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Investigation and Comparison of the Effect of Saffron Petals Hydroalcoholic Extract with Metformin and Glibenclamide on Serum Glucose, HbA1c and Insulin Levels in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Introduction: Chemical drugs administered to control and treat diabetes mellitus are mostly with side effects and this has caused a daily increasing expansion in the use of alternative treatment methods including medicinal herbs. The present study has been conducted to investigate the effect of saffron petal hydroalcoholic extract on serum glucose, insulin and glycosylated hemoglobin (HbA1c) levels and compare it with the effects of metformin and glibenclamide in diabetes-induced rats.
Materials and Methods: A total number of 35 male Wistar rats (200 ± 20 g) were assigned to seven 5-animal groups: control group (normal), diabetic group (Streptozotocin 60 mg/kg/body weight) and five other diabetic groups (receiving saffron petals hydroalcoholic extract in 100, 200 and 300 mg/kg doses and glibenclamide in 5 mg/kg and metformin in 500 mg/kg, respectively). The experiment lasted eight weeks and blood samples were collected from all the rats in the end of the study.
Results: 100 and 200 mg/kg dosages of saffron petal extract caused significant reductions in serum glucose levels in contrast to the diabetic groups (P<0.01 and P<0.05, respectively). All three dosages of saffron petals extract, 100, 200 and 300 mg/kg, were found significantly reducing serum HbA1c level in diabetic groups as well as significantly increasing serum insulin level (P<0.001, P<0.001 and P<0.05, respectively). The diabetic groups treated with glibenclamide and metformin, as well, brought about significant reductions in serum glucose and HbA1c levels (P<0.01 and P<0.001, respectively) and significant increase in serum insulin levels (P<0.001).
Results: 100 and 200 mg/kg dosages of saffron petal extract caused significant reductions in serum glucose levels in contrast to the diabetic groups (P<0.01 and P<0.05, respectively). All three dosages of saffron petals extract, 100, 200 and 300 mg/kg, were found significantly reducing serum HbA1c level in diabetic groups as well as significantly increasing serum insulin level (P<0.001, P<0.001 and P<0.05, respectively). The diabetic groups treated with glibenclamide and metformin, as well, underwent significant reductions in serum glucose and HbA1c levels (P<0.01 and P<0.001, respectively) and significant increase in serum insulin levels (P<0.001). The impact rate of saffron petal extract administered in a dosage of 100 mg/kg on serum insulin level elevation was higher than such drugs as glibenclamide and metformin.
Conclusion: Saffron petal, like glibenclamide and metformin, is interestingly capable of influencing high serum levels of glucose and HbA1c and insulin, especially in low dosages, and it can be an appropriate solution to the control of diabetes.

Key words: Diabetes mellitus, Glucose, HbA1c, Insulin, Metformin, Glibenclamide, Saffron

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INTRODUCTION

Diabetes is a type of chronic metabolic disease that is accompanied by the occurrence of disorder in glucose, lipid and protein metabolism [1]. The disease appears as hyperglycemia resulting from defective insulin secre-

tion, insulin performance disorders or both [2]. Metabolic syndrome and its components like hypertension and dyslipidemia in patients with diabetes are very common and lead to the increase in the risk of cardiovascular diseases in these patients [3,4]. Type 2 diabetes is the most common form of diabetes inflicting over 90% of the diabetics' population [1]. Being chronic in nature, diabetes causes effects on the body and mind as well as individual and social performance of the patient in such a manner that it can result in acute and durable symptoms; due to the same reason, it is considered as the most important cause of disability and death in a great many of the countries [5].

At present, the diabetes control solutions are changing the food habits and use of healthy dietary regimes, increase in physical activity and prescription of antidiabetic drugs and/or insulin [6]. It has been estimated that at least 10% of the total healthcare costs are spent on diabetes control in the majority of the countries. This is while almost one third of the world population cannot procure drugs [7]. Since the drug-based treatment of diabetes is faced with numerous constraints including high costs and side effects, their inability of perfect prevention and appropriate control of the disease symptoms as well as the reduction in the drugs' effectiveness in the course of time, researchers have been encouraged to investigate the novel solutions of controlling the disease and its side effects and the global interests in the use of supplementary and alternative medicine have increased [3,8-11]. Amongst the supplementary medicine solutions, the greatest deal of attention has been paid to the change in food patterns and the use of medicinal herbs [3].

One of these drugs is saffron (Crocus sativus L.) which belongs to Iridacea family. Saffron is a small perennial plant-the dried stigma of which is used in food and drug industry [12].

Saffron and its constituent parts have various pharmacological effects that have caused it to be proposed as a potential drug. The main ingredients of saffron are crocin, crocetin, picrocrosin and safranal [13]. Saffron is prescribed in traditional medicine for its anticonvulsive, happiness-activating, diuretic, anti-anxiety, liver and nerve strengthening and blood coagulating effects and it is also used in treating acne, pimple and dermal diseases, intracerebral hemorrhage, asthma, arthritis, maldigestion and menstrual pains [14-17].

The majority of the studies pertain to the investigation of the properties of saffron stigma which is very expensive hence not cost-effective. Therefore, the present study investigated the effect of saffron petals hydroalcoholic extract (SPHAE) on serum glucose, insulin and HbA1c levels as the blood glucose index in the long run and then compared it with the effects of such drugs as metformin and glibenclamide in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Extract preparation of saffron plants

Saffron plants were freshly collected from the fields and subjected to scientific identification following which the petals were dried in shade and milled. The obtained powder was combined with hydroalcoholic solvent (water-ethanol for a ratio of 20%:80%) and the mixture was filtered after three days. In the end, the extract was concentrated using a rotary device and kept in a refrigerator [18].

Animals' grouping and performing the injections

Thirty five male Wistar rats (200 ± 20 g) were procured from Razi Serum-Making Institution and kept in special cages under standard laboratory conditions. After animals were adapted to the conditions of the environment, streptozotocin (Enzo Life Science Co. Ltd., Farmingdale, NY, USA) was used to induce them with diabetes. To do so, the animals were injected intraperitoneally with 1.2% streptozotocin in normal saline with a dosage of 60 mg/kg/body weight. Blood samples were taken from the tail end of the rats injected with streptozotocin after three days to be subjected to serum glucose level determination. Serum glucose levels above 250 mg/dl are considered as an indicator of being diabetic [19]. Next the diabetic rats were randomly assigned to diabetic groups.

Glibenclamide and Metformin were purchased from Chemidarou Company (Tehran, Iran) and HEXAL Company (Holzkirchen, Germany), respectively. The amount of treatment given to entire groups was 1 ml that was given to the animals orally through an intragastric tube.

Various dosages of saffron petal hydroalcoholic extract were dissolved in 1 cc distilled water based on weight mean and administered in milligram per kilogram per body weight; as for glibenclamide and metformin, as well, the pills were milled in mortar considering the drug dosages in milligram per kilogram per body weight and dissolved in 1 cc distilled water for every animal following which each animal was orally fed on the specified dosage through an intragastric tube.

The treatment lasted 8 weeks. The animals were assigned to seven groups:

Diabetic Group 1: Healthy animals treated with 1 cc distilled water for every animal.
Diabetic Group 2: Diabetic animals treated with 1 cc distilled water for every animal.
Diabetic Groups 3, 4 and 5: Diabetic animals treated with SPHAE for dosages of 100, 200 and 300 mg/kg of their body weights, respectively.
Diabetic Group 6: Diabetic animals treated with glibenclamide for a dosage equal to 5 mg/kg of their body weights.
Diabetic Group 7: Diabetic animals treated with metformin for a dosage of 500 mg/kg of their body weights.

Measuring biochemical factors

In the end of the study, the animals, having been kept hungry for 12 hours, were put into anesthesia in com-

plete adherence to principles of working with laboratory animals and blood samples were taken from their hearts for an investigation of the biochemical factors. Pars Azmoun (Pars Azmoun Co, Karaj, Iran) enzymatic kits were utilized for the determination of fasting glucose levels and ELISA kits were utilized to determine insulin and HbA1c levels.

Data analysis

The data analysis was conducted in SPSS software, version 16, based on one-way variance analysis (one-way ANOVA). The statistical inference threshold was set at $P<0.05$.

RESULTS

Mean comparison of glucose levels determined for various groups in the end of the study indicated that the glucose levels of the diabetic groups receiving extract for dosages of 100 and 200 mg/kg like diabetic groups receiving glibenclamide and metformin have undergone significant decreases in respect to untreated diabetic groups. SPHAE administering for a dosage of 300 mg/kg was also found causing decreases in glucose levels but it was not significant (Figure 1).

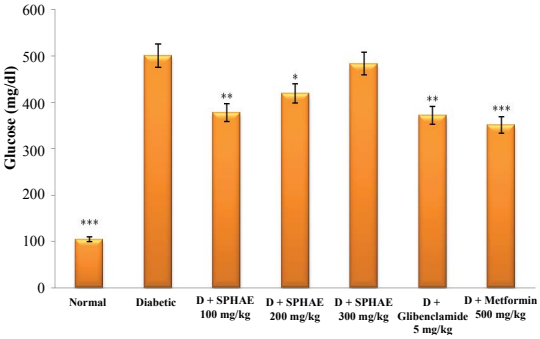

Figure 1: Comparing of fasting blood sugar the different groups with diabetic group. The significance level: P<0.05. Means ± SD for every group (n=5) (*P<0.05)

The results signify that all three dosages of SPHAE, namely, 100, 200 and 300 mg/kg, cause reductions in serum HbA1c level in diabetic groups. Treating with glibenclamide and metformin also brings about significant reductions in serum HbA1c level in diabetic groups (Figure 2).

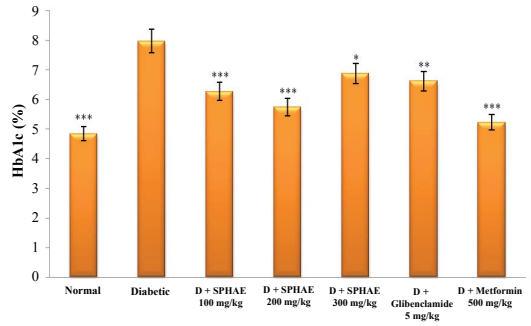

Figure 2: Comparing of HbA1c the different groups with diabetic group. The significance level: P<0.05. Means ± SD for every group (n=5) (*P<0.05)

As for serum insulin level, the results were also indicative of the significant increase in insulin levels of diabetic groups receiving SPHAE for dosages of 100 mg/kg, 200 mg/kg and 300 mg/kg in comparison to the untreated diabetic groups. The increase was also found significant for the diabetic groups receiving glibenclamide and metformin (Figure 3).

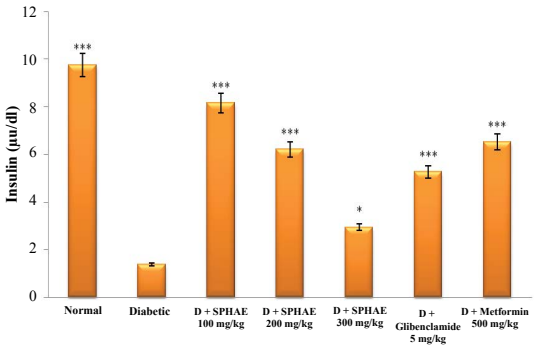

Figure 3: Comparing of insulin the different groups with diabetic group. The significance level: P<0.05. Means ± SD for every group (n=5) (*P<0.05)

DISCUSSIONS

There are applied numerous types of traditional and natural food treatments have been applied for the curing of diabetes mellitus in the course of time [20]. However, a few numbers of them have been found with acceptable effects in scientific and medical terms. Due to the same reason, WHO has recommended the performing of further evaluations for the use of traditional cures for controlling diabetes [21]. The present study indicated that saffron petals can effectively reduce high fasting blood glucose and HbA1c levels and increase insulin levels in diabetes-induced rats. These results are in consistency with the ones obtained in other studies on the investigation of hypoglycemic effects of medicinal herbs [22,23].

Secondary metabolites of all plants, including phenolic ingredients, glycosides, alkaloids, glycans, terpenoids, mucilages, polysaccharides, vitamins, saponins, glycoproteins, peptides, aminoacides and proteins, cause blood glucose reductions [24,25]. Part of the hypoglycemic effects of flavonoids can be attributed to the increase in hepatic hexokinase and glucokinase activities as well as the pseudo-insulin attributes of some of them by way of which they are capable of reducing symptoms of diabetes mellitus [26]. The existence of high amounts of them, especially high levels of terpenic ingredients and phenolic derivatives, including flavonoids, in saffron petals justifies the above-presented results [27,28].

Preliminary phytochemical screening of different petals extracts of Crocus sativus indicated the presence of alkaloids, flavonoids, carbohydrate, glycosides, tannins, terpeniods, phenol, steroids and saponins [29].

Significant reductions of fasting blood glucose levels were evidenced in diabetic rats in another study that

was conducted in 2010. The reductions were attributed to the considerable amounts of such ingredients as anthocyanins and phenolic ingredients and flavonoids existing in plant extract [30].

Moradabadi et al. dealt in a study with the hypoglycemic effects and the likely mechanism of action of the extracts of three plant species from lamiaceae race and observed that these extracts of all three plant species cause reductions in blood glucose levels. They reported that the effect is similar to Acarbose drug and that it is related to glucosidase enzyme control and reduction of intestinal glucose take-up. Such an enzyme controlling effect stems from the role played by phenolic ingredients extant in the extract of the plant [31].

Saffron is considered as a natural source of antioxidants. Antioxidants play an undeniable role in moderating diabetes and reducing its symptoms in such a way that they are said to improve endothelial performance, reduce platelets' pile-up, reduce blood glucose and exert anti-inflammatory effects [32,33]. The issue becomes important when we know that systematic inflammation is envisioned as a risk factor in a great many of etiological and pathological aspects of diabetes [34].

CONCLUSION

Saffron hydroalcoholic extracts can considerably control blood glucose in diabetic rats by reducing the serum FBS levels followed by inducing HbA1c reduction and, on the other hand, increasing serum insulin levels. However, more extensive research on saffron petal seems to be necessary for the verification of the findings of the present study.

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DISCLOSURE

The authors report no conflicts of interest in this work.

ETHICS

This study approved by the Ethics Committee of Ilam University of Medical Sciences.

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