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Original Article

The Hypoglycemic Effects of an Ethanol Extract of *Peganum harmala* in Streptozotocin-Induced Diabetic Rats

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Abstract

Peganum harmala is a plant that is traditionally used as an analgesic, anti-inflammatory, antibacterial, antioxidant, anti-helmintic, and antimutagenic agent. Moreover, it is used to treat a variety of human ailments, including depression. This study was conducted to investigate the antidiabetic activity of hydro alcoholic extract of this herb. A hydro alcoholic extract from seeds of this herb was prepared and administered at three doses of 30, 60, and 120 mg/kg to three groups of streptozotocin-induced diabetic rats. Two additional groups were used as negative (normal saline plus solvent) and positive control (metformin). Blood glucose levels in animals from all groups were measured at 2, 4, and 6 hours after intraperitoneal injection of the extract to rats. Blood glucose levels decreased in the diabetic rats in comparison with normal rats ($P < 0.05$). Our finding showed that *P. harmala* seed extract has good antidiabetic activity in streptozotocin-induced diabetic rats. Further studies are needed to isolate active compounds and to investigate their activity.

Key words: antidiabetic, diabetes, *Peganum harmala*, Rat, seed extract, STZ.

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1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin action, insulin secretion, or both. Polydipsia, weight loss, and polyurea (1) are other signs of diabetes. Some complications of diabetes include neurologic and vascular disorders, microangiopathy, generalized diabetic angiopathy, and diabetic triopathy (2). The cause of diabetes can be genetic, but environmental factors such as overweight and lack of exercise play a significant role (3).

It is estimated that the prevalence of diabetes will be 7.7 % in 2030, indicating that 439 million people will be diabetic by 2030 (4). Diabetes mellitus is classified into four groups: 1) type 1 diabetes mellitus; 2) type 2 diabetes mellitus; 3) diabetes mellitus due to other specific mechanisms or diseases; and 4) gestational diabetes mellitus (GDM) (1). Type 1, or insulin-dependent diabetes mellitus, usually occurs in children and young adults but type 2, or insulin-independent diabetes mellitus, usually occurs in adults with obesity. In type 1 diabetes, patients can't produce insulin but in the other types the body doesn't produce sufficient insulin or can't use it properly (3).

Diet, exercise, and weight loss are non-pharmacological therapeutic methods to treat diabetes. Pharmacological therapy includes drugs such as tolbutamide, metformin, and glyburide (5). Gene therapy is another method to treat diabetes (6). The final way to treat diabetes is through the use of medicinal herbs (7). Increased use of alternative medicines has recently attracted the attention of many researchers around the world (7). Due to the inability of current therapies to control all aspects of the pathology of diabetes, as well as high cost and low availability of current treatments, especially in rural areas of developing countries, alternative therapies are clearly needed (8). Before the discovery of insulin and anti-diabetic drugs, patients were treated with herbs and traditional treatments (9). Some of these herbs were recommended by physicians or taken by patients as supplements (10). Positive effect of more than 1200 medicinal plants in reducing blood sugar levels or reduce its complications has ever known (9) and can be useful as hypoglycemic agents (11). These plants may be useful as a source of new compounds for the development of oral hypoglycemic drugs or as a simple dietary supplement therapies (10). Herbal ingredients such as terpenoids, alkaloids, flavonoids, phenolic had been reported for their anti-diabetic properties (12). Some of these plants are: *Momordica charantia* L. (Cucurbitaceae), *Pterocarpus marsupium* Roxb. (Leguminoaceae), and *Trigonella foenum greacum* L. (Leguminoceae) (12). However, there is little toxicological data and their use should always be cautious (10), and patients who consume herbs should be screened by a doctor (7).

Peganum harmala is a wild plant that belongs to the family of Zygophyllaceae and it is found abundantly in the Middle East and North Africa (13). This plant has white flowers and seed capsules, each capsule capable of carrying more than 50 seeds (14). Beta –carbolin derivatives such as harmalol and harmine have been isolated from *P. harmala* (15). Other compounds that have been extracted from *Peganum harmala* are, deoxyvasicinone, L-vasicinone, vasicine , evodiamin , and fagomine (16). This plant have been used in the treatment of wide variety of human diseases (17). Some of its properties are antidepressant (18), hallucinogenic, antitumor, hypothermic (19), antibacterial, antifungal, antidermatosis (20), antioxidant, antimutagenic (21), analgesic, and anti-inflammatory (22). The present study was conducted to study the hypoglycemic effect of the ethanol extract of *P. harmala* seeds in normal and streptozotocin (STZ)-induced diabetic rats.

2. Materials and Methods

2.1. Preparation of the Ethanol Extract

The seeds of *P. harmala* were collected from Tarom district of Zanjan province, Zanjan, Iran. The plant was identified, and the reference material and a voucher specimen (No. RP-HP90) were deposited in the herbarium of the Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran. Powdered seeds (280 g) of *P. harmala* were placed in a glass percolator with ethanol (300 ml) and allowed to stand at room temperature for 24 h. This extraction process was repeated three times. The combined extracts were concentrated under vacuum using a rotary evaporator at 50°C. The weight of the extract obtained was 4 g.

2.2. Animal

Male albino Sprague–Dawley rats (age, 10 weeks; weight, 165–245 ± 5 g) were procured from the animal colony of the Institute. The following norms applied to the animal room environment: temperature, 23 ± 2°C; humidity, 50–60%; light, 300 lux at floor level with a regular 12 h light cycle; noise level, 50 decibels, and ventilation, 10–15 air changes/h. The animals had free access to a pellet diet and tap water.

2.3. In vivo Study of Hypoglycemic Effect of P. harmala

Diabetes was induced in overnight fasted rats by intraperitoneal injection of 50 mg/kg body weight (BW) streptozotocin (STZ) in 0.1 M citrate buffer (pH 4.5). An oral sucrose load of 150 g/l was given to all groups after the STZ injection (except group 5). Fasting blood glucose levels were measured after 48 h, and animals with blood glucose level > 280 mg/dl were considered diabetic. Diabetic rats with fasting blood glucose values (baseline at 0 min) from 326–591 mg/dl were included in this study. The animals were divided into six groups of five rats each. Each rat in experimental groups 1, 2, and 3 was given a suspension of the ethanol extract at a dose of 30, 60, or 120 mg/kg BW intraperitoneally. Group 4 was dosed with 100 mg metformin/kg BW. Animals in the control group (group 0) were administered 0.01 ml/g of solvent. Blood glucose levels in all animals measured at 2, 4, and 6 h after treatment. Group 5 (normal rats) received the ethanol extract at 60 mg/kg. Blood glucose levels were measured again at 2, 4, and 6 h after injection.

2.4. Statistical Analysis

Data were analyzed with the Statistical Package for the Social Sciences ver. 16 (SPSS, Inc., Chicago, IL, USA). A repeated-measures analysis of variance was used to identify differences between each group.

3. Results and Discussion

The ANOVA analysis was performed on the different groups at a particular time, and meaningful differences were seen in the mean between all groups at any time but Duncan's test results were considered to be more accurate. The hypoglycemic effect of *P. harmala* is indicated in Table 1. Group 1 in comparison with the Group 0 and 4 showed meaningful differences at zero time and 2 hour after treatment, and also shows significant difference with group 0 at 4 hour after treatment.

Zero group:

Table 1. The hypoglycemic effect of *P. harmala* extract.

Groups	Blood glucose concentration (mg/dl)				P value
	0 hrs	2 hrs	4 hrs	6 hrs	
0 (solvent)	490.0±6.000	451.2±22.526	515.4±18.236	542.6±14.747	$P < 0.001$
1 (30 mg/kg)	400.6±28.458*/€	289.8±41.058*/€	297.4±42.943*	427.0±62.702	$P < 0.001$
2 (60 mg/kg)	446.8±31.528*	366.6±15.804	425.6±45.473	566.0±14.943	$P < 0.001$
3 (120 mg/kg)	430.6±8.594	400.4±22.884	325.8±24.138*/€	325.2±11.547*/€	$P < 0.001$
4 (metformin)	484.6±41.292	397.2±17.330	430.6±36.903	483.6±29.409	$P < 0.001$
5 (60 mg/kg)	94.0±2.387	116.2±3.089	130.8±4.329	120.6±3.203	$P < 0.001$

Values are Mean ± SD; *shows meaningful difference to solvent group; € shows meaningful difference to metformin group; Group 0: solvent at 0.01 ml/g, Group 1: extract at 30 mg/kg dose, Group 2: extract at 60 mg/kg dose, Group 3: extract at 120 mg/kg dose, Group 4: metformin at 100 mg/kg dose, Group 5: extract at 60 mg/kg dose.

Blood glucose decreased 2 hours after treatment (451.2 mg/dl) compared to time zero (490 mg/dl) but increased 4 (515.4 mg/dl) and 6 (542.6 mg/dl) hours after treatment.

Group 1:

Glucose in the second hour (289.8 mg/dl) and forth hour (297.4 mg/dl) after treatment compared with zero time (400.6 mg/dl) decreased significantly, but at 6 hour after treatment (427 mg/dl) has a sharp increasing.

Group 2:

Blood glucose increased at 2 hours after treatment (366.6mg/dl) compared to time zero (446.8 mg/dl), but decreased at 4 hour after treatment (425.6 mg/dl) and then 6 hour after treatment (566 mg/dl) has been seen an increasing trend again.

Group 3:

Glucose at the whole time of experiment decreased in this group. (Blood sugar at zero: 430.6 mg/dl, 2 hours: 400.4 mg/dl, at 4: 325.8 mg/dl, at 6: 325.2 mg/dl).

Group 4:

Two hours after treatment, blood glucose levels increased (397.2 mg/dl) compared with zero time (484.6 mg/dl) but at 4 hours (430.6 mg/dl) and 6 hours (483.6 mg/ dl) after treatment increased.

Group5:

Glucose after zero time (94 mg/dl) has been increased.the blood sugar were 116.2mg/dl at the second hour after treatment and 130.8 mg/dl 4 hours after treatment but at 6 hours (120.6 mg dL) after treatment decreased. Also repeated measure have been done and the results showed meaningful differences (P <0.0001) (Table 1)

This study aimed to investigate the antidiabetic effect of alcoholic extract of *P. harmala* in streptozotocin-induced diabetic rats. Because of the doses used in this study differs from other research we cannot do a direct comparison between this study and other researches, but it can be confirmed that this herb has anti-diabetic effects. In one of the studies have been carried out experiments on mice, extract have been used in three forms of oral, parenteral and rectal (23). In

another study the anti-diabetic effect of this plant has been proven in form of oral (24). In this study, zero group, has received solvent (solvent was tween 20 plus sterile water) and the survey found that blood sugar levels in the control group at 2 h after treatment decreased and after that it has taken on an increasing trend so to investigate whether the reduction in blood glucose levels after treatment, is tween 20 or not, some papers were evaluated but a relationship between tween 20 and reduce the blood sugar was not found. Also we can't claim that solvent, thin the blood and reducing the amount of glucose per unit volume, because enough solvent has not been injected to dilute the blood. Decline in blood glucose 2 hours after treatment remains unclear and there was no justification for it. Similar experiments have been carried out on this plant; the plant's effect on blood glucose in normal rats was also measured. Previous studies have shown that the plant has hypoglycemic effect on normal rats (23-24), but according to the results of the present study *P. harmala* extract significantly increased glucose levels in normal rats at 2 and 4 hours after treatment. However, at 6 hours after treatment, blood glucose levels have been declined but the level is higher than the zero hour. According to this source, the plant collected for this study had different source from other studies, so active ingredients may also be different and this might cause different results. Although the species composition of each region should be examined separately, perhaps this will prove to the plants used in this study, compared to other studies, have different combinations and this has created such differences. It is also possible that normal rats affect by a matter, but diabetic rats affect by another matter, although this issue has not been proven. In this study the group that received extract at a dose of 30 mg/kg show significantly different with metformin and solvent groups at time zero and 2 hours after treatment.

However, in the fourth hour after treatment the only significant difference was between this group and the vehicle group. But the results in the group that received the extract at a dose of 60 mg/ kg were different. In this group, the only significant difference was at time zero and between this group and solvent group. In the group that received the extract at a dose of 120 mg/ kg, the blood sugar at 4 and 6 hours after treatment was significantly different from the vehicle group and metformin group. We can't explain why extract at a dose of 30 and 120 mg/ kg doses of the extract were able to make significant but extract at a dose of 60 mg did not work, but maybe this happened because of an error in the test. As mentioned, in the group that received the extract at a dose of 120 mg/ kg, the blood sugar at 4 and 6 hours after treatment was significantly different from the vehicle group and metformin group, whereas in the group receiving the extract at a dose of 30 mg / kg, the extract effect was reduced in the sixth hour after treatment. Perhaps this explains that the plant extract in high doses, has a longer and later effectiveness but in low doses, has a shorter and faster effectiveness.

4. Conclusion

This study aimed to investigate the antidiabetic effect of alcoholic extract of *P. harmala* in streptozotocin-induced diabetic rats. The overall goal of this study can be said that the extract is effective in reducing blood glucose levels in

diabetic rats, and of course, the most effective dose was 120 mg/ kg, while in normal rats was increased blood sugar.

Ethical Approval

All authors hereby declare that, "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) was followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethics committee of Zanjan University of Medical Sciences.

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References

[1]. Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y, Kobayashi M, Nanjo K, Sasaki A, Seino Y, Ito C, Shima K, Nonaka K, Kadowaki T. Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *Diabetes Res Clin Pract* (2002) 55(1): 65-85.

[2]. Pirart J. Diabetes Mellitus and Its Degenerative Complications: A Prospective Study of 4,400 Patients Observed Between 1947 and 1973. *Diabetes Care* (1978) 1(3): 168-88.

[3]. Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol* (2004) 92(1): 1-21.

[4]. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* (2010) 87(1):4-14.

[5]. Inzucchi SE. Oral antihyperglycemic therapy for type 2 diabetes: scientific review. *JAMA* (2002) 287(3): 360-72.

[6]. Demeterco C, Levine F. Gene therapy for diabetes. *Front biosci* (2001) 6:D175-91.

[7]. Al-Rowais NA. Herbal medicine in the treatment of diabetes mellitus. *Saudi med J* (2002) 23(11): 1327-31.

[8]. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. *Phytomedicine* (1995) 2(2): 137-89.

[9]. FallahHosseini H, Fakhrzadeh H, Larijani B, Shikhsamani A. Review on medicinal plants used in diabetes. Medicinal plants 1384 [Article in Persian].

[10]. Bailey CJ, Day C. Traditional Plant Medicines asV Treatments for Diabetes. *Diabetes Care*. (1989) 12(8): 553-64.

[11]. Shukia R, Sharma SB, Puri D, Prabhu KM, Murthy PS. Medicinal plants for treatment of diabetes mellitus *Indian J Clin Biochem* (2000) 15:169-77.

[12]. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. Antidiabetic agents from medicinal plants. *Curr Med Chem* (2006); 13(10): 1203-18.

[13]. Zargari A. *Medicinal plants*.Tehran University Press: Tehran (1989).

[14]. Ríos JL, Francini F, Schinella GR. Natural Products for the Treatment of Type 2 Diabetes Mellitus *Planta Med* (2015) 81(12-13): 975-94.

[15]. Movafeghi A, Abedini M, Fathiazad F, Aliasgharpour M, Omidi Y. Floral nectar composition of Peganum harmala L. *Nat Pros Ret* (2009) 23:301-8.

[16]. Rudolph A, Sainz J, Hein R, Hoffmeister M, Frank B, Forsti A, *et al.* Modification of menopausal hormone therapy-associated colorectal cancer risk by polymorphisms in sex steroid signaling, metabolism and transport related genes. *Endocr Relat Cancer* (2011) 18(3):371-84.

[17]. CHOPRA IC, JAMWAL KS, PILLAY PP, SANTHAKUMARI TN. Pharmacological action of Lochnera rosea linn (Rattanjot). . *Ind J Med Res*. (1957) 45: 567-57.

[18]. Farzin D, Mansouri N. Antidepressant-like effect of harmane and other β-carbolines in the mouse

forced swim test. *Eur Neuropsychopharmacol* (2006) 16(5):324-8.

[19]. Lamchouri F, Settaf A, Cherrah Y, Zemzami M, Lyoussi B, Zaid A, *et al.* Antitumour principles from Peganum harmala seeds. *Therapie* (1999) 54(6):753-8.

[20]. El-Saad El-Rifaie M. *Peganum harmala*: its use in certain dermatoses. *Int J Dermatol* (1980) 19(4):221-2.

[21]. Moura DJ, Richter MF, Boeira JM, Pêgas Jenriques JA, Saffi J. Antioxidant properties of β-carboline alkaloids are related to their antimutagenic and antigenotoxic activities. *Mutagenesis* (2007) 22(4):293-302.

[22]. Monsef H, Ghobadi A, Iranshahi M, Abdollahi M. Antinociceptive effects of Peganum harmala L. alkaloid extract on mouse formalin test. *J Pharm Pharmaceut Sci* (2004) **7**(1):65-9

[23]. Nafisi S, Asghari MH, Mohammad Nezhadi MA, Ekhtiari MS. Possible antidiabetic effect of Peganum harmala on streptozocine-induced mouse. *World Appl Sci J* (2011) 14:822-4.

[24]. Singh AB, Chaturvedi JP, Narender T, Srivastava AK. Preliminary studies on the hypoglycemic effect of peganum harmalal.seeds ethanol extract on normal and streptozotocin induced diabetic rats. *Indian J Clin Biochem* (2008) 23:391-3.

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