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Original article

Influences of *Physalis peruviana* L. and *Lupinus albus* L. fruits extracts on the levels of some biochemical parameters in brain and muscle tissues of type II diabetic rats

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Summary. New functional foods are increasingly sought to improve the treatment of diseases related to glucose and lipid metabolism. The present study was designed to investigate the possible antidiabetic and antioxidant effects of goldenberry and lupin on brain and muscle tissues of streptozotocin-induced type II diabetic rats. Type II diabetes was produced in albino rats by the streptozotocin injection. Wistar albino rats were divided into four groups, each one containing 10 rats: non-diabetic control group, STZ- type II Diabetes group, STZ- type II Diabetes+goldenberry group, and STZ- type II Diabetes+lupin group. After one week from the injection, goldenberry and lupin were injected to rats for 2 months. Malondialdehyde, glutathione, cholesterol, and fatty acid levels, which are signs of lipid peroxidation, were measured in brain and muscle tissues. In type II diabetes, malondialdehyde increased compared to the control group. Glutathione decreased in the both tissues and all of the streptozotocin-induced diabetic groups. In type II diabetes, brain cholesterol levels decreased. Treatment with similar doses of goldenberry and lupin significantly reduced oxidative stress, augments antioxidant system and altered fatty acid metabolism in these tissues, thereby maintaining favourable fatty acid distribution affected by diabetic complications. These results validate the use of goldenberry and lupin fruits as a treatment against diabetes mellitus and its complications and suggest it is suitable to continue studies for its safe therapeutic use.

Key words: diabetes mellitus, goldenberry, lupin, malondialdehyde, glutathione, brain, muscle

Introduction

Prolonged hyperglycemia is a primary cause of most long-term complications of diabetes. Miserable suffering of the diabetic patients is attributed not only to the hyperglycemic state, but also to life-threatening complications associated with the disease. These disorders can cause short-term and long-term effects which can cause physiological damage to various organs of the body (1). Microvascular (retinopathy and nephropathy) and macrovascular (atherosclerotic) disorders will develop if long-term diabetes is not effectively controlled (2, 3).

The disease is generally broken down into two major groups as insulin dependent and non-insulin dependent diabetes (4). Among all the cases of diabetes, type-II diabetes was found to be more prevalent. The pernicious effects of diabetes have been found to be mediated through oxidative stress. Oxidative stress is associated with increased production of reactive oxygen species and impaired antioxidant defense systems, which cause lipid peroxidation, alteration in antioxidant enzymes and impaired glutathione metabolism (5, 6).

Many hypoglycaemiant agents, such as biguanides and sulfonylureas, are used separately or with insulin

to treat this disease. However, these medications can cause serious side effects. These challenges have motivated researchers to find new efficacious molecules to control diabetes that cause fewer side effects in old and new sources of natural drugs (plants) (7, 8). Before the discovery of anti-diabetic drugs and insulin, diabetic patients used medicinal plants and traditional medicine (9). Medicinal plants play an important role in the treatment of diabetes mellitus, especially in the developing countries due to their cost effectiveness. Plants may operate through different mechanisms that effect on blood sugar. Some of them may have insulin kinase, some other may inhibit insulinase activity and others may increase reconstruction of pancreatic β cells (10-12).

There are known herbs and secondary metabolites that are effective in diabetes management and control. Therefore, the present study aims to examine the influence of *Physalis peruviana* and *Lupinus albus* fruits on the levels of some biochemical parameters (malondialdehyde, glutathione, total protein, fatty acid, cholesterol and vitamin) in muscle and brain of streptozotocin-induced diabetic Wistar albino rats.

Materials and methods

Animals

Animals, experimental design, and experimental protocols were approved by the local Animal Experiments Ethics Committees of Firat University (Elazig, Turkey). Animal care and experimental protocols were compatible with the NIH Guide for the Care and Use of Laboratory Animals (NIH publication no. 30.06.2011/101). Thirty-seven healthy adult male Wistar albino rats, aged 8-10 weeks were obtained from Firat University Experimental Research Centre (Elazig, Turkey). The animals were housed in the polycarbonate cages in a room with a 12-h day-night cycle, temperature of 24±3°C, and humidity of 45% to 65%. During the whole experimental period, the animals were fed with a balanced commercial diet (Elazig Food Company, Elazig, Turkey) *ad libitum*.

Experimental design

The first group was used as the control group (n=7) and the others were as follows: second group, Diabetes (n=10); third group, Diabetes+ *Physalis peruviana* group (D+PP) (n=10); fourth group, Diabetes+ *Lupinus albus* group (D+LA) (n=10) groups were made diabetic by a single intraperitoneal injection of 40 mg/kg streptozotocin (STZ) in citrate buffer (pH 4.5). Control group rats were injected intraperitoneally with buffer alone. One week after administration of STZ, the tail vein blood glucose level of all the animals was measured. Blood glucose levels of 140-200 mg/dl were considered as diabetic. The rats in D+PP group were injected intraperitoneally with 1 ml/kg *P. peruviana* fruit extract. Also the addition of 2 gr *P. peruviana* extract added to 500 ml drinking water was administered to the rats two times per week. The rats in D+LA group were injected intraperitoneally with 1 ml/kg *L. albus* fruit extract. Also the addition of 2 gr *L. albus* extract added to 500 ml drinking water was administered to the rats two times per week. These treatments continued for 8 weeks and after this period, each experimental rat was anesthetized with ether. Muscle and brain tissue samples were dissected and stored at -85°C prior to biochemical analyses.

Homogenate preparation

Tissue samples were homogenized in Tris-HCl buffer (pH 7.5) and centrifuged at 9000xgfor 20 min at 4°C. Supernatants were collected, aliquoted, and stored at -70°C until use. The supernatant obtained from the TBARS, reduced glutathione and total protein analysis, the pellets ADEK vitamins, cholesterol, and fatty acid analysis was performed.

Determination of MDA-TBA level

Lipid peroxides (TBARS) in tissues homogenate were estimated using thiobarbituric acid reactive substances by the method of Ohkawa et al. (13). To 1,0 ml tissue homogenate, 0,5 ml of 8,1% SDS, 1,0 ml of (20% acetic acid/NaOH pH 3,5), 1,0 ml of 10% TCA, 50 µl of 2% BHT and 1,0 ml of 0,8% TBA were added. The mixture was heated in a water bath at 95°C for 60 min. After cooling, 4 ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4250 rpm for 15 min, the organic layer was taken and its absorbance at 532 nm was measured. 1.1.3.3-tetramethoxypropane was used as standard. The resulting nmol/g tissue was calculated.

Determination of GSH level in tissue samples

Reduced glutathione (GSH) was determined by the method of Ellman (14). Briefly, 1 ml tissue homogenate was treated with 1 ml of 5 trichloroacetic acid (% 10) (Sigma, St. Louis, MO), The mixtures were centrifuged at 5000 rpm and the supernatant was taken. After deproteinization, the supernatant was allowed to react with 1 ml of Ellman's reagent (30 mM 5, 5'-dithiobisnitro benzoic acid in 100 mL of 0.1% sodium citrate). The absorbance of the yellow product was read at 412 nm in spectrophotometer. Pure GSH was used as standard for establishing the calibration curve.

Lipid extraction

Lipid extraction of tissue samples were extracted with hexane-isopropanol (3:2 v/v) by the method of Hara and Radin (15). A tissue sample measuring 1 g was homogenized with 10 ml hexane-isopropanol mixture. Fatty acids in the lipid extracts were converted into methyl esters including 2% sulphuric acid (v/v) in methanol (16).

The fatty acid methyl esters were extracted with 5 ml n-hexane. Analysis of fatty acid methyl ester was performed in a Shimadzu GC-17A instrument gas chromatograph equipped with a flame ionization detector (FID) and a 25 m, 0.25 mm i.d.permabond fused-silica capillary column (Macherey-Nagel, Germany). The oven temperature was programmed between 145-215°C, 4°C/min. Injector and FID temperatures were 240 and 280°C, respectively. The rate of nitrogen carrier gas was at 1 ml/min. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. *Class GC 10* software version 2.01 was used to process the data. The resulting mg/g tissue was calculated.

Saponification and extraction

Alpha-tocopherol and cholesterol were extracted from the lipid extracts by the method of Sanchez-Machado et al. (17) isopropyl alcohol mixture was treated with 5 ml of KOH solution (0.5 M in methanol), which was immediately vortexed for 20 s. The tubes were placed in a water bath at 80°C for 15 min. Then after cooling in iced water, 1 ml of distilled water and 5 ml of hexane was added, and the mixture was rapidly vortexed for 1 min, then centrifuged for 5 min at 5000 rpm. The supernatant phase was transferred to another test tube and dried under nitrogen. The residue was re-dissolved in 1 ml of the HPLC mobile phase (68:28:4 (v/v/v) methanol:acetonitrile:water). Finally, an aliquot of 20 µL was injected into the HPLC column. Before injection, the extracts were maintained at -20°C away from light.

Total protein assay

Total protein contents in brain tissue were determined as Lowry's method described. The procedure for measuring protein was followed according to Lowry et al. (18) using BSA (Bovin serum albumin) as standard. The absorbance was read at 750nm using a spectrophotometer.

Statistical analysis

One-way analysis of variance (ANOVA) and Post Hoc Tukey-HSD test were used to determine differences between groups. Results are presented as mean ± S.E.M. Values were considered statistically significant if $p<0.05$. The SPSS/PC program (Version 15.0; SPSS, Chicago, IL) was used for the statistical analysis.

Results

Values of muscle tissue in type-2 diabetes

The levels of MDA, GSH and total protein in the muscle tissue of control and diabetic rats are presented in the Table 1. Compared to control group, a significant increase was observed in MDA level in the diabetic group ($p<0.001$). While a partial decrease was found in D+PP group ($p<0.05$), any difference did not occur in D+LA group ($p>0.05$). As diabetic group was compared with D+PP and D+LA groups, a significant decreasing difference was determined between both groups ($p<0.001$).

GSH level in the diabetic group had a significant difference in decreasing tendency compared to control group ($p<0.01$) (Table 1). While a partial increase was observed in D+LA group ($p<0.05$), a significant increase was observed in D+PP group ($p<0.01$). It was seen that there was a significant difference in increas-

Table 1. The MDA, GSH and protein levels in the muscle tissue of control and type II diabetic rats

Groups	MDA (nmol/g)	GSH (µg/g)	Protein (µg/g)
C	3,94±0,17	199,97±7,77	3,53±0,24
D	6,85±0,22[d]	171,21±9,21[c]	2,75±0,37[c]
D+PP	4,55±0,11[b]	228,21±5,55[c]	3,07±0,24[b]
D+LA	4,90±0,24[a]	205,13±1,81[b]	3,60±0,48[a]

a: p>0.05, b: p<0.05, c: p<0.01, d: p<0.001

ing tendency between diabetic group and D+PP and D+LA groups (p<0.001) (Table 1).

It was found that protein levels significantly decreased in the diabetic group compared to control group (p<0.01), partially decreased in the D+LA group compared to control group (p<0.05), and had no difference between in the control group and D+PP group (p>0.05). When diabetic group was compared to D+PP and D+LA groups, a distinct increase was determined between both groups (p<0.001) (Table 1).

Table 2 shows vitamins A, D, E, and K and cholesterol levels in muscle tissue of control and type II diabetic rats. Compared to control group, K-1, K-2, D-2, and D-3 levels decreased in the diabetic group. A significant difference was seen in α-tocopherol, δ-tocopherol, cholesterol and retinol levels (p<0.001, p<0.01, respectively). D-2 and δ-tocopherol levels increased in D+LA group. Compared to control and diabetic groups retinol amounts decreased significantly in plant extract groups (p<0.001). An evident difference in K-1, K-2, D-2, and retinol levels (p<0.01) were seen in D+PP group. Compared to diabetic group, a significant increase in D-2 level (p<0.01) and a decrease significantly in α-tocopherol, δ-tocopherol and retinol levels (p<0.001) were observed in D+PP group. In D+LA group, there was a significant decrease in levels of K-2, α-tocopherol, δ-tocopherol, cholesterol and retinol (p<0.01); whereas, a relative increase in D-2 and D-3 levels was determined.

Table 3 shows fatty acid levels in muscle tissue of control and type II diabetic rats. Compared to control group, there were an evident increase in 18:0 (Stearic acid), 18:2 n-6 (Linoleic acid) levels (p<0.001) and 16:0 (Palmitic acid), 16:1 n-7 (Palmitoleic acid) (p<0.01), 18:1 n-9 (Oleic acid) (p<0.001), 20:4 n-6 (Arachidonic acid) and 22:6 n-6 (Docosahexaenoic acid) levels (p<0.05) relative decrease in the diabetic group. In D+PP group, an evident increase in 18:0 level was observed (p<0.01); whereas, a significant decrease was determined in 16:1 n-7, 18:2 n-6, 20:4 n-6, and 22:6 n-6 levels (p<0.001). A partial decrease was found in 16:1 n-7 and 18:1 n-9 levels (p<0.05). 20:3 n-6 level was determined to get close to C group (p>0.05). While 16:0, 16:1 n-7, 18:1 n-9, 18:2 n-6, 20:4 n-6 levels significantly decreased in D+LA group (p<0.001), 20:3 n-6 level got close to C group (p>0.05). Compared to diabetic group, a significant difference in 20:3 n-6 and 22:6 n-6 levels (p<0,01) and an evident difference in 16:0,16:1 n-7, 18:0, 18:1 n-9,18:2 n-6 and 20:4 n-6 levels (p<0,001) were observed in D+PP group. In D+LA group, 16:0, 16:1 n-7, 18:0, 18:1 n-9,

Table 2. The A, D, E, K vitamins and cholesterol levels in the muscle tissue of control and type II diabetic rats (µg/g)

Vitamins	C	D	D+PP	D+LA
K-1	0,52±0,20	0,38±0,07[c]	0,25±0,04[c]	0,40±0,04[b]
K-2	0,60±0,31	0,32±0,10[d]	0,38± 0,13[c]	0,20±0,10[d]
α-Tocopherol	7,60±0,48	9,21±0,34[c]	6,92±0,48[a]	6,54±0,47[a]
δ-Tocopherol	0,08±0,15	0,28±0,04[d]	0,08±0,02[a]	0,26±0,15[d]
D-2	0,18±0,13	0,15±0,04[b]	0,35±0,10[c]	0,40±0,17[d]
D-3	0,32±0,07	0,30±0,04[a]	0,32±0,06[a]	0,38±0,09[b]
Cholesterol (µmol/g)	0,86±0,06	0,98±0,02[c]	0,89±0,03[a]	0,88±0,04[a]
Retinol	0,36±0,13	0,48±0,01[d]	0,15±0,04[c]	0,11±0,02[d]

a: p>0.05, b: p<0.05, c: p<0.01, d: p<0.001

Table 3. The fatty acid levels in the muscle tissue of control and type II diabetic rats (mg/g)

Fatty Acids	C	D	D+PP	D+LA
16:0	4,98±0,72	3,13±0,25[b]	1,62±0,08[c]	1,35±0,12[d]
18:0	0,27±0,25	2,92±0,17[d]	0,44±0,03[c]	0,45±0,02[c]
∑SFA	5,25±0,97	6,05±0,42	2,06±0,11	1,80±0,14
16:1, n-7	1,91±0,21	0,88±0,13[c]	0,39±0,03[d]	0,44±0,03[d]
18:1, n-9	4,88±0,45	1,97±0,21[d]	3,05±0,23[b]	2,61±0,19[c]
∑MUFA	6,79±0,66	2,78±0,44	3,44±0,26	3,05±0,22
18:2, n-6	5,82±0,27	8,14±3,98[d]	3,21±0,23[c]	2,78±0,19[d]
20:3, n-6	0,12±0,01	0,11±0,01[a]	0,13±0,02[a]	0,10±0,04[a]
20:4, n-6	1,46±0,14	1,26±0,05[b]	0,68±0,03[c]	0,43±0,07[d]
22:6, n-3	0,58±0,05	0,53±0,03[b]	0,35±0,01[d]	0,50±0,23[c]
∑PUFA	7,98±0,47	10,04±4,07	4,37±0,29	3,81±0,53
∑USFA	14,77±1,13	12,82±4,51	7,81±0,55	6,85±0,58

a: p>0.05, b: p<0.05, c: p<0.01, d: p<0.001

18:2 n-6 and 20:4 n-6 levels decreased significantly (p<0,01); whereas, an evident difference was not found in 20:3 n-6 and 22:6 n-3 levels (p<0.001).

Values of brain tissue in type-2 diabetes

The levels of MDA, GSH and total protein in the brain tissue of control and diabetic rats are presented in the Table 4. Compared to control group, a significant increase was observed in MDA level in the diabetic group (p<0.001). While a partial decrease was found in D+PP group (p<0.05), a significant decrease was observed in D+LA group (p<0.01). When diabetic group was compared with D-PP and D+LA groups, a significant decreasing was determined between both groups (p<0.001) (Table 4).

GSH level in the diabetic group had a significant difference in decreasing tendency compared to control group (p<0.001). While a partial decrease was observed in D+PP group (p<0.05), any difference did not occur in D+LA group (p>0.05). It was seen that there was a significant difference in increasing tendency between diabetic group and D+PP and D+LA groups (p<0.001) (Table 4).

It was found that protein levels increased in the diabetic group compared to control group (p<0.05), significantly decreased in D+PP and D+LA groups

Table 4. The MDA, GSH and protein levels in the brain tissue of control and type II diabetic rats

Groups	MDA (nmol/g)	GSH (µg/g)	Protein (µg/g)
C	7,71±0,36	278,99±0,64	3,67±0,80
D	10,63±0,26[d]	246,18±0,78[d]	4,03±0,13[b]
D+PP	6,40±0,28[b]	255,63±0,37[b]	2,95±0,10[b]
D+LA	5,79±0,34[c]	281,44±0,90[a]	2,58±0,12[c]

a: p>0.05, b: p<0.05, c: p<0.01, d: p<0.001

(p<0.05). No statistical difference was observed between the D+PP and D+LA groups (p>0.05). When diabetic group was compared to D+PP and D+LA groups, a distinct decrease was determined between both groups (p<0.001) (Table 4).

Table 5 shows vitamins A, D, E, K and cholesterol levels in brain tissue of control and type II diabetic rats. While a significant decrease was observed in cholesterol level of diabetic group compared to control group (p<0.01), a significant difference was determined for its K-1, K-2 and α-tocopherol levels (p<0.01, p<0.05, p<0.05, respectively). D-3 level was found to get closer to values of group C (p>0.05). Compared to control group, retinol level increased in the diabetic group (p<0.001). An evident difference in K-1 and K-2 levels (p<0.01) were seen in D+PP group. However, com-

Table 5. The A, D, E, K vitamins and cholesterol levels in the brain tissue of control and type II diabetic rats (µg/g)

Vitamins	C	D	D+PP	D+LA
K-1	6,28±0,30	7,06±0,14[c]	7,21±0,17[c]	6,42±0,19[a]
K-2	9,15±0,18	10,14±0,18[b]	11,27±0,26[c]	9,18±0,19[a]
α-Tocopherol	13,77±0,44	14,29±0,28[b]	13,44±0,41[a]	12,48±0,52[b]
D-3	1,02±0,44	0,99±0,94[a]	1,24±0,09[a]	1,07±0,13[a]
Cholesterol (µmol/g)	8,32±0,50	6,98±0,17[c]	7,94±0,23[a]	7,70±0,38[b]
Retinol	0,21±0,04	1,30±0,14[d]	0,24±0,02[a]	0,23±0,02[a]

a: p>0.05, b: p<0.05, c: p<0.01, d: p<0.001

pared to control group, α-tocopherol, D-3, cholesterol and retinol levels not significant difference in D+PP group (p>0.05). While a partial decrease was observed in α-tocopherol and cholesterol levels (p<0.05), any difference did not occur K-1, D-3 and retinol levels in D+LA group (p>0.05).

Compared to diabetic group, it was seen that α-tocopherol and retinol levels in D+LA group significantly decreased (p<0.01, p<0.001, respectively), there was not a significant difference in K-2 and D-3 levels (p>0.05). In D+PP group, K-2 and cholesterol levels were observed to increase significantly (p<0.01), K-1 and D-3 levels had not a significant difference (p>0.05). Compared to diabetic group, K-2 and cholesterol levels increased in the D+PP group (p<0.01, p<0.001, respectively).

Table 6 shows fatty acis levels in brain tissue of control and type II diabetic rats. Compared to control group, a significant increase was observed in 16:1 n-7 (Palmitoleic acid), 18:0 (Stearic acid), 18:2 n-6 (Linoleic acid), 20:4 n-6 (Arachidonic acid) and 22:6 n-3 (docosahexaenoic acid) levels in diabetic group (p<0.01, p<0.05). It was determined that changes observed in 18:1 n-9 (Oleic acid) and 16:0 (Palmitic acid), levels were statistically insignificant (p>0.05). While a significant increase was observed in 16:0 and

Table 6. The fatty acid levels in the brain tissue of control and type II diabetic rats (mg/g)

Fatty Acids	C	D	D+PP	D+LA
16:0	2,10±0,08	2,56±0,22[a]	3,08±0,14[d]	3,14±0,12[d]
18:0	2,09±0,15	2,33±0,29[b]	3,00±0,39[d]	3,07±0,12[d]
∑SFA	4,19±0,23	4,89±0,51	6,08±0,50	6,21±0,24
16:1, n-7	0,11±0,01	0,17±0,01[b]	0,20±0,02[c]	0,22±0,02[c]
18:1, n-9	2,68±0,51	2,00±0,40[a]	3,72±0,13[d]	3,79±0,12[c]
∑MUFA	2,79±0,52	2,89±0,41	3,92±0,15	4,01±0,14
18:2, n-6	0,10±0,01	0,13±0,01[b]	0,17±0,01[c]	0,13±0,01[b]
20:3, n-6	0,10±0,04	0,08±0,01[b]	0,14±0,01[c]	0,11±0,01[a]
20:4, n-6	1,69±0,12	1,99±0,01[b]	2,19±0,22[c]	2,56±0,10[d]
22:6, n-3	2,65±0,22	3,24±0,15[b]	3,35±0,25[c]	4,18±0,13[d]
∑PUFA	4,54±0,30	4,55±0,18	5,85±0,49	6,98±0,248
∑USFA	7,33±0,91	7,44±0,60	9,77±0,64	10,99±0,398

a: p>0.05, b: p<0.05, c: p<0.01, d: p<0.001

18:1 n-9 amounts in D+PP group ($p<0.001$), a evident difference in 16:1 n-7, 18:2 n-6, 20:3 n-6, 20:4 n-6 and 22:6 n-6.While a significant difference was seen in 16:0, 18:0,18:1 n-9, 20:4 n-6 and 22:6 n-3 levels for D+LA group ($p<0.001$), 16:1 n-7 and 18:2 n-6 levels were determined to have a partial increase ($p<0.05$). Changes observed in 20:3 n-6 level were determined to be statistically insignificant ($p>0.05$).

Compared to diabetic group, it was seen that there was a distinct difference 20:3 n-6 level ($p<0.001$) and a significant difference in 16:0, 16:1 n-7, 18:0, 18:1 n-9, 18:2 n-6 and 20:4 n-6 levels ($p<0.01$) in D+PP and D+LA groups. Compared to diabetic group, an important difference in 22:6 n-3 level ($p<0,001$) were found in D+LA group (Table 6).

Discussion

Epidemiological studies and *in vitro* and *in vivo* tests in animals and humans show that diets based on the consumption of vegetables can have a hypoglycemic effect and reduce the risk of chronic diseases (19-21). This action is exerted by biologically active substances such as proteins, oils, dietary fibres, phytosterols and saponins (22-25).

Lupinus albus, is the one that has the highest protein content in its composition apart from being a good source of fibres (26), giving it great potential for consumption. *L. albus* exerted hypoglycemic effects and an increase in the level of serum insulin in normal and diabetic subject as well as in normal and alloxan-diabetic animals (27-29). Many people are using *L. albus* as a part of their integral food as well as hypoglycemic agents. *Physalis peruviana* has called attention to increased interest worldwide due to its nutritional composition and the presence of biologically active compounds that provide health benefits and reduce the risks of certain diseases such as cancer, malaria, asthma, hepatitis and rheumatism (30, 31). *P. peruviana* fruit contains 15% soluble solids, mainly sugars and its high level of fructose makes it valuable for people with diabetes (32). *Physalis* polyphenols may, therefore, prevent the damage and death of pancreatic β-cells and/or stimulate the regeneration of this type of cells in diabetic rats. It has been reported that the administration of polyphenols, such as quercetin and epicatechin, to surviving diabetic rats protect the architecture of pancreatic β-cells, preserves the secretion of insulin and stimulates the regeneration of this type of cells (33,34).

In the present study, we showed that *P. peruviana* and *L. albus* fruits could influence on the levels of some biochemical parameters (malondialdehyde, glutathione, total protein, fatty acid, cholesterol and vitamin) in muscle and brain of streptozotocin-induced diabetic Wistar albino rats.

The chronic hyperglycemias cause an imbalance in the oxidative status of the nervous tissue, and the resulting free radicals damage the brain through a peroxidative mechanism. In diabetes, brain tissue is more vulnerable to oxidative stress (35, 36). Oxidative stress that leads to an increased production of reactive oxygen species (ROS) and finally cellular lipid peroxidation (LPO) has been found to play an important role in the development of diabetes mellitus LPO is one of the cellular features of chronic diabetes (37).

Reactive oxygen species and the end products of advanced glycosylation are significant in the onset and development of complications in chronic diabetes (38). It has been suggested that cell membrane lipid peroxidation is involved in the etiology of neurodegenerative diseases; lipid peroxides may cause oxidative damage to the myelin sheath surrounding the nerve. Therefore, oxidative stress may predispose diabetic patients to the development of neuropathy by a mechanism involving increased lipid peroxidation (39). The brain exhibits numerous morphological and functional alterations during diabetes. Muscle is susceptible to oxidative deterioration due to a combination of lipid oxidation catalysts and membrane lipid systems that are high in unsaturated fatty acid. To prevent or delay oxidation reactions, several endogenous antioxidant systems are found in muscle tissue.

The results of the present study revealed that while a distinct increase of MDA was determined in diabetic groups compared to control group, it generally decreased in other tissues. Lipids are particularly vulnerable to oxidation because the brain is rich in polyunsaturated fatty acids (40). Additionally, then it was observed that MDA levels in D+PP and D+LA groups had a value close to control group and a decrease compared to diabetic group after the adminis-

tration. We suggest that the induction of antioxidant enzymatic and non-enzymatic defense systems and suppression of MDA by *Physalis* could be effective in preventing apoptosis activation which might be supported by previous finding (33,41,42). In a study conducted on goldenberry, malondialdehyde (MDA) levels of diabetics were examined and positive outcomes were obtained as a result of administration. The effect of *Physalis* was shown upon effect of anti-free radicals in pancreatic beta cells (33).

Oxidative stress acts a main role in the complications and the pathogenesis of diabetes. Hyperglycemia leads to overproduction of oxygen free radicals, which contributes to the complications of diabetes and its progression. Several investigations have shown that STZ produces imbalance between plasma oxidant and antioxidant content, resulting in the progression of diabetes and its complications (44). The results of the present study revealed that the level of GSH in diabetic group was lower than the control group. However, GSH level was high in the plant extract groups. The progression of variable measurements in STZ-diabetic rats after plant extracts treatment might offer a protective influence of *Physalis* and *Lupinus* against STZ action that could be induced through suppression of oxygen free radicals mediated by STZ. The GSH reacts with free radicals and is a vital substrate for glutathione peroxidase and glutathione-S-transferase, which participate in the cellular defense mechanisms against intermediate oxygen products (45, 46). When the GSH molecule neutralizes the free radicals, the GSH molecule is converted to oxide form (GSSG). The GSSG is again converted to GSH use to NADPH by the GSH reductase. The conservation and formation of NADPH in the cells are realized by the activity of pentose-phosphate pathway and malic enzyme (47). With an insufficient insulin level, the activities of glucose-6-phosphate dehydrogenase in pentose phosphate shunt decreased and glutathione reductase led to impairment of GSH regeneration and increased the level of GSSG. Declined GSH level in the diabetic group may be associated with the lack of insulin. Increased GSH level in the plant extract groups may be associated with the consequences of antioxidants.

Compared to the control group, lipophilic vitamins and cholesterol level significantly changed in the diabetic group. Lipophilic vitamins are necessary to maintain the physiological functions of the organism, support immune and growth activity and also undertake an important role in stimulating digestion and synthesis. In addition, several studies tend to concur that vitamin deficiency increases in some diseases (48).

The polyunsaturated fatty acids in cholesterol esters, phospholipids, and triglycerides are subject to free radical-initiated oxidation and contribute in chain reactions that amplify damage to biomolecules. Brain tissue is rich in cholesterol. In the present study the level of cholesterol in the brain tissue of diabetic rats decreased compared to the control group, and the plant extract in the brain tissue of diabetic rats increased levels of cholesterol. Earlier studies showed that diabetes produces a global suppression of the enzymes of cholesterol synthesis and their master transcriptional regulator SREBP-2 in the brain. *P. peruviana* and *L. albus* application may be inhibitive or stimulative effects on the activities of the enzymes responsible for the transcription of cholesterol which result in differences in cholesterol quantities. Sirtori et al. (25), reported that lupin proteins were capable of stimulating the activity of LDL receptors and increasing the capture of LDL from the plasma to the cells. On the other hand, the inhibition of HMG-CoA reductase, a key enzyme in the synthesis of cholesterol, regulated by the action of SREBP-2, could also reduce the concentration of LDL cholesterol in plasma (49). Bettzieche et al. (50), described distinctive effects for different species of lupin proteins in the lipid metabolism. The cultivar vitabor of lupin (*Lupinus angustifolius* L.) administered to rats reduced the triglycerides and total cholesterol through the reduction of the expression of genes SREBP-1c and HMG-CoA reductase (22). Martins et al. (52) who administered whole lupin (*L. angustifolius*) to pigs as experimental models, also reported a reduction of cholesterol and an increase in the excretion of cholesterol and bile acids in the feces caused by the components of legumes such as fibers and phytosterols.

The mammalian central nervous system contains a high proportion of PUFAs, and significant changes in brain lipid composition occur with aging and in conditions such as diabetes and neurological disorders. The results of the present study revealed that the levels of palmitic and stearic acid in D, D+PP and D+LA

groups was higher than the control group in brain tissue. Expression of this increase may be due to a lack of insulin. Because the palmitic and stearic acid biosynthesis, acetyl-CoA carboxylase and fatty acid synthase enzyme activity occurs. Brain tissue of diabetic rats, increased levels of palmitic and stearic acids was determined to be in compliance with previous studies (53). The present study showed that the level of oleic acid in fatty acid composition of brain tissue was low in the diabetic group. Oleic acid is in mono unsaturated fatty acids and stearic acid is substrate of oleic acid. Oleic acid is synthesized with activity of stearyl CoA desaturase. The activity of this enzyme is dependent on insulin. Oleic acid levels decreased in the diabetic group compared to the control group, and it was determined to prevent the changes, which emerged from plant extracts, in diabetic rats.

Fatty acid metabolism composed of synthesized fatty acid and SCD enzyme is also known as endogenous fatty acid and essential fatty acid. Linoleic and linolenic acids known as essential fatty acids. Essential fatty acid is composed of Δ^6 and Δ^5 desaturase enzyme and Δ^6 desaturation is added to this acid. The results of activation of these enzyme are γ-linoleic, eicosatrienoic, arachidonic, docosapentaenoic and docosahexaenoic acids. Christie (54) and Rule et al. (55) have reported linoleic acid and linolenic acid need to be supplied by diet, and arachidonic acid would be synthesized from 18:2 and 18:3. Polyunsaturated fatty acids are important structural components by conferring fluently and selective permeability to membranes (56). Deficiencies in both docosahexaenoic (22:6) and arachidonic acids (20:4) were associated with disorders of neuro-visual development. Brenner (57) reported that in experimental type I diabetes mellitus, the depressed delta-6 desaturase was restored by insulin, which stimulated transcription of the gene. We found that the arachidonic and docosahexaenoic acid levels increased in the brain tissue. Our results confirm that *P. peruviana* and *L. albus* treatment have benefical effects on the D-6 desaturase system and unsaturated fatty acid levels in brain tissue. Antioxidants (phytosterols, phytochemicals and polyphenol) may have protective effects on unsaturated fatty acids and that insulin exerts important effects on enzymes in the D-6 pathway (58,59). They may also reduce oxidative stress and thus impair progression to LDL oxidation, cell membrane lipid peroxidation and decreased endoneurial blood flow, thereby reducing peripheral nerve and vascular dysfunction.

Conclusions

Physalis peruviana and *Lupinus albus* fruits could be considered as a potential candidates for developing a new anti-diabetic agents. Through, offers promising antidiabetic effects that may be mainly attributed to its potent antioxidant potential. We speculate that such an anti-diabetogenic effect is achieved by the combined actions of *Physalis* and *Lupinus* components. It can be asserted that goldenberry and lupin decreased lipid peroxidation levels and increased glutathione level. However, it was observed that they were not sufficiently effective on especially enzyme activities at molecular level. As a result of the obtained results, it is concluded that these plants can be used for diabetic patient follow-up and recovery process. This will be important as an indication of the potentially medicinal and economical utility of *P. peruviana* and *L. albus* as new sources of bioactive phytochemicals and functional foods.

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