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IMPACT OF AQUEOUS EXTRACT OF BLACK MULBERRY (*Morus nigra)* ON LIVER AND KIDNEY FUNCION OF DIABETIC MICE

Hemmati AA[1,2]*, Jalali MT[3], Rashidi I[4], Kalantar Hormozi T[1]

[1]*Department of Pharmacology and Toxicology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

[2]*Physiology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

[3]*Department of Clinical Biochemistry, School of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

[4]*Department of Pathology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

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Abstract

Several reports claiming a hypoglycemic effect for Black Mulberry (*Morus nigra folium*) extract. Carbohydrate metabolic disorders have serious acute and chronic complications resulting in high mortality and morbidity rate. In this study along with hypoglycemic effect of extract of black mulberry its toxic effect on liver and kidney have also been investigated in mice. Diabetes was induced in mice using streptozocin (100mg/kg single intraperitoneal injection). Extract of Black Mulberry leave was prepared with maceration method. The treatment schedule was set for oral administration of aqueous extract of mulberry leave (100mg/kg twice daily) for 42 days. Serum creatinine, glutamate-pyruvate Transferase (SGPT), glutamate-oxalate Transferase (SGOT) activities were measured. Mulberry extract could reduce the blood sugar in animals. However; it caused a significant rise in the above mentioned factors in both control and diabetic animals. Based on the data obtained from this study, it is concluded that caution should be taken with the use of mulberry extract or drugs containing this herb in diabetic patients. In the case of chronic use of this herbal product, liver and kidney function need to be monitored.

Keywords:

Morus nigra, Diabetes Mellitus, Streptozocin, SGPT, SGOT, Creatinine.

Introduction

Diabetes mellitus is one of the major endocrine disorders responsible for renal failure, blindness or diabetic cataract, poor metabolic control, increased risk of cardiovascular disease including atherosclerosis and AGE (advanced glycation end) products (1). Medicinal plants used to treat hypoglycemic or hyperglycemic conditions are of considerable interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of hypoglycemic and anti-hyperglycemic activity (2). The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Several species of medicinal plants are used in the treatment of diabetes mellitus.

*Email: Hemmati_aa@yahoo.com

Traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications (3). Black mulberry (*Morus nigra*) a species of mulberry, native to southwestern Asia is one of these plants in which its hypoglycemic effects has been reported in both human and animals (2, 4). Its edible fruit is dark purple, almost black, when ripe, 2-3 cm long, a compound cluster of several small drupes; it is richly flavoured, similar to the red mulberry (*Morus rubra*). Black mulberry has long been cultivated for its edible fruit, and is planted and often naturalised to west across much of Europe, including the Ukraine, and east into Iran and China (5, 6). Chemical compositions of mulberry include: phenolic compounds, flavonoides, ascorbic acid and quercetin. The major fatty acids in mulberry are linoleic acid, palmitic acid and oleic acid. Mineral compositions of the mulberry are: N, P, K, Ca, Mg, Na, Fe, Cu, g, Mn and Zn (7). The mulberry has a long history of medicinal use in Chinese medicine, almost all parts of the plant are used in one way or another. Recent researches have shown various effects therapeutic effects for this plant. Analgesic, emollient and sedative effects have been calimed for black mulberry. The leaves have antibacterial, astringent, diaphoretic, hypoglycaemic and odontalgic propertes. The stems are antirheumatic, diuretic, hypotensive (5, 8). The fruit has a tonic effect on kidney. It is used in the treatment of urinary incontinence, tinnitus, premature greying of the hair and constipation in the elderly. It can be used as a colouring and flavouring in both food and pharmaceutical industires. The root bark is antitussive, diuretic, expectorant and hypotensive (8). In vitro studies suggest that extracts of black mulberry and green, tea could interfere with carbohydrate and triglycerides absorption via their ability to inhibit alpha-amylase, alpha-glucosidase, sodium-glucose transporters, and pancreatic lipase (9, 10). Aqueous extract of black mulberry leaves can reduce the blood sugar in diabetic mice (4). Despite the several reports about its therapeutic effect in diabetes, the unwanted effects of the plant extract on other organs has been neglected. This study was performed to evaluate the effect of sub-chronic use of aqueous extract of black mulberry leaves in normal and diabetic mice.

Material and methods

Albino wistar mice of either sexes weighing 20-35g were used during the study. The animals were housed in polycarbonate cages (10 in each cage) under controlled conditions (12h light/dark cycle, 55% humidity, at 23±2°C). All animals had free access to water and normal pellet diet (Pars Khorak Daam, Shushtar, Iran) during the experiments. Mice were acclimatized for 5 days before experiments. All experiments were performed in accordance with the guidelines and consent of Ethical Committee of Ahvaz University of Medical Sciences. Animals were randomly allotted in four groups (n=10).

Experimental groups

Group one was considered as control. Group two was used to induce diabetes by streptozocin (100mg/kg single peritoneal injection). Group three as non-diabetic was treated with mulberry extract and the fourth group, received streptozocin to induce diabetes and then treated with mulberry extract. The treatment schedule was set for oral administration of aqueous extract of mulberry leave (100mg/kg twice daily) for 42 days in groups three and four.

Preparation of the herb extract

leaves of mulberry was collected during the summer and dried in shade. The dried leaves were powdered and extracted by maceration technique. The extract was then filtered. The filtrates then were concentrated in vacuum rotary evaporator.

The density of concentrated extract was determined by a picnometer and appropriate dose was prepared for administration to animals.

After the end of treatment course (42 days) animals were sacrificed. Blood sample was collected from carotid artery. Serum creatinine, SGPT, SGOT, glucose concentration were measured by autoanalyzer employing enzymatic techniques for SGPT/SGOT and Jaff reaction for creatinine. Liver and the kidney tissues were removed and fixed in formalin solution (10%). The tissues then processed and cut into 5micron sections and stained with H&E for light microscopic investigation.

Statistical analysis

The results were examined by analysis of variance (ANOVA) and significant differences among the means of groups were accepted at the $p<0.05$ level. Further analyses were performed using Duncan's multiple range or student's t-test .

Results

Streptozocin, caused significant increase in blood glucose concentration which confirmed the diabetes in animals (Fig 1)

As shown in figure 2, the extract of Morus nigra has significantly ($P<O.05$) decreased the fasting blood glucose level in both normal and diabetic mice.

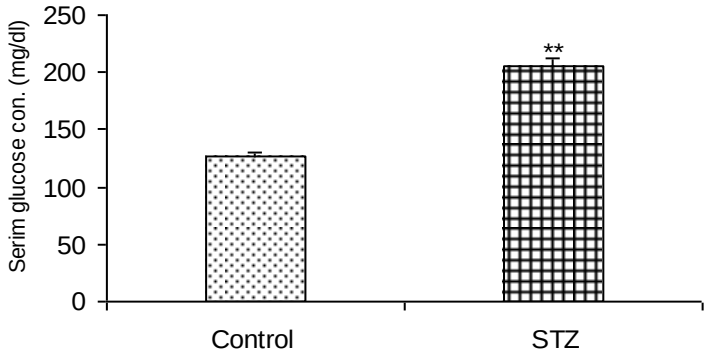

Fig. 1: Effect of streptozotocinon on serum glucose level in mice. Significant increase of serum glucose is indicated ** ($p<0.01$).

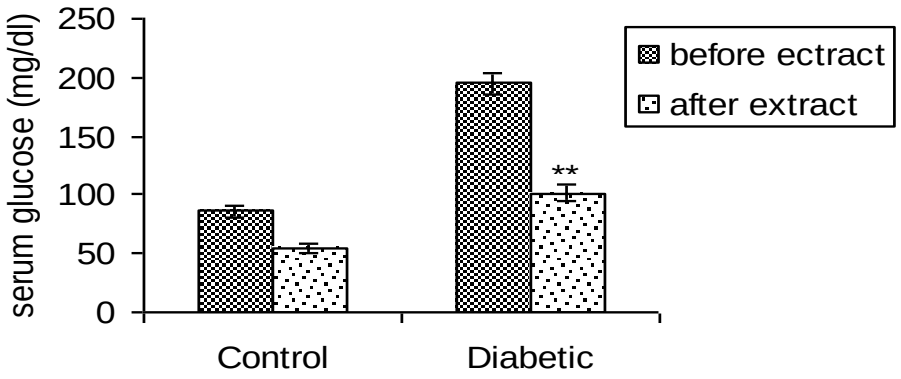

Fig. 2: Effect of mulberry extract on fasting serum glucose in normal and diabetic mice. Significant reduce of serum glucose is indicated ** ($p<0.01$).

The extract increased serum creatinine concentration , SGPT and SGOT activities in both control and treatment groups at a significant level ($p<0.01$ &0.05) (figs 3-5). Histopathologic investigation on liver showed no pathologic changes in the control group during the period of study. Liver of diabetic animals without extract showed slight congestion of portal space with few lymphocytes infiltration.

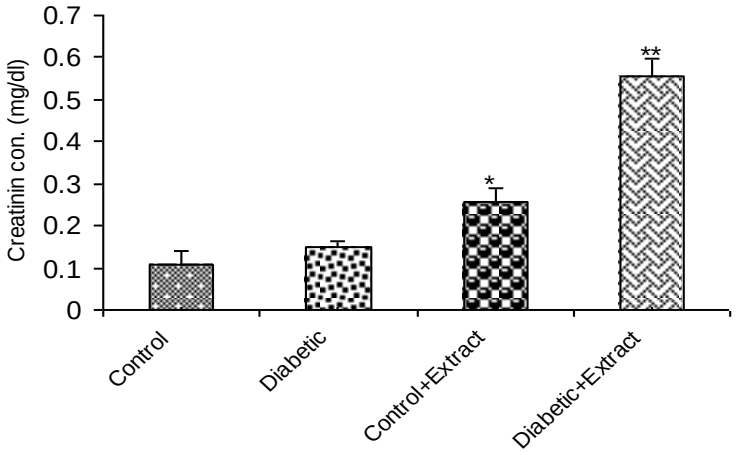

Fig. 3: comparison of creatinine levels in four groups of mice. Significant increase of creatinine level compare to control is indicated * ($p<0.05$) and ** ($p<0.01$).

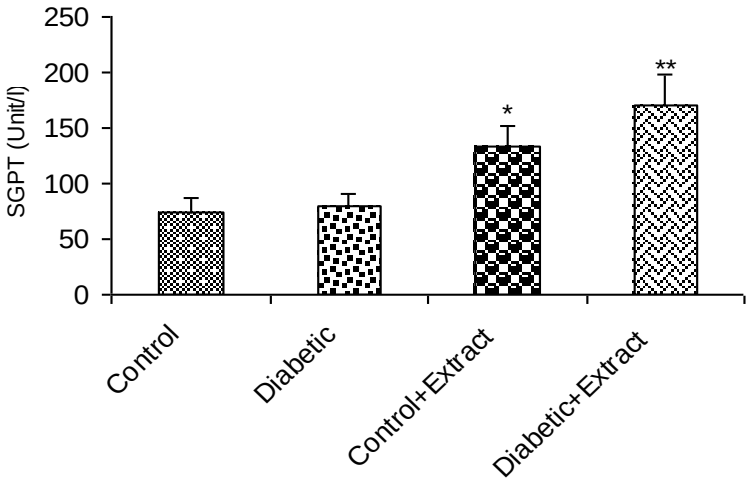

Fig. 4: Comparison of SGPT activity in four groups of mice. Significant increase of SGPT compare to control is indicated * ($p<0.05$) and ** ($p<0.01$).

Liver of control animals which treated with extract showed vascular dilation in sinusoids and portal vein. Lymphocyte infiltration into the portal space was evident. Lymphocyte accumulation was also seen in all lobes and caused focal necrosis, but fibrosis was not seen. Effect of mulberry extract on diabetic mice showed more intensive congestion. In this group vascular dilation in sinusoids was associated with lymphocyte infiltration and formation of triadite. Necrosis was also seen without signs of fibrosis (figs 6 & 7).

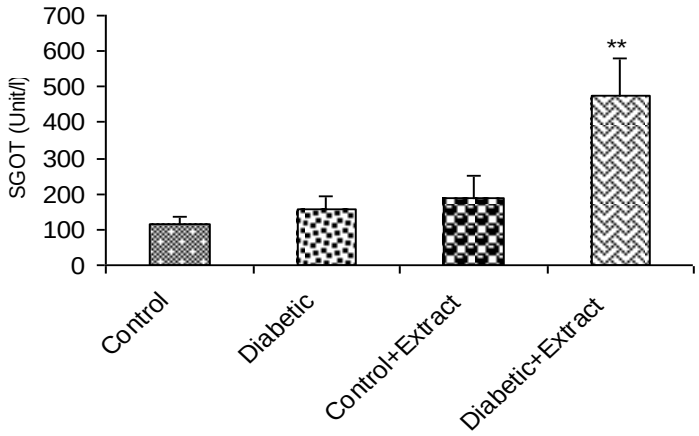

Fig. 5: Comparison of SGOT activity in four groups of mice. Significant increase of SGOT compare to control is indicated ** ($p<0.01$).

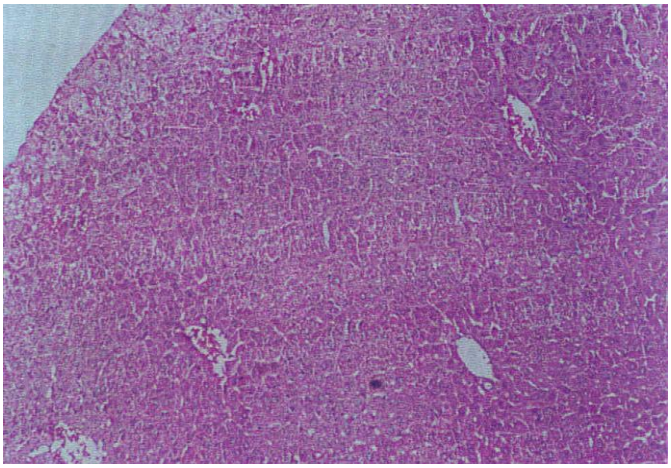

Fig. 6: Photomicrograph of liver section of normal mouse. Hepatocytes, portal spaces and sinosoides are seen with a normal structures (H&E x40).

In kidney tissues of normal animals no abnormality was observed after the end of experiments. In diabetic group slight and sparse vascular congestion was seen in interstitium. Streptozocin treatment also caused slight glomerular proliferation. In normal group treated with extract vascular dilation and congestion was exhibited. Tubular dilation was accompanied with inflammation and degeneration of in lateral cells. A low degree mesenchymal proliferation has occurred in some glomerules which appear to be hyperplasia. In kidney of diabetic animals which received extract congestion was associated with interstitial nephritis. Tubular epithelial degeneration and glomerular proliferation was characteristic in few sections (figs 8 & 9).

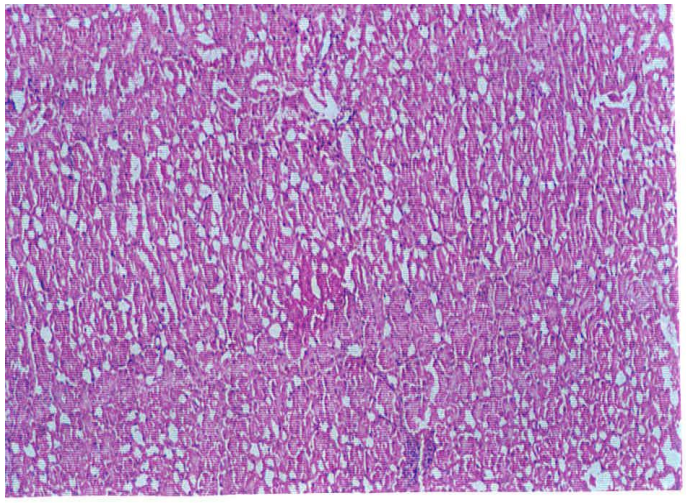

Fig. 7: Photomicrograph of liver section of diabetic mouse, after treatment with mulberry extrct. Infiltration on lymphocytes in portal spaces and adjacent to hepatocytes, are seen (H&E x40).

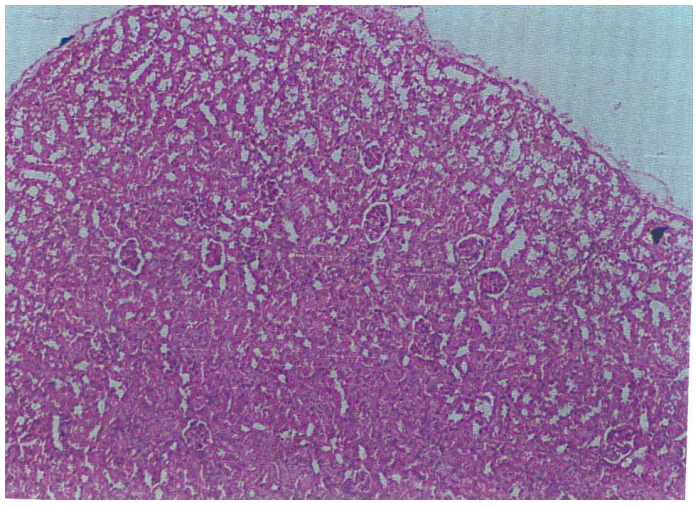

Fig. 8: Photomicrograph of kidney section of normal mouse indicating intact glumeroles and vascular structure (H&E x40).

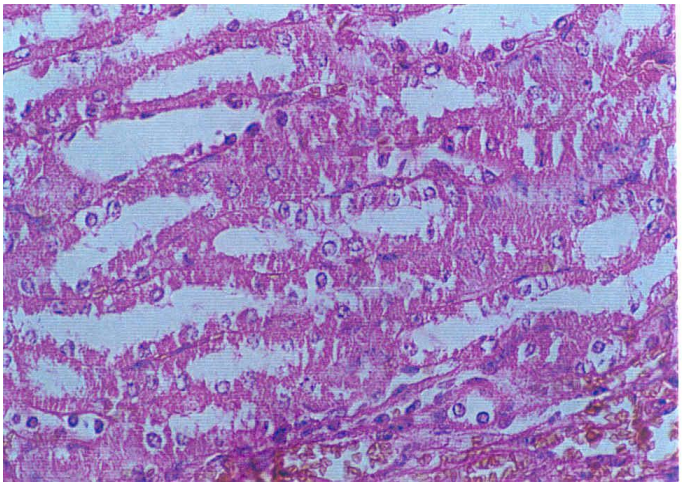

Fig. 9: Photomicrograph of kidney section of diabetic mouse, after treatment with mulberry extrct. Tubular degeneration and vascular congestion associated with lymphocytes infiltration are seen (H&E x100).

Discussion

Disturbed regulation of blood glucose level is associated with serious detrimental outcomes acutely and chronically resulting high mortality and morbidity rate. The cornerstone of all this pathological processes is hyperglycemia. Therefore all kind of efforts have been directed toward the correction of this phenomenon. Among the employed measures for this purpose, we may mention herbal remedies including black mulberry. Recent researches have mainly focused on the hypoglycemic effect of this herb even in clinical trial (11,12). However diabetes is chronic disease and long term use of drugs for treatment may bring about unwanted effects during such treatment. Therefore any herbal remedy which is suggested to be used in diabetes should be examined from the adverse effects point of view. In this work the extract of black mulberry (*Morus nigra)* has been studied for this purpose. The results indicate a significant glucose lowering effect for this herb as mentioned earlier. However the obtained results from biochemical parameter such as creatinine, SGPT and SGOT and also histopathological investigations depicted a relatively toxic effects for this herb with the used dosage. However; we may notice that streptozocin which is used to induce diabetes has its own adverse effect on kidney and liver (13). Such impact may potentiate the unwanted effects of extract observed in diabetic animals.

The significant difference between the groups received extract of *Morus nigra* and the control groups indicate the toxic effect of this herb extract. Toxicities which have been created by extract may be due to different components of the herb which can not be verified by this work. However; the results of this work alert us that the long term use of mulberry extract may produce serious consequences on liver and kidney. Therefore the herbal product containing mulberry extract should be used with a caution and in a period as short as possible.

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