Effects of Aqueous Extract of Gunnera (*Gundelia tournefortii* L.) on the Blood Serum Sugar Levels and Changes in the Streptozotocin-induced Diabetic Pancreatic Tissue of Rat

Mohammad Alimoradi¹, Cyrus Jalili², Syran Kakeh-Baraei³, Ahmad Tajehmiri⁴, Reza Khodarahmi⁵

¹Researcher, Human Physiology, Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran, ²Associate Professor, Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran, ³Researcher, Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, ⁴Researcher, Medical Sciences, Kermanshah, Iran, Nosocomial Infection Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran, ⁵Professor, Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran, Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran,

Abstract

Introduction: Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostasis resulting in severe diabetic complications including retinopathy, angiopathy, nephropathy, and neuropathy causing neurological disorders due to perturbation in utilization of glucose. *Gundelia tournefortii* L. is a medicinal plant, native to Asia, used as an occasional food source, and its extracts have been used for prevention and treatment of diabetes disease.

Aim: The aim of this study was to effects of aqueous extract of gunnera (*Gundelia tournefortii* L) on the blood serum sugar levels and changes in the streptozotocin-induced diabetic pancreatic tissue of rat.

Methods: A sum of 36 male Wistar rats were categorized into six study groups. In a 21 day study period, aqueous extract were given every day throughout the study period whereas. On day 21, fasting blood samples, body mass and pancreatic tissues were collected and processed for blood sugar estimation and the determined values were also used to assess further and Hematoxylin-Eosin staining was applied to evaluate the changes of pancreatic tissues. SPSS One-way ANOVA was used to analyses the data and multiple comparison was done, interpreted based on Tukey's test.

Results: Streptozotocin significantly increased the levels of glucose in the body in the 2, 5 and 6 group ($p\leq0.001$). Beneficial effect were seen when rats were treated with G. tournefortii at dose of 400 mg/kg/day. This lead to a significant decrease in the levels of glucose in tissue pancreas.

Conclusion: A significant decrease in glucose levels was observed in G. tournefortii treatment in 400 mg/kg/day and according to the histological results, the destroyed tissues (Langerhans islets) were also reconstructed. The aqueous extract of Gundelia tournefortii L exhibited marked protection against Diabetes mellitus and also recovery for tissue pancreas in Wistar rats.

Key words: Tissue pancreas, Hyperglycemia, Histopathology, Diabetes mellitus, Drug induced hyperglycemia

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by the insufficient secretion and resistance to the action of

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insulin. It leads to diseases like; neuropathy, retinopathy, nephropathy and cardiovascular disease [1]. Decrease in insulin secretion from the pancreatic beta cells and/or decreased sensitivity of target cells to insulin leads to an increase in the blood glucose level. According to the reports of the Iranian Diabetes Society, more than 8 percent of the Iranian people have diabetes and the disease affects more than three million people [2]. Medicinal plants have been used in the treatment of diabetes since ancient times. Nowadays the use of the medicinal plants for treatment is increasing because of the low cost, less side-effects and the diversity of effective chemical compounds. Medicinal

Corresponding Author: Reza Khodarahmi, Professor, Medical Biology Research Center (MBRC), Kermanshah University of Medical Sciences, Kermanshah, Iran. E-mail: rkhodarahmi@mbrc.ac.ir

plants are rich with natural antioxidants. These plants are used to control and treat many diseases in traditional medicine. In recent years, numerous experimental and clinical studies have been performed related to the effects of medicinal plants in order to reduce the rates of disease all over the world. However, the functional mechanism of the most widely used plants is still elusive to most scientists. The therapeutic effects of compounds derived from the medicinal plants have been considered, but treatment has not been verified by evidence [3-4]. Gunnera (Gundelia tournefortii L.) belongs to the Asteraceae family. It is a thistle herb that grows in some countries including Iran, Armenia, Azerbaijan, Anatolia and Iraq. Gunnera is used to treat vitiligo and diabetes mellitus [5]. The Gunnera extract is also used in the treatment of liver diseases [6-7]. The antioxidant properties of this plant have been reported and its protective effects on liver have been confirmed [8-9]. The Gunnera extract reduces the plasma lipid level and has phenolic compounds such as quercetin with powerful antioxidant effects [10-11]. The antioxidants protect sperm cells from damage by free radicals and improve sperm quality. Antioxidant treatment is a protective defense against oxidative stress and helps in improving fertility parameters [12-13]. Artichoke leaves contain phenolic compounds, flavonoids and acidic compounds including: caffeic acid, esters of caffeic and kainic acid, chlorogenic and pseudo chlorogenic acid, neochlorogenic acid, cynarin and di-caffeil kainic acid [14-15]. The effects of the Gunnera plant on damaged pancreas tissue was not evaluated. This article studies the effects of Gunnera extract on the blood serum sugar levels and histo-morphological changes in the streptozotocin-induced diabetic pancreatic tissue of male rats.

MATERIALS AND METHODS

Experimental Animals

In This experimental study we use sum of 36 male Wistar with 8-10-week old, weighing 200-250 gram were obtained from Medical College of Kermanshah University, they were housed under standard conditions of temperature and they rats maintained at 22±3°C, 30 to 55% of relative humidity on standard protocol 12 hours day and night cycle was maintained food and water was provided ad libitum [16]. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health [17]. For induction of diabetes, 45 mg/kg¹-Intra peritonea single dose, streptozotocin with citrate buffer was injected after 12 hours fasting. The diabetic animal was determined using a sample from the tail of rats, 3 days after the STZ injection. Rats who had fasting blood sugar than 250 mg/dl with polydipsia, polyuria and weight loss were determined as diabetic animals. Rats were categorized into six study randomized groups: First group was control group that received normal food and water without an injection of STZ. Second group was the diabetic control group without treatment. Third group received 200 mgkg⁻¹ extract of Gunnera. Fourth group received 400 mgkg⁻¹ extract of Gunnera. Fifth group was diabetic and received 200 mgkg⁻¹ extract of Gunnera and sixth group was diabetic and received 400 mgkg⁻¹ extract of Gunnera. Gavage for all groups was done for a period of 21 days.

Plant Tissue Extraction

Naturally grown *G. tournefortii* plants were collected from Songhor Kolyaei location, Kermanshah Province. The plant shoots were washed and dried at temperature of 20-25°C in the shade. The dried shoots were then powdered using electrical mills. Was added 1000 ml of distilled water to the plant powder and it was placed in a water bath at 60°C for one hour. The resulting mixture was filtered and incubated at 40°C until the gel of extract was formed. The efficiency of extraction was 20.2% [18].

Measurement of Fasting Blood Sugar and Tissue Passage

In a 21 day study period, aqueous extract were given every day throughout the study period whereas. On day 21, the animals were anesthetized with ether, Heart blood sample was taken with a syringe in order to collect blood serum. Blood samples were then placed in a water bath. After using the applicator, the samples were centrifuged at 7000 rpm for 15 minutes. Then the blood serum was stored at -70° C. Fasting blood samples and body were collected and processed for blood sugar estimation and the determined values, for tissue passage, firstly the samples were marked and were placed in 10% formalin, then the samples were fixed and were impressed using paraffin. Continuous sections with 5-7 micrometer thickness were prepared using microtome. They were then stained with hematoxylin - eosin stain. The diameter of Islets of Langerhans was measured using a calibrated lens. The parameters that were measured included number and size of islets, cell edema and atrophy measured in the endocrine pancreas. Magnification photo (40X) micrographs of cross-sections of pancreas were prepared and evaluated using an optical microscope [19].

Statistical Analysis

SPSS software, one way ANOVA and Tukey's range test were used for statistical analysis at 0.05% probability level.

3. RESULTS

Body Weight

As shown in the Figure 1, there were no significant differences among weights of animals in the baseline

($p \le 0.005$). The weight of the animals in the groups 1, 3 and 6 increased significantly compared to the group 2 in the first and second weeks ($p \le 0.001$). The weight of the rats in the five groups increased significantly compared to the group 2 during the fourth week ($p \le 0.001$).

Blood Sugar

As shown in figure 2, there was a non-significant difference for blood serum sugar levels of non-diabetic rats in the groups 1, 3, 4 and diabetic rats in the groups 2, 5 and 6 during the start of the study and the first week of the experiment ($p \le 0.001$). The blood serum sugar levels of the group 2 increased significantly compared to other groups in the second and third weeks ($p \le 0.001$).

Tissue Sections

In photomicrographs of the cross-sections of the pancreas of first, second and third group, the structure of the tissue and pancreatic islets were normal (Figures 3-5). The necrotic changes and severe degradation and decrease the size of the pancreatic islands in groups 5 and 6 that received 200-400 mg/kg/day of plant extract were less than as seen in the all groups (Figures 6-8).

DISCUSSION

The form of diabetes treatment is not only about the treatment of the disease itself, but rather reducing the



Figure 1: Weight of diabetic and non-diabetic rats under different treatment of Gunnera plant extract levels. *significant difference at 0.05% of probability level, **significant difference at 0.01% of probability level, **significant difference at 0.05% of probability level



Figure 2: Means of streptozotocin-induced diabetic pancreatic rats under different Gunnera plant extract levels. *significant difference at 0.01% of probability level



Figure 3: Photo micrograph of cross-sections of the pancreas in group 1Staining: H/E 40X magnification



Figure 4: Photo micrograph of cross-sections of the pancreas in group 3 (200 mg/kg/day of plant extract) Staining: H/E 40X magnification



Figure 5. Photo micrograph of cross-sections of the pancreas in group 4 (400 mg/kg/day of plant extract). Staining: H/E 40X magnification, *White arrows showing Islets of Langerhans

complications and symptoms of the disease has created extensive research still, researchers are looking for new and better ways, The treatment of this phenomenon is physiologic. This experimental study indicates the hypoglycemic effects of aqueous extract of *Gundelia tournefortii* L in Streptozotocin-induced diabetic rats. These effects were dose dependent. The hypoglycemic effects included the improvement of the pancreas, increasing the number and average diameter of the Islets of Langerhans, insulin secretion, significant glucose level reduction and improvement in body weight. Streptozotocin produces



Figure 6: Photo micrograph of cross-sections of the pancreas in group 2 (STZ), Staining: H/E 40X magnification



Figure 7: Photo micrograph of cross-sections of the pancreas in group 5 (200 mg/kg/day of plant extract) Staining: H/E 40X magnification



Figure 8: Photo micrograph of cross-sections of the pancreas in group 6 (400 mg/kg/day of plant extract) Staining: H/E 40X magnification, *Yellow arrows indicating the Islets of Langerhans and distribution of lymphocytes in the pancreas. Black arrows shows edema, fatty tissue and the subsequent necrosis in pancreas

free radicals that cause inflammation and destruction of the pancreatic beta cells [20]. At zero time, there was non-significant difference among the body weight of animals, but in the first and second weeks, difference is beginning between group 2 (diabetic) and groups 1, 3 and 6. During the 21 days, five groups had higher weight compared to group 2 (1, 3, 4, 5, and 6 vs. 2). Probably this weight increase, is a natural process of healthy animals, but the decrease in the weight in diabetic animals is due to Streptozotocin. Blood serum sugar levels were not significantly different among the non-diabetic groups including 1, 3 and 4 and diabetic groups including 2, 5 and 6 at zero time and first week. These results were predictable. Streptozotocin within 48 hours after injection had an impact on the pancreatic beta cell membranes and the detachment of the DNA strands. Therefore, it causes necrosis of the cells and the induction of diabetes [11]. Blood serum sugar levels in five groups decreased significantly compared to group 2 in the second and third weeks. Phenolic compounds such as quercetin reported in Gunnera [21]. Quercetin is the most abundant natural non-carbohydrate flavonoid that can be found in plants and vegetables. Low carbohydrate flavonoids are more powerful compared to glycoside flavonoids [21-22]. Quercetin has powerful antioxidant effects [23]. It is concluded that Gunnera has powerful antioxidant effects as reported by another study [7]. Cynarin, chlorogenic acid and luteolin have antioxidant effects [24]. Antioxidants protect pancreatic beta cells against the free radicals of Streptozotocin and also by stimulating insulin secretion. Also, they decrease the intestinal absorption of glucose by inhibiting the digestive enzymes of carbohydrates [25].

Stained tissue sections revealed that Streptozotocin caused necrotic changes along with degradation and reduction in the size of the pancreatic islets. These changes were lower in the treatment group with 400mgkg⁻¹ of the gunnera plant extract. Recent research shows that under certain conditions there is also the possibility of proliferation of mature pancreatic beta cells [26-27-28]. Above mentioned antioxidants caused an increase in the number of beta cells [26]. This increase happens through many possible ways including differentiated beta cell proliferation in the islets of Langerhans, progenitor cells to beta cells, stem cells and differentiate pancreatic duct cells to beta cells [29-30]. In a diabetic animal, new islets were produced by the cells differentiation of progenitor cell of the islets [31]. Histopathologic study of pancreatic sections revealed the lymphocytic infiltration and edema. Changes in blood glucose and insulin levels, which is a reflection of pancreatic beta cell dysfunction, swelling of the pancreas and degeneration of pancreatic beta cells were due to induced diabetes. These signs were also mentioned by others [32]. So, it is possible according to this study, administration of plant extract stimulates the proliferation of beta cells in the diabetic pancreas of rats. Our study indicates the usefulness of G. tournefortii extract as hypoglycemic in diabetic treated rat.

CONCLUSION AND RECOMMENDATIONS

Changes in blood glucose levels in diabetic patients can be reversible, and the use of medications derived from nature has less side effects and more positive effects than chemical drugs. The results of this study showed in dose 400 mg/kg/day aqueous extract of *Gundelia tournefortii* significant decrease in glucose levels and increase body weight was observed and according to the histological results, the destroyed tissues (Langerhans islets) were also reconstructed, The use of this plant is recommended to decrease the level of glucose but mode of action of aqueous extract, type of its effective compound and it's mechanism in the cell levels has not been determined In the next studies, these problems can be considered. We hope to continue this research on humans to confirm its application in drugs for human consumption.

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