



Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (5):18-21
(<http://scholarsresearchlibrary.com/archive.html>)



Anti-diabetic effect of aqueous leaf extract of *Morusnigra* on streptozotocin induced Diabetic rats

Benattouche Zouaoui¹, Benhalima Rabab² and Benharrat Assia²

¹Laboratory of Bioconversion, Microbiology Engineering and Alimentary Security, Department of Biology, Mascara University, Algeria

²Department of Biology, Faculty of Science, Mascara University, Algeria

ABSTRACT

The antidiabetic effect of aqueous leaves extracts of *Morusnigra* was studied in Streptozotocin-induced diabetic rats (50 mg/kg) with phytochemical composition. Rats were administered *Morusnigra* extract at dose rate of (150 mg/kg body weight per day for a period of 21 days). Blood glucose concentration was measured by AccuChek Glucometre test kit and compared with a patent drug glibenclamide at a dose rate of 100 mg/kg b.wt. The data were compared statistically by using analysis of variance (ANOVA). Preliminary phytochemical screening of the aqueous leaf extract of *M. nigra* revealed the presence of tannins, flavonoids, heterosides, terpenes and saponins. The herbal preparation of *Morusnigra* significantly ($P < 0.05$) decreased blood glucose when compared with patent drug. The present study clearly indicated anti-diabetic activity of *M. nigra* and supports the traditional usage of the herbal preparations for the therapy of diabetics.

Keywords: *Morusnigra*, glibenclamide, streptozotocin, diabetes, rats.

INTRODUCTION

Diabetes mellitus is going to become an epidemic disease all over the world. Diabetes is characterized by a loss of glucose homeostasis resulting in high blood glucose level, accompanied by an alteration of lipid parameters. The persistence of hyperglycemic state cause enhanced oxidative stress, leading to the development of atherosclerosis, coronary artery disease and other diabetic complications [1]. Nowadays, various medicinal plants are becoming very popular in the treatment of different diseases all over the world. The hypoglycemic activity of a number of plant products has been evaluated and confirmed in animal models [2].

Morusnigra L, belonging to the family Moraceae is a deciduous, fast growing, small to medium-sized tree up to 10-20 m tall. The leaves are generally 25 cm long. Several *Morus* spp have been reported to possess medicinal properties including antibacterial, astringent, hypoglycemic, anti atherosclerotic, ophthalmic and diuretic [3]. *Morus* spp are known to contain a variety of antidiabetic phytochemical constituents, such as flavonoids, tannins, triterpenoids, saponins, anthocyanins, anthroquinones, sterols and phenolic compounds [4].

Plants which had been shown to have hypoglycaemic action, act on blood glucose through several mechanisms. Some of them may inhibit endogenous glucose production [5] or interfere with gastrointestinal glucose absorption [6]; some may have insulin-like substances [7,8]; some may inhibit insulinase activity and some may increase secretion of insulin from β cells of the pancreas (pancreatotrophic action) [9,10,11], while others may proliferate β cells in pancreas by activating regeneration of these cells [12]. Very few traditional treatments for diabetes mellitus have been scientifically examined. The aim of this study was to evaluate the possible anti-diabetic action of *C. owariensis* and to examine the safety and efficacy of this plant in the management of diabetes mellitus.

MATERIALS AND METHODS

Collection and acclimatization of rats

Male Wistar albino rats aged 3 months, with average weight of 300 g were collected from the Animal House of the Mascara University, Algeria. Rats were grouped into five groups, each containing nine animals. Each group of rats was housed at serene bottomed wire cages arranged in rows. The animals were housed in standard environmental conditions of temperature ($25 \pm 5^\circ\text{C}$) and a 12 h light-dark cycle. The animals were fed with palletized feed and water. The rats were maintained in this condition for a period of three weeks to acclimatize them prior to experimental uses.

Collection and preparation of plants extract

Fresh leaves of *Morusnigra* were collected from garden of university in the month of May and June. The plant was botanically identified and authenticated by Doctor Zehafi El Bachir of the Laboratory of Ethno Botanical Research, agronomy Department of Mascara University, Algeria. The fresh leaves were lyophilized. The leaf powder (250 g) was then suspended in 500 ml of distilled water overnight and filtered to remove the residue.

Phytochemical screening of the extract

The phytochemical study for the presence of flavonoids, terpenoids, tannins, saponins and C-heterosids in the aqueous extract was done according to the method described by [13].

Experimental design

From the thirty six rats collected, twenty seven were rendered diabetic after single intraperitoneal injections of streptozotocin in a dose of 50 mg/kg [14] (in 0.1 M citrate buffer, pH 4) body weight. The rest (untreated rats) served as non-diabetic rats (control group). The diabetic and non diabetic rats were divided into five groups of rats and were treated as follows: Group 1, non diabetic rats (control group) non-treated; Group 2, non diabetic treated rats (toxicity control); Groups 3, diabetic rats (diabetic control) non-treated; Groups 4, diabetic rats were orally given the aqueous extract of *M.nigra* at doses of 150 mg/kg body weight, for 21 days; Group 5, diabetic rats were given single oral dose of anti-diabetic drug glibenclamide as a reference drug at 100 mg/kg body weight for 21 days. Rats induced with streptozotocin were administered 5% glucose solution during the first 24 h [15]. Blood glucose levels in STZ treated mice were measured after 48 h. Values of 250 mg/dl or above were considered diabetic for the study.

The administration (p.o) was started from the same day, except normal control and diabetic control groups for a period of 21 days. During this period, animals in all groups had free access to standard diet and water. The blood glucose was measured at 0, 3, 14, and 21 days.

RESULTS

Phytochemical composition

The phytochemical analysis of the aqueous leaf extract of *Morusnigra* showed that it contains in high concentration, phytochemicals like saponins, tannins, terpenoids and flavonoids but C-heterosids were found in moderately high concentration (Table 1).

Antihyperglycemic activity

There was a significant decrease ($p < 0.005$) in the levels of FBG in diabetic rats treated with aqueous extract of *Morusnigra* leaves 150 mg/kg from 21 days, diabetic control rats showed marked hyperglycemia throughout the experimental period (Table 2). The diabetic rats fed with the extract and glibenclamide exhibited remarkable glycemic control as evident by significant decrease ($p < 0.005$) in FBG levels.

Toxicity studies

Toxicity studies revealed that administration of the doses of *Morusnigra* aqueous leaf extract (150 mg/kg) produced no adverse effect on the general behavior. All the rats survived during the experimental period.

DISCUSSION

Phytochemical analysis of the aqueous extract of *M. nigra* leaves extract revealed the presence of *flavonoids*, *saponins*, *tannins*, *terpenoids*, *C-heterosids* as shown in Table 1. Secondary plant metabolites like polysaccharides, *coumarins*, *flavonoids*, *terpenoids*, *arginine* and glutamic acid are known to have hypoglycaemic effects in various experimental animal models [16, 17, 18, 19]. Tannin containing drugs have also been shown to demonstrate anti-diabetic activity [20, 21]. Effect of the flavonoids on pancreatic β cells leading to their proliferation and secretion of more insulin have been proposed by [22, 23] as the mechanism by which they reduced *hyperglycaemia* in diabetic

rats. STZ-induced diabetic animals represent a good experimental diabetic state with residual or remnant insulin production by the β cells. The diabetic state in these animals is, therefore, not the same as that obtained by total pancreatectomy, as daily administration of insulin is not required for survival of STZ-induced diabetic rats. Further, the STZ-induced diabetic animals may exhibit most of the diabetic complication. The presence of *flavonoids* in the crude extract of *M. nigra* may also be acting similarly, thereby decreasing the high blood glucose levels of diabetic rats. The observed reduced activity of the treated mice in the initial period of extract administration showed that the extract possessed depressing effect. After 21 days administration of *M. nigra* leaf extract, blood glucose concentration decreased significantly ($P < 0.05$) in comparison with day zero. Glibenclamide also decreased blood glucose concentration. After 21 days of administration of glibenclamide, the blood glucose concentration reduced significantly ($P < 0.05$) which agreed with the report of [24]. Signs of regeneration of β cells, potentiating insulin secretion from surviving β cells of the islets of Langerhans and decrease of blood glucose concentration have been reported following consumption of some plant extracts [12,11]. *M. nigra* leaf may have some chemical components that exerts regenerative effects on β cells, stimulate these cells to produce more insulin (pancreatotropic action) or may have some insulin-like substances, and induction of regenerative stimulus in diabetic state triggers pancreatic regenerative processes, thereby restoring functional activities of the pancreases [25,19]. It has been reported that flavonoids and tannins present in plants extracts possess antidiabetic activity [19,21]. In the present study, the observed anti-diabetic potential of our test extract may be due to the presence of similar phytochemical constituents which was evident by our preliminary phytochemical screening.

Anti-diabetic effect of aqueous leaf extract of *Morus nigra* on streptozotocin induced Diabetic rats

Table 1. The phytochemical composition of the aqueous leaf extracts of *Morus nigra*

Phytochemical	Aqueous leaf extract
C-heterosids	++
Flavonoids	+++
Tannins	+++
Saponins	+++
Terpenoids	+++

+++ = Present in high amount, ++ = present in moderately high amount, + = present in trace amount, - = absent.

Table 2. Effect of aqueous leaf extract and glibenclamide tablet on blood glucose in normal and streptozotocin treated diabetic rats

Groups	Blood glucose concentration (g/dl)			
	Day 0	Day 3	Day 14	Day 21
1 Normal control non treated	159.00 $\pm$ 0.52	136.00 $\pm$ 0.56	173.00 $\pm$ 0.09	160.00 $\pm$ 0.28
2 Normal treated	159.00 $\pm$ 1.41	159.00 $\pm$ 0.55	100.00 $\pm$ 0.51*	125.00 $\pm$ 0.36
3 Diabetic control non treated	356.00 $\pm$ 2.82	335.00 $\pm$ 0.14	340.00 $\pm$ 0.08	342.00 $\pm$ 0.08
4 Diabetic treated (a. l. extract)	369.50 $\pm$ 6.03	369.00 $\pm$ 0.11	206.00 $\pm$ 0.13	175.00 $\pm$ 0.44
5 Diabetic treated (Glibenclamid)	352.00 $\pm$ 7.00	262.14 $\pm$ 2.42	207.50 $\pm$ 9.19*	178.6 $\pm$ 24.2 *

PO = Per Os (orally), values are mean $\pm$ SD for each group of five rats. *Means significantly different at $P < 0.05$.

CONCLUSION

From this study, we can state that the aqueous extract of *Morus nigra* has beneficial effects on blood glucose concentration. Further pharmacological investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

Acknowledgements

We are grateful to the technical staff in the department of biology, faculty of science, Mascara university, Algeria.

REFERENCES

- [1] Hunt J V, Smith C C & Wolff S P. *Diabetes*. (1988) 39: 1420-1424.
- [2] Ruzaidi A, Amin I, Nawalyah A G, Hamid M & Faizul H A. *J Ethnopharmacol*. (2005) 64: 211-217.
- [3] Bown D. *Encyclopaedia of herbs and their Uses*. Dorling Kinderley, London. (1995).
- [4] Mukherjee PK, Maiti K, Mukherjee K & Houghton P J. *J Ethnopharmacol*. (2006) 106: 1-28.
- [5] Eddouks M, Jouad H, Maghrani M, Lemhadri A, Burcelin R. *Int. J. Phytother. Phytopharmacol*. (2003) 10(6-7): 594-599.
- [6] Musabayane CT, Bwititi PT, Ojewole, JAO. *Method Find Exp. Clin. Pharmacol*. (2006) 28(4): 223-228.
- [7] Collier E, Watkinson A, Cleland CF, Roth J. *J. Biol. Chem*. (1987) 262: 6238-6241.

-
- [8] Gray AM, Flatt PR. *Br. J. Nutr.* (1999) 81: 203-208.
- [9] Khan A, Bryden NA, Polasky MN, Anderson RA. *Biol. Trace Elem. Res.* (1990) 24: 183-188
- [10] Trivedi NA, Mazumder B, Bhatt JD, Hemavathi KG. *Indian J. Pharmacol.* (2004) 36: 373-376.
- [11] Yadav JP, Saini S, Kalia AN, Dangi AS. *Indian J. Pharmacol.* (2008) 40(1): 23-27.
- [12] Shanmugasundaram ER, Gopith KI, Radha SK, Rajendram VM. *J. Ethnopharm.* (1990) 30: 265-269.
- [13] Harbone JB. *Phytochemical methods. A guide to modern techniques of plant analysis.* 2nd edition. Chapman and Hall, London. (1984) 4,26: 140-149.
- [14] Borges G.R., Oliveira M., Salgado H.C. and Fazan R. *Cardiovascular Diabetology* (2006) 5: 26.
- [15] Sathishesekar D, Subramanian S. *Asia Pac J Clin Nutr.* (2005) 14,2: 153-158.
- [16] Akah PA, Okafor CL. *Phytother. Res.* (1992) 6: 171-173.
- [17] Marles RJ, Farnsworth NR. *Phytomed.* (1995) 2: 137-189.
- [18] Ross I A. *Medicinal Plants of the World – Chemical Constituents, Traditional and Modern Medicinal Uses.* Humana Press Inc. New Jersey, USA. (2001) pp. 30-45.
- [19] Ojewole JAO (2002). *J. Ethnopharmacol.* (2002) 81: 231-237.
- [20] Iwu MM (1980). *Planta Medica*, (1980) 39: p. 247.
- [21] Iwu MM. *Fitoterapia*, (1983) 54: 243-248.
- [22] Mahesh T, Menon PV. *Phytother. Res.* (2004) 18: 123-127.
- [23] Sri Balasubashini M, Rukkumani R, Viswanathan P, Menon PV. *Phytother. Res.* (2004) 18: 310-314.
- [24] Sharma VK, Kumar S, Patel HJ, Hugar S. *Int. J. Pharm. Sci. Res.* (2010) 1(2): 18-22.
- [25] Adewole SO, Ojewole JAO. *Clin. Pharmacol.* (2007) 29(7): 447-455.