The therapeutics effects and toxic risk of *Heracleum persicum* Desf. extract on streptozotocin-induced diabetic rats☆

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ABSTRACT

There is an increasing interest against to fight of diabetes by using hypoglycemic plants in the world. The public thinks that *Heracleum persicum* (HP) has antidiabetic effect local consumer in Turkey. As far as our literature survey, no studies have been reported so far on antidiabetic effects and toxic risk potential of the HP lyophilized extract supplementation used in this study. The aim of this study, for the first time, was to investigate the therapeutic effects of diabetic complications, antioxidant properties and toxic risk potential of HP against experimentally streptozotocin (STZ) induced diabetes in rats, which were evaluated by measuring the level of serum biomarker releated diabetes complications changes such glucose, insülin, c-peptide, lipid profile (LP), hepatic and renal damage biomarkers (HRDB), glucosylated hemoglobin (HbA1c), antioxidant defense system constituents (ADSCs), malondialdehyde (MDA) content measured in erythrocyte, brain, kidney and liver tissues, and α-glucosidase activitiy of small intestine. The plant aqueous extract was allowed to freeze-dried under a vacuum at $-54\,°C$ to obtain a fine lyophilized extract. The study was performed on STZ-induced diabetic rats (45 mg/kg, body weight (bw), intraperitonally) designed as normal control (NC), diabetic control (DC), diabetes + acarbose (DAC) (20 mg/kg, bw), diabetes + HP (100 mg/kg, bw) (DH1), diabetes + HP (200 mg/kg, bw) (DH2) and diabetes + HP (400 mg/kg, bw) (DH3)] groups. The experimental process lasted 21 days.

According to results; the levels of blood glucose (BG), glucosylated hemoglobin (HbA1c) and malondialdehyde (MDA) of DC group increased significantly ($p < 0.05$) compared to NC group, whereas these parameters of the groups treated with oral administrations of HP plant lyophilized extract were observed significant ($p < 0.05$) declines compared to DC. The biochemical analyses showed a considerable decrease in insulin and c-peptide levels and the fluctuated ADSCs in the DC group as compared to control group, whereas the extract supplementations diet restored the diabetic complications parameters towards to the NC. On the other hands, liver damage serum enzymes as serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were increased significantly ($p < 0.05$) in the plant extract supplementations groups as compared to NC and DC groups. It was concluded that while the extracts of HP have had therapeutic effects on some complications caused by diabetes, but might be caused hepatocyte damage changes as the transport functions and membrane permeability of these cells, thus causing enzymes to leak.

1. Introduction

Diabetes is a chronic endocrine disease characterized by persistent hyperglycemia and associated with abnormalities of carbohydrate, protein, and lipid metabolism and the disease is caused by a decrease or deficiency of insulin secretion and/or from increased cellular resistance [1]. Diabetes mellitus, rapidly increasing disease prevalence all over the world, leads to life-threatening, microvascular and macrovascular complications and even deaths in the advanced stages, has recently become a global health problem and on September 14, 2011, the

International Diabetes Federation announced that 336 million people worldwide now have type 2 diabetes, and that the disease is responsible for 4.6 million deaths each year, or one death every seven seconds [2].

The increased blood glucose levels in diabetes produce superoxide anions, which generate hydroxyl radicals via Haber Weiss reaction, resulting in peroxidation of membrane lipids and protein glycation causing oxidative damage of cell membranes. These radicals further damage other important biomolecules including carbohydrates, proteins and deoxyribonucleic acid [3]. Oxidative stress plays an important role in the pathogenesis of diabetes and its complications. Findings

about involvement of reactive oxygen species (ROS) not only in defense processes, but also in a number of pathologies, stimulated discussion about their role in etiopathogenesis of various diseases. Yet questions regarding the role of ROS in tissue injury, whether ROS may serve as a common cause of different disorders or whether their uncontrolled production is just a manifestation of the processes involved, remain unexplained. Dogmatically, increased ROS formation is considered to be responsible for development of the so-called free-radical diseases [4]. Also, ROS are produced constantly in living organisms as a result of aerobic processes. Under physiological conditions, the production of ROS is low and is related to physiological cell procedures as, for example, ROS can act like signaling molecules. However, under pathological situations, ROS production is significantly increased, and as a result, adverse impact and toxic effects can result. Thus, each organism has a defense system against ROS production. This system includes antioxidant molecules and antioxidant enzymes [5]. Eukaryotic cells generally function in a reduced state, but an amount of reactive species is essential for several biochemical processes. The antioxidant network is the defensive mechanism that occurs when the concentration of reactive species exceeds a threshold. Polyphenolic compounds present in plant extracts are potent antioxidants in vitro, but they may promote oxidative stress when administered in animals and humans, especially when given as supplements in exercise, a modality usually adopted as an oxidant stimulus [6]. Further, antioxidant products are important in the prevention of many oxidative stress related diabetes, arteriosclerosis, cardiovascular illnesses, cancer and several neurodegenerative disorders properties previously [7].

The WHO estimates that more than 1200 plant species are used to treat diabetes, primarily in the developing world [8]. These species represent more than 725 genera in 183 families ranging from marine algae to higher plants [9]. However, only about 350 of them are documented to present antihyperglycemic activity [10] and few have been subjected to rigorous scientific evaluation for safety and efficacy in humans [11,12]. Controlling blood glucose level is essential for preventing diabetic complications and for improving the health of patients with diabetes. However, currently available drugs for diabetes have a number of limitations, such as unwanted side effects that include hypoglycemia, cell death, and high rates of secondary failiure [13]. Recent efforts for the complementary treatment of diabetes have focused on functional foods and their bioactive compounds [14].

There is a growing interest of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and the increased consumer perception of this problem in recent years [15]. Recently, some researches have revealed several benefits of *Heracleum persicum* (*H. persicum*). In consequence of atherosclerosis antioxidant capacities of H.persicum Desf, species from Apiaceae family were evaluated by determining their effects on DPPH (2,2′-diphenyl-1-picrylhydrazyl) radical scavenging and lipid peroxidation inhibition as well as their total phenolic contents [16,17]. Pimpinellin, isopimpinellin, bergapten, isobergapten and sphondin are furanocoumarins which are reported from roots of this plant. H.persicum fruits are a rich source of furanocoumunaris which might act as an immunostimulatory agent [18]. *H. persicum* has stimulated both humoral, as well as cellular arms of the immune system. Hexyl butyrate (%565), octyl acetate (%165), hexyl 2-methylbutanoate (%5,2) and hexyl isobutyrate (%3,4) were identified as the major constituents of *H. persicum* essential oil. Hydroalcoholic extractive of *H. persicum* includes several furanocoumarins. Due to presence of these components, the plant is used medicinally to relieve flatulence, stomachaches as well as flavoring as an antiseptic [19]. Moreover, some antioxidant of glutathione category has also been seen in this plant [20]. A plant medicine researcher has shown that *H. persicum* have antioxidant, anticonvulsant, analgesic, antiinflammatory, immune modulatory and cytotoxic effects [19]. In traditional medicine it was observed that use of *H. persicum* during the sexual cycle stops progression of ovarian phase in females [21]. Dalouchi et al showed that the extract of *H.*

persicum in association with cyclo phosphamide significantly improved sperm parameters. Seminal plasma of infertile men has worse antioxidant levels in comparison with fertile men, especially those who have poor sperm motility [22]. Dehghan et al showed that the extract of *H. persicum* exhibited significant antidiabetic activities in α-amylase and α-glucosidase assays, more effective than acarbose concentrations that cause 50% inhibition and has radical scavenging activity as *in vitro* [23,24]. Further, studies repoted that this plant reduces LDL cholesterol [25] and has strong anti-inflammatory, analgesic, anticonvulsant, antibacterial, cytotoxic, antifungal, and immunomodulatory effects [26]. Another study showed that different parts of *H. persicum* contain terpenoids, terry terpenes, furanocoumarins and flavonoids, alkaloids, and volatile metabolites [19].

There is a growing interest of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and the increased consumer perception of this problem in recent years. There is an increasing interest against to fight of diabetes by using hypoglycemic plants in the world. In addition, the public thinks that the plant has antidiabetic effect local consumer in Turkey. As far as our literature survey, no studies have been reported on the healing effects of diabetic complications, antioxidant role against oxidative stress causing by diabetes and toxic risk potential of *H. persicum* lyophilized extract supplementation used in this study model. Also, the objective of this study was to determine healthful potentials of *H. persicum* lyophilized the plant extract against induced diabetic complications as oxidative stress, hepatotoxicity and nephropathy and hyperlipidemia. Thus, in the present study, we have extensively studied the mentioned properties of *H. persicum* using *in vivo* models. For this aim, the treatments of *H. persicum* lyophilized extract were done orally as extract of *H. persicum* because the effect of the functional plant represents a well characterized in nutrition and widely used as consumption by human in our country and worldwide. Therapeutic potential, antioxidant capacity and toxic risk of the *H. persicum* were evaluated by evaluated by serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH), insulin, c-peptide, glucose, lipid profile [total trigliseride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c)], creatinine (CRE), blood urea nitrogen (BUN), urea, and the amount of glucosylated hemoglobin of total blood (HbA1c) were determined. Moreover, as an indicator of the efficacy of antioxidant capacity, activities of antioxidant defense system constituents (ADSCs) such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), glutathione reductase (GR) and reduced glutathine (GSH) levels and malondialdehyde (MDA) levels were measured in erythrocyte, brain, kidney and liver tissues. In addition, α-glycosidase activity in small intestine tissue samples was investigated.

2. Materials and methods

2.1. Plant materials and preparation of lyophilized extract

Heracleum persicum was collected in Bitlis (Hizan town) TURKEY during May 2015. This was authenticated by Dr. Fevzi ÖZGÖKÇE (Department of Botany, Yuzuncu Yil University, Turkey) and the herbarium number of *H. persicum* was determined to be165061. A sample of plant has been stored at the Yuzuncu Yil University Science and Art Faculty Herbarium (VANF). The extract was prepared according to the methods modified by Dalar et al. [27]. The aqueous extract was prepared with the help of magnetic stirrer 100 g of powder in 500 mL of distilled water (dH$_2$O) for 24 h following the traditional method. The mixture is centrifuged for 20 min at 7000 rpm. The filtrate was combined and the solvent evaporated under reduced pressure at 37 °C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The viscous extract was allowed to falcon tube and freeze-dried under a vacuum at −54 °C to obtain a fine lyophilized powder.

2.2. Experimental animals

Wistar albino female rats 3–4 months of age with an average weighing 200–300 g were provided by the Experimental Animal Research Center, Yuzuncu Yil University, and were housed in 6 groups, each group contained 7 rats. Rats were in housed controlled humidity (60%–70%) and temperature (25 ± 1 oC) conditions with a 12 h light/dark cycle. All animals were fed a group wheat-soybean-based diet and given water ad libitum in stainless cages. They received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by the Ethic Committee of the Yuzuncu Yil University (Protocol number 27552122-142).

2.3. Chemicals

Thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), metphosphoric acid, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), trihydroxymethyl aminomethane (Tris), 1-chloro-2,4-dinitrobenzene (CDNB), oxidized glutathione (GSSG), β-Nicotinamide adenine dinucleotide phosphate (NADPH), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), sodium dihydrogen citrate anhydrous ($C_6H_7NaO_7$) and streptozotocin (STZ) (Sigma, USA) of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Kits for an- tioxidant enzymes analysis were supplied by Randox Laboratories ltd. and α-Glycosidase Activity Colorimetric Assay Kit (Catalog #K690-100, BioVision, USA) was used for small intestine tissue samples.

2.4. Acute toxicity test

Twelve rats were grouped into three groups each containing four rats. Acute toxicity test was determined using the method described by the method of Lorke [28] and Ibeha and EzeaJa [29]. The rats were saturated with 250, 500 and 1000 mg/kg rat body weight (bw) of the extract orally via gastric gavage. It was no observed for signs of toxicity or mortality at period of 72 h.

2.5. Induction of experimental diabetes mellitus

Rats were fasted for 12 h prior to the induction of diabetes. STZ freshly prepared in citrate buffer (0.1 M, pH 4.5) was administered intraperitoneally at a single dose of 45 mg/kg, bw. The rats with blood glucose 200 mg/dl or over were regarded as diabetic subject.

2.6. Experimental design

The experimentally rats were divided into six groups containing seven rats.

Normal Control (NC): the rats received citrate buffer (1 ml/kg, bw, pH 4.5) which is dissolved STZ intraperitonally and fed with standard pellet diet as *ad libitum*.

Diabetic Control (DC): the rats received STZ (45 mg/kg, bw) dissolved citrate buffer (pH 4.5) at a single dose intraperitonally and fed with standard pellet diet as *ad libitum*.

Diabetes + Acarboz (DAC): the rats received STZ (45 mg/kg, bw) dissolved citrate buffer (pH 4.5) at a single dose intraperitonally and treated to acarbose supplement (20 mg/kg, bw) orally via gastric gavage during 21 days experimental period per day.

Diabetes + Heracleum persicum (DH1): the rats received STZ (45 mg/kg, bw) dissolved citrate buffer (pH 4.5) at a single dose intraperitonally and treated to the plant extract supplement (100 mg/kg,

bw) orally via gastric gavage during 21 days experimental period per day.

Diabetes + Heracleum persicum (DH2): the rats received STZ (45 mg/kg, bw) dissolved citrate buffer (pH 4.5) at a single dose intraperitonally and treated to the plant extract supplement (200 mg/kg, bw) orally via gastric gavage during 21 days experimental period per day.

Diabetes + Heracleum persicum (DH3): the rats received STZ (45 mg/kg, bw) dissolved citrate buffer (pH 4.5) at a single dose intraperitonally and treated to the plant extract supplement (400 mg/kg, bw) orally via gastric gavage during 21 days experimental period per day.

2.7. Preparation of tissues supernatant and erythrocyte pellets

At the end of the 21 days experiments, the rats were anesthetized intraperitoneally injection of 5 mg/100 g body weight ketamine. Blood samples were obtained from a cardiac puncture using syringe for the determination of biochemical analysis. Serum samples were obtained by centrifuging of blood samples at 4000*g* for 15 min at 4 °C, and biochemical parameters were measured in these serum samples. For biochemical analysis of erythrocytes, blood samples were put immediately into two silicon disposable glass tubes with EDTA as an anticoagulant. The first tubes were used for the determination of glucosylated hemoglobin (HbA1c) levels. Second tubes were centrifuged at 4000*g* for 15 min at 4 °C and erythrocyte pellets were obtained. Then, the pellets were washed tree times with physiological saline (0.9% NaCl).

The tissues such as small intestine, brain, kidney and liver were dissected and put in petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept at −78 °C during the analysis. The tissues were homogenized for 5 min. in 50 mM icecold KH_2PO_4 solution (1:5 w/v) using stainless steel probe homogenizer (20 KHz frequency ultrasonic, Jen-cons Scientific Co.) for 5 min, and then centrifuged at 7000*g* for 15 min. All processes were carried out at 4 °C. Supernatants and erythrocyte pellets were used to determine ADS constituents and MDA contents [30,31]. Also, α-glycosidase activity was investigated in small intestine tissue supernatant samples.

2.8. Biochemical analysis

The erythrocyte and tissues MDA concentration was determined using the method described by Jain et al. [32] based on TBA reactivity. Erythrocyte and the tissues GSH level were measured using the method described by Beutler et al. [33]. GST activity of erythrocyte and the tissues were assayed by following the conjugation of glutathione with CDNB at 340 nm as described by Mannervik and Guthenberg [34]. GR activity was assayed according to Carlberg and Mannervik [35] based on that of the decrease in absorbance of NADPH at 340 nm. GPx activity was measured using the method described by Paglia and Valentine [36] which is GPx catalyses the oxidation of glutathione by cumene hydroperoxide. SOD activity was measured at 505 nm by calculating inhibition percentage of formazan dye formation [37]. CAT activity was determined using the method described by Aebi [38] based on that of the rate of H_2O_2 consumption and as the decrease in absorbance at 240 nm. α-Glycosidase activity in small intestine was measured colorimetrically at 410 nm by α-glycosidase hydrolyzes the substrate mix to release the p-nitrophenol (BioVision kits, USA).

2.9. Measurement of biochemical parameters

AST, ALT and LDH, lipid profile [total triglyceride (TG), total cholesterol (TC), LDL-cholesterol (LDL-c) and HDL-cholesterol (HDL-c)], creatinine (CRE), blood urea nitrogen (BUN) and urea were measured by an auto analyzer (COBAS 8000/ROCHE/Germany/Serial No 1296-08) using the Roche kits. On the other hand, insulin (CEA448Ra, USA) and c-peptide (CEA447Ra, USA) levels were measured in the 450

absorbance by ELISA Enzyme-linked Immunosorbent Assay Kit.

2.10. Measurement of blood glucose levels

Fresh blood samples were collected from the tail vein of the rats. The blood glucose levels were determined with a blood glucose meter (ACCU-CHEKr Active, Roche). The blood glucose level measurements were performed in 0th, 1th and 3th hand at 0th, 7th, 14th and 21th days after treatments of the plant extract.

2.11. Analysis of data

All data were expressed as mean ± standard deviation (SD). The statistical analyses were made using the Minitab 13 for windows packet program. Means and Standard deviations were calculated according to the standard methods for all parameters. One-way analysis of variance (ANOVA) statistical test was used to determine the differences between means of the experimental groups accepting the significance level at $p \leq 0.05$.

3. Results

3.1. Acute toxicity observations

Animals showed a good tolerance to testing tree (250, 500 and 1000 mg/kg, bw) doses of the plant lyophilized extract. The highest dose of extract did not show any noticeable signs of toxicity or mortality after once daily administration orally for 3 days.

3.2. Heracleum persicum extract effect on body weight and blood glucose levels

Table 1 summarizes the levels of glucose at the different periods and body weight of the groups. During the study period, the normal control rats gained weight, while STZ-induced diabetic rats exhibited a loss body weight compared to initial animals. A significant ($p < 0.05$) body weight loss was observed in finally DC and DH1 groups compared to beginning, whereas body weight loss was not significantly ($p > 0.05$) observed in the other groups. On the other hand, the increased levels of plasma glucose in diabetic groups were lowered by the plant extract administration as compared to beginning day at the end of 21 days.

3.3. Effect of the Heracleum persicum extract on liver and renal serum biomarkers of experimental groups

As shown in Table 2, the serum levels of ALT, AST, BUN and urea were significantly ($p < 0.05$) increased in the diabetic control rats compared with the normal control rats. Also, the serum levels of ALT and AST were significantly ($p < 0.05$) increased in the treated with H. persicum extract compared with the diabetic and normal control groups. Renal serum biomarkers of the treatment groups such as urea and BUN were significantly ($p < 0.05$) different compared with NC group, but did not different as compared to DC group.

3.4. Effect of the Heracleum persicum extract on lipid profile

Following the end of the experiment, the serum levels of TC, HDL were significantly ($p < 0.05$) decreased in the diabetic group compared with the normal control group, whereas the plant extract supplementations diet restored the parameters towards to the normal control group (Table 3).

3.5. Effect of the Heracleum persicum extract on HbA1c, serum insulin, c-peptide and α-glycosidase activity in small intestine tissue

As shown in Table 4, at the end of the experiment, the serum insulin and c-peptide levels of diabetic control rats were significantly ($p < 0.05$) decreased compared to those of normal control rats, whereas significantly ($p < 0.05$) increased these parameters of the plant extract administrations groups as compared with the diabetic control rats. Further, while HbAlc level significantly ($p < 0.05$) increased in diabetic control group, but decreased in the plant extract supplementation groups as compared with the diabetic control rats.

3.6. Effect of the Heracleum persicum extract on lipid peroxidation and antioxidant defense systems

According to the results, while STZ-induced diabetes caused a significant ($p < 0.05$) increase in MDA content levels as a result of oxidative stress condition and fluctuated in the ADS constituents in the tissues of rats, the plant extract supplementations diet restored these diabetic complications parameters towards to the normal control (Table 5).

Table 1

Effects of *Hearcleum persicum* aqueous extract supplements on body weight and glucose level of experimental groups during 21 days.

	GRUPLAR					
	NC Mean 7SD	DC Mean 7SD	DAC Mean 7SD	DH1 Mean 7SD	DH2 Mean 7SD	DH3 Mean 7SD
Body weight (g)						
Beginning	213,85 ± 5,55	213,71 ± 797	20,571 ± 11,81	236,42 ± 4,27	227,42 ± 7,02	228,14 ± 12,58
Finally	21,957 ± 1021	20,357 ± 5,74[a]	196,14 ± 831	22,357 ± 12,63[a]	21,857 ± 11,31	21,857 ± 12,85
Three hour period Blood glucose (mg/dL) 0th Hour (Fasting blood glucose)	8423 ± 9,28	330,47 ± 159,32	388,81 ± 150,71	220,85 ± 78,06	207,01 ± 66,81	187,14 ± 63,72
1th Hour (After consumption)	8595 ± 8,39	363,61 ± 142,04	390,09 ± 145,41	200,85 ± 54,61	201,61 ± 70,73	195,66 ± 56,06
3rd Hour (After consumption)	9023 ± 661[b]	388,14 ± 131,79	409,33 ± 128,70	199,47 ± 67,18	186,61 ± 79,22	174,76 ± 62,01
21 day period Blood glucose (mg/dL)						
0th Day	8442 ± 12,76	261,42 ± 68,73	225,85 ± 21,16	209,28 ± 9,44	218,42 ± 16,97	246,14 ± 54,98
7th Day	8291 ± 9,85	363,71 ± 146,21	472,14 ± 105,51[c]	258,66 ± 50,77[c]	264,38 ± 47,05[c]	235,76 ± 43,04
14th Day	8866 ± 8,41[c]	377,04 ± 145,34	393,01 ± 134,72[c]	215,76 ± 59,45	192,33 ± 37,95	188,23 ± 40,35[c]
21th Day	8885 ± 5,33	341,47 ± 146,66	323,09 ± 140,51	145,33. ± 31,20[c]	138,52 ± 62,85[c]	133,57 ± 48,05[c]

Data are expressed as mean ± SEM, n = 7.

[a] When finally body weights were compared with beginning body weight ($p \leq 0.05$).

[b] When 1th and 3rd hours were compared with 0th ($p \leq 0.05$).

[c] When 7th, 14th and 21th days were compared with 0th ($p \leq 0.05$).

Table 2

Effects of *Heracleum persicum* aqueous extract supplements on liver and renal serum biomarkers of experimental groups.

GROUPS						
Parameters	NC Mean 7SD	DC Mean 7SD	DAC Mean 7SD	DH1 Mean 7SD	DH2 Mean 7SD	DH3 Mean 7SD
ALT U/L	70,94 ± 18,57	176,55 ± 19,91[a]	139,88 ± 19,91[ab]	268,81 ± 54,83[ab]	309,91 ± 70,71[ab]	829,12 ± 131,94[ab]
AST U/L	146,81 ± 30,33	239,61 ± 71,33[a]	248,57 ± 17,84[a]	451,65 ± 125,55[ab]	488,81 ± 65,83[ab]	467,15 ± 77,99[ab]
Urea mg/dL	49,21 ± 4,62	58,52 ± 6,09[a]	87,24 ± 1,85[ab]	59,61 ± 3,73[a]	59,01 ± 8,53[a]	88,52 ± 11,89[ab]
CRE mg/dL	36,10 ± 4,02	40,01 ± 5,71	52,01 ± 5,40[ab]	3828 ± 3,45	3828 ± 3,19	50,01 ± 2,64[ab]
BUN mg/dL	23,14 ± 2,03	27,42 ± 2,82[a]	40,57 ± 113[ab]	28,75 ± 1,95[a]	27,71 ± 4,19[a]	41,42 ± 528[ab]
LDH U/L	1623,5 ± 274,6	1610,2 ± 247,9	1630,1 ± 211,7	1707,2 ± 448,3	1992,7 ± 375,2	1577,01 ± 336,5

Data are expressed as mean ± SEM, n = 7.
[a]When DC, DAC, DH1, DH2 and DH3 groups were compared with NC group (p ≤ 0.05).
[b]When DAC, DH1, DH2 and DH3 groups were compared with DC group (p ≤ 0.05).

Table 3

Effects of *Heracleum persicum* aqueous extract supplements on lipid profile of experimental groups.

GROUPS						
Parameters	NC Mean 7SD	DC Mean 7SD	DAC Mean 7SD	DH1 Mean 7SD	DH2 Mean 7SD	DH3 Mean 7SD
TG (mg/dL)	13,407 ± 31,06	110,62 ± 33,02	78,71 ± 16,05[ab]	74,38 ± 18,72[ab]	86,62 ± 20,63[a]	68,91 ± 12,23[ab]
TC (mg/dL)	6805 ± 5,81	43,88 ± 9,33[a]	69,52 ± 2,31[b]	58,21 ± 8,95[ab]	63,92 ± 9,59[b]	71,84 ± 7,45[b]
HDL-c (mg/dL)	5152 ± 5,06	38,35 ± 5,83[a]	57,64 ± 6,69[b]	44,47 ± 8,01	49,41 ± 10,66[b]	53,52 ± 664[b]
LDL-c (mg/dL)	558 ± 0,99	5,94 ± 1,41	11,42 ± 2,23[ab]	7,02 ± 1,24[a]	7,41 ± 1,56	11,07 ± 2,23[ab]
VLDL (mg/dL)	2828 ± 4,61	20,71 ± 4,28	15,82 ± 323[ab]	17,71 ± 464[a]	20,14 ± 3,02[a]	14,42 ± 3,40[ab]

Data are expressed as mean ± SEM, n = 7.
[a]When DC, DAC, DH1, DH2 and DH3 groups were compared with NC group (p ≤ 0.05).
[b]When DAC, DH1, DH2 and DH3 groups were compared with DC group (p ≤ 0.05).

4. Discussion

Diabetes mellitus treatment methods do not completely remove the disease, but rather control blood glucose and prevent complications caused by the disease. So, recent efforts for the complementary treatment of diabetes have focused on functional foods and their bioactive compounds [14]. The discovery of phytochemical components may be a source of new generation drugs. On the other hand ethnopharmacological researches on traditionally used plants and evaluation of these plants in pharmacognosy are crucial for the success of the results in the treatment of diseases leading to many complications such as diabetes.

The aim of the study was to evaluate the antidiabetic potential, antioxidant capacity, antihyperlipidemic and toxic risk of the *H. persicum* in STZ induced diabetes in rats. It is known that Diabetes mellitus causes a disturbance in glucose metabolism as well as uptake of glucose. A dose of STZ as low as 50 mg/kg bw produces an incomplete destruction of pancreatic β-cells even if the rats become permanently diabetic [39]. After treatment with such as a low dose of STZ many β-cells survive and regeneration is also possible [40].

Weight loss is a very common condition in diabetes in diabetes mellitus. In this study, the plant extract treatment groups were not observed significant changes in the weight loss except for DH1, whereas decreased significantly in the DC group. The loss of body weight is caused by increase in muscle wasting and catabolism of fat and proteins [41]. Elevated muscle wasting and decrement of tissue proteins may have given rise to body weight loss in STZ- treated group [42]. The reversal of weight loss in the plant extract and the acarbose-treated diabetic groups indicate that the restorative effect of the plant extract and acarbose may be by the reversal of gluconeogenesis and glycogenolysis [15].

The increased levels of plasma glucose in diabetic rats were lowered by *H. persicum* extract supplementations in the all groups at the edn of 21 days as comparet with beginning day. The antihyperglycemic action of the *H. persicum* extract results from the potentiation of insulin from existing beta cells of the islets of Langerhans [43]. Also, the cause of this decline may be caused by the fact that the flavanoids found in plants exert protective effects against hyperglycaemia by stimulating the release of pancreatic insulin or by regenerating the functions of islet

Table 4

Effects of *Heracleum persicum* aqueous extract supplements on HbA1c serum insulin, C-peptide and α-Glucosidase activity in small intestine levels of experimental groups.

GROUPS						
Parameters	NC Mean 7SD	DC Mean 7SD	DAC Mean 7SD	DH1 Mean 7SD	DH2 Mean 7SD	DH3 Mean 7SD
Insülin (pg/mL)	59,417 ± 44,74	415,28 ± 30,82[a]	516,76 ± 103,42	418,63 ± 71,53[a]	618,51 ± 104,21[b]	654,65 ± 108,22[b]
HbA1c (%)	445 ± 0,64	7,29 ± 142[a]	6,31 ± 1,46	4,42 ± 022[b]	464 ± 1,29[b]	431 ± 131[b]
α-Glucosidase (U/g)	3255 ± 5,88	56,35 ± 9,66[a]	52,74 ± 321[a]	69,64 ± 3,75[ab]	64,96 ± 901[a]	73,12 ± 13,89[a]
C-Peptide (pg/mL)	57,776 ± 67,77	465,51 ± 33,58[a]	582,16 ± 86,48[b]	445,03 ± 46,29[a]	512,42 ± 89,22	670,87 ± 89,14[b]

Data are expressed as mean ± SEM, n = 7.
[a]When DC, DAC, DH1, DH2 and DH3 groups were compared with NC group (p ≤ 0.05).
[b]When DAC, DH1, DH2 and DH3 groups were compared with DC group (p ≤ 0.05).

Table 5

Effect of *Heracleum persicum* aqueous extract supplements on lipid peroxidation and antioxidant defense systems of experimental groups.

Parameters		GROUPS					
		NC Mean 7SD	DC Mean 7SD	DAC Mean 7SD	DH1 Mean 7SD	DH2 Mean 7SD	DH3 Mean 7SD
Erythrocyte (U/ml)	MDA	9,49 ± 2,15	125 ± 179[a]	1010 ± 247[b]	6,47 ± 108[b]	10,06 ± 3,38[b]	9,17 ± 180[b]
	GSH	4,56 ± 0,50	3,77 ± 047[a]	492 ± 0,69[ab]	3,98 ± 0,30[a]	3,70 ± 0,70[a]	3,22 ± 0,56[a]
	GST	9,10 ± 2,40	1010 ± 1,07	10,01 ± 2,01	6,60 ± 1,20[ab]	950 ± 1,90	950 ± 140
	GR	0,17 ± 0,07	0,11 ± 0,05	0,09 ± 002[a]	006 ± 0,03	0,11 ± 006	0,07 ± 002[a]
	CAT	371,16 ± 41,70	384,80 ± 53,23	460,65 ± 53,93[ab]	261,79 ± 42,28[ab]	412,55 ± 47,86	414,23 ± 5753
	GPX	2298,83 ± 527,79	2485,72 ± 307,60	2261,19 ± 256,81	2326,95 ± 140,39	1749,4 ± 167,95[ab]	1633,89 ± 479,91[ab]
	SOD	2284,78 ± 25,51	2270,41 ± 28,29	2262,58 ± 21,40	2295,06 ± 14,11	2284,72 ± 15,70	2287,27 ± 7,34
Brain (U/g)	MDA	3219 ± 6,53	72,62 ± 3,13[a]	38,11 ± 8,49[b]	34,97 ± 8,64[b]	16,15 ± 2,17[ab]	44,77 ± 2,06[b]
	GSH	2459 ± 5,99	28,39 ± 9,79	25,65 ± 664	34,04 ± 950[a]	33,07 ± 724[a]	34,30 ± 1393
	GST	125 ± 179	13,78 ± 0,76	13,21 ± 2,58	14,40 ± 1,25[a]	13,87 ± 1,64	13,23 ± 108
	GR	098 ± 0,21	0,92 ± 012	088 ± 0,18	0,73 ± 0,26	0,62 ± 0,24[b]	0,66 ± 0,21[ab]
	CAT	2943 ± 1,57	25,84 ± 1,52	35,65 ± 7,15	18,66 ± 1,61[a]	18,90 ± 176[a]	15,55 ± 1,75[a]
	GPX	2604,26 ± 75,97	2607,28 ± 38,34	2642,33 ± 46,03	2422,99 ± 56,42[ab]	2632,38 ± 64,85	2428,61 ± 120,07[ab]
	SOD	217,507 ± 14,92	2196,48 ± 67,47	2166,92 ± 46,73	2136,43 ± 65,42	2121,13 ± 22,78[ab]	1863,84 ± 46,56[ab]
Kidney (U/g)	MDA	16,921 ± 38,81	231,02 ± 21,54[a]	212,42 ± 53,09	146,73 ± 27,15[b]	125,73 ± 23,67[b]	135,54 ± 14,24[ab]
	GSH	5174 ± 2,67	45,71 ± 12,33[a]	43,63 ± 12,48	43,37 ± 10,73	38,24 ± 4,84[a]	46,08 ± 884
	GST	823 ± 0,85	8,72 ± 1,07	14,06 ± 8,77	11,81 ± 1,22[ab]	12,12 ± 1,38[ab]	11,65 ± 2,09[ab]
	GR	080 ± 022	047 ± 0,10[a]	0,62 ± 0,10[b]	0,59 ± 0,19	0,58 ± 008[ab]	0,50 ± 0,04[a]
	CAT	31,875 ± 22,59	154,59 ± 30,84[a]	205,80 ± 44,58[ab]	194,31 ± 23,13[ab]	313,96 ± 52,31[b]	247,91 ± 33,05[ab]
	GPX	1587,17 ± 292,42	1879,62 ± 170,63[a]	186,448 ± 240,02	1802,62 ± 244,76	1791,80 ± 125,48	1797,42 ± 148,23
	SOD	216,604 ± 49,06	2189,9 ± 26,28	2235,34 ± 43,27[ab]	2184,42 ± 52,14	2183,60 ± 27,44	2211,88 ± 50,61
Liver (U/g)	MDA	5310 ± 9,92	70,12 ± 13,88[a]	44,13 ± 4,00[ab]	52,18 ± 6,35[b]	38,21 ± 6,75[b]	36,54 ± 1,89[ab]
	GSH	5029 ± 4,43	51,64 ± 5,15	56,34 ± 2,31[ab]	51,63 ± 247	54,64 ± 3,06	56,04 ± 1,25[a]
	GST	61,76 ± 10,52	69,75 ± 7,48	74,03 ± 4,82	78,19 ± 8,61[a]	79,64 ± 5,77[ab]	75,19 ± 9,49[a]
	GR	047 ± 0,11	0,48 ± 0,09	0,37 ± 008	0,16 ± 0,05[ab]	0,20 ± 006[a]	0,26 ± 0,01[a]
	CAT	44,606 ± 62,27	319,70 ± 62,47[a]	319,70 ± 58,84[a]	458,02 ± 21,65[b]	443,19 ± 60,08[b]	460,18 ± 47,60[b]
	GPX	1394,66 ± 238,55	1233,72 ± 204,32	829,23 ± 130,39[ab]	1164,51 ± 263,80	925,70 ± 132,34[a]	364,16 ± 52,56[ab]
	SOD	210,289 ± 47,71	1985,05 ± 67,79[a]	1945,73 ± 67,84[a]	2146,16 ± 73,77[b]	1973,47 ± 99,41[a]	1991,90 ± 69,91[a]

Data are expressed as mean ± SEM, n = 7.

[a] When DC, DAC, DH1, DH2 and DH3 groups were compared with NC group (p ≤ 0.05).

[b] When DAC, DH1, DH2 and DH3 groups were compared with DC group (p ≤ 0.05).

cells over time. Moreover, we found that the all of the extract treatments decreased the inceased levels of HbAlc. An increase in the level of HbA1c in the diabetic control group of rats is due to the presence of large amount of blood glucose which reacts with hemoglobin to form glycosylated hemoglobin [44]. In a conducted study, it had been repoted that a decrease in the treatment groups while an increase in the HbA1c levels of the diabetic control group. It has been stated that this result may be due to the improvement in insulin release [1]. On the other hand, the treatments of extract increased the levels of insulin and c-peptide levels decreased in DC. The reasons for such effect of the plant extract additions are not understood at the present. Nevertheless, this may be an indication of the extract regulate pancreas function and normalize insulin secretion. This result may be due to additional constituents of *H. persicum* including pimpinellin, isopimpinellin, bergapten, isobergapten, sphondin and furanocoumarins.

The results showed that STZ induced diabetes caused a significant increase in the AST and ALT levels in comparison to those of control rats whereas the extract supplementations caused a significant increase in these serum marker enzymes in comparison to those of NC and DC groups. Our results accordance with some earlier studies noted increases in AST and ALT activity in the serum of STZ-induced diabetic rats caused by hepatocyte damage changes, the transport functions and membrane permeability of these cells [43]. The rise in serum levels of AST, ALT and ALP has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [45]. The enzyme leakage due to the damage may have led to an increase in these parameters in diabetic and the extract treated groups. Thus, the elevated plasma levels of ALT and AST may indicate hepatotoxicity.

It is known that hypertriglyceridemia and hypercholesterolemia are

major risk factors in diabetes with respect to the development of atherosclerosis and coronary heart disease, which are secondary complications of diabetes [1]. In this study, it was observed that the serum levels of TC and HDL-c levels were significantly decreased in the diabetic group as compared to the normal group, but the plant extract supplementations diet restored the parameters towards to the normal control group. The reasons for such effect of the plant extract additions are not understood at the present exactly. However, the decrease of TC and HDL-c in the diabetes and the increase in the extract treatment may associate with insulin concentrations. Insulin may result in inhibition of lipolysis and decreased plasma triglyceride and cholsterol levels [39]. Some studies suggest that the antihyperglycemic action of traditional antidiabetic plant extracts may be due in part to decreased glucose absorption in vivo [46]. This explanation may also apply to the actions of *H. persicum* extract in lowering the HDL-c and total cholesterol level.

STZ is usually used for experimental induction of type I diabetes mellitus, causing pancreatic islet β-cell cytotoxicity [47]. Hyperglycemia in diabetic conditions is believed to cause oxidative stress and toxicity in pancreatic β-cells, leading to their dysfunction and the eventual development of insulin resistance in peripheral tissues. Hyperglycemia generates abnormally high levels of free radicals by autoxidation of glucose and protein glycation, and oxidative stress has been reported to be a positive factor of cardiovascular complications in STZ-induced diabetes mellitus [48]. Hyperglycemia is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to heart, kidney, eyes, nerves, liver, small and large vessels and gastrointestinal system [49]. In this study, the results demonstrated that the plant aquatic extract could have had antioxidant capacity in diabetes-induced oxidative stress. This resolution was evident from our investigation that the consequence of the extract

supplementation *in vivo*, the MDA content of HP supplementation diabetic groups differed from the DC group. As the obtained results, MDA contents appreciable increased in the tissues of STZ-diabetic rats, whereas the tissues MDA levels significantly decreased in the plant extract supplementation groups compared to DC group. The increased MDA concentration might have resulted from an increase of ROS as a result of oxisidative stress condition in the diabetic rats. There are increasing evidences that the complications associated with diabetes mellitus may be related to oxidative stress by the production of free radicals [50]. Some studies have shown the elevation MDA levels in the diabetic state [51,52]. Furthermore, the experintal study demonstrated that a similar trend of significantly decreased some ADS constituents in the Diabetes mellitus group compared to control, but the extract supplementations did not restored precisely the fluctuate ADS constituents towards to control group (Table 5). It was reported in previous studies that STZ-induced Diabetes mellitus could cause a reduction in ADSC level such as GSH, GR, GST and CAT [51,52]. The reasons for such effect of functional plant's supplementations are not understood at the present. However, the reasons for such effect of *H. persicum* supplements may be due a rich source of additional antioxidant constituents of *H. persicum* as pimpinellin, isopimpinellin, bergapten, isobergapten sphondin and furanocoumarins, and may be a promising alternative to synthetic substances as natural compound with high antioxidant and antiproliferative activities [7]. Because of the plant extracts antioxidant contents, the supplemental treatments have all been found to prevent these effects, indicating that they could provide protection to β-cells [1,46,53]. Studies had reported that a significant increase of ROS and lipid peroxidation in experimental diabetic rats and diabetic patients [54]. On the other hands, it has been determined that the total phenolic composition of HP extract has had lipid peroxidation inhibitory and free radical scavenging effect [17]. The results suggest that *H. persicum* extract-based treatments may be able to healing power of oxidative stress on β-cells and the other tissues. Since allantoin is the main component of *H. persicum*, this or other antioxidants molecules in *Heracleum persicum* may directly or indirectly preserve and regenerate β-cells. It is known that some furanokumarins present in the HP structure are antioxidant [55]. Recent studies have shown that *H. persicum* plant has antioxidant, analgesic, anticonvulsant, anti-inflammatory and immunomodulatory effects too [20]. So far, no study examining the antidiabetic potential, antioxidant capacity, antihyperlipidemic and toxic risk of the *H. persicum* supplementations on STZ induced diabetes as in vivo has been made on rat. Therefore, we had no chance to compare our results with the previous ones. In addition, because of high variability in analyzing serum enzymes-chemicals interaction in vitro and in vivo, and inconsistent factors like treatment time and manner, the setting of studies and species tissue differences etc., it is difficult to compare the present data to different studies regarding the healing and preventive properties.

5. In conclusion

The present study demonstrates the therapeutic properties of *Heracleum persicum* lyophilized extract in STZ-diabetic with evident hepatotoxic effects. We have seen that *Heracleum persicum* extract can have antidiabetic beneficial effects as evidenced from the changed levels of diabetic biomarkers such as blood glucose, HbA1c, insulin and c-peptide levels, due to the improved function of pancreatic β-cells, towards to control. Further, the present study demonstrated that the the plant extract supplementation could have antioxidative role in diabetic rats. This was obvious from our observation that MDA concentration of the tissues of the plant supplemented diabetic groups lower from diabetic control group. In short, the results suggest that moderate intake of the HP extract may be useful for diabetes-related complications. However, it might be caused hepatocyte damage changes as the transport functions and membrane permeability of hepatocytes.

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