INVESTIGATION OF ANTIHYPERGLYCEMIC EFFECT OF *MORUS NIGRA* ON BLOOD GLUCOSE LEVEL IN STREPTOZOTOCIN DIABETIC RATS

Hassan Fallah Hoseini [1], Soodabeh Saeidnia [2], Ahmad R. Gohari [2*], Mojgan Yazdanpanah[2], Abbass Hadjiakhoondi [2,3]

1- Department of Pharmacology, Institute of Medicinal Plants ACECR, Tehran , Iran
2- Medicinal plants Research center, Tehran University of Medical Sciences, Tehran, Iran, P. O. Box 14155-6451, Tel: +98-21-66959090, Fax: +98-21-66461178, goharii_a@tums.ac.ir
3- Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Summary

In this report, the effect of *Morus nigra* hydro alcoholic extract on the blood glucose level was evaluated in rats. After inducing diabetes via streptozotocin injection, the animals were orally received various concentrations of *M. nigra* extract (5, 10, 100, 200, 400, 600, 800 and 1000 mg/kg) for one week. Three groups which received 200, 400 and 600 mg/kg of extract were selected to continue for two months. Results showed that mulberry extract at the dose of 400 mg/kg (in short period) and 600 mg/kg (in long period) of the extract cause a significant decreasing in the blood glucose levels in the treated rats compared to control.

Keywords: *Morus nigra*, diabetes mellitus, streptozotocin, blood glucose

Introduction

Plants have been the most important sources of medicine since ancient times. Traditional medicine and other literatures mention the use of plants in treatment of various human ailments (1). Diabetes mellitus is the major endocrine disorder responsible for renal failure, blindness or diabetic cataract, poor metabolic control, increased risk of cardiovascular disease including atherosclerosis and AGE (advanced glycation end) products (2-4).
Morus nigra (Moraceae), named Shatoot in Persian, has been cultivated for so long. It is thought to come from Asia or possibly the Middle East. It was brought to Europe by the Romans and the Greeks who grew it for its bittersweet fruit. The black mulberry is best known for its edible fruits which are dark shade of purple when ripe, they appear almost black, and provide the tree with its name. The leaves of *Morus nigra* are most often heart-shaped but can vary and some have distinct lobes instead. Mulberry leaves always have a rough hairy feel to them; they are dark green when mature and can grow to a fulsome 20cm in length (5).

Recently, the antioxidant activity of three different extracts of *Morus nigra* fruit on haemoglobin glycosylation, peroxidative damage to human erythrocytes, liver hepatooytes of rats and human low-density lipoprotein (LDL) were reported. The results show that it could inhibit haemoglobin glycosylation induced by glucose to differing degrees (6). Antioxidant activity of *Morus nigra* was reported from Jordan which was in agreement with its anti-diabetic consumption in traditional medicine (7).
The present paper reviews the effects of *Morus nigra*, which has been used in the Iranian traditional medicine for anti-diabetic activity, on the blood glucose level in streptozotocin diabetic rats.

Material and methods

Plant material

Leaves of *Morus nigra* were collected in June 2008 from Tehran province. A voucher specimen was preserved for further reference at the Herbarium of Jahad-Daneshgahi Institute.

Extraction

Dried Leaves of the plant (3 kg) were cut in to small pieces then powdered and percolated with hydro-alcoholic (water: EtOH, 2:8) for 24 hours. After filtering, the solutions were concentrated under reduced pressure to obtain (990 g) extract. The yield of extraction was 33% based on the dried weight of plant material.

Animals

Adult Wistar rats weighting 200-250 g (prepared from Seromsazi-Razi Institute, Iran) were kept in the animal house under standard condition in 12h / 12h light dark cycle (23 ± 3 °C). The animals received standard pellet diet and water *ad libitum*. Animal handling was performed as per *Good Laboratory Practice*. Research proposal was prepared based on the CPCSEA (Committee for the Perpose of Control and Supervision of Experiments on Animal) and approved by IAEC (Institutional Animal Ethical Committee) of Tehran University of Medical Sciences.

Induction of diabetes in rats

In order to induce the diabetes, the animals were injected by streptozotocin at the dose of 50 mg/kg of the body weight intraperitoneally. Streptozotocin induces diabetes within 10 days by destroying the beta cells. Diabetic animals and non-diabetic control group were kept in metabolic cages individually and separately and under feeding and metabolism control. Glucose in the blood of diabetic rats exceeded that of the non-diabetic control ones. Food and water consumption were measured in terms of gram and milliliter respectively. Urine volume was measured in terms of milliliter on a daily basis. Glucose in blood serum was also measured, so that chemical diabetes was verified in rats with fasting blood sugar more than 200 mg/dl (8).

Administration of the plant extracts

The rats were randomly selected and divided in to 10 groups of 10-12 in each. The normal group was normal and non diabetic animals. The positive control rats were received glybenclamide 10 mg/kg/day. Eight groups were treated orally (via gavage) the different doses of the hydro-alcoholic *Morus* extract (5, 10, 100, 200, 400, 600, 800 and 1000 mg/kg) for short (one week) and long period (8 weeks). In all animals, blood glucose was measured.

Statistical analysis

The data were expressed as Mean ± SEM. One-way ANOVA was used for comparison of the data followed by Duncan's Multiple Range Test and P values less than 0.05 were considered significant.

Results and Discussion

Streptozotocin (STZ) is an antibiotic which cause pancreatic β-cell destruction and widely used for inducing insulin-dependent diabetes mellitus (IDDM) or type 1 diabetes mellitus (T1DM) in mice and rats. These models for diabetes can be employed for assessing the mechanisms of T1DM, screening potential therapies for the treatment of this condition, and evaluation of therapeutic options (9).

In this study, we evaluated the effect of the black mulberry leaves extract on blood glucose of the diabetic rats. The results of short period (one week) consumption of *Morus* extract is summarized in table 1.

Table 1. The results of the blood glucose for the short period treated rats (with *Morus nigra* extract) compared to control.

groups	N	Blood glucose (Mean)	Standard Deviation
control	12	127.7500	27.45616
10 mg/kg	12	145.1667	43.41310
50 mg/kg	12	129.1667	33.92192
100 mg/kg	10	130.5000	30.20761
200 mg/kg	10	146.5000	33.49378
400 mg/kg	10	105.2000*	17.52966
600 mg/kg	10	162.0000	60.55301
800 mg/kg	10	164.7000	43.29242
1000 mg/kg	10	169.4000	35.05298

* The mean difference is significant at the 0.05 level ($P < 0.05$); Control: The normal and non diabetic rats which received nothing; Treated rats: The diabetic rats which received different doses of the *Morus nigra* extract.

Table 2. The results of the blood glucose for the long period treated rats (with *Morus nigra* extract) compared to control.

	Groups	N	Blood glucose (Mean)	Standard Deviation
In the beginning of treatment	Control	12	141.7500	17.62294
	Glybenclamide	12	140.5833	19.44904
	200 mg/kg	12	147.0000	35.84563
	400 mg/kg	12	138.5000	17.17556
	600 mg/kg	12	145.8333	32.27111
After 2 month treatment	Control	12	131.4167	21.63103
	Glybenclamide	12	79.8333*	26.19102
	200 mg/kg	12	121.2500	23.19924
	400 mg/kg	12	127.8333	19.61833
	600 mg/kg	12	109.4167*	9.81148

* The mean difference is significant at the 0.05 level ($P < 0.05$); Control: The normal and non diabetic rats which received nothing; Glybenclamide: The diabetic rats which received 10 mg/kg/day of Glybenclamide; Treated rats: The diabetic rats which received different doses of the *Morus nigra* extract.

As it can be seen, the results of short period treatment of the rats with *Morus* extract (table 1) indicated that the mulberry extract can decrease the blood glucose level in comparison of both diabetic and non diabetic control at the dose of 400 mg/kg ($p<0.05$). There for, three groups which received 200, 400 and 600 mg/kg of extract selected to continue the long period study. The biostatistics analysis showed that only 600 mg/kg of the extract could cause a significant decreasing in the blood glucose levels in the treated rats. The result of this study is in agreement with the antioxidant activity of *Morus nigra* and its inhibitory effect on haemoglobin glycosylation (6, 7).
In conclusion, the *Morus nigra* extract has potentially the anti-diabetic constituents. There is no document to report the active compound(s) of the mulberry so we suggest carrying out a pharmacological guided fractionation on the effective extract.

Acknowledgments

This research has been supported by Tehran University of Medical Sciences and Health Services grant (No.6639).

References

1. Grover JK, Yadav S, Vats V, Medicinal plants of India with anti-diabetic potential. J. Ethnopharm 2002; 81: 81-100.

2. Thylefors B, The WHO program for the prevention of blindness. Int J Ophthal 1990; 14 :211.

3. Yokozawa T, Nakagawa T. Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. Food Chem Toxicol 2004; 42: 975-981.

4. Burke JP, Williams K, Narayan KMV, Leibson C, Haffner SM, Stern MP. A population perspective on diabetes prevention: Whom should we target for preventing weight gain? Diabetes Care 2003; 26: 1999-2004.

5. http://apps.kew.org/trees/?page_id=114

6. Naderi GA, Asgary S, Sarraf-Zadegan N, Oroojy H, Afshin-Nia F. Antioxidant activity of three extracts of *Morus nigra*. Phytother Res 2004; 18: 365 – 369.

7. Ahmed H., Al-Mustafa and Osama Y., Al-Thunibat. Antioxidant Activity of Some Jordanian Medicinal Plants Used Traditionally for Treatment of Diabetes. Pak J Biol Sci 2008; 11: 351-358.

8. Karunanayake EH, Hearse D J, Mellows G. The metabolic fate and elimination of streptozocin. Biochem Soc Trans 1975; 3: 410-14.

9. Kenneth KW, Youming H. Streptozotocin Induced Diabetic Models in Mice and Rats. Current Protocols in Pharmacology 2008, DOI: 10.1002/0471141755.ph0547s40.