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Original article

Effects of *Physalis peruviana* and *Lupinus albus* on malondialdehyde, glutathione, cholesterol, vitamins and fatty acid levels in kidney and liver tissues of diabetic rats

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Summary. In this study, the effects of goldenberry and lupin on lipid peroxidation and antioxidant system parameters of liver and kidney tissues of streptozotocin-induced diabetic rats were investigated. Type II diabetes was produced in rats by the streptozotocin intraperitoneal injection. Albino rats were divided into four groups, each one containing 10 rats: non-diabetic control group, STZ-Diabetes type II group, STZ-Diabetes+goldenberry type II group, and STZ-Diabetes+lupin type II group. After one week from the injection, goldenberry and lupin were intraperitoneal injected to rats for 2 months. Malondialdehyde, glutathione, cholesterol, and fatty acid levels, which are signs of lipid peroxidation, were measured in these tissues. At the beginning and end of the study, postprandial blood glucose levels and weights of the rats were measured. In type II diabetes, malondialdehyde increased compared to the control group. Glutathione decreased in the other tissues and all of the streptozotocin-induced diabetic groups. In type II diabetes, liver cholesterol levels increased. Treatment with similar doses of goldenberry and lupin extracts significantly reduced postprandial hyperglycemia, oxidative stress, and augmented antioxidant system. The results of the present study showed that the herb suspensions exerted anti-hyperglycemic effects and consequently may alleviative liver and kidney damage caused by streptozotocin-induced diabetes.

Key words: Diabetes mellitus, goldenberry, lupin, malondialdehyde, glutathione, cholesterol, fatty acid

Introduction

Diabetes mellitus is one of the most common and complex problems which have caused many economic and social problems in modern societies. International Diabetes Federation (IDF)'s most recent estimates indicate that 8.3% of adults (382 million people) suffer from diabetes, and the number of diabetic people is predicted to rise beyond 592 million by 2035 (1,2). Diabetes is a metabolic disease characterized by a disorder in the regulation of carbohydrate metabolism. It leads to high blood glucose concentration or hyperglycemia (3). Long-term increase of glucose in diabetes is thought to be the main cause of some disorders like microangiopathy, macroangiopathy, poor antioxidant defense system, and impaired lipid metabolism. These disorders can cause short-term and long-term effects which can cause physiological damage to various organs of the body (4-6).

Type I diabetes results from defects in insulin secretion; whereas, the pathogenesis of type II diabetes is associated with a course of progressive insulin resistance in liver and peripheral tissues, reduced β-cell mass, and impaired insulin secretion (4,7,8). Although new anti-diabetic agents with unique properties have been introduced into the therapy of type 2 diabetic patients in the last few years, prevention and treatment of diabetes type 2 remain an unresolved problem. Unfortunately, none of the currently used anti-diabetic

agents provides all the required advantages for successful management including adequate hypoglycemic activity, modification of insulin secretion, peripheral insulin resistance, and sufficient safety.

Plants have always been a good source of drugs. Some plants are well known in traditional herbal medicine for their hypoglycemic effect. There are more than 800 plant species showing hypoglycemic activity (9,10). Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having anti-diabetic effect (11-13). Plant products with anti-diabetic activity are cheaper and more available and have lesser side effect than medicine. Numerous traditional herbal remedies are used by diabetic patients, especially in the third World countries (14) and may, therefore, represent new avenues in the search for alternative hypoglycemic drugs.

In recent times, *Physalis peruviana* and *Lupinus albus* are very much appreciated by researchers worldwide for their effect as hypoglycemic agents. Medically, *Physalis peruviana* and *Lupinus albus* have been used as a medicinal herb to cancer, leukemia, asthma, hepatitis or diabetes (3, 9, 15, 16). Besides, there is no information about if exposure to *Physalis peruviana* and *Lupinus albus* extracts of biochemical parameters in kidney and liver tissues of diabetic rats. The present study was conducted to determine the anti-diabetic and biochemical activity of *Physalis peruviana* and *Lupinus albus* in kidney and liver tissues of diabetic 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, diabetes (n=10); third, Diabetes+ *Physalis peruviana* group (D+PP) (n=10); fourth, Diabetes+ *Lupinus albus* group (D+LA) (n=10) groups were made diabetic by a single intraperitoneal injection of 40mg/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*. 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*. 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. Liver and kidney tissue samples were collected 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. (17). 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 (18). 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'sreagent (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 (19). 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 (20).

The fatty acidmethyl 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 25m, 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. (21) 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 of brain tissue were determined as Lowry's method described. The procedure for measuring protein was followed according to Lowry et al. (22) 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

Changes in blood glucose levels

Even though fasting blood glucose levels measured at the 1st, 4th, and 7th (at the end of study) weeks were within normal limits in the control group during the study, a continuous increase was observed in the diabetic group (Table 1). A decrease was determined in time in D+PP and D+LA groups (Table 1).

Changes in body weight

During the study, it was observed that the weight change measured at the 1st, 4th, and 7th (at the end of study) weeks was as follows; a regular increase in the control group and a significant decrease in the diabetic

group (Table 2). A weight increase was determined in D+PP and D+LA groups compared to diabetic group (Table 2).

Values of liver tissue in type-2 diabetes

The levels of MDA, GSH and total protein in the liver of control and diabetic rats are presented in the Tables 3. Compared to control group, a significant increase was observed in MDA level in the diabetic group ($p<0.01$). 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 3). While a partial increase was observed in D+PP group ($p<0.05$), a significant increase was observed in D+LA group ($p<0.001$). 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 3).

It was found that protein levels significantly decreased in the diabetic group compared to control group ($p<0.01$), partially increased in the diabetic group compared to D+PP group ($p<0.05$), and had no difference between in the diabetic group and D+LA 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 3).

Table 4 shows vitamins A,D,E, and K and cholesterol levels in liver tissue of type-2 diabetic rats. Compared to control group, K-1, K-2, α-tocopherol, and cholesterol levels increased significantly in the diabetic group ($p<0.001$). A significant difference was seen in δ- tocopherol, D-2, D-3, and retinol levels ($p<0.01$). K-1, K-2, and α-tocopherol levels increased significantly in D+PP group ($p<0.001$). A significant difference in D-2 and cholesterol levels ($p<0.01$) and a par-

Table 1. Changes in blood glucose concentration in rats

Blood Glucose Levels	C	D	D+PP	D+LA
	61,83±3,86	138,17±29,46	162,55±27,15	153,80±29,96
2 Weeks	66,83±2,61	141,42±13,63	138,91±8,51	150,00±15,13
3 Weeks	67,00±8,11	144,17±19,24	132,73±7,21	149,40±9,12
4 Weeks	96,50±12,34	152,75±19,38	129,27±9,45	144,90±7,41
5 Weeks	106,50±15,17	155,00±23,94	124,82±9,54	136,20±3,40
6 Weeks	108,00±10,34	211,75±43,33	121,00±15,53	135,80±7,06
End Applications	109,33±2,58	249,00±28,31	113,73±17,65	124,20±5,61

Table 2. Body weight of the rats at the various stages

Period	C	D	D+PP	D+LA
After STZ	192,17±12,06	200,67±8,08	200,18±6,04	204,40±6,46
2 Weeks	204,17±10,50	195,08±11,77	203,00±6,39	197,90±9,94
3 Weeks	214,67±10,54	193,50±11,01	211,18±7,26	219,10±7,59
4 Weeks	225,50±9,45	192,83±11,36	221,18±7,63	223,00±8,46
5 Weeks	229,17±10,47	190,25±12,02	226,64±10,09	224,50±7,33
6 Weeks	236,83±10,85	199,83±11,85	228,18±9,17	226,70±8,43
End Applications	242,50±10,95	186,58±12,12	231,09±10,11	231,00±8,53

Table 3. Type-2 diabetes in the rat liver tissue MDA, GSH and protein levels

Groups	MDA (nmol/g)	GSH (µg/g)	Protein (µg/g)
C	20,36±0,30	596,97±21,79	25,33±0,24
D	26,00±0,47[c]	409,76±32,12[c]	13,42±0,24[d]
D+PP	17,11±0,18[b]	666,19±17,26[b]	28,30±0,41[b]
D+LA	19,46±0,25[a]	708,66±19,72[d]	24,58±0,18[a]

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

tial difference in δ- tocopherol and D-3 levels occurred (p<0.05). An evident difference in K-1, K-2, D-2, and D-3 levels (p<0.001) and a significant difference in α-tocopherol and δ- tocopherol levels (p<0.01) were seen in D+LA group. It was also observed that cholesterol level partially increased (p<0.05), and the change in retinol level was statistically insignificant (p>0.05). Compared to diabetic group, a significant increase in δ- tocopherol level (p<0.01) and a partial decrease in K-1, K-2, α- tocopherol, D-2, D-3, cholesterol and retinol levels (p<0.05) were observed in D+PP group. In D+LA group, there was a significant decrease in levels of D-2, D-3, cholesterol and retinol (p<0.01); whereas, a relative difference in K-1, K-2, α- tocopherol, and δ- tocopherol levels was determined (p<0.05).

Table 5 shows vitamins fatty acid levels in liver tissue of type-2 diabetic rats. Compared to control group, there were an evident increase in 16:0 (Palmitic acid), 18:0 (Stearic acid), 18:1 n-9 (Oleic acid), 18:2 n-6 (Linoleic acid), 20:4 n-6 (Arachidonic acid), and 22:6 n-6 (Docosahexaenoic acid) levels (p<0.001) and a significant increase in 16:1 n-7 (Palmitoleic acid) and 20:3 n-6 (Eicosatrienoic acid) levels (p<0.01) in the diabetic group. In D+PP group, an evident increase in 20:3 n-6 level was observed (p<0.001); whereas a significant difference 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 increase was found in 18:0 level (p<0.05). 18:1 n-9 level was determined to get close to C group (p>0.05). While 16:0, 16:1 n-7, 18:0,18:1 n-9, 18:2 n-6, 20:3 n-6, and 22:6 n-6 levels partially decreased in D+LA group (p<0.05), 20:4 n-6 level got close to C group (p>0.05). Compared to diabetic group, a significant difference in 20:3 n-6, 20:4 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, and 18:2 n-6 levels (p<0,001) were observed in D+PP group. In D+LA group, 16:1 n-7, and 18:1 n-9 levels decreased significantly (p<0,01); whereas, an evident difference was found in 16:0, 18:0, 18:2 n-6, 20:3 n-6, 20:4 n-6, and 22:6 n-6 levels (p<0.001).

Values of kidney tissue in type-2 diabetes

The levels of MDA, GSH and total protein in the liver of control and diabetic rats are presented in the Tables 6. Compared to control group, a significant increase was observed in MDA level in the diabetic and D+PP groups (p<0.001; p<0.01). No statistical difference was observed between the control and D+LA groups (p>0.05). As diabetic group was compared with

Table 4. Type-2 diabetes in the rat liver tissue A,D,E,K vitamins and cholesterol levels (µg/g)

Vitamins	K	D	D+PP	D+LA
K-1	7,76±0,14	16,94±0,19[d]	14,01±0,35[d]	13,27±0,14[d]
K-2	3,06±0,33	12,42±0,29[d]	10,75±0,17[d]	10,31±0,19[d]
α-Tocopherol	18,97±0,33	44,99±0,59[d]	42,07±0,19[d]	28,43±0,44[c]
δ-Tocopherol	1,86±0,18	0,73±0,12[c]	0,82±0,17[b]	0,79±0,23[c]
D-2	1,87±0,23	1,33±0,12[c]	1,30±0,17[c]	0,27±0,07[d]
D-3	0,69±0,06	0,86±0,12[c]	0,76±0,14[b]	0,44±0,10[d]
Cholesterol (µmol/g)	3,62±0,06	6,41±0,24[d]	5,49±0,14[c]	4,22±0,26[b]
Retinol	1044,40±42,73	1312,85±13,71[c]	1166,36±17,59[b]	1005,69±19,70[a]

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

Table 4. Type-2 diabetes in the rat liver tissue A,D,E,K vitamins and cholesterol levels (µg/g)

Vitamins	K	D	D+PP	D+LA
K-1	7,76±0,14	16,94±0,19[d]	14,01±0,35[d]	13,27±0,14[d]
K-2	3,06±0,33	12,42±0,29[d]	10,75±0,17[d]	10,31±0,19[d]
α-Tocopherol	18,97±0,33	44,99±0,59[d]	42,07±0,19[d]	28,43±0,44[c]
δ-Tocopherol	1,86±0,18	0,73±0,12[c]	0,82±0,17[b]	0,79±0,23[c]
D-2	1,87±0,23	1,33±0,12[c]	1,30±0,17[c]	0,27±0,07[d]
D-3	0,69±0,06	0,86±0,12[c]	0,76±0,14[b]	0,44±0,10[d]
Cholesterol (µmol/g)	3,62±0,06	6,41±0,24[d]	5,49±0,14[c]	4,22±0,26[b]
Retinol	1044,40±42,73	1312,85±13,71[c]	1166,36±17,59[b]	1005,69±19,70[a]

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

Table 5. Type-2 diabetes in the rat liver tissue fatty acid levels (mg/g)

Fatty Acids	C	D	D+PP	D+LA
16:0	3,60±0,09	7,79±0,12[d]	4,21±0,20[b]	4,14±0,18[b]
18:0	2,83±0,16	6,02±2,02[d]	3,77±0,19[b]	3,26±0,12[b]
∑SFA	6,43±0,25	13,81±2,14	7,98±0,40	7,40±0,30
16:1, n-7	0,41±0,02	0,69±0,01[c]	0,25±0,01[c]	0,33±0,02[b]
18:1, n-9	0,92±0,02	0,54±0,03[d]	1,07±0,01[a]	1,34±0,10[b]
∑MUFA	1,33±0,04	2,63±0,14	1,32±0,02	1,67±0,12
18:2, n-6	2,32±0,30	7,10±0,16[d]	3,14±0,12[c]	3,03±0,18[b]
20:3, n-6	0,16±0,01	0,54±0,02[c]	0,62±0,02[d]	0,22±0,01[b]
20:4, n-6	4,94±0,15	11,36±0,30[d]	6,03±0,17[c]	6,72±0,12[a]
22:6, n-3	0,90±0,08	3,31±0,11[d]	1,75±0,14[c]	1,46±0,13[b]
∑PUFA	8,32±0,54	22,31±0,59	11,54±0,45	9,43±0,44
∑USFA	9,65±0,58	24,94±0,73	12,86±0,47	11,1±0,56

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

Table 6. Type-2 diabetes in the rat kidney tissue MDA, GSH and protein levels

Groups	MDA (nmol/g)	GSH (µg/g)	Protein (µg/g)
C	14,36±0,29	246,96±13,69	10,01±0,21
D	22,47±0,29[d]	176,91±10,74[c]	8,92±0,33[c]
D+PP	18,56±0,32[c]	202,45±6,58[b]	9,51±0,31[a]
D+LA	15,40±0,64[a]	304,87±21,04[c]	9,88±0,20[a]

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

D-PP and D+LA groups, a significant decreasing was determined between both groups (p<0.01) (Table 6).

GSH level in the diabetic group had a significant difference in decreasing tendency compared to control group (p<0.01). While a partial decrease was observed in D+PP group (p<0.05), a significant increase was observed in D+LA group (p<0.01). 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 6).

It was found that protein levels significantly decreased in the diabetic group compared to control group (p<0.01), 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 increase was determined between both groups (p<0.001) (Table 6).

Table 7 shows vitamins A,D,E,K and cholesterol levels in kidney tissue of type-2 diabetic rats. While a significant decrease was observed in K-2 and cholesterol levels of diabetic group compared to control group (p<0,001), a significant difference was determined for its K-1, α-tocopherol, δ-tocopherol, and D-2 levels (p<0.01). D-3 level was found to get closer to values of group C (p>0.05). While α-tocopherol level was observed to increase significantly in D+PP

Table 7. Type-2 diabetes in the rat kidney tissue A,D,E,K vitamins and cholesterol levels (μg/g)

Vitamins	C	D	D+PP	D+LA
K-1	1,54±0,16	1,81±0,14[c]	1,83±0,12[c]	4,09±0,10[d]
K-2	14,14±0,39	5,71±0,28[d]	12,62±0,32[c]	16,64±0,48[a]
α-Tocopherol	29,48±0,44	22,75±0,67[c]	37,31±0,42[d]	27,64±0,89[a]
δ-Tocopherol	0,39±0,09	0,54±0,04[c]	0,57±0,08[c]	0,92±0,16[d]
D-2	0,21±0,01	0,37±0,04[c]	0,25±0,33[a]	0,21±0,04[a]
D-3	0,30±0,04	0,23±0,05[a]	0,39±0,06[a]	0,52±0,09[c]
Cholesterol (μmol/g)	3,24±0,03	2,70±0,03[d]	3,22±0,07[a]	3,39±0,06[b]
Retinol	3,25±0,25	2,71±0,16[b]	8,91±0,25[c]	11,50±0,37[d]

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

group (p<0.001), a significant difference was observed for K-1, K-2, δ- tocopherol, and retinol levels (p<0.01). D-2, D-3, and cholesterol levels were determined to get closer values of group C (p>0.05). In D+LA group, a significant difference was observed in K-1, δ- tocopherol, and retinol levels (p<0.001), a significant increase in D-3 level was observed (p<0.01). K-2, α-tocopherol, and D-2 levels were determined to get closer values of group C (p>0.05). Compared to diabetic group, it was seen that α-tocopherol level in D+PP group significantly increased (p<0.001), there was a significant difference in K-2, D-2, D-3, cholesterol, and retinol levels (p<0.01). K-1 and δ- tocopherol levels were found to get closer values of group C (p>0.05). In D+LA group, α- tocopherol and δ- tocopherol levels were observed to increase significantly (p<0.01), K-1,K-2,D-2, D-3, cholesterol, and retinol levels had a significant difference (p<0.001).

Table 8 shows vitamins fatty acis levels in kidney tissue of type-2 diabetic rats. Compared to control group, a significant decrease was observed in 16:0 (Palmitic acid), 16:1 n-7 (Palmitoleic acid), 18:0 (Stearic acid), 18:1 n-9 (Oleic acid), 18:2 n-6 (Linoleic acid) and 20:4 n-6 (Arachidonic acid) levels in diabetic group (p<0,01). It was determined that changes observed in 20:3 n-6 (Eicosatrienoic acid) and 22:6 n-6 (Docosahexaenoic acid) levels were statistically insignificant (p>0.05). While a significant decrease was observed in 16:0,18:2 n-6 and 20:4 n-6 in D+PP group (p<0.001), a significant difference in 16:1 n-7 and 22:6 n-6 levels (p<0.01) and a partial difference in 18:0 and 20:3 n-6 levels were determined (p<0.05). 18:1 n-9 level was observed to get closer values of group C (p>0.05). While a significant difference was seen in 16:0, 18:0, 18:2 n-6 and 20:4 n-6 levels for D+LA group (p<0.001), 16:1 n-7 and 22:6 n-6 levels were determined to have a partial increase (p<0.05). Changes observed in 18:1 n-9 and 20:3 n-6 levels were determined to be statistically insignificant (p>0.05). Compared to diabetic group, it was seen that there was a distinct difference 18:2 n-6 and 22:6 n-6 levels (p<0.001) and a significant difference in 16:0, 16:1 n-7, 18:0, 18:1 n-9 and 20:4 n-6 levels (p<0.01) in D+PP group. An important difference in 16:0,16:1 n-7, 18:0,18:1 n-9 and 18:2 n-6 levels (p<0,001) and a significant difference in 20:4 n-6ve 22:6 n-6 levels (p<0,01) were found in D+LA group. Change observed in 20:3 n-6 levels in D+PP and D+LA groups was determined to be statistically insignificant (p>0.05).

Discussion

Diabetes mellitus is a disorder of carbohydrate, fat, and protein metabolism mainly caused due to attenuate production of insulin or its inhibitory action. Before there was no synthetic drug, natural cure was used and they can still be used, today. Although dietary modification has long been recommended as typical treatment modalities for diabetes, it is not clearly decided yet about the use or avoidance of particular food (23,24). Recently, there has been an increasing

evidence that some dietary components can potentially affect the initiation of diabetes in animal models. In the present study, we showed that *Physalis peruviana* and *Lupinus albus* could prevent development of streptozotocin-induced type-2 diabetes in the rat liver and kidney tissues.

It is known that the single high-dose streptozotocin-induced diabetes mellitus in rats that arises from irreversible destruction of the β-islet cells of the pancreas, causing degranulation or reduction of insulin secretion results in hyperglycemia and decreased body weight (25). Structural proteins have an important effect on body weight but weight loss is experienced in diabetes due to degradation occurring in structural proteins. Weight loss is observed in diabetic rats because of catabolic reactions in protein and fat metabolism, and dehydration. It was determined that body weights of STZ diabetic rats decreased as a result of 4 - 6 week experiment (26,27).

In this study, diabetic groups exposed to rapid weight loss due to effect of STZ were then observed to start to gain weight by means of goldenberry and lupin extracts given via injection. Our findings are considerably compatible with previous data.

The pancreas plays an important role in glucose homeostasis (28). The role of oxidative stress is implicated in the decline of pancreatic function in diabetes mellitus. The diabetic effe ct of STZ is due to an excess in the production of reactive oxygen species (ROS). This excess leads to toxicity in pancreatic cells, which, in turn, reduces the synthesis and release of insulin while concurrently affecting other organs such as liver and kidney (29). In the present study it was observed that blood glucose levels increased in diabetic groups, but blood glucose levels were observed to reduce again by means of goldenberry and lupin extracts given by injection. In the study conducted with goldenberry, it was shown that it controlled glucose level by absorbing carbohydrates owing to its fibrous structure (30,31). Blood glucose level was also observed to decrease after lupin was given to diabetic rats (31). The polyphenols content of the fresh *Physalis* juice (70mg/100ml) (32,33). *Physalis* polyphenols may, therefore, prevent the damage and death of pancreatic-cells and/or stimulate the regeneration of this type of cells in diabetic rats.

The obtained results of *Physalis* were agreed with Estakhr and Javdan (34) who recorded that oral administration of ethanolic extract of *Physalis* normalized the levels of blood glucose. The presence of potent anti-diabetic active principles as physalin, citric acid and vitamin C in the extract inhibits glycogen phosphorylase enzyme that catalyzes the process of gly-

Table 8. Type-2 diabetes in the rat kidney tissue fatty acid levels (mg/g)

Fatty Acids	C	D	D+PP	D+LA
16:0	3,40±0,27	1,88±0,02[c]	0,95±0,02[d]	6,51±0,33[d]
18:0	1,40±0,02	1,29±0,02[c]	0,51±0,05[b]	1,85±0,16[d]
∑SFA	4,80±0,29	3,17±0,04	1,46±0,07	8,36±0,49
16:1, n-7	0,65±0,05	0,18±0,02[c]	0,21±0,02[c]	0,94±0,02[b]
18:1, n-9	2,55±0,64	1,33±0,20[c]	2,58±0,17[a]	2,62±0,18[a]
∑MUFA	3,25±0,69	1,51±0,22	2,79±0,19	3,56±0,20
18:2, n-6	2,59±0,14	1,85±0,07[c]	1,18±0,05[d]	1,23±0,03[d]
20:3, n-6	0,16±0,01	0,15±0,01[a]	0,13±0,04[b]	0,16±0,02[a]
20:4, n-6	3,10±0,25	2,92±0,19[c]	2,24±0,14[d]	2,26±0,13[d]
22:6, n-3	0,23±0,01	0,20±0,01[a]	0,46±0,01[c]	0,64±0,03[b]
∑PUFA	6,08±0,41	5,19±0,28	4,01±0,24	4,29±0,21
∑USFA	9,28±1,10	6,63±0,50	6,80±0,43	7,85±0,41

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

cogenolysis thereby inhibiting glucagon on feedback inhibition in the production of insulin.

Oxidative stress that leads to an increased production of ROS and finally cellular lipid peroxidation has been found to play an important role in the development of diabetes mellitus. LPO is one of the cellular features of chronic diabetes (35). Extensive evidence has demonstrated that the increase of lipid peroxidation plays an important role in the progression of diabetes by altering the transbilayer fluidity gradient which could hamper the activities of membrane-bound enzymes and receptors (36). Previous study reported increased levels of lipid peroxidation in diabetic rats (37). However, the administration of physalis to the diabetic group of rats significantly reverted back MDA levels to near normal values which showed the anti-lipid peroxidative property of physalis in the experimental diabetes.

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. 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 administration. 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 (38,39). 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 (9).

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. GSH is the most prevalent low molecular weight antioxidant within cells and protects cellular constituents from oxidative damage by reacting directly with oxidants or acting as the substrate for glutathione peroxidase to scavenge peroxides (40). 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 enzyme. The conservation and formation of NADPH in the cells are realized by the activity of pentose-phosphate pathway and malic enzyme (41). 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 (42). Other antioxidants such as vitamin E can protect cells from oxidative damage by cleaning. In the present study the level of α-tocopherol in the kidney tissue of diabetic rats decreased compared to the control group, and the plant extract in the kidney tissue of diabetic rats increased levels of α-tocopherol.

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. The present study indicated that cholesterol level in liver was high in the insulin treated diabetic group. Liver is a major site of insulin action, and insulin sensitivity may be related to the cholesterol level of liver lipids. Insulin increases activity of the 3-hydroxy- 3-methylglutaryl coenzyme A *reductase* enzyme (HMG-CoA), which is a key enzyme in cholesterol biosynthesis. Therefore, cholesterol synthesis increases and facilitates the storage of fuels and macromolecules in liver, muscle, and fat (43). In the present results, the cholesterol level was also found to be high in the insulin and plant extract treated diabetic groups. This augmentation might be a result of insulin administration. However, the level of cholesterol in the kidney tissues of D+PP group was lower than the control group. This decrease may be due to the effects of the administered *P. peruviana* and *L. albus* on choles-

terol metabolism. Sirtori et al., (44) 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 (45). Bettzieche et al., (46) 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 (47). Martins et al. (48) 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.

Lipid profile abnormalities form 40% of cases of diabetes and diabetic complications are one of the most common. Fatty acid is required for both the structure and function of every cell in the body and forms an important component of cell membranes. Fatty acid composition is changed in humans (49) and animals with diabetes (50). Liver tissue, palmitic and stearic acids were shown to increase in diabetic group. A major metabolic role of the liver is the synthesis of fuel components for utilization by other organs. The liver is a major site for fatty acid synthesis (51,52). Additionally, liver obtains much of its fatty acid complement from circulating fatty acid; thus, liver fatty acid composition may be influenced greatly by plasma lipid fatty acid composition (53). In rats diabetes induced an increase of stearic acid among free fatty acids (54).

In the FA composition of kidney tissue, palmitic acid and stearic acid levels in diabetic and D+PP groups were lower than the control group. In mammalian cells, palmitic and stearic acids are major products of *de novo* synthesis by the activity of cytoplasmic FA synthase (55).

The present study showed that the level of 18:1 n-9 in fatty acid composition of liver and kidney tissues was low in the diabetic group. Desaturases are key enzymes in the biosynthesis of the monounsaturated and polyunsaturated fatty acids and thereby contribute to the control of the fatty acid-dependent structure and disorder of the membrane. A relationship has been observed between the 16:0/16:1 and 18:0/18:1 ratios usually used as an index of *in vivo* Δ9-desaturase activity. The significant increase in the ratios of 16:1, n-7 and 18:1, n-9 may reflect higher activity of Δ9 desaturase in the cell. An upsurge in the Δ9 desaturase activity has been reported to be related to hyperinsulinemia. It has been found to induce an over-expression of Δ9 desaturase by the effect of insulin in animals (55-57). The level of 18:1 in both liver and kidney tissues of insulin treated diabetic groups was lower than control group. While oleic acid is in monounsaturated fatty acids, stearic acid is the substrate of oleic acid. Oleic acid is synthesized with activity of stearoyl CoA desaturase enzyme. This enzyme activity 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

The level of PUFA in liver tissues was found to be higher than in kidney. The higher proportion of PUFA in liver may be associated with the synthesis of these fatty acids by liver. Christie (58) and Rule et al. (59) 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 (60). Deficiencies in both docosahexaenoic (22:6) and arachidonic acids (20:4) were associated with disorders of neuro-visual development. Brenner (61) 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 acid level decreased but docosahexaenoic acid level increased in the kidney tissue. Changes in the fatty acid composition of rat liver, heart, kidney, testis, spleen, and brain phospholipids were examined at various stages of STZ-induced diabetes. Brenner et al. (62) reported that STZ-induced diabetes depressed Δ-9, Δ-6 and Δ-5 fatty acid desaturases, decreased arachidonic acid level and increased linoleic acid levels. Desaturase activities in Δ-6 pathway may be increased by insulin and diet restriction (63).

Conclusions

Consequently, possible effects of diabetes on various tissues as well as protective and therapeutic properties of goldenberry and lupin in these tissues were investigated in this study. It can be asserted that goldenberry and lupin decreased blood glucose and 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.

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