Effects of hydro-alcoholic extract of *Prangos ferulacea (L.) Lindle* on histopathology of pancreas and diabetes treatment in STZ- induced diabetic rats

Khosro Soltani band*[1], Najme Kafash Farkhad[1], Farah Farokhi[2], Amir Togmechi[3]

Abstract

Objectives: This study investigates the effects of hydro-alcoholic extract of *Prangos ferulacea (L.) Lindle* (P.f) on rats' pancreas structure changes and diabetic treatment after streptozotocin injection.

Materials and Methods: In this research forty male Wistar rats with body weights of 100±20 gram, were randomly divided into 5 groups with 8 rats per each group. Diabetes was induced in rats by streptozotocin at a dose of 75 mg/kg body weight (B.W) injected intraperitoneally. Root and leaves with stems hydroalcoholic extract of P.f at a dose of 100 mg/kg B.W have been injected orally in diabetic rats, daily for a month.

Results: Significant decrease in blood glucose, WBC and HbA1c and increase in body weight were observed in treated diabetic rats. Histopathologic results of diabetic rats revealed reduction in number of pancreatic islets as well as their number of β-cells and insulitis with lymphocytes infiltration. Regeneration of pancreatic islets and β-cells, along with a reduction in the number of infiltrated lymphocytes were present in plant extract –treated diabetic rats.

Conclusion: The roots' hydro-alcoholic extract of P.f seems to be capable to regenerate the islets of Langerhans in the treated rats in comparison with the untreated diabetic rats. This property can be due to some components of the plant that can increase insulin secretion.

Keywords: Insulitis, Diabetes mellitus, *Prangos ferulacea (L,) Lindle*

1- Histology and Embriology, Department of Biology, Faculty of Science, Urmia University, I. R. Iran
2- Department of Biology, Faculty of Science,Urmia University, I. R. Iran
3- Department Artemias research,Faculty of Veterinarity, Urmia University, I. R. Iran
*Corresponding author: Tel: 09356575771
E-mail: Bandsoltani@yahoo.com

Introduction

Diabetes is one of the endocrine illnesses in the world including dysfunction in carbohydrate, fat and protein metabolism. According to WHO report, prevalence of diabetes is about 35 percent (Bastaki, 2005 and Tomas, 2009). Nowadays there are 150 million diabetic patients in the world. It is predicted that this amount will increase to 300 million until 2025 (Bastaki, 2005). There fore it is essential to research on drugs and new interventions for treating and controlling this metabolic illness. Although there are many drugs for treating and managing diabetes, these are very expensive for developing countries and may have side effects such as hypoglycemia and weight gain (Alarcon et al., 1998). In the diabetes process, long periods of hypoglycemia can result in increase of free radicals production specially ROS. It is shown that glucose auto-oxidation and protein glycosylation and numerous conditions in all tissues can disrupt the balance between ROS production and cell defending mechanism. This disruption in the balance results in a cell dysfunction and changes in cell function and damaging the tissues specially in pancreas (Halliwell et al.,1989 and Kaneto et al., 2007). Currently, there is a high interest in recognition of antioxidant compounds that have pharmacologically potential without any possible side effects or at least less side effects,which can be used in medicine and the food industry (Srivastava et al., 2003).

In cellular defense system, scavenging of free radicals is an important issue which is related to utilization of both exogenous and endogenous antioxidant (Eddouks et al., 2002). Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity (Srivastava et al., 2003). Ayurveda, supposed to be the oldest medical system in the world, provides potential leads to find active and therapeutically useful compounds from plants. Therefor it seems that plants particularly those with high levels and strong antioxidant compounds have an important role in improvement of disorders involving oxidative stress such as diabetes mellitus (Nazirogilu et al., 2005). The ethnobotanical information reports about 800 plants that may possess antidiabetic potential, but antidiabetic effects of many plants yet are unknown (Kazerooni et al., 2006). In this aspect, Prangos ferulacea (L.) Lindle is a plant native to the mountains of southern Iran (Fars province). In French it is called Oppoponax and in Persian Jashir. Its green sticks and leaves are used in different ways, e.g., boiled in churned sour milk and yoghurt or processed vinegar. The leaf is used for gastrointestinal disorders and has also demonstrated an absence of toxicity (Teiler et al., 1983). Recent studies showed that this plant is an excellent source of antioxidant and have a great antioxidant potential that is more than of α-tocopherol (vitamin E) (Coruh et al., 2007). In this research for the first time we investigated the antidiabetic effects of hydroalcoholic extract of this plant.

Material and Methods
Chemicals and drugs

Streptozotocine (STZ) and gliben_clamid and chloroform were purchased from Sigma Aldrich, Germany. Normal saline and citrate buffer from Iran Darupakhsh Company, ethanol from Pakdis Company, Iran and other materials were purchased from Merk Company, Germany.

Plant material

Fresh, green P.ferulacea plants were collected from the Shahidan Mountains of West Azerbaijan in northwest of Iran in frontier localities between Iran and Turkey

in May 2010 and authenticated with a professor from the Department of Biology at Uremia University. The samples (roots separately, and green leaf and stems had weight rate of 1: 1) were dried in shadow for 7 days.

Preparation of extract

Collected samples were dried and ground by an electrical mill. 100 gram of both powder samples was added to 1000 ml of alcohol. First, ethanol 96% was used and after 24 h both solutions were filtered and in the second step ethanol 70% was added to the remained of dry materials. After 24 h solutions were filtered and then both filtered solutions were mixed together and then evaporated repeatedly to half the first volume by rotary evaporator in 50 c and 70 rpm. Concentrated extracts were dried on water bath at 40º c temperature to yield 6% w/w dry extract. For the preparation of injected extract, this powder was solved in specific volume of normal saline (Kazerooni et al., 2006).

Preparation of diabetic rats

STZ dissolved in saline was injected to rats intraperitoneally at dose of 75 mg/kg body weight (B.W). After a fortnight, rats with marked hyperglycemia (serum glucose more than 200 mg/dl) were selected and used for the study (Dhandapani et al., 2002).

Experimental plan

Forty male Wistar rats with B.W of 100±20 gram were purchased from Pasteur Institute, Iran and kept in animal houses of Urmia University. They were kept at 20±5^0 C, relative humidity of 30±5% and light/dark cycle for 12h. All the animals were fed with rodent pellet diet and water was allowed ad-labium under strict hygienic conditions. These rats were randomly divided into 5 groups with 8 rats per each group, as follows: group 1(C: control group) administrated 0,5 ml saline, group 2 (D: untreated diabetic rats), group 3 (D+S1) diabetic rats receiving roots`

hydro-alcoholic extract of P,f at 100 mg/kg B.W in saline, group 4 (D+S2) diabetic rats receiving leaves and stems` hydro-alcoholic extract of P,f at 100 mg/kg B.W in saline, group 5 (D+S3) diabetic rats receiving gliben_ clamid at 5 mg/kg. The treatment period was 4 weeks and all extracts were administered orally in rats by intragastric tube.

Tissue preparation and biochemical stimation

At the end of experiment, the rats were weighed, anesthetized by diethyl ether and their pancreases were taken out and fixed in 10% natural buffer formalin. After tissue processing, the samples were blocked in cylindrical paraffin blockers and then stained by Hematoxilin- eosin (each sample`s diameter was 5-6 microns) (Dhandapani et al., 2002). At first, WBC count was measured from Rats' blood collected from heart left ventricle and then serum samples were collected for estimation of biochemical parameters from all the experimental rats: serum glucose and HbA1c. For the measurement of diameters of pancreatic islets a binocular-micrometer was used that was connected to the microscope. In each sample, some of the Langerhans islet were investigated randomly , in Unit level (0.25 mm^2).

Statistics

All values are expressed as Mean±SEM. The differences were compared using one way analysis of variance (ANOVA) followed by Tukey´s multiple comparison tests. p values <0.05 were considered statistically significant.

Result

The obtained results showed that in D group glucose, WBC and HbA1c values were elevated to high levels during the study, where Langerhans islet diameters and body weight were decreased significantly (p<0.05). Chronic treatments with roots hydro-alcoholic extract of P.f

causes decrease in serum glucose in 2 and 4 weeks as shown in Figure 1. Also as shown in Figures.2, 3, 4 and 5 in D+S1 and D+S3 group at the end of treatments period, Langerhans islet diameters and B.W were increased significantly, where WBC levels and HbA1c were decreased.
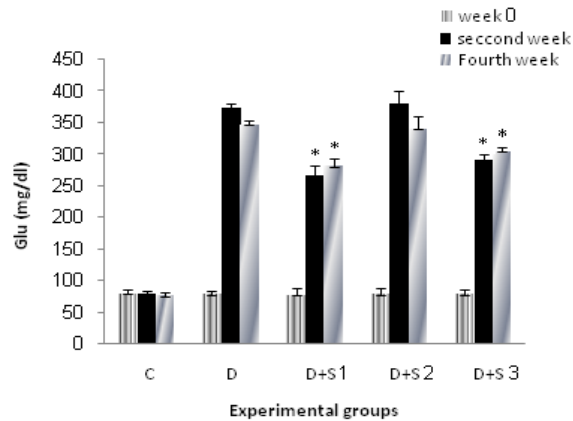
Mean± SEM (n=8). Statistical comparisons between each group were carried out by one way ANOVA followed by Tukey´s multiple comparison tests.*p<0.05 in comparison with diabetic values.

Figure 1. Effect of hydroalcoholic extract of P.f on serum glucose level in experimental groups on 0,14 and 28 days. All values are expressed as Mean± SEM (n=8). Statistical comparisons between each group were carried out by one way ANOVA followed by Tukey´s multiple comparison tests.*p<0.05 in comparison with diabetic values.
C: control group, D: diabetic group, D+S1: diabetic rats treated with roots hydroalcoholic extract of P.f (100 mg/kg)
D+S2: diabetic rats treated with stems&leaves hydroalcoholic extract of P. f (100 mg/kg)
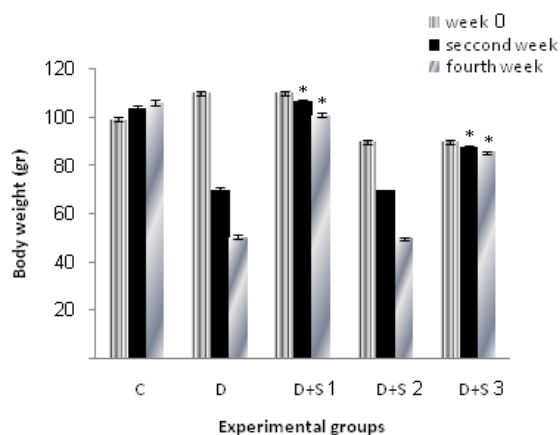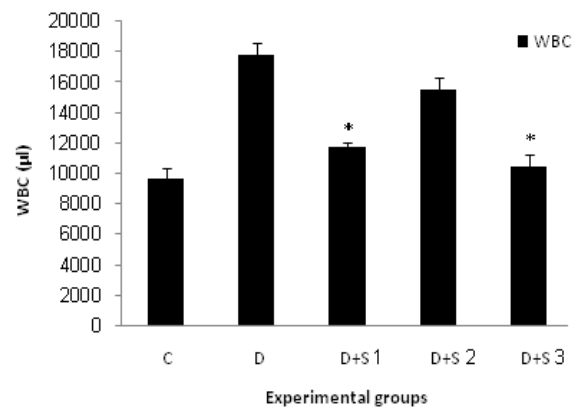D+S3:diabetic rats treated with gliben clamid (5mg/kg)

Figure 3. Effect of hydroalcoholic extract of P.f on WBC level in experimental groups on 28 days. All values are expressed as Mean± SEM (n=8). Statistical comparisons between each group were carried out by one way ANOVA followed by Tukey´s multiple comparison tests.*p<0.05 in comparison with diabetic values.
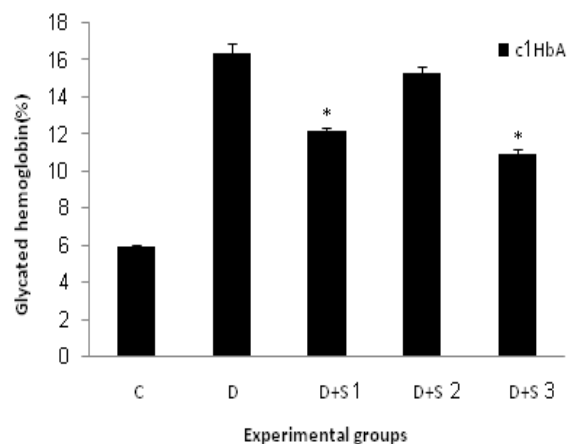

Figure 4. Effect of hydroalcoholic extract of P. f on HbA1c level in experimental groups on 28 days. All values are expressed as Mean± SEM (n=8). Statistical comparisons between each group were carried out by one way ANOVA followed by Tukey´s multiple comparison tests.*p< 0.05 in comparison with diabetic values.

Figure 2. Effect of hydroalcoholic extract of P. f on Body weight in experimental groups on 0, 14 and 28 days. All values are expressed as
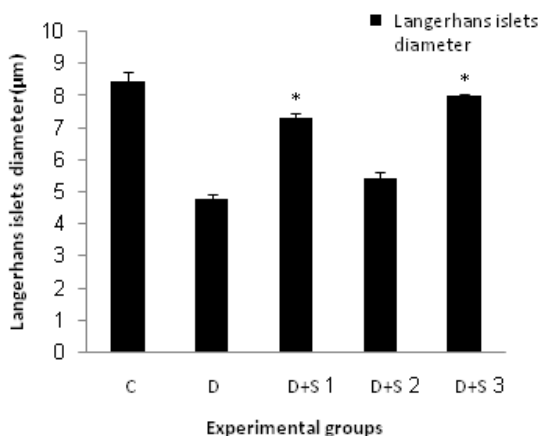
Figure 5. Effect of hydroalcoholic extract of P. f on Langerhans islets diameter in experimental groups on 28 days. All values are expressed as Mean± SEM (n=8). Statistical comparisons between each group were carried out by one way ANOVA followed by Tukey´s multiple comparison tests.*p< 0.05 in comparison with diabetic values.

Histopathological findings

Histopathologic study of diabetic rats revealed the reduction of number and diameters of pancreatic islets as well as the number of β-cells and insulitis (inflammation of pancreatic islets) with lymphocytes infiltration and β-necrotic cells with some vacant space beside the islets that was resultant of islets disruption. Regeneration of pancreatic islets and β-cells, along with the number of infiltrated lymphocytes and increase in the pancreatic islets diameters, in D+S1 and D+S3 groups were observed. In D+S2 group all of the signs were similar to the D group and treatment with extract had no significan effects on pancreatic injury induced by diabetes. Overall, as shown by microscopic images, in D+S1 group under the infiltration of lymphocytes and macrophages and the lymphocytic invasion in comparison with diabetic group was decreased significantly, which could be due to increased insulin secretion and reconstruction and repair of pancreatic islets by the extract.
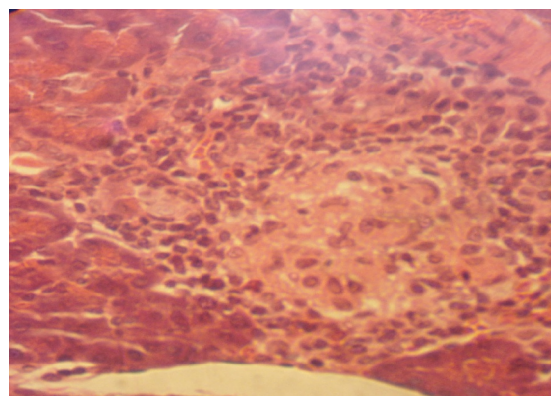

Figure 6. Diabetic control rats (H&E×400) showing lymphocytes inflammation and shrinkage of islets with atrophic islets and necrotic β cells.
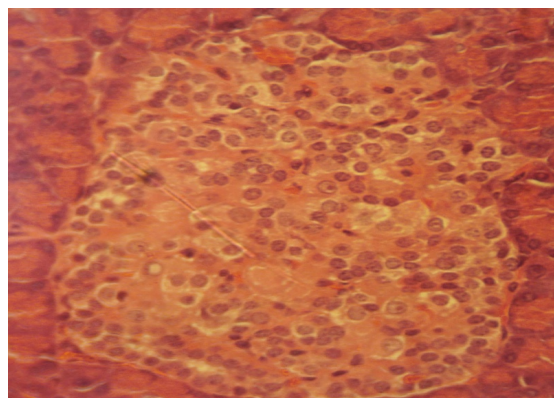

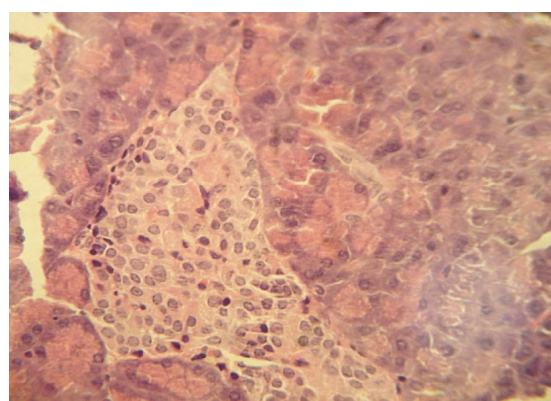Figure 7. Normal rat pancreas (H&E×400) showing normal islet cells and hypertrophic β cells.

Figure 8. Diabetic+Roots hydroalcoholic extract of P. f (100 mg/kg) treated rat pancreas (H&E×400) showing reduction in lymphocytes inflammation and necrotic cells, and pancreatic islets within normal limit.
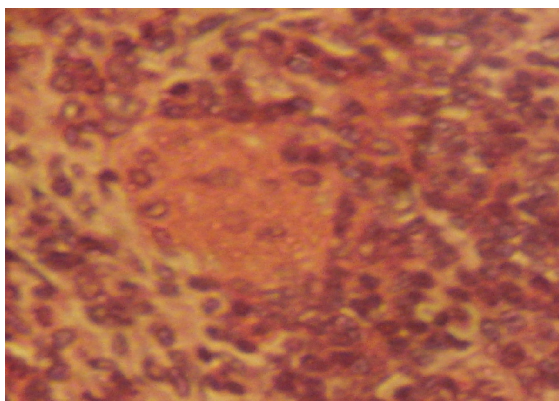
Figure 9. Diabetic+Stems & leaves hydroalcoholic extract of P. f (100 mg/kg) treated rat pancreas (H&E×400) showing reduction in Langerhans islets diameter,and insulitis(inflammation of pancreatic islets).
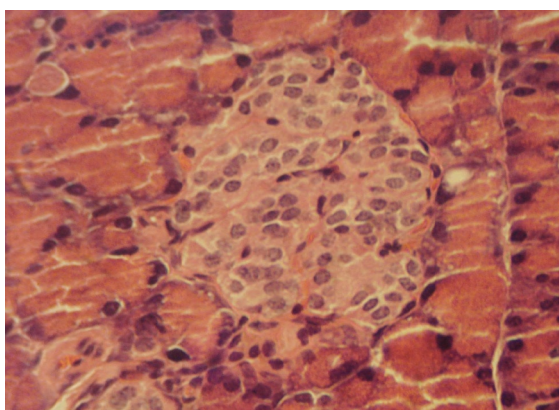

Figure 10. Diabetic+gliben- clamid (5 mg/kg) treated rat pancreas (H&E×400) showing reduction in necrotic β cells and insulitis.

Discussion

It has been proved that streptozotocin damages pancreatic cells selectively and causes hyperglycemia (Larkins et al., 2004). Although the pathogenesis of chemical drugs induced diabetes and the role of insulitis has not been clarified totally, STZ can cause DNA damage and β-cells disruption by producing of free radicals (Ramesh et al., 2005). STZ creates a diabetes similar to type 1 diabetes whith final symptoms such as insulin deficiency (Jamshidian et al., 2000). In this study pancreatic injury in diabetic rats was exactly like the people with IDDM. Lymphocytic infiltration can be a representative of autoimmunization response that results in a disruption of Langerhans islet in most of the β cells. In the current research, insulitis and lymphocytic infiltration were obvious and treating with roots hydro-alcoholic extract of P.f decreased lymphocytic infiltration. On the other hand serum glucose reduction showed improvment and rebuilding of Langerhans islet damaged by STZ-injection.

Scientists are eager in finding novel ways to treat diabetes because of increasing in number of diabetic patients. Beside the modern medicine science and medicinal research, traditional medicine has been successful by using medicinal plants (Mc Gee et al., 1997-2001). Hyperglycemic effects in many of these plants refer to their ability in stimulation of insulin secretion.This function causes Langerhans islet restoration and pancreas function improvement(Walid et al., 2009). In general there is little biological knowledge on the specific modes of action in the diabetic treatment, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having anti diabetic effects (Diabetes Care., 2009). In this study, we investigated anti diabetic effects of Prangos ferulacea (L.) Lindle in STZ-induced diabetic rats. The genus of Prangos with the common Persian name of (jashir) includes 15 species which are growing widely in many regions of Iran. Some species are distributed in Anatolia, Central Asia and Caucasian. Prangos ferulacea (L.) Lindle is a native plant from southern mountains of Iran (Fars province). Previous phytochemical studies of this plant indicated the presence of coumarines, Alkaloyides, flavenoides and terpenoyides. More importantly some components such as Umbelliferone, Frundenole, Fruliden, Prangon and Penthyl coumarins were detected in the roots of this plant (Nazirogilu et al., 2005).

The mechanism by which STZ brings about its diabetic state includes selective destruction of pancreatic insulin secreting β cells, which make cells less active and lead to poor glucose utilization by tissues (Ramesh et al., 2005). Umbelliferon(7-hydroxy coumarin), an excellent natural antioxidant, is an abundant compound in roots of P, f. Earlier studies showed that this compound had a protective effects on membrane structure of fatty acids and can increase insulin secretion from Langerhans islet (Eddouks et al., 2002). So it has antihyperglycemic and antioxidant properties. In the previous studies the parent compound coumarin has been reported to reduce plasma glucose. Previous studies by Rameshet al in 2005 showed that treatment with Umbelliferone and gliben_clamide reduced the changes in the pancreas, which supports the biochemical analysis (Ramesh et al., 2005). Treatment with Umbelliferone and glibenclamide reduced the changes in the pancreas, which supports the biochemical analysis. Chronic hyperglycemia causes elevated concentrations of reactive oxygen species accompanied by lowered enzymatic and nonenzymatic cell antioxidant defenses (Eddouks et al., 2002). Reactive oxygen species have been suggested to be involved in β cell dysfunction and insulin resistance (Eddouks et al., 2002). Antioxidant property of many plant extracts has been attributed to their phenol content (Hikino et al., 1989). As earlier studies showed P.f is an excellent source of antioxidant compounds such as phenol content, coumarins and alkaloids that can be useful to decrease oxidative stress and to elevate the antioxidant potential of this plant (Hikino et al., 1989). Regarding the results that Umbelliferon can stimulates the insulin secretion and having in mind that this compound is the main element of P.f, we suggest regeneration and treatment of pancreatic islets in treated diabetic rats with roots` hydro-alcoholic extract of P.f which can cause be due to the elevation of insulin secretion. Regeneration of pancreatic islets

decreased number of WBC, having protective effects on pancreas.

Conclusion

In summary, the results of this research showed that roots` hydro-alcoholic extract of P.f can influence the changes of serum glucose levels and prevent the histopathological changes of pancreases related to STZ diabetes in rats and the effect can be due to the flavenoides, Umbelliferone and their antioxidant features.

Acknowledgment

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