

ORIGINAL ARTICLES

Effect of *Morus Alba* Linn extract on Enzymatic Activities in Diabetic Rats

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ABSTRACT

Morus alba has been used as a therapeutically agent and beneficial effects. This study was investigated the effect of *Morus alba* on lipid peroxidation and hepatic glucose-regulating enzymes in streptozotocin (STZ) induced diabetic rats. Four groups of rats were classified as: control group (I); diabetic group (II) injected intraperitoneal (*i.p.*) with single dose STZ as a diabetogenic agent; control treated with *M. alba* group (III); diabetic treated with *M. alba* after STZ-induced diabetes group (IV). The results of this study showed that lipid peroxidation was increased in diabetic rats; whereas *M. alba* treated diabetic rats reduced lipid peroxidation. *Morus alba* treating diabetic rats prevent glycogen depletion and avoided lactate overproduction as compared with normal treated *M. alba* group. The activities of hexokinase, glucose 6 phosphate dehydrogenase and lactate dehydrogenase significantly raised in *M. alba* treated diabetic rats, where as glutathione s transferase and glucose 6 phosphatase activity reduced. *M. alba* treating diabetic rats reduce hyperglycemia by control oxidative stress, increasing glycogen levels and prevent anaerobic glycolysis and improve hepatic carbohydrate metabolism.

Key words: *Morus*; Streptozotocin; Glycogen, Lactate, MDA, Glutathione, GST, Hexokinase, G6Pase, G6PD, LDH.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic disorder of carbohydrate, fat and protein metabolism that results from defects in both insulin secretion and/or insulin action. The disease is associated with reduced quality of life and increased risk factors for morbidity and mortality. The long term hyperglycemia is an important factor in the development and progression of micro- and macrovascular complication, which include cerebrovascular diseases (Altan, 2003 and Strojek, 2003) neuropathy, nephropathy and cardiovascular (Shim *et al.*, 2011).

Streptozotocin (STZ), an extract from *Streptomyces achromogenes*, has been used for inducing diabetes mellitus by its toxic action to islet β cells of pancreas (Szkudelsk, 2001), possibly by a free radical mechanism. The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and non-enzymatic scavenger systems (Halliwell and Gutteridge, 1994); the levels of which are altered in diabetes (Wohaieb and Codin, 1987).

Several drugs such as biguanides, sulfonyleurea and thiazolidenediones are presently available to reduce hyperglycemia in diabetes mellitus (Jung *et al.* 2006 and Matsui *et al.*, 2006). The use of these drugs is accompanying side effects (Donath *et al.*, 2006 and Noor *et al.*, 2008). The medicinal plants may provide the useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapies (Kavishankar *et al.*, 2011).

Morus alba, known as mulberry, has been considered to possess many different medicinal properties such as antiphlogistic, diuretic, expectorant and antidiabetic effects (Hikino *et al.* 1985). Andallu *et al.* (2001) reported that the oral administration of mulberry leaves powder could decrease blood and urine glucose, triacylglycerid (TG), LDL-cholesterol and VLDL-cholesterol and fatty acid in type-2 diabetes patients. *Morus alba* leaves contained active ingredients such as flavones, steroids, triterpenes, amino acid, vitamins, 6 N-containing sugars, Iminosugar 1-deoxynojirimycin(DNJ) and 2-O- α D-galactopyranosyl- DNJ (GAL-DNJ) and fagomine have been claimed to be the most potent antihyperglycemic effects (Nakagawa *et al.*, 2010 and Chen *et al.*, 1995). Furthermore, the piperidine alkaloid and glycoproteins from the *Morus* root bark and/or leave extract have been used for antidiabetic agents (Hikino *et al.*, 1985).

The purpose of this study was to investigate the effect of *M. alba* extract on the activities of hepatic enzymes of glucose metabolism in normal and STZ-induced diabetic rats.

Materials and Methods

Preparation of M. alba leaves extract:

Two hundred gram of *M. alba* leaves (It was identified through the plant Department of the Faculty of Science, Fayoum University, Egypt.) were extracted in a round flask with distilled water (3000 ml) at 100°C for 1h. The aqueous extracts were subsequently filtered, evaporated in a Rota vapor at 40-50°C under reduced pressure and freeze-dried as a powder. 100 mg of *M. alba* leave extract was suspended in 0.5 ml distilled water and administered orally to the rats through an intragastric tube at a dose of 100 mg /kg body weight (Kim *et al.*, 2006).

Experimental animals:

Forty eight adult male *Sprague Dawely* rats, weighing 200-265 g, were purchased from the breeding unit of Egyptian Organization for Biological Products and Vaccines (Abbassia, Cairo). Rats were housed in steel mesh cages and maintained for one week acclimatization period on commercial pellet diet and drinking water *ad libitum* (Revees *et al.*, 1993). Rats were randomly classified into four groups, each group comprising 12 rats as follows: Group I (control group): Rats were injected *i.p.* with citrate buffer solution (0.1M pH 4.5), Group II (Diabetic group), Group III (control *M. alba* group): Rats were treated with *M. alba* 4 days/week for 4weeks and Group IV (diabetic + *M. alba* group): Rats were treated with *M. alba* 4 days/week for 4 weeks after STZ-induced as a diabetes inductor (Jaslin *et al.*, 2010) .

Induction of experimental diabetes:

Experimental diabetes was induced by a single intraperitoneal (*i.p.*) injection of 75 mg/kg streptozotocin (STZ) (Sigma, St Louis, MO, USA) to overnight fasted rats (Singh *et al.*, 2001). STZ was dissolved in citrate buffer (0.1M, pH 4.5) and freshly prepared immediately before injection. Rats with blood concentration of higher than 200 mg % after 48 hours of STZ injection were considered diabetic (Kedziora-Kornatowska *et al.*, 1998). Blood glucose was measured using a diagnostic kit obtained from Boehringer Mannheim (GmbH, Germany).

Sample preparation:

After 4 weeks of experiment, the rats were anthesized then sacrificed by decapitation under ethical committe. Blood was collected and centrifuged at 3000 rpm. for 10 min at 37°C the serum was separated and kept in a florid vial at -20°C until assay. Livers were immediately excised, washed with ice cold saline and kept frozen at -70°C until use. Liver tissue (1g) were homogenized using Ultra-Turrax homogenizer in 10 ml a saline and centrifuged 8000 \times g for 15 min. at 4°C and the resulting supernatants were subjected to assay of different parameters.

The protein content was measured by the method of Bradford (1976) using Coomassie brilliant blue G-250. Calibration curve was prepared with bovine serum albumin.

Assay of glycogen:

Liver glycogen was determined by anthrone method as described by Sandham and Kleinberg (1969).

Assay of lactic acid:

Its concentration was measured using a commercial kit from Eli-Tech Diagnostics (France) (Artiss *et al.*, 2000).

Assay of Malondialdehyde (MDA):

It was assayed by thiobarbituric acid method as described by (Buege and Aust, 1978).

Assay of Reduced glutathione (GSH):

It was determined according to Jakoby (1985).

Assay of Glutathione -s transferase (GST):

It determine the formation of the conjugate of glutathione GSH and 1- chloro - 2,4- dinitrobenzene (CDNB) (Habig *et al.*, 1974). One unit will conjugate 1.0 μ mole of 1-chloro-2,4-dinitrobenzene with reduced glutathione per minute at pH 6.5 at 25°C.

Assay of Hexokinase:

It is based on the reduction of NAD^+ through a coupled reaction with glucose 6 phosphate dehydrogenase (Brandstrup *et al.*, 1957). One unit of hexokinase activity is defined as 1nmol of reduced NAD^+ formed per min. under the standard assay condition.

Assay of Glucose 6 phosphatase (G6Pase):

It catalyzes the conversion of glucose 6 phosphate to glucose and assayed by the method described by (Baginsky *et al.*, 1974). The enzyme activity unit was expressed as nmol phosphate per minute under standard assay condition.

- Assay of Glucose 6 phosphate dehydrogenase (G6PD):

Glucose 6 phosphate dehydrogenase assay is based on the rate of increase of absorbance of the formation of reduced NADP^+ (Deutsch, 1983). One unit of glucose 6 phosphate dehydrogenase is defined as 1 nmol of reduced NADP^+ reduced under the assay condition.

Assay of Lactate dehydrogenase (LDH):

Its activity was determined in liver tissue by following, the initial rate of reduction of pyruvate to lactate (Pesce, 1989). One unit of enzyme activity is defined as the amount of enzyme, which oxidizes one \square mol of NADH in 1 min under the standard assay condition.

Results and Discussion

STZ selectively destroys insulin-producing β -cells of the pancreas by inducing high levels of DNA strand breaks in these cells, causing activation of poly (ADP-ribose) polymerase (PARP), resulting in reduction of cellular NAD^+ , and cell death (Bolzan and Bianchi, 2002).

The effect of *M. alba* on body weight and fasting serum glucose levels of normal and STZ induced animals are presented in Table (1). Rats treated with STZ were hyperglycemic and lost weight over experimental period. The body weight in diabetic along with *M. alba* treated group was increased significantly at the end of the experimental period when compared with diabetic rats. The concentration of blood glucose was significantly increased in diabetic *M. alba* treated rats as compared with *M. alba* group whereas was significantly decreased as compared with diabetic rats.

Oxidative stress is one of the metabolic events associated to diabetes and its complications (Baynes, 1991). Our experimental results showed that liver MDA level (an index of lipid peroxidation) significantly increased in STZ-treated rats as compared to the control, but no significant change in the *M. alba* treated diabetic animals in comparison with both control and *M. alba* control groups (Table 2). *Morus alba* diminished hepatic MDA level induced by STZ. Our data is in coincidence with previous reports (Chang *et al.*, 1993).

There is evidence that hyperglycemia can lower both the activity of antioxidant enzymes including SOD (West, 2000) and glutathione synthesis (Yoshida *et al.*, 1995). Glucose is preferentially used in the polyol pathway that consumes NADPH which is necessary for GSH regeneration by the glutathione reductase enzyme (Lee *et al.*, 1999). Hyperglycemia is therefore indirectly the cause of GSH depletion and these results in oxidative stress (Paolisso *et al.*, 1992). However the observation that patients have lowered antioxidant defense is almost as the observation of increased of oxidative damage (West, 2000). In this study, liver glutathione was significantly decreased in both diabetic and *M. alba* treated diabetic animals in comparison with control and *M. alba* groups. Table (3) showed that the activity of rat hepatic GST were significantly increased in diabetic and *M. alba* -treated diabetic animals in comparing with control group, and significantly decreased in *M. alba* treated diabetic animals comparing to with diabetic group. Therefore, these results suggest that *M. alba* have protective effects on antioxidant defenses improve glucose metabolism.

Our finding showed that STZ-induced diabetic rat's significantly deplete glycogen storage in the liver, promoting its conversion into lactate. Crover *et al.* (2000) reported that hepatic glycogen depletion after STZ induced diabetic rats. When blood glucose concentration increase, hexokinase begins to phosphorylate glucose

to glucose 6 phosphate for glycogen synthesis (Venkateswaran and Pari, 2003). In the present study, diabetic animals showed significantly decrease in liver glycogen concentrations and decreased in *M. alba* diabetic animal as compared to control and *M. alba* groups. No significant difference in Liver glycogen concentration was observed between *M. alba* and *M. alba* treated diabetic animal (Table 2). *M. alba* was able to increase glycogen storage in diabetic rats by 2 folds and prevented its break down to lactate, thus reducing the intracellular acidosis associated with anaerobic glycolysis, may be due to *M. alba* contain iminosugars 1-deoxynojirimycin, DNJ and DNJ, 1-deoxynojirimycin; GAL–DNJ, 2-O- α -D-galactopyranosyl–DNJ and fagomine (Nakagawa *et al.*, 2010). These having some β -glucosidase inhibitory activity (Asano *et al.*, 2001) or other unique effects (e.g., insulin secretion induced by fagomine (Taniguchi *et al.*, 1998).

Our results showed that Hexokinase, G6PD were significantly decreased in diabetic and *M. alba* -treated diabetic animals in comparing with control group, and significantly increased in *M. alba* treated diabetic animals comparing to with diabetic group (Table 3). The decrease of the activities of hexokinase and glucose 6 phosphate dehydrogenase in STZ-induced diabetic rats was consistent with other studies on glucose 6 phosphate dehydrogenase (Diaz-Flores *et al.*, 2006) and hexokinase (Latha and Pari, 2003).

Table 1: Mean \pm S.D of body weight and blood glucose concentrations for all experimental groups.

Groups (n=12)	Initial body weight (g)	Final body weight (g)	Blood glucose (mg %)	
			At the start ⁽¹⁾ of experiment	At the end of experiment
Control group I % Change	224 \pm 12.42	289 \pm 4.64 ^b ↑29%	149 \pm 21.4	156 \pm 14.2 ^b ↑4.7 %
Diabetic (STZ) group II % Change	238 \pm 16.73	213.8 \pm 5.06 ^a ↓10.2%	386 \pm 42	437 \pm 54.3 ^a ↑13.2 %
<i>M. alba</i> group III % Change	231 \pm 14.46	286 \pm 3.50 ^b ↑24%	154 \pm 34	163 \pm 28.4 ^b ↑5.84 %
<i>M.alba</i> +STZ group IV % Change	235 \pm 11.72	253 \pm 5.41 ^{bc} ↑7.7%	379 \pm 48	289 \pm 61.2 ^{bc} ↓23.75 %

¹Start at end of 48 hours after STZ-induced diabetes. $p < 0.05$ is considered significant, $p > 0.05$ is considered non significant (NS). ^a: $p < 0.05$ compared with control, ^b: $p < 0.05$ compared with diabetic rats, ^c: $p < 0.05$ compared with *M. alba* group.

Table 2: Mean \pm S.D Rat hepatic glycogen, lactate, MDA and glutathione levels in the different groups.

Parameters	Control group I	Diabetic group II	<i>M. alba</i> group III	<i>M. alba</i> +STZ group IV
Glycogen (mg/g tissue) % Change	29 \pm 3.4	10.4 \pm 2.1 ^{a*} ↓64.138 %	29 \pm 3.6 ^{a NS, b*} No change	21 \pm 3.8 ^{a*, b*, c NS} ↓27.586 %
Lactate (nmol/g tissue) % Change	3.9 \pm 0.46	8.3 \pm 0.78 ^{a*} ↑112.821 %	4.1 \pm 0.34 ^{a NS, b*} ↑5.128 %	5.8 \pm 0.62 ^{a*, b*, c*} ↑48.718 %
MDA (nmol/g tissue) % Change	984 \pm 92	1373 \pm 102 ^{a*} ↑39.533 %	965 \pm 84 ^{a NS, b*} ↓1.931 %	1054 \pm 113 ^{a NS, b*, c NS} ↑7.114 %
Glutathione (mg/g tissue) % Change	3.1 \pm 0.06	2.4 \pm 0.5 ^{a*} ↓20.072 %	3.2 \pm 0.6 ^{a NS, b*} ↑7.107 %	2.6 \pm 0.5 ^{a*, b NS, c*} ↓15.484 %

MDA: Malondialdehyde. % Change of groups compared with control group. * $p < 0.05$ is considered significant, $p > 0.05$ is considered non significant (NS). a: Groups compared with control group I, b: Groups compared with diabetic group II, c: Groups compared with group III.

Glucose 6 phosphatase (G6Pase), a key enzyme in gluconeogenesis or glycogenolysis, in which it catalyzes the hydrolysis of glucose 6 phosphate (G6P) to glucose and phosphate, plays an important role in glucose homeostasis in the liver. Glucose is transported out of the liver to increase blood glucose concentration. STZ increases the expression of G6Pase activity (Liu *et al.*, 1994; Massillon *et al.*, 1996). In the present study, administration of *Morus* enhanced the reversal of high G6Pase activity in diabetic rats (Table 3). The reduction in G6Pase can lead to a decrease in gluconeogenesis and blood glucose concentration (Dijk *et al.*, 2001).

This study showed that liver lactate concentration was significantly increased in diabetic and in *M. alba* -treated diabetic animals as compared to control and *M. alba* groups, respectively (Table 2). On the other hand, there was a significant decrease in liver tissue LDH in diabetic group, however, it was slightly elevation of LDH activity in diabetic rats treated with *M. alba* and re-elevated with significant p value < 0.05 (Table 3). Kim *et al.* (2006) used the activities of GOT, GPT and LDH in the circulation as indicators of hepatic damage due to diabetes. They found that treated groups with experimental plant extracts effectively reduced plasma GOT, GPT and LDH activities in diabetic rats, suggesting that the aqueous extracts of experimental plants may prevent hepatic injury associated with diabetes.

In conclusion, our results showed that *M. alba* markedly reduced hyperglycemia in STZ-induced diabetic rats by control oxidative stress and increasing hexokinase activity, glycogen synthesis to reduce lactate formation and G6PD in the liver. These finding suggested that *M. alba* extract is useful in the control of diabetes

mellitus. Appropriate *M. alba* therapy improve glucose metabolism, representing a fundamental property of this complementary medical approach.

Table 3: Mean \pm S.D Rat hepatic enzymes specific activities in the different groups.

Parameters	Control group	Diabetic group	<i>M. alba</i> group	<i>M. alba</i> +STZ group
GST (U/mg protein)	514.8 \pm 74.8	755.3 \pm 105.3 ^{a*}	578.2 \pm 100.2 ^{a NS, b*}	625.6 \pm 131.7 ^{a*, b*, c NS}
% Change		\uparrow 46.707 %	\uparrow 12.31 %	\uparrow 21.519 %
Hexokinase (U/ mg protein)	258 \pm 19.3	114 \pm 6.4 ^{a*}	254 \pm 20.1 ^{a NS, b*}	163 \pm 18.6 ^{a*, b*, c*}
% Change		\downarrow 55.8 %	\downarrow 1.55 %	\downarrow 36.8 %
G6Pase (U/mg protein)	192 \pm 17.5	247 \pm 14.6 ^{a*}	168 \pm 11.9 ^{a NS, b*}	204 \pm 18.3 ^{a NS, b NS, c NS}
% Change		\uparrow 28.65 %	\downarrow 12.5 %	\uparrow 6.25 %
G6PD (U/ mg protein)	4.97 \pm 0.2	2.53 \pm 0.18 ^{a*}	4.8 \pm 0.2 ^{a NS, b*}	3.78 \pm 0.2 ^{a*, b*, c*}
% Change		\downarrow 49.1 %	\downarrow 2.8 %	\downarrow 23.9%
LDH (U/ mg protein)	14.03 \pm 3.1	8.6 \pm 1.87 ^{a*}	12.4 \pm 3.2 ^{a NS, b*}	10.14 \pm 1.69 ^{a*, b*, c*}
% Change		\downarrow 38.5 %	\downarrow 11.75%	\downarrow 27.7 %

GST: glutathione s transferase, G6Pase: glucose 6 phosphatase, G6PD: glucose 6 phosphate dehydrogenase and LDH: Lactate dehydrogenase. % Change of groups compared with control group. * $p \leq 0.05$ is considered significant, $p > 0.05$ is considered nonsignificant (NS). a: Groups II, III, and IV compared with control group I, b: Groups III, and IV compared with diabetic group II.

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