The effect of the hydroalcoholic extract of Echium amoenum on glycemic control and body weight in streptozotocin-induced diabetic male Rats

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Abstract

Introduction: Diabetes mellitus is one of the main metabolic disorders across the world. Numerous medicinal herbs are currently used for the management of diabetes mellitus. The present study was conducted to assess the effect of the hydroalcoholic extract of Echium amoenum (EA) on glycemic control and weight in diabetic male rats.

Materials and Methods: The study was conducted on 30 male Wistar rats randomly divided into five groups of six rats each, including a control group, a diabetic group receiving no treatments and three diabetic groups treated with 100, 400 or 600 mg/kg of the hydroalcoholic extract of Iranian EA. Diabetes was induced in the rats by the intraperitoneal administration of a single dose of Streptozotocin (60 mg/kg). The rats' blood glucose and weight were measured at baseline and then on days 10, 20 and 30 of the study. Their insulin and superoxide dismutase (SOD) activity levels were determined at the end of the study.

Results: In all the groups treated with the extract, there was a significant increase in the mean weight and a significant decrease in the mean blood glucose levels compared to the diabetic group (P<0.05). Insulin levels increased significantly in the rats treated with a 600 mg/kg dose of the extract compared to the diabetic group. SOD activity also increased significantly in the groups receiving 400 and 600 mg/kg doses of the extract compared to the diabetic group (P<0.05).

Conclusion: The study demonstrated that the hydroalcoholic extract of Iranian EA has hypoglycemic effects and can be used to prevent weight loss due to diabetes. These effects might be due to the presence of compounds such as flavonoids and saponins and some of the plant's antioxidant properties.

Keywords: Diabetes Mellitus, Echium Amoenum, Blood Glucose, Streptozotocin, Rats

Introduction

Diabetes mellitus is a metabolic disorder identified with chronic increase in blood sugar, and caused by insulin secretion disorder, insulin function or both (1). This disease is associated with moderate to severe hyperglycemia and failure to metabolize carbohydrates, lipids, and proteins, and can cause retinopathy, nephropathy, neuropathy, and cardiovascular diseases in the long-term (2). According to the published statistics by the World Health Organization in 2014, the prevalence of diabetes among people older than 18 years is about 9%, and the number...
of people with diabetes worldwide is anticipated to reach 600 million by 2035 (3). According to statistics by the World Diabetes Federation, the prevalence of diabetes in Iran, irrespective of type, is about 6%, and the number of people with diabetes mellitus was reported 3 million in 2010 (4).

Reducing chronic hyperglycemia in diabetes will prevent or postpone its complications such as weight loss, cardiovascular diseases, ocular damage, etc(5). High oxidative stress, resulting from an imbalance between oxygen free radicals and antioxidant capacity, is highly associated with diabetes and its complications. Diabetes-induced oxidative stress affects progress of dysfunction of pancreatic beta cells and exacerbates complications of diabetes (6). Given that insulin injection and hypoglycemic medications are the main treatment for type I diabetes mellitus (7), and since long-term use of insulin and most blood sugar lowering medications entails several side-effects; finding modern treatments and research to obtain harmless and effective medications is essential (8). As such, an effective way may be use of herbal medications. It has been found that herbal antioxidants are necessary for stimulation of response and coping with effects of oxidative stress in body tissues, including pancreas (9). More than 800 different types of herbs are used as traditional drugs for treatment of diabetes, and hypoglycemic effects of a large number of them have been investigated and approved in animal samples and clinical studies (10).

With a scientific name "Echium amoenum. L", borage is a plant indigenous to Iran from boraginaceae family, which grows naturally in northern parts of Iran, including Gilan and Mazandaran provinces, and Chalus, Kelardasht, and Kandovan heights (11). Photochemical examination of the leaves of this plant reveals various amounts of flavonoids, saponins, polyphenolic compounds, quinines and quinoforans, antioxidant compounds, and low amounts of mucilage. Presence of large amounts of omega-3 and omega-6 (linoleic acid) and compounds such as potassium nitrate and calcium salts in this plant has been demonstrated. Borage is considered one of the most popular medicinal herbs in Iranian traditional medicine, with flowers possessing anti-inflammatory, analgesic, anxiolytic, antidepressant, anti-palpitation properties, and also effective as a remedy for kidney diseases. Anti-microbial and anti-cancer properties of this plant have recently been demonstrated (12, 13).

Considering that no domestic or foreign report has found on the effects of Iranian borage on diabetes and its complications, including weight loss, this study aims to investigate effects of three different doses of hydroalcoholic extract of this plant on control of sugar, weight, as well as insulin level and activity of superoxide dismutase in experimentally-induced diabetes via streptozotocin, so as to determine the best dose and also use results in management of blood sugar and weight loss in diabetic people.

Materials and Methods

In this experimental study, 30 male Wistar rats weighing 250 grams to 300 grams that had been reproduced in the animal house of Hamedan Islamic Azad University were used. Animals were housed in standard cages at 20-22 °C, relative humidity of 50% to 60%, and alternate 12-hourly light/dark. Animals had free access to tap water and special rat feed (Pars animal feed Company). In all stages of the experiment, animals were treated according to the International Convention for Laboratory Animal Care and Use (14). Iranian borage flowers were procured from Ebn-Sina Medicinal Plants Center (following approval by the center's expert, Herbarium No 9322), and were fully dried for a few days in the shade and normal weather. Dried flowers were turned into powder using an electric mill (BL-335...
Kenwood). Enough alcohol 80% was added to 100 grams of the powder in an Erlenmeyre flask to cover the powder. After a few weeks, the solution was filtered, and pure extract was separated using a rotary device, and was then fully dried in laboratory ambiance, and kept at 4 °C until use. In this way, 5.93 g of pure extract powder was obtained from every 100 grams of borage powder. Doses used in this study were based on those in previous studies. Since previous studies revealed no toxic effects of normal doses (40-800 mg/kg) of Iranian borage flower extract in rats (15), in this study, 100, 400, and 600 mg/kg doses were prepared for use by dissolving dry pure extract in normal saline (sodium chloride 0.9%). Following adaptation to environment, rats were divided into five groups of six, including healthy control group (healthy animals receiving normal saline), diabetic control group (diabetic animals receiving normal saline), and three diabetic groups treated with 100, 400, and 600 mg/kg doses. Experimental model of type I diabetes mellitus was induced in four groups of rats by a single intraperitoneal administration of 60 mg/kg of streptozotocin (Sigma-Aldrich, USA). Diabetes mellitus in rats was confirmed after a week by observation of symptoms including polydipsia, bulimia, polyuria, and fasting blood sugar (for 10 hours) more than 200 mg/dl (16). In all groups, the extract was administered intraperitoneally. Animals' weight and blood sugar were measured before inducing diabetes, one week after (before injecting the extract), and 10, 20, and 30 days after injection, using a digital scale (Sartorius) and Glucometer device (Chek Accu) respectively. According to previous studies, blood sugar measured by glucometer has the least mean difference compared to the standard method (enzymatic method) (17). By the end of the 30th day, blood samples were taken from animals' hearts, and insulin level was measured using ELISA method (Monobind kit) and superoxide dismutase, using spectrophotometry (with Ransod-Randox Co kit).

Statistical analysis of data was performed in SPSS-19. Normal distribution of data was verified and confirmed using Kolmogorov-Smirnov test. Statistical differences between groups were assessed using one-way ANOVA and Tokey post hoc test. The difference in groups before and after the experiment was determined with paired t-test. P<0.05 was considered statistically significant.

Results
According to results from variance analysis and Tokey post hoc test, following streptozotocin-induced diabetes, mean fasting blood sugar in all diabetic groups significantly increased compared to the control group (P<0.01). This increase in mean blood sugar in diabetic control group progressively persisted throughout the course of study (Table 1).

In diabetic groups receiving the extract, an increase in blood sugar on days 10, 20, and 30 stopped and then began a decreasing trend. On day 10 following injection, no significant mean blood sugar decrease was observed in the three extract receiving groups compared to diabetic control group. But, on day 20, mean blood sugar in groups receiving 400 mg/kg and 600 mg/kg doses of extract showed a significant decrease compared to diabetic control group (P<0.05). By the end of the course (day 30), mean blood sugar in all three extract receiving groups significantly reduced compared to diabetic control group (P<0.05). The results from paired t-test showed mean blood sugar at the start of the study (before injection) in diabetic groups receiving 100, 400, and 600 mg/kg dose of extract was 515.8±10.4 mg/dl, 518.6±13.03 mg/dl, and 513.4±9.6 mg/dl respectively, and by the end of experiment (day 30) reduced to 495.2±6.05 mg/dl, 410.6±5.9 mg/dl, and 389.8±4.3 mg/dl respectively.

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respectively, and this decrease was significant in groups treated with 400 mg/kg and 600 mg/kg (P<0.05). No significant difference was observed in animals between their weights at the start and before inducing diabetes (table 2). According to variance analysis and Tukey post hoc test, animals in all diabetic groups after inducing diabetes showed significant mean weight loss compared to control group (P<0.05). In diabetic control group, this weight loss progressively persisted throughout the course of the study. On days 10, 20, and 30 following administration of the extract, diabetes-induced weight loss stopped, and a relative weight gain was observed in groups receiving different doses of the extract, but on day 10, this weight gain in diabetic control group was not significant compared to the control group. On days 20 and 30, mean weight of animals in all diabetic groups receiving 100, 400, and 600 mg/kg doses of the extract showed a significant gain compared to diabetic control group (P<0.05). However, no significant difference was found between mean final weights of rats in the group receiving 600 mg/kg extract compared to the control group.

According to paired t-test results, mean weight of animals at the start of the study (before injection) in the three extract-receiving groups was found 238.2 ± 5.01 grams, 232.2 ± 3.14 grams, and 236.5 ± 6.7 grams respectively, and by the end of experiment (day 30) increased to 240.2 ± 6.5 grams, 248.6 ± 4.7 grams, and 260.8 ± 2.3 grams. This increase was significant in the group treated with 600 mg/kg dose (P<0.05). Results also revealed that 600 mg/kg was the most effective dose for reducing blood sugar and weight control in different groups.

Table 1: The effect of Iranian borage flower extract on mean blood sugar of rats (mg/dl) in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Diabetic control</th>
<th>Control</th>
<th>Extract (100mg/kg)</th>
<th>Extract (400mg/kg)</th>
<th>Extract (600mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before diabetes</td>
<td>124.5 ± 8.1</td>
<td>121.3 ± 11.7</td>
<td>118 ± 8.3</td>
<td>120.1 ± 5.3</td>
<td>122.7 ± 10.9</td>
</tr>
<tr>
<td>Before extract</td>
<td>126.4 ± 7.4</td>
<td>504.8 ± 14.1**</td>
<td>515.8 ± 10.4**</td>
<td>518.6 ± 13.03**</td>
<td>513.4 ± 9.6**</td>
</tr>
<tr>
<td>Day 10</td>
<td>117.3 ± 10.7</td>
<td>515.6 ± 11.3**</td>
<td>525.2 ± 4.2**</td>
<td>510.3 ± 3.6**</td>
<td>502.8 ± 4.08**</td>
</tr>
<tr>
<td>Day 20</td>
<td>121.6 ± 9</td>
<td>542.2 ± 8.1**</td>
<td>521.1 ± 5.4**</td>
<td>495.7 ± 7.5**#</td>
<td>470.2 ± 6.9**#</td>
</tr>
<tr>
<td>Day 30</td>
<td>125.2 ± 6.3</td>
<td>550.8 ± 7.7**</td>
<td>495.2 ± 6.05**#</td>
<td>410.6 ± 5.9**#a</td>
<td>389.8 ± 4.3**a</td>
</tr>
</tbody>
</table>

**: Significant difference with the control group (P<0.01)
#: Significant difference with the diabetic control group (P<0.05)
a: Difference compared to before extract injection

Table 2: The effect of Iranian borage flower extract on mean weight of rats (grams) in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Diabetic control</th>
<th>Control</th>
<th>Extract (100mg/kg)</th>
<th>Extract (400mg/kg)</th>
<th>Extract (600mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before diabetes</td>
<td>263.3 ± 1.2</td>
<td>258.7 ± 6.03</td>
<td>260.4 ± 4.6</td>
<td>262.6 ± 2.3</td>
<td>255.2 ± 3.7</td>
</tr>
<tr>
<td>Before extract</td>
<td>270.2 ± 9.6</td>
<td>247.3 ± 2.3*</td>
<td>238.2 ± 5.01*</td>
<td>232.2 ± 3.14*</td>
<td>236.5 ± 6.7*</td>
</tr>
<tr>
<td>Day 10</td>
<td>275.3 ± 3.3</td>
<td>215.6 ± 8.8*</td>
<td>232.2 ± 10.1*</td>
<td>225.3 ± 7.2*</td>
<td>239.8 ± 18.4</td>
</tr>
<tr>
<td>Day 20</td>
<td>281.6 ± 5.6</td>
<td>195.2 ± 12*</td>
<td>235.1 ± 5.03*#</td>
<td>238.7 ± 2.1*#</td>
<td>253.4 ± 4.01*#</td>
</tr>
<tr>
<td>Day 30</td>
<td>289.2 ± 7.5</td>
<td>176.8 ± 3.6*</td>
<td>240.2 ± 6.5*#</td>
<td>248.6 ± 4.7*#</td>
<td>260.8 ± 2.3#a</td>
</tr>
</tbody>
</table>

*: Significant difference compared to the control group (P<0.05)
#: Significant difference compared to the diabetic control group (P<0.05)
a: Difference compared to before extract injection (P<0.05)
Table 3 shows the effect of Iranian borage flower extract on insulin level and activity of serum superoxide dismutase. According to results of variance analysis and Tokey post hoc test, inducing diabetes led to a significant decrease in insulin level in diabetic groups compared to the control group. In the group receiving 600 mg/kg dose of the extract, mean insulin level showed a significant increase compared to the control group (P<0.05). In all diabetic groups, the activity of superoxide dismutase significantly reduced compared to the control group (P<0.05). However, treatment with 400 and 600 mg/kg doses of extract led to a significant increase in the activity of superoxide dismutase compared to the diabetic control group (P<0.05).

Table 3: The effect of borage flower extract on insulin level (U/mlµ) and activity of serum superoxide dismutase in different groups, by the end of study

<table>
<thead>
<tr>
<th>Group</th>
<th>Diabetic control</th>
<th>Control</th>
<th>Extract (100mg/kg)</th>
<th>Extract (400mg/kg)</th>
<th>Extract (600mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>12.33 ± 1.23</td>
<td>5.7 ± 0.71*</td>
<td>6.4 ± 1.01*</td>
<td>5.9 ± 0.93*</td>
<td>8.1 ± 0.66*#</td>
</tr>
<tr>
<td>Superoxide</td>
<td>135.2 ± 8.6</td>
<td>72.3 ± 7.03*</td>
<td>81.2 ± 3.7*</td>
<td>96.5 ± 9.14*#</td>
<td>101.6 ± 5.7*#</td>
</tr>
<tr>
<td>dismutase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Significant difference compared to the control group (P<0.05)
#: Significant difference compared to the diabetic control group (P<0.05)

Discussion
The present study results showed that intraperitoneal injection of 60 mg/kg body weight of streptozotocin induced diabetes in rats, resulting in a significant decrease in insulin level and a significant increase in blood glucose compared to the control group. Streptozotocin decreases insulin level with subsequent increase in animals' blood glucose through damaging pancreatic beta cell membrane, fragmenting DNA, increasing expression of mRNA associated with glucose enzyme liver-phosphatase-6, and reaction with enzymes such as glucokinase (18). Results also showed that experimentally induced diabetes in rats caused significant weight loss compared to the control group. In type I diabetes mellitus, the body is unable to metabolize blood sugar due to defective insulin synthesis, and instead uses other sources such as lipids, and occasionally proteins; thus, diabetic patients become very thin and frail (19). Amount of superoxide dismutase decreased following induced diabetes in rats compared to control group. Studies have shown that streptozotocin-induced diabetes results in inhibition of free radicals-reducing enzymes. This reduction in enzymes together with increased oxidative stress leads to increased destruction of pancreatic beta-cells (20).

According to the results, mean final blood sugar reduced in diabetic groups receiving 400 and 600 mg/kg doses of the extract compared to the start of the study and also to the diabetic control group. By the end of the study, all extract receiving groups gained weight compared to the diabetic control group, and the group treated with 600mg/kg dose showed a significant weight gain compared to the start of the study. Results also showed that a month's injection of the extract had led to increase in insulin and activity of serum superoxide dismutase compared to the diabetic control group.

Various studies have confirmed analgesic (21), antidepressant, antimicrobial, and anti-cancer (12,13) effects of borage flower. But, to date, no study had been conducted on anti-diabetes and hypoglycemic properties of Iranian borage flower. Various studies have also confirmed anti-diabetes effects of other
species of the boraginaceae family. In 2012, Mohammad & Ari confirmed anti-diabetes and blood sugar lowering effects of Anchusa strigosa species in diabetic rats. They attributed anti-diabetes effect of this species of boraginaceae family to compounds such as flavonoids contained in this plant and argued that herbal flavonoids are able to reduce blood sugar in diabetic patients through various mechanisms, including 1) increasing activity of liver hexokinase and glucokinase; 2) insulin-like properties in some of them; 3) increasing glucose absorption by muscle and liver cells and fat-through different mechanisms from insulin (22).

Andred et al. (2007) studied hypoglycemic effects of extract from Tourefortia hirsutissima species of boraginaceae family in diabetic rats, and observed reduction in blood sugar in these animals. They attributed these positive effects to flavonoids and polyphenolic compounds in this plant, and asserted that perhaps polyphenols reduce blood sugar by increasing expression of glucose transporters (GLUT) in muscles (23).

Ashwini et al. (2012) studied anti-diabetic effects of another species of this family called Rotula aquatica and concluded that flavonoid compounds such as quercetin contained in this plant are able to reduce blood sugar in diabetic animals by 50%, and saponins in this plant effectively prevent diabetes-induced weight loss (24). Previous studies results indicate that compounds such as saponins cause weight gain in adult rats. Saponins have been found to cause weight gain in adult rats by affecting circadian rhythm feeding habits and increasing appetite (25). Studies have shown that Iranian borage flower contains certain amounts of fatty acids such as linoleic acid (26) and antioxidant compounds such as rosmarinic acid (27). Linoleic acid has been found to cause weight gain (28). Thus, it may be asserted that, perhaps antioxidant compounds in Iranian borage flower extract improve this disease by treating oxidative stress caused by diabetic hyperglycemia. Some herbal extracts can increase insulin release from pancreatic beta-cells (not damaged by streptozotocin action) and reduce blood sugar in diabetic people by increasing insulin (29). Some anti-diabetic medicinal plants do not interact with insulin receptor and stimulate insulin action through interaction with tyrosine kinase (insulin receptor) (30).

Accordingly, it can be said that insulin increasing and blood sugar lowering properties of borage flower extract occur through stimulation of insulin synthesis and release from Langerhans islets beta-cells.

Increased production of free radicals due to glucose oxidation and reduced antioxidants defense mechanisms leads to increased oxidative stress in diabetic patients, causing oxidative damage to tissues and cells (including pancreatic beta-cells) and progressive reduction in insulin synthesis in these patients (31). Use of herbal antioxidants has been found an effective way to deal with oxidative stress in various tissues, especially pancreatic cells (32). Previous studies suggest that natural antioxidants such as phenolic compounds and flavonoids are able to reduce oxidative stress caused by streptozotocin-induced diabetes and prevent destruction of pancreatic beta-cells by increasing the activity of antioxidant enzymes such as superoxide dismutase. Furthermore, these compounds readjust pancreatic beta-cells causing increased insulin level with subsequent reduced blood sugar (34).

According to previous studies, the main therapeutic effect of Iranian borage flower can be attributed to its effective compounds such as flavonoids, saponins, polyphenolic compounds, antioxidants such as rosmarinic acid and fatty acids (13, 12, and 35). Although this study was not designed to find the effective substance and its mechanism of effect, according to results obtained, positive properties of
borage flower can probably be attributed to its chemical compounds that have been able to show their hypoglycemic effects through various mechanisms such as insulin-like properties, the effect on enzymes associated with metabolism of sugars, stimulation of insulin secretion, and increase in glucose absorption by cells. Positive effects of this extract in preventing weight loss can be partly attributed to saponins and linoleic acid contained in the extract, and regulation of metabolism of sugars and fats. Borage extract imposes its protective effects on pancreatic beta-cells, thereby improves their functions due to antioxidants contained such as phenolic compounds and flavonoids, probably through increased activity of superoxide dismutase and reduced oxidative stress.

Further studies in this area appear necessary. To find the exact mechanism of Iranian borage flower extract in improving diabetic condition; it is recommended that each element of the extract be separately assessed in experimental and clinical studies. The effect of this plant on restoration of Langerhans islets should also be studied.

Conclusion

Iranian borage flower extract lowers blood sugar in diabetic rats and prevents weight loss as a common diabetes complication. These beneficial properties can probably be attributed to compounds such as flavonoids, saponins, and antioxidants contained in this plant. The results obtained also suggested that the 600 mg/kg dose of the extract is the best and most effective dose used in this study.

Conflict of interest

None was expressed by authors.

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