Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

The Cytoprotective Effects of Morus Alba Leaves in Cultured Fetus Fibroblast Cells against Hyperglycemia

Mohammadreza Shams-Ardekani,[1] Abbas Barin,[2] Naiema Vakili-Saatloo,[3] Parisa Sadighara*[3]

1. Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran
2. Department of Clinical Pathology, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran
3. Department of Environmental Health, Food Safety Division, School of Public Health, Tehran University of Medical Science, Tehran, Iran

Article information

Article history:
Received: 29 Aug 2012
Accepted: 14 Nov 2012
Available online: 28 May 2013.
ZJRMS 2013; 15 (11): 52-54

Keywords:
Morus alba
Cytoprotective
Hyperglycemia

*Corresponding author at:
Department of Environmental Health, Faculty of Public Health, Tehran University of Medical Science, Tehran, Iran
E-mail:
sadighara@farabi.tums.ac.ir

Abstract

***Background*:** Diabetes is a chronic disease characterized by high blood glucose levels. Medicinal plants have useful source of hypoglycemic compounds and play important role in preventing the progress of this disease. Morus alba leaves is natural therapeutic agent shown hypoglycemic effects. In this study, the protective effects of alcoholic extract Morus alba leaves on fetus fibroblast cells under hyperglycemic condition were assessed.

***Materials and Methods*:** In this experimental study the cells were treated with six different concentrations of extracts. The nontoxic concentration of plant extract was measured with MTT assay in fetus fibroblast cells. Then, the fetus fibroblast cells were incubated with high dose of glucose and plant extract at concentrations of 1000 ppm. DNA synthesis, as marker of cell proliferation was determined.

***Results*:** In this study treated with plant extract showed an increase in the level of DNA content. There were significant changes in cell proliferation in treated group with plant extract. Morus alba leaves could cause cell attachment and proliferation. The attachment of cells to culture plates has been shown to be affected by plant extract.

***Conclusion*:** The data obtained from this study approved that Morus alba leaves has cytoprotective effects against hyperglycemia.

Introduction

Diabetes is still a serious health problem. The worldwide figure of people with diabetes is set to rise from 150 million in the year 2000 to 300 million in 2025 [1]. It is associated with hyperglycemia in both type 1 and type 2. Furthermore, diabetes mellitus is a complex disease. Major complications of diabetes mellitus have been associated with increased oxidative stress, retinopathy, nephropathy, neuropathy and coronary artery disease [2].

Diabetic people have lower bone density. They lack healthy bone. Therefore, high rate of bone fracture are observed in these people. Both type I and II diabetes are risk factor for coronary artery disease and stroke. More than 75% of all hospitalizations for diabetic complications are attributable to cardiovascular disease [3].

Increased free radicals and impaired antioxidant defenses are widely accepted participant in development of diabetes. Glucose oxidation is believed to be the main source of free radicals. These free radicals may dangerous for beta-cells. These cells have the lowest levels of intrinsic antioxidant defense [1].

This alteration induced to decrease beta-cells function. Furthermore, free radicals attack the macromolecules like lipid, proteins and nucleic acids. In normal condition, these free radicals inactive by antioxidant defense system. But, during diabetes is impaired the endogenous antioxidant defense system. Therefore, alteration in the oxidant/antioxidant equilibrium crates oxidative stress in diabetic people. The variety of antioxidant exits to inactive free radicals. Common these antioxidants include the vitamins, glutathione, and enzymatic antioxidants. Other antioxidants include carotenoids, bioflavonoids that are found in plants. A number of herbs have a long history for traditional use in treating and preventing diabetes. Morus Alba leaves are useful for prevention of diabetes [4, 5].

This plant has a suppressive effect on the blood glucose response and on the secretion of insulin [4]. Chlorogenic acid, 1-deoxynojirimycin, rutin, isoquercitrin, astragalin, caffeic acid, quercetin were identified in this plant. Chlorogenic acid was major component showed stronger maltase and sucrase inhibitory activity [6]. In our studies, a potent antioxidant activity of Morus Alba leaves was observed in fetus fibroblast cells. Thus, a fundamental question addressed that whether Morus Alba leaves has cytoprotective effects in addition to decrease blood glucose level. Therefore, this study was conducted to assess the protective effects of Morus Alba leaves on fetus fibroblast cells under hyperglycemia condition. In previous studies, fetus fibroblast cells were used to show DNA synthesis [7].

Materials and Methods

Dulbecco´s Modified Eagle´s Medium (DMEM) supplement and fetal calf serum were purchased from Razi Institute of Iran. L-Glutamine, penicillium, streptomycin were purchased from Sigma.

In this experimental study, the fetus fibroblast cells were obtained from Pasteur Institute of Iran (NIH-3T3D4). Cells were maintained in DMEM supplement with 10% fetal calf serum, 2 mM L-Glutamine, 50 U/ml penicillium, 50 µg/ml streptomycin. The cells were cultured at 37ºC in a humidified atmosphere and 5% CO_2. Morus Alba leaves were separated, air dried in the shade and powdered. The extract was prepared in ethanol-water (2:1).

Then, it was transferred to vials, and kept at 4ºC. Plant extract was filtered through filter before using. For determination nontoxic concentration of plant extract, the fetus fibroblast cells were seeded in a 96-wel microplate, cells were exposed to various concentrations of the plant extract (10^5-10^{-1}) in fresh serum-free medium. Following removal of the plant extracts from each well, cells were washed in phosphate-buffered saline. The viable cell was assayed with MTT [3-(4, 5-dimethythiazol-. 2-yl)-2,5-diphenyl tetrazolium bromide] method. MTT was added to each well and incubated for a further 3 h. Then, the medium was removed and the cells were incubated with isopropanol to dissolve the formazan crystals. The optical density was measured at 492 nm with 620 nm as a reference and cell viability was normalized as a percentage on control.

The fetus fibroblast cells were exposed to medium with hyperglycemia (35.5 mM glucose) for seven days in a flask that its cells had been reached confluence. Medium was renewed two times after washing with PBS in this period. After this time, two stocks of medium were prepared: (a_1) 35.5 mM glucose; (a_2) 35.5 mM glucose + plant extract (1000 ppm). Then, the cells were trypsinized in flask. The equal volume of this cell suspension was added to both of stocks (a_1) and (a_2). These cells were seeded in 24-well plate and incubated for 24 h. The resistance to hyperglycemia was performed with determining cell proliferation. Cell proliferation was assessed by measuring DNA amount. Briefly, DNA was extracted with High Pure PCR Template Preparation Kit (Roche) according to the manufacturer`s instructions. Total DNA amounts were determined at 260 nm by spectrophotometer (Beckman, USA). The results analyzed using SPSS-17. All values were expressed as the mean±standard error (SEM).

Results

The MTT assay in cells that treated with Morus Alba leaves indicated that the viability have not been changed in the presence of extraction. The optical density was shown in table 1. All of concentrations expect 10^5, had no detectable toxicity on cells according to MTT test. Their amounts were similar to control group (0.107±0.021). Therefore, these concentrations don't cause cellular death (expect 10^5). Randomly, the plant extract was chosen 1000 ppm in this experiment.

The cytoprotective effect of plant extract against hyperglycemia was determined by DNA content. The cells in plant extract group were attached after seeding and rate of growth was considerable. The DNA content in this group (a_2) was 0.05±0.04 (compared with the (a_1) group, -1.73±0.12).

Table 1. Effects of different concentration of plant extract on the viability of fibrobroblast cells

Concentration of plant(ppm)	Absorbance unit
Control(0)	0.107±0.021
10^5	0.018±0.008
10^4	0.140±0.027
10^3	0.102±0.014
10^2	0.103±0.014
10^1	0.113±0.013
10^{-1}	0.116±0.017

The data is expressed as the Mean±SEM. The difference higher than 95 % ($p \leq 0.05$) was considered significant. Their amounts expect 105 were similar to control group and different between treatment groups (expect 105) were not significant.

Discussion

In this study, the cytoprotective effects against hyperglycemia were observed by Morus Alba leaves. The finding indicates that Morus Alba leaves increases the ability of cells to develop resistance to hyperglycemia. The effects of hyperglycemia are often irreversible and lead to progressive cell dysfunction and cell death. The several studies show that hyperglycemia inhibited cell proliferation [3]. In this study, the attachment and proliferation was considerable as result of treatment with plant extract in cells. The marker increase in the rate of DNA synthesis is as result of increasing of cell proliferation. Similar study showed leaves extract increase DNA concentration in diabetic rat pops. They concluded that the ameliorating effect of leaves on these diabetic pops may be attributed to its flavonoids content, which shows potential anti-oxidative activity and has potential hypoglycemic effects [8]. Recent evidence demonstrated that methanolic extracts cause cell cycle progression. Moreover, in previous study increasing in protein synthesis was observed [9].

The free radicals are mutagenic factor for DNA damage. Antioxidants are the components of the cellular defense mechanism against these molecules [10]. The highest antioxidant properties of water extraction of Morus Alba were approved by ferric reducing/ antioxidant power [11]. Nine flavonoids have been isolated from the leaves of Morus Alba that quercetins exhibited significant radical scavenging effect [12]. These flavonoids exerted a significant inhibitory effect on the growth of the human promyelocytic leukaemia cell line [13]. Abdulla et al [14] have showed that oral administration of plant extract had cytoprotection effect against gastric ulcer. The potent antioxidant effects of plants may be attributed to inhibition of reactive oxygen species and free radicals leading to enhancement of ulcer healing. These reactive oxygen species are highly cytotoxic and can induce tissue damage. Decreased levels of glutathione and elevated concentrations of thiobarbituric acid are observed in diabetes. In an experimental study, the concentration of thiobarbituric decreased by treatment with antioxidants such as vitamins C, E and β-carotene. These

normalization effects are seen in kidney, liver, heart, brain, intestine, lung, pancreas, plasma, red blood cells, lens and retina [15]. In other study, antioxidant defenses limit atherosclerosis and its clinical manifestations like stroke and myocardial infarction [16]. Therefore, the results of the present study suggest a direct protective effect of Morus Alba extracts on cells under hyperglycemic condition may be due to antioxidant properties.

Many traditional have been recommended in the alternative system of medicine for diabetes mellitus. In order to decrease complications related diabetes, it is necessary to develop processed foods that do not increase blood glucose and secretion of insulin. These foods have health benefits. Morus Alba leaves is a nontoxic natural therapeutic agent that could be used in processed foods for diabetic people as lowering glucose and a natural antioxidant sources [17]. The reducing oxidative stress seems to be an effective method to improve beta-cells function and survival [1]. Therefore, regular training of diabetic patient for consuming the food which containing this plant could also decrease susceptibility and improve resistance to hyperglycemic and delay diabetic complications. However, further investigation especially in vivo studies and clinical trials are recommended.

Acknowledgements

The authors sincerely acknowledge their gratitude to the efforts of Clinical Pathology Department, Faculty of Veterinary Medicine of Tehran University.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Tehran University of Medical Sciences.

References

1. Lupi R, Del Guerra SD, Mancarella R, et al. Insulin secretion defects of human type 2 diabetic islets are corrected in vitro by a new reactive oxygen species scavenger. Diabetes Metab 2007; 33(5): 340-345.
2. Phillips M, Cataneo RN, Cheema T and Greenberg J. Increased breath biomarkers of oxidative stress in diabetes mellitus. Clin Chim Acta 2004; 344(1-2): 189-194.
3. Aronson D Rayfield EJ. How hyperglycemia promotes atherosclerosis: molecular mechanisms. Cardiovasc Diabetol 2002 ;(1): 1-10.
4. Oku T, Yamada M, Nakamura M, et al. Inhibitory effects of extractives from leaves of Morus alba on human and rat small intestinal disaccharidase activity. Br J Nutr 2006; 95(5): 933-938.
5. Takahiko A, Koichi T, Hiromi O, et al. Maltase, sucrase and ALPHA-amylase inhibitory activity of Morus leaves extract. Food Preserv Sci 2004; 30(5): 223-229.
6. Takahiko A, Koich T, Hiromi O, et al. Maltase, sucrose and alpha amylase inhibitory activity Morus Alba leaves extracts. Food Preservation Science 2004; 30(5): 223-229.
7. Holley RW, Kiernan JA. Control of the Initiation of DNA Synthesis in 3T3 Cells: Serum Factors. Proc Natl Acad Sci 1974; 71(7): 2908-2911.
8. El-Sayyad HI, El-Sherbiny MA, Sobh MA, et al. Protective Effects of Morus alba Leaves Extract on Ocular Functions of pups from Diabetic and Hyper cholesterolemic mother rats. Int J Biol Sci 2011; 7(6): 715-728.
9. Nade VS, Kawale LA, Yadav AV. Protective effect of Morus Alba leaves on haloperidol-induced orofacial dyskinesia and oxidative stress. Pharm Biol 2010; 48(1): 17-22.
10. Diakowska D, Lewandowski A, Kopeć W, et al. Oxidative DNA damage and total antioxidant status in serum of patients with esophageal squamous cell carcinoma. Hepatogastroenterology 2007; 54(78): 1701-4.
11. Wattanapitayakul SK, Chularojmontri L, Herunsalee A, et al. Screening of antioxidants from medicinal plants for cardioprotective effects against doxorubicin toxicity. Basic Clin Pharmacol Toxicol 2005; 96(1): 80-87.
12. Kim SY, Gao JJ, Lee WC. Antioxidative flavonoids from the leaves of Morus Alba. Arch Pharm Res 1999; 22(1): 81-5.
13. Kim S, Gao J, Kang H. Two flavonoids from the leaves of Morus Alba induces differentiation of the human promyelocytic leukaemia (HL-60) cell line. Biol Pharm Bull 2000; 23(4): 51-55.
14. Abdulla MA, Ali H, Abdul-Aziz K, et al. Evaluation of the anti-ulcer activities of Morus Alba extracts in experimentally-induced gastric ulcer in rats. Biomed Res 2009; 20(1): 35-39.
15. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants. J Biochem Mol Toxicol 2003; 17(1): 24-38.
16. Gobind Rai G, Naresh N, Kapil Dev KD, et al. Modulation of oxidative stress, serum lipids and renal dysfunction by benazepril in diabetes mellitus. J Med Educ Res 2010; 12(1): 11-14.
17. Mohammadi J, Naik P. The histopathologic effects of Morus Alba leaf extract on the pancrease of diabetic rats. Turk J Biol 2012; 36: 211-216.

Please cite this article as: Shams-Ardekani M, Barin A, Vakili-Saatloo N, Sadighara P. The cytoprotective effects of morus alba leaves in cultured fetus fibroblast cells against hyperglycemia. Zahedan J Res Med Sci (ZJRMS) 2013; 15(11): 52-54.