The histopathologic effects of *Morus alba* leaf extract on the pancreas of diabetic rats

Jamshid MOHAMMADI\(^1\), Prakash R. NAIK\(^2\)

\(^1\)Department of Physiology, Herbal Medicine Research Center, College of Medicine, Yasouj University of Medical Sciences, Yasouj - IRAN

\(^2\)Endocrinology Laboratory, Department of Zoology, University of Mysore, Mysore, 570006 - INDIA

Received: 11.08.2010

**Abstract:** Many traditional treatments have been recommended in the alternative system of medicine for the treatment of diabetes mellitus. Mulberry (*Morus alba*) is a nontoxic natural therapeutic agent shown to possess hypoglycemic, hypotensive, and diuretic properties. The purpose of this study was to examine the histopathologic effects of *Morus alba* leaf extract on the pancreas of diabetic rats. The animals were treated with mulberry leaf extract at dosages of 400 and 600 mg/kg body weight for 35 days. The various parameters studied included blood glucose, the relative body weight of the pancreas, the diameter of islets, and the number of β cells in all groups. Blood glucose level, the diameter of the islets, and the number of β cells were increased in treatment groups as compared to the diabetic group. According to the histological and biochemical results obtained, it was concluded that the extract of this plant may reduce blood glucose levels by regeneration of β cells.

**Key words:** *Morus alba*, type 1 diabetes, islets, β cell, glucose

**Introduction**

Diabetes is a chronic disease characterized by high blood glucose levels and abnormal metabolism of carbohydrates, proteins, and fat associated with a relative or absolute insufficiency of insulin secretion and with various degrees of insulin resistance (1). Such alterations result in increased blood glucose, which causes long-term complications in many organs. Despite important progress in the management of diabetes using synthetic drugs, many traditional plant treatments are still being used throughout the world. Plants are valued in indigenous systems of medicine for the treatment of various diseases (2). Medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as dietary supplements to existing therapies (3). Some of the plants that are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the World Health Organization’s expert committee on diabetes recommends that this area warrants further attention (4).

Recent evidence shows that leaves and shoots from the mulberry tree possess several medicinal properties, including hypoglycemic, hypotensive, and diuretic effects (5). Mulberry root bark or leaf extracts were shown to possess hypoglycemic effects in animal models of type 1 diabetes mellitus (6). We recently reported that the extract of *Morus alba* leaves promoted significant hypolipidemic activity in experimental animals (7). The aim of the present study was to clarify the effect of *M. alba* leaf extracts on blood glucose and to determine any possible effect on pancreatic tissue.
The histopathologic effects of *Morus alba* leaf extract on the pancreas of diabetic rats

### Materials and methods

#### Animals

Adult Wistar rats were procured from the animal house of the Department of Zoology. Irrespective of sex, the animals had body weights ranging between 150 and 200 g. Protocols of the experiment were approved by the Institutional Animal Ethics Committee. The animals were maintained under standard conditions, with a temperature of 20 ± 5 °C, a regular 12-h light and 12-h dark cycle, and a humidity level of 50%-68%. The rats were allowed free access to standard laboratory food and water ad libitum throughout the experiment.

#### Procurement and preparation of the plant material

The mulberry leaves used in this experiment were collected from the garden of the Sericulture Department of the University of Mysore. The fourth and fifth leaves were plucked from the apex of healthy plants, washed thoroughly under running tap water, shade-dried for 5 days, and eventually ground to a fine powder in an electric grinder. The powdered plant material (1250 g) was extracted twice, 24 h each time, with 90% ethanol at room temperature. The extract was filtered with Whatman filter paper No 1. The filtrate was evaporated using a Soxhlet evaporator until dry, and 135.5 g of extract was obtained. The dried powder was diluted in distilled water at concentrations of 400 and 600 mg/kg body weight.

#### Experimental protocols

The animals were classified into 5 groups, each containing 8 rats. These groups were identified as follows: control group (I), control group with *mulberry* leaf extract treatment (II), diabetic control group (III), diabetic group treated with 400 mg/kg per day *mulberry* leaf extract (IV), and diabetic group treated with 600 mg/kg per day *mulberry* leaf extract (V). The animals in groups III, IV, and V were rendered diabetic by a single intraperitoneal injection of streptozotocin (STZ; 60 mg/kg) freshly prepared in 0.1 mol citrate buffer (pH 4.5). The animals in groups I and II were injected with the buffer alone. Next, 72 h after the injection, blood was drawn from the tail of conscious rats in order to estimate their glucose levels using a glucometer. Blood glucose was similarly estimated every week until autopsy. Ten days after the STZ injection, animals in groups II and IV began to receive 400 mg/kg of the *mulberry* leaf extract daily; those in group V received 600 mg/kg of the extract daily. Doses were administered orally for 5 weeks. Body weight was recorded at the beginning and end of the experiment in every group. At the end of the experimental period, animals were fasted overnight and autopsied under light ether anesthesia.

#### Morphometric analysis

Pancreatic tissue was taken from all groups. The weight of the pancreas was also recorded in every group. The tissue samples were washed, fixed in Bouin-Holland, and dehydrated with alcohol in Bouin-Holland for 18-20 h. Serial sections of 4-5 μm in thickness were cut using a microtome, and every fifth slide was stained using the chrome alum hematoxylin and phloxine (CHP) method. The slides were mounted using DPX mountant, and 100 islets were measured from 100 randomly selected cross-sections of the pancreas from each rat. The β cells were also counted (8). The relative weight of the pancreas was calculated using the following formula: relative weight of pancreas = weight of pancreas/weight of animal × 100.

#### Statistical analysis

Results were analyzed statistically using analysis of variance (ANOVA) and represented as mean ± standard error (SE). Wherever the variance value was found to be significant at 5%, Duncan’s multiple range test was applied.

#### Results and discussion

The effect of *M. alba* leaf extract on the blood glucose levels of experimental rats is shown in Figure 1. Throughout the experimental period, the control rats (I) did not show any significant variation in blood glucose level with group II. The administration of STZ (60 mg/kg) led to a more than 3.5-fold elevation of blood glucose levels, which was maintained over a period of 5 weeks. At a daily dosage of 400 mg/kg, the *mulberry* leaf extract significantly reduced hyperglycemia compared to the diabetic group, although this amount failed to restore the level to that of the control group. Daily treatment with 600 mg/kg, however, almost reached the levels demonstrated by the control group (I) (P < 0.05).
Figure 2 shows the changes of the relative weight of the pancreas in all groups. The relative weight of the pancreas with type 1 diabetes did not differ significantly across groups I, II, IV, and V, but there was a significant increase in the relative weight of the pancreas in diabetic group III (P < 0.5).

Changes in the diameter of islets in all groups are given in Figure 3. The diameter of islets did not differ significantly in groups I and II of the experimental animals. Since the β cells were damaged due to the induction of diabetes, the islet size decreased significantly in group III of the experimental animals (P < 0.5). The administration of the mulberry leaf extract to groups IV and V of the experimental animals resulted in the recovery of damaged β cells and the restoration of the diameter of the islets to that of the control group (I).

A decrease in the number of β cells of the islets of Langerhans was observed in the diabetic group in comparison to the control group (Figure 4). The number of β cells in animals from groups I and II did not show significant variation. When compared to group III, the number of β cells seen in specimens from groups IV and V increased significantly (P < 0.5) after daily treatment with 400 and 600 mg/kg mulberry leaf extract, respectively.

Figure 5A shows an islet of Langerhans from an animal in the control group (I). The islet shows a large number of β cells (hematoxylin-stained blue cells) distributed throughout the islet. A few α cells (phloxine-stained pink cells) can also be observed. The islet of Langerhans shows that the β cells did not differ in group II, neither in number nor in diameter, from those of the control group (I).
In the diabetic group, a decrease in the number of β cells of the islets of Langerhans was observed in comparison to the control group (Figures 5A and 5B). The damage or necrosis of β cells was caused by the streptozotocin used to induce diabetes. Few functional β cells were observed and α cells were more prominent.

Figures 5C and 5D show the islets of Langerhans from the diabetic groups treated with *M. alba* leaf extract at concentrations of 400 and 600 mg/kg body weight, respectively. The damaged β cells seen after the initial induction of diabetes were no longer observed after treatment with *M. alba* leaf extract. The recovery of necrotic β cells was especially more pronounced after treatment with 600 mg/kg of *M. alba* leaf extract than in the group treated with 400 mg/kg of the extract.

This study was carried out histologically by light microscopy and biochemically in order to detect whether this plant decreased blood glucose and whether it had an effect on the pancreatic tissue. The *M. alba* leaf extract was found to be effective in controlling hyperglycemia in diabetic rats. Although many suggestions have been made about plants’ roles, the precise mechanism is unclear. Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances, some may inhibit insulin activity, and others may increase β cells in the pancreas by activating the regeneration of these cells. The fiber of plants may also interfere with carbohydrate absorption and thereby affect blood glucose (9-12).

Different studies have demonstrated that certain plant extracts (*Scoparia dulcis*, *Teucrium polium*, and *Salvadora oleoides*) effectively increased the number of β cells in streptozotocin- and alloxan-induced diabetes mellitus (13-15). The diminished islet size and β cell number resulted in the histopathology of the diabetic pancreas. A recent report showing that adult β cell numbers are formed by self-duplication/self-proliferation supports our finding of an increase in the β cell number in diabetic islets after mulberry treatment (16).

Taniguchi et al. (17) showed that the compounds of mulberry leaf, particularly fagomine, are capable of inducing insulin secretion in isolated rat islet cells. Kim et al. (18) observed that aqueous extracts of *C. coronarium* and *M. alba* effectively decreased blood glucose in alloxan-induced diabetic rats.

Shirwaikar et al. (19) reported on histopathology studies in which STZ was suspected of partially
destroying the pancreas. Diabetic rats showed reduced islet cells, which were restored to near normal upon treatment with the extract. Al-Eryani and Naik (20) observed the effects of *T. cordifolia* extract on high fat-fed type 2 diabetic rats; in that study, the extract significantly restored the diameter of islets and the number of β cells in diabetic rats.

Similarly, Yin et al. (21) observed that high-dose STZ in diabetes indicates β cell destruction. After 120 days of normoglycemia, a statistically significant 3.7-fold increase in β cell mass was observed.

This study supports the possibility of regeneration as a means for generating new β cells, even in severely diabetic animals or individuals with significantly reduced β cell mass. As other possible mechanisms, the extract from mulberry leaves may increase β cells and sensitize the insulin receptor to insulin, or stimulate the stem cells of the islets of Langerhans in the pancreas of STZ-induced diabetic rats.

In conclusion, the histopathologic studies undertaken on the islets demonstrated the recovery of damaged islets and an improvement in the number of β cells after treatment with the plant extract. It can thus be assumed that *M. alba* leaf extract has a therapeutic effect that alleviates diabetes mellitus. The actual chemical compound that is responsible for this effect requires further investigation.

**Acknowledgments**

The first author gratefully acknowledges Yasouj University of Medical Sciences, Iran, and the Chairman of the Department of Zoology, University of Mysore, Mysore, India, for support and access to facilities.

**Corresponding author:**

Jamshid MOHAMMADI  
Department of Physiology,  
Herbal Medicine Research Center,  
College of Medicine,  
Yasouj University of Medical Sciences,  
Yasouj - IRAN  
Email: jamshidm2005@yahoo.com
The histopathologic effects of *Morus alba* leaf extract on the pancreas of diabetic rats

References