The Effects of Hypericum Extract on Blood Factors in Diabetic Rats

Afshin Roostaie[1], Kambiz Roshanaei[1,*], Mohammad Reza Yazdian[1] and Ali Akbar Malekirad[2,3]

[1]*Islamic Azad University, Qom Branch, Qom, Iran*

[2]*Biology Department, Payame Noor University, Iran*

[3]*Environmental Toxicology Scientific Group, Iran*

Abstract: Diabetes mellitus comprises a heterogeneous group of metabolic diseases characterized by chronic hyperglycemia and impaired metabolism of carbohydrates, lipids and proteins caused by defects in insulin secretion or function. It is said to be the sixth factor of mortality and its complications, if untreated, include: atherosclerosis, nephropathy, neuropathy, foot ulcers, disability and death. To treat diabetes either insulin is administrated or some chemicals that lower blood sugar levels are prescribed. However, due to their various complications, the use of medicinal herbs has been increasingly taken into consideration. In this study, the effects of Hypericum extract on blood parameters in diabetic rats-male Wistar rats-were studied.

Methods: In this study, male rats (n =36) were randomly divided into six groups: control, 21 day treated control, 28 day treated control, diabetic, 21 day treated diabetic rats, and 28 day treated diabetic rats. To induce diabetes, streptozotocin was used intraperitoneally at a single dose of 60 mg per 1kg body weight. The treated groups were gavaged with the extract at a dose of 60 mg per kg body weight in a period of 3-4 weeks. After this period, blood serum parameters were measured and the data were analyzed using multivariate analyze of variance

Results: The results showed that there is a significant difference between the six groups in parameters FBS, Total P, and Na in blood, and PU, FBSU, KU in urine.

Conclusion: The administration of Hypericum extract in the experimental models of diabetes mellitus has improved the biochemical parameters in diabetic rats.

Keywords: Diabetes, Hypericum, blood factors, rat.

INTRODUCTION

Diabetes is one of the most common disorders of the endocrine system and it is estimated that its prevalence in the human population will increase in the future [1]. Currently, the prevalence of the disease in Iran, is about 5-6 percent, and about 4 million of Diabetes (Diabetes Type I and II) have been detected and more people are prone to it [2]. Relative insulin deficiency or shortage is involved in the pathogenesis of acute metabolic complications such as ketoacidosis and osmolar coma or metabolic disorders, and chronic and long-term complications such as retinopathy, renal vascular involvement, neuropathy and heart failure. Moreover, the decrease in insulin secretion in diabetes type I and the insulin resistance in diabetes type II are associated with the increase in the osmolarity resulted from organic molecules (sugars, fats, proteins) and ions (such as sodium and potassium). This is followed by increased urine secretion which is resulted from the high concentration of glucose excretion. The high concentration of glucose in the urine osmolarity together with increased fecal excretion can be the major cause of decreased blood volume and its increased concentration. These two factors stimulate chemoreceptors and baroreceptors in the lateral hypothalamic nuclei activation and Polydipsia, which, together with blood hypersmolarity, if untreated, can increase the cardiovascular, kidney, liver and brain problems of diabetic patients. Currently the most effective treatments for diabetes mellitus are the use of insulin and hypoglycemic agents. However, these compounds have several undesirable effects such as increased fat deposits, adipose tissue atrophy at the site of injection and the incidence of hypoglycemic shock, and have no effects on long term debilitating complications of diabetes [1]. As the human knowledge about heterogeneity of this disease increases, the need to find more effective combinations for the treatment of diabetes with fewer side effects increases, too [1-4].

Medicinal herbs and their derivatives have always been used in the treatment of diabetes mellitus and its complications. However, many are uncertain about the effectiveness of them due to the scarcity of valid research and evidence. The plant Hypericum (Hypericum perforatum) from the family of Clusiaceae, is herbaceous and perennial, with a glabrous stem, spoon-shaped leaves with no tail. The plant grows in various areas of Alborz, northern Iran, Lahijan,

*Address correspondence to this author at the Islamic Azad University, Qom Branch, Qom, Iran; E-mail: Kambizroshanaei@gmail.com

E-ISSN: 2308-8044/14

Khorasan, Boroojerd and Western parts of Iran [5-6]. Hypericum has been proposed to have anti-depression and anti-anxiety, anti-viral, anti-inflammatory and vulnerary effects as well as improving on some types of emotional disorders [5]. Hypericum has also been shown to have antioxidant [17], analgesic [8], anticonvulsant [7], and anti-cancer properties [9]. The plant contains chemicals such as polyphenols and flavonoids like quercetin, isoquercetin and rutin whose anti-diabetic activities have been studied in various plants. For example, the rutin can increase insulin secretion and reduce blood glucose in diabetic animals and is beneficial in preventing diabetic complications [10] and quercetin has anti-diabetic effects and its hypoglycemic effects have been shown in experimentally induced diabetes in animals [11-12]. It has also been shown that flavonoids are non-competitive inhibitors of intestine glucose transporter GLUT2 and reduced intestinal glucose uptake [13]. In animals receiving a diet high in fat, Hypericum has had hypocholesterolemic_effects [14]. As the increase in glucose levels and other factors markedly occur in diabetes and there is no cure for the disease and there are also complications, in this study, the effects of Hypericum extract on blood factors in an experimental model of streptozotocin-induced diabetes were studied for 3 and 4 weeks.

METHOD

In this work, 36 male Wistar rats (Pasteur Institute of Iran) were used. All animals were kept at a temperature of 20-24° C, relative humidity of 25 to 30% in 12 hour light and 12 hour darkness, and they had free access to food (Pars Feed) and water. Before starting treatment, the rats spent 7 days to remove the stress and adapt to the new conditions.

Hypericum extract was prepared by Karaj Jihad University Complex and identified and confirmed by the Botanical Department of Biology in Qom Azad University. The plant was crushed by an electric mill. The powder was soaked for 48 hours in methanol and the extraction was performed using Perculator. The solvent in the extract was removed by Rota evaporator and was finally dried by freeze dryer at a temperature of -50 °C. Extraction efficiency was 8/11%, respectively.

In this study, rats were randomly divided into six groups: control, control-21-day treated, 28 day treated control, diabetic, 21 day treated diabetic rats and the 28 day treated diabetic rats**.** The treated groups were gavaged the extract with the dose of 60 mg per kg body weight in a period of 3-4 weeks. Diabetes was induced by intraperitoneal injection of a single dose of streptozotocin (STZ) 60 mg per 1 kg body weight dissolved in a solution of cold saline.

After 5 days of STZ injection, blood glucose was measured and rats with glucose above 250 g per dl were considered diabetic. After 21 and 28 days, fasting rats were anesthetized and their heart blood and serum samples were collected, separated and kept at -20° C. Measurement of blood factors was performed by Spectrophotometric assay kits (Biosystems kits made in Spain). For statistical analysis multivariate analyze of variance was applied. All data shown as mean+SDP <0.05 were considered as statistically significant.

RESULTS

According to the data from Table **1**, there are significant differences between the six groups in parameters FBS, Total P, Na in blood, and PU, FBSU, KU in urine (p<0/05). No significant difference was detected in other parameters.

Table 1: Effect of the Extract on Blood Glucose Levels and other Blood Factors

F	df	P value	Diabetic			Non-diabetic			gruop
			Treated+28 day	Treated+21 day	Untreated	Treated+28 day	Treated21 day	Untreated	
5.376	5	0.004	382.4±135.46	464±2.92	376.6±123.11	120±28.15	202.75±22.47	221.8±69.62	FBS(mg/dl)
2.48	5	0.073	58.2±3.42	54±3	58.33±2.88	66±3	59.75±3.4	62.5±4.65	TOTALP(g/l)
2.904	5	0.045	143.4±3.2	138.33±2.88	145.33±4.72	150.33±3.21	147.5±3.69	148±2.94	Na(mmol/l)
0.872	5	0.520	7.68±1.76	7.03±0.85	6.86±1.59	6.56±0.709	6.65±0.834	6.77±0.457	K(mmol/l)
0.441	5	0.814	49.2±11.38	50.33±12.5	50.66±15.3	56.66±3.78	51.25±8.1	56.5±7.32	CHLO(mg/dl)
1.014	5	0.440	24.6±5.31	23±7	21.33±6.8	23.66±1.52	22.25±0.5	25±3.55	HDL(mg/dl)
1.026	5	0.434	2.18±0.441	2.21±0.145	2.38±0.051	2.39±0.089	2.3±0.367	2.26±0.065	CHLHD
21.601	5	0.000	16.06±10.87	37.27±7.85	51.94±14.09	7.44±9.52	5.83±2.96	2.39±2	PU(g/24h)
38.537	5	0.000	6.23±3.45	12.67±1.74	17.93±3.19	1.54±1.92	0.595±0.393	0.045±0.041	FBSU(g/24h)
10.247	5	0.000	1.23±0.562	2±0.306	2.78±0.5	0.53±0.455	0.607±0.351	0.75±0.918	KU(mmol/l)

DISCUSSION

The results of this study showed that the administration of Hypericum extract has significantly decreased Total P, Na in blood, and PU, FBSU, KU in three or four weeks treated diabetic group compared with the diabetic group, Based on previous findings, in streptozotocin-induced diabetic rats significant adverse changes in blood glucose levels and other plasma factors are evident [16-15]. These changes were also observed in diabetic rats in this study. Hypericum plant contains phytochemical compounds such as flavonoids, which have antioxidant effects [11-17]. In this regard, it has been found that these compounds have lipid-lowering effects [14-18]. Some of these flavonoids which are administered to streptozotocin-diabetic rats have significantly decreased serum glucose levels in a dose-dependent form. While these flavonoids haven't had any significant effects on blood glucose levels in normal animals [20]. Perhaps, the beneficial and hypoglycemic effects of flavonoids can partially be related to the increased activity of hepatic hexokinase and glucokinase, and even to the increased density of beta cells in the islets of Langerhans due to their antioxidant effects [20-21]. On the other hand, some flavonoids in medicinal plants act as an antioxidant and have insulin-like properties and in this way they can reduce the symptoms of diabetes and restore normal levels of serum parameters. It has been found that oral administration of them can increase the absorption of glucose by the cells of liver, fat and muscle. However, their mechanism of action is different from that of insulin [21-23]. Furthermore, administration of some polyphenols enhances glucose transporters in muscle cells [19-10] which somewhat justifies the hypoglycemic effect in the experimental model of diabetes in present study. In diabetes, impaired glucose homeostasis increases the volume and excretion of metabolites which results in the disruption of the balance of electrolytes. In this study, serum levels of sodium and potassium increased in diabetic rats. While some reviews have shown that sodium and potassium levels in STZ diabetic rats had decreased [25, 26], other studies reported that electrolyte levels significantly increased in diabetes [24-28]. Sodium is the most important matter in the extracellular fluid (ECF). So, osmolarity of the plasma is highly dependent on sodium concentration and its increase or decrease will have a significant impact on plasmaosmolarity [29]. Potassium is essential for muscle function and plays a role in maintaining cell polarity and transmitting electrical impulses [30]. Increased glucose oxidation in the presence of transition metals causes membrane damage, by lipid peroxidation and protein glycosylation which could be the reason for the change in electrolyte balance that increases the concentration of extracellular sodium and potassium in rats with STZ diabetes [31]. Studies on plants containing compounds like flavonoid, polyphenols, terpenes have shown that these compounds can bring about electrolyte balance by changing the levels of glomerular filtration and influencing the transport processes in the renal tubular as the impact of their protective and antioxidant effects [32-34]. Possibly Hypericum extract which contains flavonoids and other compounds operates through a similar mechanism.

In summary, administration of Hypericum extract in experimental models of diabetes mellitus has improved the biochemical parameters in diabetic rats. To identify the mechanism of the action of the extract, we suggest analyzing the chemical composition of the extract at different doses and durations.

ACKNOWLEDGMENTS

We acknowledge the members of the Department of Biology in Qom Azad University for their sincere help in doing the present study.

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Received on 15-07-2013 Accepted on 21-09-2013 Published on 06-03-2014

DOI: http://dx.doi.org/10.12970/2308-8044.2014.02.01.2