by biochemical analyzer Microlab 300 (Elitech, France) and commercial kits from Elitech diagnostic (France) according to the standard protocols provided by manufacturers.

To estimate lipid peroxidation and enzymes activity liver and kidney were excised, rinsed in ice-cold physiological saline and homogenized with teflon pestle at 4˚C in 5 volumes of 50 mM Tris-HCl buffer (150 mM NaCl, 1 mM EDTA, 250 mM sucrose) pH 7.4. Homogenates were first centrifuged at 600×g, 4°C for 10 min and then at 15000×g for 10 min and supernatants were used for biochemical analysis.

The malondialdehyde (MDA) content was quantified with thiobarbituric acid (Satoh, 1978). The levels of Schiff bases and conjugated dienes, activity of superoxide dismutase (SOD) and catalase were determined according to previously described methods in (Kostiuk *et al.*, 1984; Misra and Fridovich, 1971; Korolyuk *et al.*, 1988). The activities of glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) were estimated according to methods described by Vlasova, 1990. The total proteins were measured using Bradford assay (Bradford, 1976).

Statistical analysis

The data of biochemical estimations were reported as mean ± SEM for ten animals in each group. Statistical analyses were performed using one-way analysis of variance (ANOVA). Differences were considered to be statistically significant when P was < 0.05. All statistical analyses were performed with statistically available software (SPSS 16 for WINDOWS).

RESULTS AND DISCUSSION

The blood glucose and glycosylated hemoglobin concentrations in control and experimental groups of rats are represented in Fig 1. Glucose concentration in blood of the untreated fasting diabetic animals was significantly higher than in the control group (Fig 1, A). Diabetic rats treated with aqueous extract from *P. vulgaris* pods had significantly lower blood glucose level compared to untreated diabetic rats. The blood glucose levels in control rats treated with plant extract and in untreated control animals were statistically comparable.

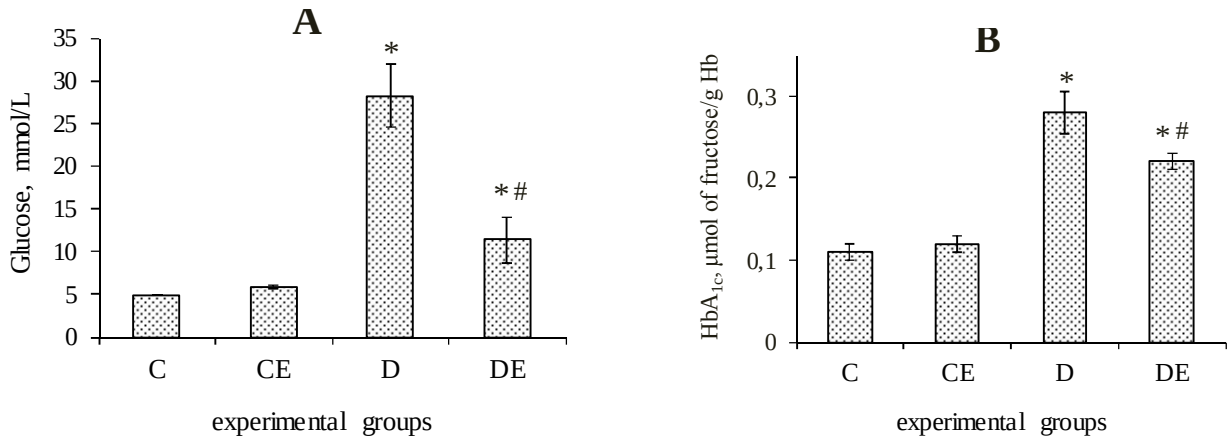

Fig. 1: Effect of *Phaseolus vulgaris* pods extract on (A) blood glucose and (B) glycosylated hemoglobin (HbA_{1c}) levels. Given data are mean ± SEM for ten animals in each group: C – control rats; CE – control rats treated with extract; D – untreated diabetic rats; DE – diabetic rats treated with extract. Values are statistically significant at P<0.05. *Significantly different from the control rats; #significantly different from the diabetes control rats.

Table 1: Effect of *Phaseolus vulgaris* pods extract on serum levels of liver and kidney enzyme markers.

Group	ALT ($U*L^{-1}$)	AST ($U*L^{-1}$)	GGT ($U*L^{-1}$)	Creatinine ($\mu mol*L^{-1}$)	Uric acid ($\mu mol*L^{-1}$)	Urea ($mmol*L^{-1}$)
Control	56.08±8.23	182.25±15.16	4.28±0.93	63.05±3.70	111.23±13.48	7.00± 0.74
Control+Extract	59.30±7.88	200.45±28.04	2.93±0.48*	64.45±3.18	122.88±13.73	6.38±1.20
Diabetes	94.80±6.00*	347.68±9.62*	10.85±0.65*	53.45±1.07*	155.40±10.47*	8.63±1.51
Diabetes+Extract	65.13±6.09#	269.77±22.20*#	8.07±0.38*#	50.87±3.96*	97.53±10.89#	15.33±3.10*#

Given data are mean ± SEM for ten animals in each group. Values are statistically significant at P<0.05.
*Significantly different from the control rats; #significantly different from the diabetes control rats.

Table 2: Effect of *Phaseolus vulgaris* pods extract on the concentration of MDA, conjugated dienes and Schiff bases in liver and kidney.

Group	MDA ($\mu mol*mg\ prot.^{-1}$)		Conjugated dienes ($nmol*mg\ prot.^{-1}$)		Schiff bases ($nmol*mg\ prot.^{-1}$)	
	liver	kidney	liver	kidney	liver	kidney
Control	6.75±0.64	11.11±1.17	0.101±0.01	0.144±0.04	83.92±6.87	98.20±5.92
Control + Extract	7.25±0.33	11.00±0.04	0.111±0.03	0.134±0.07	84.35±1.83	92.31±6.49
Diabetes	10.67±0.43*	14.95±0.15*	0.131±0.01*	0.288±0.01*	143.41±13.44*	175.61±1.88*
Diabetes + Extract	8.66±0.45*#	11.66±0.66#	0.094±0.01#	0.189±0.03#	116.23±3.62*#	118.35±6.65*#

Given data are mean ± SEM for ten animals in each group. Values are statistically significant at P<0.05.
*Significantly different from the control rats; #significantly different from the diabetes control rats.

Table 3: Effect of *Phaseolus vulgaris* pods extract on the activities of SOD and catalase in liver and kidney.

Group	SOD ($U*min^{-1}*mg\ prot.^{-1}$)		Catalase ($nmol\ H_2O_2*min^{-1}*mg\ prot.^{-1}$)	
	liver	kidney	liver	kidney
Control	92.13±3.18	85.95±6.11	2.03±0.34	0.61±0.10
Control + Extract	95.94±12.18	84.61±12.21	2.17±0.33	0.55±0.08
Diabetes	45.41±6.78*	70.59±5.76*	1.06±0.26*	1.05±0.19*
Diabetes + Extract	88.69±5.57#	127.88±9.33*#	1.89±0.29#	0.40±0.12#

Given data are mean ± SEM for ten animals in each group. Values are statistically significant at P<0.05.
*Significantly different from the control rats; #significantly different from the diabetes control rats.

Table 4: Effect of *Phaseolus vulgaris* pods extract on the activities of the glutathione-dependent enzymes in liver and kidney.

Group	GPx ($\mu mol\ GSH*min^{-1}*mg\ prot.^{-1}$)		GST ($\mu mol\ CDNB*min^{-1}*mg\ prot.^{-1}$)		GR ($\mu mol\ NADPH*min^{-1}*mg\ prot.^{-1}$)	
	liver	kidney	liver	kidney	liver	kidney
Control	4.08±0.36	6.01±0.22	2.76±0.17	0.66±0.05	0.56±0.05	0.66±0.08
Control + Extract	4.80±0.29	5.19±0.28	2.62±0.26	0.61±0.03	0.57±0.06	0.69±0.05
Diabetes	2.43±0.31*	3.67±0.16*	2.07±0.15*	0.42±0.06*	0.65±0.06	0.95±0.09*
Diabetes + Extract	2.85±0.13*	4.55±0.12*#	2.69±0.17	0.86±0.05*#	0.55±0.04	0.99±0.12*

Given data are mean ± SEM for ten animals in each group. Values are statistically significant at P<0.05.
*Significantly different from the control rats; #significantly different from the diabetes control rats.

A key pathogenic indicator of long-term hyperglycemia is the level of glycosylated hemoglobin (HbA_{1c}) (Voĭtenko *et al.*, 2013). Our results showed that both groups of diabetic animals had a significantly higher level of HbA_{1c} in comparison with the control group (Fig 1, B). However, HbA_{1c} content in rats treated with aqueous extract from *P. vulgaris* pods was decreased by 20% compared with untreated diabetic group. The level of HbA_{1c} in *P. vulgaris* treated control rats was found to be similar to that in the group of untreated control rats. Thus, it can be argued that *P. vulgaris* pods extract affects glucose metabolism only under conditions of high glucose concentration that occurs in diabetes.

Chronic hyperglycemia causes a number of metabolic disorders, which in turn can contribute to functional dysfunction of different organs. In our research we focused on the investigation of liver and kidney functional state as particularly important organs involved in development and progression of diabetes and diabetes-related diseases. Liver is a central inner organ where the metabolic processes take place. Liver along with skeletal muscle and adipose tissue is a major consumer of insulin and play a key role in the metabolism of carbohydrates (Tolman *et al.*, 2007). Liver dysfunction may cause or contribute to development of diabetes complications. One of the most prominent diabetic complications is nephropathy. This kidney condition occurs only in people with DM and results in progressive damage of glomeruli (small filtering units of kidney). This, eventually, leads to increased amount of protein in urine, high blood pressure and declining kidney function. Diabetic nephropathy is an important cause of kidney failure (Anil Kumar *et al.*, 2014).

In order to examine the effect of *P. vulgaris* pods extract on the hepatic and renal functions serum levels of liver and kidney biochemical markers were determined (Table 1). There are three liver enzymes, which are commonly included in serum chemistry screening profiles: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT). Measured levels of these enzymes in the serum of all experimental groups of rats are shown in Table 1. Significant elevation in levels of ALT, AST and GGT were observed in serum of the untreated diabetic rats compared to values in control group. The elevation of the serum enzyme levels attributed to liver dysfunction may result from rupture of hepatocytes, as far as it is well known that damage or destruction of liver cells leads to release of marker enzymes into the bloodstream, which subsequently leads to necrosis or changes in cell membrane permeability (Adeyemi *et al.*, 2014).

Creatinine, blood urea level and uric acid are markers for routine analysis of the renal function. These parameters can be used as indicators of normal biological or pathologic processes in kidney as well as the way of monitoring renal disease progression. Data represented in Table 1 show that the value of creatinine was decreased while the value of uric acid was significantly increased in serum of untreated diabetes rats compared to control group. The level of blood urea was statistically the same for both groups. A high level of uric acid in the blood, or hyperuricemia, is associated with diabetes conditions and due to reduced excretion by the kidneys or other deficiencies of renal functions (Gowda *et al.*, 2010). The decreased value of creatinine was also reported in kidney disease (Almdal *et al.*, 1988). Above listed changes of studied biochemical parameters in diabetic animals bear evidence of renal failure development.

In the diabetic rats treated with *P. vulgaris* pods extract serum levels of ALT, AST, GGP and uric acid were significantly lower compared to untreated diabetic rats. However, it was observed that creatinine level in extract treated diabetic rats was similar to that in rats from untreated diabetic group when blood urea value was elevated by 43% compared with untreated diabetic group. Studied biochemical parameters of extract treated control rats were not statistically different from untreated control group with exception of GGT level that was decreased in 1.5 times compared with level of untreated control group of rats. Based on our results it is clear that the *P. vulgaris* pods extract had a positive influence on the blood biochemical parameters in diabetic rats.

One of the most important factors in the development of diabetic complications is increased lipid peroxidation level. Elevated endogenous peroxides may initiate uncontrolled lipid peroxidation, which leads to cellular infiltration and cell damage. Table 2 shows concentration of MDA, Schiff bases and conjugated dienes, parameters that characterize intensity of free radical production, in liver and kidney tissues of all experimental groups. There was a significant elevation in tissues MDA, conjugated dienes and Schiff bases in diabetes compared to the control group. In the diabetic rats treated with aqueous extract of *P. vulgaris* pods tissues levels of MDA, conjugated dienes and Schiff bases were significantly lower compared to those in the group of untreated diabetic rats, but some parameters (liver MDA, Schiff bases in liver and kidney) were higher than control data.

Free radical homeostasis in cells and tissues is ensured by balance between enzymatic and non-enzymatic systems of generating ROS and systems of their neutralization (Ahmed *et al.*, 2005). Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of superoxide radical (O^{2-}) and thus protects membranes and other cellular structures from oxygen free radicals (Otitoju *et al.*, 2008). Catalase is a hemoprotein that catalyzes the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals (Rjasekaran *et al.*, 2005). Together, SOD and catalase are the two major enzymes that remove the toxic free radicals *in vivo*.

Table 3 illustrates the activities of SOD and catalase in liver and kidneys in examined groups of rats. We observed significant reduction in activity of SOD and catalase in untreated diabetic rats compared with control. Reduced activity of the antioxidant enzymes could be a result of their modification with glucose and ROS under DM conditions. Interestingly, catalase activity in kidney of diabetic rats was raised in 1.7 times compared with its activity in kidney of control rats. Catalase over activation under diabetes condition can be explained by compensation of decreased SOD activity. In the group of diabetic rats treated with aqueous *P. vulgaris* pods extract liver catalase activity and SOD activities in kidney were significantly higher whereas the kidney

catalase activity was lower compared to untreated diabetic rats. Moreover, we found no significant differences in studied enzymes activities between extract treated diabetic group and control groups.

Another important player in regulation of free radical disposal is glutathione system. Reduced glutathione (GSH) is one of the main components of antioxidant defense system capable to react with the products of lipid peroxidation (Chugh *et al.*, 1999). GSH maintaining carried out by several glutathione-dependent enzymes.

Glutathione peroxidase (GPx) eliminates products of lipid peroxidation catalyzing glutathione oxidation and subsequent hydrogen peroxide deactivation. The reverse glutathione reduction is ensured by glutathione reductase (GR). Glutathione-S-transferase (GST) carries out reduction of macromolecular hydrophobic hydrogen peroxides such as polyunsaturated fatty acids using reduced glutathione (Cnubben *et al.*, 2001; Hayes and McLellan, 1999).

Activity of glutathione-dependent enzymes in liver and kidney are represented in Table 4. There was a significant reduction in activity of GPx and GST in liver of diabetic rats in comparison with control group of rats, but no significant difference was observed between liver GR activities of these experimental groups. Comparing the glutathione-dependent enzymes activities in kidney tissue of untreated diabetic and control animals we observed that the both GPx and GST activities were reduced in the group of diabetic rats. GR activity was higher in comparison with control. In the group of diabetic rats treated with *P. vulgaris* pods extract, activities of GPx and GST in kidney were significantly higher compared to untreated diabetic rats. It must be noted that kidney GST activity was significantly higher in extract treated diabetic rats compared to control values. We did not observe any difference between GR activity in liver as well as in kidney of treated diabetic rats in comparison with untreated diabetic rats.

CONCLUSION

Plants are extensively used by traditional medicine trough out the world for relieving diabetes mellitus symptoms. In Eastern European countries *P. vulgaris* due to its natural abundance in this geographic area has been used for same purpose since early times. Our experimental data in accordance with previous studies performed on animals (Román-Ramos *et al.*, 1991) demonstrated that long-term administration of *P. vulgaris* pods extract decreases blood glucose level in fasting diabetic rats. We also showed that aqueous extract of this plant has a positive influence on the number of other blood biochemical parameters. Moreover, it was revealed that *P. vulgaris* pods extract modulates free radical production and activates antioxidant enzymes under diabetic conditions. This activity contributes to the protection against oxidative damage in streptozotocin-induced diabetes. Taking all together, one may suggest that long-term oral administration of *P. vulgaris* pods extract might have beneficial effect in treatment of diabetes.

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