Biosciences, Biotechnology Research Asia Vol. **5**(1), 139-144 (2008)

Hypoglycemic and metabolic activity of aqueous extract of *Morus alba* in streptozotocin-diabetic rats

MOSAAD A. M. ABOU-SEIF and EL-SAYED M. KAMEL*

Biochemistry and Pharmacology* Departments, Faculty of Medicine,
Seventh of October University, Misuratah (Libya)

(Received: January 12, 2008; Accepted: April 06, 2008)

ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia. Clinical research has confirmed the efficacy of several plant extracts in the amelioration of diabetic status. There for the possible hypoglycemic activity of the aqueous extract of morus alba was investigated in streptozotocin (STZ)- induced diabetic rats. A single dose of STZ(60 mg./kg. B.W) produced a decrease in insulin secretion , hyperglycemia .,decrease hepatic glycogen content, hepatic glucose oxidation, were as hepatic glucose 6- phosphatase (gluconeogenesis) activity was increase an aqueous extract of mours alba (200 mg./kg.) or glicalzide (100mg./kg) was administer orally once dally for two weeks to STZ - induced diabetic rats ameliorated hyperglycemia and restored the metabolic enzymes of glucose to the normal values in the liver of STZ - treated rats .In addition the administration of mours alba induce the secretion of insulin from rat pancreas. The effect of produced by morus alba extract were found to be comparable with that of glicalizde. The present results suggested that the morus alba extract could be used as ant diabetic adjuvant in treatment of DM. This may be related to its induction of insulin secretion.

Key words: *Morns alba*, streptozotocin, insulin, glucose, metabolic enzymes of glucose.

INTRODUCTION

Diabetes mellitus is probably the fastest growing metabolic disorder in the world and it is a major source of morbidity in developing countries. Once regarded as a single disease entity, diabetes mellitus is now regarded as a heterogeneous group of diseases characterized by a state of chronic hyperglycemia, which causes a number of secondary complications like cardiovascular, renal, neurological and ocular disorders[1]. Diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism[2].

Liver tissues are insulin dependent tissues, which play a pivotal role in glucose and lipid homeostasis and are severely affected during diabetes mellitus[3]. Decreased glycolysis, impeded glycogenesis ,increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver[4].

Many medicinal plants are considered useful means to prevent and/or ameliorate certain disorders, such as diabetes mellitus,atherosclerosis and other metabolic disorders[5]. Among these plant resources, the green leaves of morus alba are selected for the present study. The morus alba are cultivated in Libya. Therefore, this study was thus initiated with the aim of evaluating the possible hypoglycemic activity of an aqueous extract of the green leaves of morns alba in streptozotocin-induced diabetic rats.

MATERIAL AND METHODS

Preparation of plant extract

The green leaves of morus alba were left for drying in the shade and then reduced to a powder. A total of 200 g of the dried plant material was extracted with 0.5 liter of distilled water by the method of continuous hot extraction. The extract was evaporated to dryness in a wide container over

a boiling water bath in front of an air current. A semisolid residual extract was obtained (3.7 g). It was stored at 4 'C until used. When needed, the residual extract was dissolved in distilled water and used in the study [6]. Drugs and chemicals used Streptozotocin, thiobarbituric acid (TPA), phenazin methosulphate (PMS), nitroblue tetrazolium (NBT), NADH, NADPH and glucose- 6-phosphate were purchased from Sigma Chemical Company (St. Louis., MO, USA). Gliclazide (Servier Laboratories, France) was purchased from local pharmacies. All other chemicals were of high purity grade.

Experimental animals

All experiments were performed using adult male albino rats, with an average body weight of 150 to 180 g. purchased from animal house of faculty of medicine , seventh of October University, Misuratah, Libya. The rats were housed in steel mesh cage and provided with commercial standard diet and tap water ad libitum. Induction of experimental diabetes The rats were fasted for 12 hours before the induction of diabetes with STZ. The rats were injected intraperitoneally with freshly prepared solution of STZ (60 mg STZ/kg body weight; STZ was dissolved in 0.05 M sodium citrate buffer, pH 4.5)[14]. Seventy two hours after diabetes induction, blood samples were collected from the tail vein for measuring blood glucose levels by One-Touch blood glucose meter from Lifescan (Johnson & Johnson Company, USA).

Experimental procedure

Toxicity test of an aqueous extract of the Green leaves of morus alba: Fifty male rats were divided into five groups of 10 each and were administered orally with aliquot doses of the aqueous extracts of morus alba (200-1000 mg/kg). Mortality was observed after 72 hours[8].

Hypoglycemic action of morus alba

A total of 25 rats (15 STZ-diabetic surviving rats and 10 normal rats) were used in this experiment the rats were divided into 5 groups of 5 rats each as follows:

- Group 1 Normal control rats.
- Group 2 Normal rats treated with morus alba extract (200 mg/kg body weight) daily by an intragastric tube for two weeks.
- Group 3 STZ-diabetic control rats.
- Group 4 STZ-diabetic rats treated with gliclazide (100 mg/kg body weight) daily by an intragastric tube for two weeks.
- Group 5 STZ-diabetic rats treated with mours, alba extract (200 mg/kg body weight) an intragastric tube daily for two weeks;

At the end of the two weeks, the rats were deprived of food overnight and sacrificed by decapitation under ether anesthesia. Blood samples were collected and the livers were immediately dissected out, washed in ice-cold saline, blotted dry and weighed for measuring various biochemical parameters.

Preparation of homogenates

An accurately weighed piece of liver tissue was homogenized in ice-cold 0.9 % saline using a Teflon pestle connected to a homogenizer motor. The liver homogenate was diluted to yield a 5 %(W/V)liver homogenate the homogenate was centrifuged at 5000rpm for 30 minutes at 4 C to remove cell debris andnuclei. The resulting supernatant was used for biochemical analysis.

Biochemical analysis

Serum glucose concentrations were estimated by the method of Trinder9 using a commercial available diagnostic kit (Diamond Diagnostics, Egypt). Glycogen content in tissue homogenates was determined as described by the method of Damsbo *et al.,*[10]. Hepatic glucose-6-phosphate dehydrogenase activity was measured b,, applying the method of Chan *et al.,*[11]. Hepatic glucose-6- phosphatase activity was determined according to the method of' Rossetti *et al.,*121.

Statistical analysis

The results are expressed as means ± SE. The Statistical analysis was performed according to the method of Murray[13]. Data were analyzed using unpaired Student's t-test. P values of < 0.05 were considered to be statistically significant.

RESULTS

The acute oral toxicity of the green leaves of morus alba extract showed neither toxicity nor mortality up to 1000 mg/kg. Thus, the maximum tolerated dose of the extract was found to be 1000

mg/kg body weight. Also no toxic signs were observed over 72 hours of administering the extract. Table 1, demonstrates serum blood glucose levels in normal control, STZ-diabetic control, STZ-diabetic treated and normal-treated rat with the aqueous extract of morus alba. An extremely significant (P<0.00 l) increase in serum blood glucose levels was observed in STZ-diabetic control rats compared with that of normal-control rat group.

In contrast, an extremely significant (P<0.001) decrease in serum blood glucose levels was observed in either gliclazide (100 mg/kg) or morus alba alba extract (200 mg/kg) STZ-diabetic treated rat group as compared to STZ-diabetic control rat group. No significant changes were observed in serum blood glucose levels of normal-treated rats compared with the corresponding levels of normalcontrol rat group upon treatment with 200 mg/kg of morus alba extract (Table 1).

Table 1: Blood glucose levels in normal and STZ-diabetic rats treated with *Morus alba* extract for two weeks treatment

Groups	Blood Glucose(mg/dl)
Normal-control rats	81.70 ± 12.70
STZ diabetic control rats	371.26 ±24.**o22
STZ-diabetic rats treated witliuiciamle(I 00mg/kg)	152.26 ± 5.26"*
STZ-diahelic rats treated with *Morus alba* (200mg/kg)	158.25 ± 4.49***
Normal rats-treated with *Morus alba* (200mg/kg)	82.57 ± 3.03

The results are expressed as mean ± SD for five rats in each group.
Extremely significant (P < 0.001) compared to normal-control.
Extremely significant (P<0.001) compared to STZ-diabetic control rat groups.
NS: Not significant compared to normal-control rat group.

Table 2: Effect of two weeks treatment with mortis alba extract on hepatic glycogen content, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase activities in the liver of different experimental rat groups

Groups	Glycogen Content (g/100 g wet tissue)	Glucose-6-phosphatede hydrogenase (U/mg protein)	Glucose-6-phosphatase (1111ol Pi/lllill/ g wet tissue)
Normal-control rats	17.86 ± 0.75	132.93 ± 9.03	0.804 ± 0.08
ST/-diabetic control rats	5.04 ± 0.86***	43.02 ± 10.23*0*	1.192 ± 0.05"
STZ'-diabetic rats treated with glclazide(100mg/kg)	10.99 ± 0.72	133.42 ± 7.08	0.801± 0.04
STZ-diabetic treatedwith *Morus alba* ext. (200mg/kg)	23.07 ± 0.78	114.53 ± 6.62	0.924 ± 0.05***
Normal rats-treated with *M. alba* (200mg/kg)	17.69 ± 0.85 "'	132.48 ± 5.26 N, 'S	0.744 ± 0.05 NS

The results are expressed as mien ± SD for five rats in each group. ***- Extremely significant (P< 0.001) compared to normal-control. *** : Extremely significant (P<0.001) compared to STZ-diabetic control rat groups
NS: Not significant compared to normal-control rat group

Table 2, illustrates Hepatic glycogen content, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase activities of different experimental rat groups. There were an extremely significant (P<0.001) decrease in glycogen content and glucose-6-phosphate dehydrogenase activity, and an extremely significant (P<0.001) increase in glucose-6- phosphatase activity in the liver homogenates of STZ-diabetic control rats compared with that of normal-control rat group. In contrast, an extremely significant (P<0.00 I) increase in hepatic glycogen content and glucose-6-phosphate dehydrogenase activity, and an extremely significant (P<0.001) decrease in glucose-6-phosphatase activity were observed in either gliclazide (100 mg/kg) or mous alba extract (200 mg/kg) STZ-diabetic treated rat groups as compared to STZ-diabetic control rat group. No significant changes were observed in glycogen content and glucose- 6-phosphate dehydrogenase and glucose-6-phosphatase activities of normal-treated rats compared with the corresponding values of normalcontrol rat group upon treatment with 200 mg/kg of mortis alba extract (Table 2).

Table 3, illustrates the effect of morus alba extract on the insulin levels in various experimental rat groups. The results of Teble 3 showed that the insulin levels of normal control rat group, STZdiabetic rat group treated with morus alba extract (200 mg/kg) and STZ-diabetic rat group treated with gliclazide (100 mg/kg) were significantly higher than that of STZ-diabetic untreated rat group.

Table 3: Effect of ' two weeks treatment with tnol-iis alba extract on insulin levels in the serum of normal and STZ-diabetic rat

Rat group	Insulin level (ul/ ml)
Normal-control rats . R	2.3 + 0,03***
STZ-diabetic control rat	0.6+0.01
STZ-diabefic rats treated with mornsalba (200mg/kg)	1.9+0.01***
Normal rats-treated with morns alba(200ing/kg)	3.5 +0.03***

The results are expressed as mean + SE for five rats in each group. *** Extremely significant (P<0.001) compared to STZ-diabetic control rat.

DISCUSSION

Hypoglycemic effect of the aqueous extract of morus alba The discovery and development of new and more effective anti diabetics from plants is one of the main goals of present day and chemical research15. In the present study, a detailed account has been given to the hypoglycemic effect of the morus alba extract in STZ-induced diabetic rats. The expanded hypoglycemic effect of morus alba till two weeks may be attributed to its long efficacy on glucose uptake by the liver cells when compared to the effect of gliclazide (a known hypoglycemic drug).

In diabetes mellitus, the disorders in carbohydrate metabolism play a predominant role in diabetic complications16. This fact has been observed in the present work in which decreased the levels of hepatic glycogen content, glucose-6-phosphate dehydrogenase activity (glucose oxidation), and increased the activity of hepatic glucose-6-phosphatase (gluconeogenesis) when compared to normal control rat group. The hepatic glycogen contents were significantly lowered in STZ-control rat group when compared to the normal control rat group. The decrease in glycogen contents in the liver homogenates of STZ-diabetic untreated rats may be attributed to the depression of hlycogenesis pathway in the liver of STZ-diabetic untreated rats17.

In the present study, it was observed that the activity of G6PD was significantly decreased in

the liver homogenates of STZ-diabetic untreated rats when compared to the corresponding activity of G6PD in the liver homogenates of the normal control rat group. The deficiency of the hepatic G6PD activity (as shown in Table 2) in STZdiabetic ratsI may be attributed to the decrease in blood insulin levels which enhances the glucose uptake by the liver cells leading to stimulate glucose oxidation by pentose phosphate pathway (Glucose-6-phosphate dehydrogenase (G6PD) is the rate limiting enzyme of the pentose phosphate pathway). These findings are in consistence with the previous studies which reported that, G6PD activity was decreased in STZ-induced diabetic rats[18].

Furthermore, the deficiency of G6PD activity may participate in the building up of glucose and consequently increase the Susceptibility of type 2 diabetes mellitus as a result to the reduction in blood insulin levels[19]. Glucose-6-phosphatase (G6Pase), an enzyme located mainly i n the liver and catalyzes the terminal step in both gluconeogenesis and glycogenolysis. Thus, an increase in G6Pase activity (Table 2) in the liver homogenates of STZ-diabetic untreated rats cells may cause a marked increase in the rate of glucose formation and also decreases glucose usage20. Therefore, the elevated G6Pase activity may become a compensatory pathway for building up glucose to compensate the requirements of liver cells for energy from glucose. These findings are in accordance with that of Ashokkumar and Pari[21] who reported that, the activity of G6Pase was significantly increased, whereas the activity of G6PD was significantly decreased in acute or neonatal STZ-induced diabetic rats. The present results demonstrated that, the treatment with morus alba significantly ameliorated the adverse influence of streptozotocin, as: lowered blood glucose levels leading to increased hepatic glycogen contents, and a regulation in the metabolic enzymes of glucose utilization as compared to rats administered with streptozotocin alone. These results are consistent with those of other studies using different other hypoglycaemic plants[22-24]. The present study showed that the aqueous extract of morus alba stimulated the secretion of insulin from pancreatic α-cells in different rat groups. Therefore, the hypoglycemicaction of morus alba may be similar to the action of gliclazide.

CONCLUSION

From the results of the present study ,it could be concolouded that the improvement of glucose metabolism and its blood levels after morus alba treatment of STZ-injected rats might a treating influence of' morus alba against STZ action. In addition, Our study indicates that, the aqueous extract of the morus alba possesses an anti diabetic effect in the STZ - induced diabetic rats .The hypoglycemic activitv of morus alba may be attributed to either its regulation of metabolic enzymes of glucose utlization or enhance ment of insulin secretion. Further clinical investigations are required prior utilization mours alba as a safe oral ant diabetic agent.

REFERENCES

1. Thornally PJ, McLellan AC, Lo TW: Negative association between reduced glutathione concentration and diabetic complications. *Med Sci,* **91**: 575-580 (1996).
2. Arley RA: Clinical correlates of metabolic derangements of diabetes mellitus. In: Kozak GP (ed.), Complications of Diabetes mellitus, Saunders WB. Philadelphia, pp 16-20 (1982).
3. Seifter ,S ., England S.: Energy metabolism. In: The Liver; Biology and Pathology, Arias, I.; Papper, M. and Schacter, D. (eds.), New York, Reven Press, 219-249 (1982).
4. Baquer NZ: Glucose over utilization and under utilization in diabetes and effects of antichabetic compounds. *Ann Real Acad Farm* **64**: 147-80 (1998).
5. IDRC (International Development Research Centre). Environmental Management of Fuelwood Resources in Wadi Allaq' (Egypt), Final submitted File gypt), Final Report,

submitted to IDRC File 92-1001-01 (1995).

6. Montilla P, Barcos M, Munoz MC. Munoz-Castaneda JR, Bujalance I Tunez I: Protective effect of 'Montilla-.\1o!-*11e'-~ appellation red wine on oxidative stress induced by streptozotocin in the rat. *J Nutr Biochem* **15**(11): 688-693 (2004).
7. Litchfield JT, Wilcoxon FA: Simplified method of evaluating dose effect experiments. *J Pharmacol Exp Ther* **96**: 99-133 (1949).
8. Trinder P: Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* **6**: 24-27 (1969).
9. Damsbo P, Vaag A, Hother-Nielsen 0, Beck-Nielsen H: Reduced glycogen synthase activity in skeletal muscle from obese patients with and without type 2 (non-insulin dependent) diabetes mellitus. *Diabetologia* **34**(4): 239-245 (1991).
10. Chan TK, Todd D Wong CC: Tissue levels in erythrocyte glucose-6-phosphate dehydrogenase deficiency. *J Lab Clin Med* **6**: 936-940 (1965).
11. Rossetti L, Lee YT, Ruiz J. Aldridge S, Shamoon H, Boden G: Quantitation of glycolysis and skeletal muscle glycogen synthesis in humans. *Am J Physiol* **295**: 761-769 (1993).
12. Lowery OM, Rosenbrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin-phenol reagent. *J Biol Chem* **193**: 265-276 (1951).
13. Murray RS: Schaurn's Outline Series of Theory and Problems of Probability and Statistics. Singapore, McGraw-Hill Book Company, **8**: 265-298 (1982).
14. Tiwari AK, Madhusudana RJ: Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. *Current Science* **83**: 30-38 (2002).
15. Luzi L: Pancreas transplantation and diabetic complications. *New England J Med* **339**: 115-117 (1998.
16. Sridhar SB, Sheetal UD, Pai MRSM, Shastri MS: Preclinical evaluation of the antidiabetic effect of *Eugenia jambolana* seed powder in streptozotocin-diabetic rats. *Brazilian J Med Biol Res* **38**(3): 463-470 (2005).
17. Maiti R, Jana D, Das UK Ghosh D: Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin induced diabetic rats. *J Ethnopharmacol* **92**(1): 85-91 (2004).
18. Gastrin RS, Estwicl< D, Peddi R: G6PD deficiency: its role in the high prevalence of hypertension and *Diabetes mellitus. Ethn Dis,* **11**: 749-754 (2001).
19. Aiston S, Trinh KY, Lange AJ, Newgard CB, Agius L: Glucose-6- phosphatase overexpression lowers glucose 6-phosphate and inhibits glycogen synthesis and glycolysis in hepatocytes withoutaffecting glucokinase trans location. Evidence against feedback inhibition of glucokinase. *J Blot Chem* **274**: 24559-24566 (1999).
20. Ashokkumar N, Pari L: Effect of N-benzoyl-D-phenylalanine and metformin on carbohydrate metabolic enzymes in neonatal streptozotocin diabetic rats. *Clin Chim Acta* **351**(1-2): 105-113 (2005).
21. Ahmed I, Lakham MS, Gillett M, John A, Raza 1-1: Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic Momordica charantia (karela) fruit extract in streptozotocin-induced diabetic rats. *Diabetes Res. Clin. Pract.* **51**(3): 155-161 (2001).
22. Cho SY, Park JY, Park EM, Choi MS, Lee MK, Jeon SM, Jang MK, Kim MJ, Park YB: Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocininduced diabetic rats by supplementation of dandelion water extract. *Clin. Chim. Acta.* **317**(1-2): 109-117 (2002).
23. Prince PSM, Kamalakkannan N, Menon VP: Antidiabetic and anti hyperli pi daern ic effect of alcoholic Syzigium curnim seeds in alloxan induced diabetic"albino rats. *J Ethnopharmacol* **91**: 209-213 (2004).
24. Opara EC: Oxidative stress, micronutrients, *Diabetes mellitus* and its complications. *J R Soc Health,* **122**(1): 28-34 (2002).