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Research Paper

Antidiabetogenic action of *Morus rubra* L. leaf extract in streptozotocin-induced diabetic rats

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Abstract

Objectives Researchers all over the world are exploring herbal supplements to control diabetes and its complications. This study evaluated the antidiabetic action of *Morus rubra* L. aqueous leaf extract through its effect on hyperglycaemia, dyslipidaemia and oxidative stress in streptozotocin-induced diabetic rats.

Methods The extract was orally administered to diabetic rats (100, 200 and 400 mg/kg body weight) daily for 21 days. Fasting blood glucose was measured on days 0, 7, 14 and 21. At the end of the experiment, blood samples were drawn to measure glucose tolerance, glycosylated haemoglobin, insulin, C-peptide and lipid parameters. Antioxidant enzymes (superoxide dismutase and catalase), reduced glutathione and lipid peroxides were determined in blood and liver tissue. Histopathological examination of pancreatic tissue was also performed.

Key findings The extract showed a dose-dependent fall in fasting blood glucose. Treatment with 400 mg/kg extract produced a significant reduction in glycosylated haemoglobin with a concomitant elevation in plasma insulin and C-peptide levels. The altered serum lipids in diabetic rats were significantly restored following treatment with the extract. In erythrocytes, as well as liver, the activity of antioxidant enzymes and content of reduced glutathione were found to be significantly enhanced, while levels of serum and hepatic lipid peroxides were suppressed in extract-fed diabetic rats. Histopathological examination of pancreatic tissue revealed an increased number of islets and β-cells in extract-treated diabetic rats.

Conclusions *M. rubra* aqueous leaf extract leads to control over hyperglycaemia and dyslipidaemia. The study also demonstrates its antioxidant nature, and hence it may be protective against diabetic complications.

Keywords diabetes mellitus; dyslipidaemia; hyperglycaemia; *Morus rubra*; oxidative stress

Introduction

Diabetes is an epidemic disease and the number of diabetic patients is increasing year by year. Worldwide, a 122% rise is projected from a total of 135 million in 1995 to 300 million in 2025.[1] Diabetes is characterized by a loss of glucose homoeostasis resulting in a high blood glucose level, accompanied by an alteration in lipid parameters.[2] The persistence of the hyperglycaemic state causes enhanced oxidative stress leading to the development of atherosclerosis, cardiovascular disease and other diabetic complications.[3,4]

Despite the presence of established antidiabetic medicines in the pharmaceutical market, there is growing interest in herbal remedies throughout the world as they have few or no side effects. Traditional medicinal plants having antidiabetic properties can provide a useful source for the development of safer and effective oral hypoglycaemic agents as pharmaceutical entities or simple dietary adjuncts to existing therapies. Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycaemic activity.[5–7] The hypoglycaemic activity of a number of plants and plant products has been evaluated and confirmed in animal models[8–10] as well as in human beings.[11–13] In India, several indigenous plant products have been used by

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practioners of the Ayurvedic system to treat diabetes.[14] This study places emphasis on the Indian medicinal plant *Morus rubra*.

Morus rubra L., or red mulberry, belongs to the family Moraceae. It is a deciduous, fast growing, small-to-medium sized tree. Several *Morus* species have been reported to possess medicinal properties, including antibacterial, astringent, hypoglycaemic, anti-atherosclerotic, ophthalmic and diuretic effects.[15] *Morus* species are known to contain a variety of antidiabetic phytochemical constituents such as flavonoids, alkaloids, triterpenes, saponins, sterols, glycosides and phenolics.[16,17] Leaves from several mulberry varieties are reported to contain a very high concentration of sugar-mimic glycosidase inhibitors known to have antidiabetic properties, such as 1,4-dideoxy-1,4-imino-D-arabinitol, 1-deoxynojirimycin and 1,4-dideoxy-1,4-imino-D-ribitol.[18,19] Rutin and quercetin are the other main components of mulberry leaves.[20] Four new flavonoid compounds, namely rubraflavones A, B, C and D, have been isolated from *M. rubra*.[21] The presence of these compounds shows the potential of the *Morus* species in the treatment of diabetes. The antidiabetic activity of leaves of *M. alba* and *M. indica* is evident from literature.[22–24] This study was designed to evaluate the antidiabetic potential of *M. rubra* leaves. We have investigated the effect of an aqueous extract of *M. rubra* leaves on hyperglycaemia, dyslipidaemia and oxidative stress in streptozotocin (STZ)-induced diabetic rats. Moreover, the cytotoxic effect of the plant extract was also determined.

Materials and Methods

Experimental animals

Male Wistar albino rats, 160–200 g, were procured from the Central Animal House of University College of Medical Sciences (UCMS), Delhi, India. The rats were housed in standard conditions of temperature (22 ± 2°C) and with a 12-h light–dark cycle. The rats were fed with commercial diet (Hindustan Lever Ltd., Mumbai, India) and allowed free access to water. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of UCMS, Delhi, India. All experimental procedures were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain.[25]

Experimental induction of diabetes

A freshly prepared solution of STZ (45 mg/kg in 0.1 M citrate buffer, pH 4.5) was injected intraperitonially to overnight-fasted rats.[26] The rats exhibited hyperglycaemia within 48 h of STZ administration. The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.

Preparation of the extract

The leaves of *M. rubra* were dried in the shade and ground in an electric grinder to a moderately coarse powder. The leaf powder (250 g) was suspended in 500 ml of cold distilled water overnight and filtered through muslin cloth to remove the residue. The dark brown filtrate thus obtained was lyophilized in a lyophilizer to achieve a powdered form.

Analysis of high-performance liquid chromatography fingerprint

High-performance liquid chromatography (HPLC) was performed using a Shimadzu system (SPD-M20A) consisting of a binary HPLC pump and UV detector. The HPLC profile was obtained using a C18 column (250 × 4.60 mm, 5 μm). The flow rate was set at 1 ml/min and 50 μl of the sample (1 mg/ml in milli Q water) was injected on to the column. Phosphate buffer (20 mM, pH 7.2) was used as a mobile phase. The chromatogram was captured at λ = 240 nm.

Experimental design

The experiment was carried out in five groups of five rats each: group 1, normal control; group 2, diabetic control; group 3, diabetic treated with extract (100 mg/kg); group 4, diabetic treated with extract (200 mg/kg); group 5, diabetic treated with extract (400 mg/kg); group 6, diabetic treated with glibenclamide (600 μg/kg).

Control rats (groups 1 and 2) received vehicle (distilled water). Groups 3, 4 and 5 received the extract dissolved in 1 ml of distilled water and group 6 received the standard antidiabetic drug glibenclamide suspended in 1 ml of distilled water. The treatment was given daily for a period of 21 days using a standard orogastric cannula. FBG was measured at 0, 7, 14 and 21 days. Initial and final body weight was recorded. All other parameters were determined at the end of the experiment. To perform the glucose tolerance test, glucose (2 g/kg in 1ml of distilled water) was administered and blood was withdrawn after 1 h of glucose load to determine peak blood glucose.

Sample collection

Blood was drawn from the retro-orbital plexus of overnight-fasted rats by using a micro-capillary technique.[27] Blood samples were collected in anticoagulant (sodium fluoride and potassium oxalate) vials to estimate FBG and peak blood glucose after glucose load in plasma. The whole blood in EDTA was used to perform glycosylated haemoglobin (GHb), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) and separated plasma was used to assay insulin and C-peptide. The blood, collected in plain vials, was allowed to clot for separation of serum. Serum was used to estimate lipid profile parameters and malondialdehyde (MDA). To obtain clear plasma and serum, blood samples were centrifuged at 4000 rev/min for 10 min.

After blood withdrawl, the rats were decapitated. The liver and pancreas were excised immediately. Liver was rinsed in ice-chilled normal saline and stored at −70°C for MDA, CAT and GSH estimation. Pancreatic tissue was preserved in 10% neutral formalin and processed for paraffin embedding, sectioned at 5 μm and stained with haematoxylin–eosin for microscopic observation of histopathological changes.

Analytical methods

Blood glucose was determined by the glucose oxidase–peroxidase method of Barham and Trinder.[28] GHb was assayed by the method of Goldstein *et al*.[29] Insulin and C-peptide levels were estimated by enzyme-linked immunosorbent assay (ELISA) using commercially available kits from Mercodia

(Uppsala, Sweden) and DRG (Marburg, Germany), respectively. Triglycerides (TG) were measured by the method of Fossati and Prencipe.[30] Total cholesterol (TC) was assayed using the method of Allain *et al*.[31] High-density lipoprotein (HDL) was determined by the method of Burstein *et al*.[32] Low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were calculated by using the formula of Friedewald *et al*.[33] Lipid peroxides were measured in serum as MDA by using the standard method of Satoh.[34] MDA was estimated by reaction with thiobarbituric acid in the presence of *n*-butanol and measuring the absorbance spectrophotometrically, at 530 nm, of the pink-coloured chromogen formed. The activity of SOD in erythrocytes was determined according to the method of Marklund and Marklund[35] with some modifications as described by Nandi and Chatterjee.[36] This method is based on the inhibition of pyrogallol autoxidation in the presence of SOD. CAT in red blood cells was assayed, using the method of Sinha,[37] by the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide. The chromic acetate thus formed was measured at 570 nm. GSH content in erythrocytes was assayed by the method of Beutler *et al*.[38] This method is based on the development of a yellow colour when 5,5′-dithiobis (2-nitro benzoic acid) is added to sulfhydryl compounds.

MDA in liver tissue was determined according to the method of Ohkawa *et al*.[39] Hepatic GSH was estimated using the method described by Ellman.[40] CAT activity in liver was assayed by the method of Aebi.[41]

Acute toxicity study

To evaluate the toxicity of high exposure to the aqueous leaf extract of *M. rubra*, three groups of fasted healthy rats (five per group) were orally administered the extract at a dose of 2, 4 and 6 g/kg (5, 10 and 15 times the effective dose) and one group, taken as control, was given distilled water. The rats were observed for 1 h continuously and then hourly for 4 h and finally after every 24 h up to 14 days for any physical signs of toxicity, such as writhing, gasping, palpitation and decreased respiratory rate or mortality. Liver function tests, such as serum glutamate phosphotransferase (SGPT) and alkaline phosphatase (ALP), as well as kidney function tests, such as urea and creatinine levels, were performed in serum at the end of the study using standard methods.

Statistical analysis

Values are expressed as mean ± SEM for five rats in each group. The data was analysed by using repeated-measure analysis of variance followed by Dunnett's multiple comparison test and one-way analysis of variance followed by Tukey's multiple comparison test. The results were considered significant at $P < 0.05$.

Results

HPLC fingerprint profile

Figure 1 shows the HPLC fingerprint profile of *M. rubra* aqueous leaf extract.

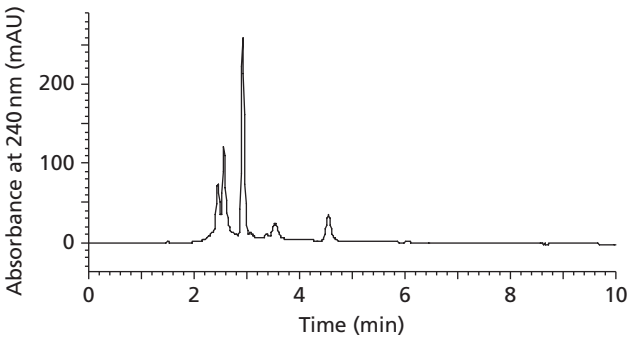

Figure 1 HPLC fingerprint profile of *Morus rubra* aqueous leaf extract

Glycaemic control

Table 1 shows the changes in the levels of FBG of normal and diabetic rats at various time intervals. There was a significant decrease ($P < 0.001$) in the levels of FBG in diabetic rats treated with aqueous extract of *M. rubra* leaves (100, 200 and 400 mg/kg) and glibenclamide from day 7 to day 21, whereas diabetic control rats showed marked hyperglycaemia throughout the experimental period. As the extract produced a dose-dependent fall in FBG, further studies were carried out with the dose of 400 mg/kg.

Table 2 depicts the effect of *M. rubra* leaf extract on oral glucose tolerance test, GHb, insulin, C-peptide and body weight of diabetic rats. Diabetic control rats showed significant elevation ($P < 0.01$) in the level of GHb as compared with normal rats. The plasma insulin and C-peptide levels were also significantly decreased ($P < 0.001$) in diabetic control rats compared with normal. The diabetic rats fed with the extract exhibited remarkable glycaemic control as demonstrated by the significant decrease ($P < 0.001$) in GHb and increase ($P < 0.001$) in the levels of insulin and C-peptide. Glibenclamide-treated diabetic rats also showed significant reduction ($P < 0.01$) in GHb with a concomitant elevation ($P < 0.001$) in insulin and C-peptide levels.

As shown in Table 2, the extract- and glibenclamide-fed diabetic rats exhibited significant gain ($P < 0.001$) in body weight, whereas diabetic control rats continued to lose weight.

β-Cell protective effect

Pancreatic sections from STZ-induced diabetic control rats (Figure 2a) showed extensive reduction in the number of islets, with a reduced number of β-cells as compared with sections from normal rats (Figure 2b). The islets were seen to be atrophic. However, sections from extract-treated diabetic rats (Figure 2d) revealed an increase in the number of islets and β-cells. An increased number of islets was also observed in the pancreatic sections taken from diabetic rats supplemented with glibenclamide (Figure 2c).

Lipid-lowering effect

Table 3 shows the effect of *M. rubra* leaf extract on the serum lipid profile of diabetic rats. Diabetic control rats showed a significant increase ($P < 0.001$) in the levels of TG, TC and LDL with a concomitant decrease ($P < 0.001$) in

Table 1 Effect of different doses of *Morus rubra* aqueous leaf extract on fasting blood glucose in rats at various time intervals

Group	Dose (per kg)	Fasting blood glucose (mg/dl)			
		Day 0	Day 7	Day 14	Day 21
Normal control	n.a.	81.6 ± 2.6	80.8 ± 1.3	83.4 ± 2.0	78.4 ± 1.8
Diabetic control	n.a.	271.4 ± 12.4	263.4 ± 11.6*	280.0 ± 9.0*	282.8 ± 14.0*
Diabetic + glibenclamide	600 μg	274.8 ± 13.7	217.6 ± 10.1*	194.4 ± 6.4*	169.4 ± 9.6*
Diabetic + *Morus rubra*	100 mg	282.6 ± 12.9	256.8 ± 10.9*	230.4 ± 8.3*	198.6 ± 7.5*
Diabetic + *Morus rubra*	200 mg	285.8 ± 16.1	244.2 ± 13.6*	213.2 ± 14.2*	173.4 ± 16.5*
Diabetic + *Morus rubra*	400 mg	273.6 ± 16.5	222.6 ± 12.8*	189.2 ± 12.7*	154.0 ± 12.5*

n.a., not applicable. Data are expressed as mean ± SEM of five rats in each group and evaluated by repeated-measure analysis of variance followed by Dunnett's test. *$P < 0.001$ compared with day 0.

Table 2 Glycaemic control by *Morus rubra* aqueous leaf extract (400 mg/kg) and its effect on body weight in diabetic rats after 21 days of treatment

Group	GTT (mg/dl)	GHb (%)	Insulin (μU/ml)	C-peptide (pmol/ml)	Body weight (g)	
					Initial	Final
Normal control	114.8 ± 2.6	3.01 ± 1.3	10.30 ± 0.56	0.170 ± 0.004	180.0 ± 5.7	197.0 ± 5.1
Diabetic control	417.2 ± 12.4+	7.36 ± 11.6+	3.12 ± 0.24+	0.039 ± 0.002+	165.0 ± 4.7	146.0 ± 3.7+
Diabetic + glibenclamide	235.0 ± 13.7**	5.62 ± 10.*	7.50 ± 0.29**	0.117 ± 0.007**	164.0 ± 3.3	178.0 ± 4.9**
Diabetic + *Morus rubra*	205.6 ± 12.9**	4.93 ± 10.9**	7.74 ± 0.36**	0.124 ± 0.007**	168.0 ± 4.6	183.0 ± 3.4**

GTT, glucose tolerance test; GHb, glycosylated haemoglobin. Data are expressed as mean ± SEM of five rats in each group and evaluated by one-way analysis of variance followed by Tukey's test. Data on body weight was analysed by repeated-measure analysis of variance followed by Dunnett's test. +$P < 0.001$ compared with normal rats; *$P < 0.01$, **$P < 0.001$ compared with diabetic control rats.

HDL compared with the normal group. These changes were significantly reversed in extract-treated diabetic rats. The atherogenic index, TG/HDL, was significantly elevated ($P < 0.001$) in diabetic control rats compared with normal. Following administration of the extract to the diabetic rats, this ratio was significantly decreased ($P < 0.001$). Glibenclamide-fed diabetic rats also showed a significant reduction in the levels of TG, TC, LDL and TG/HDL. However, an increase in HDL was not found to be statistically significant following glibenclamide treatment.

Antioxidant activity

Table 4 demonstrates the effect of *M. rubra* leaf extract on the antioxidant parameters in the blood and liver of diabetic rats. A significant elevation ($P < 0.001$) in the level of serum MDA was observed in diabetic control rats as compared with normal. Administration of the extract and glibenclamide significantly decreased ($P < 0.001$) serum MDA level in diabetic rats. The activity of erythrocyte antioxidant enzymes, SOD and CAT, in diabetic control rats was found to be significantly lower ($P < 0.001$) than in the normal group. The protective effect of *M. rubra* leaf extract on the antioxidant defence system was indicated by the significant increase ($P < 0.001$) in the activity of SOD and CAT in erythrocytes of extract-fed diabetic rats. The effect of glibenclamide on the antioxidant enzymes was less than that of the extract. Diabetic control rats showed significantly lower ($P < 0.001$) levels of erythrocyte GSH than normal rats. However, GSH was found to be significantly elevated ($P < 0.01$) in erythrocytes of extract-treated diabetic rats. The increase in GSH following glibenclamide treatment was not found to be statistically significant.

As shown in Table 4, a significant rise in MDA, with a concomittant depletion in the activity of CAT and the GSH level, was also noticed in hepatic tissue of diabetic control rats compared with normal rats. However, diabetic rats given the extract showed significant improvement in these parameters. Glibenclamide also produced significant reduction in MDA and elevation in GSH content in the liver of diabetic rats, while the activity of hepatic CAT was not significantly increased.

Acute toxicity study

Toxicity studies revealed that the administration of graded doses of *M. rubra* aqueous leaf extract (up to a dosage of 6 g/kg) produced no adverse effect on the general behaviour or appearance of the rats and all the rats survived during the whole experimental period. As presented in Table 5, no significant change was observed in the levels of SGPT and ALP in the treated groups when compared with the control group. The levels of urea and creatinine were also not significantly changed in treated rats compared with controls (Table 5).

Discussion

The major classes of oral hypoglycaemic agents currently available for the treatment of diabetes include sulphonylureas,

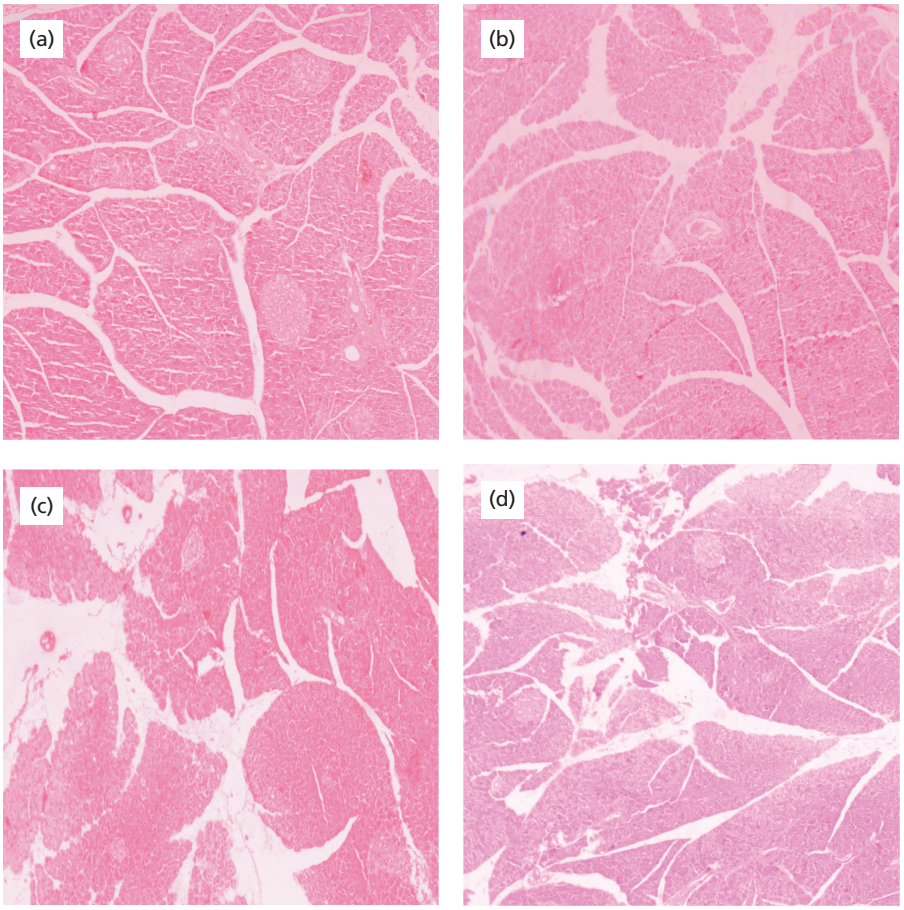

Figure 2 Pancreatic histopathological findings of diabetic rats treated with aqueous leaf extract of *Morus rubra* (400 mg/kg) for 21 days. (a) Normal control rats (haematoxylin and eosin (H-E) stain, original magnification 40 ×), (b) Diabetic control rats (H-E stain, original magnification 40 ×), (c) Diabetic rats treated with glibenclamide (H-E stain, original magnification 40 ×), (d) Diabetic rats treated with *M. rubra* aqueous leaf extract (H-E stain, original magnification 40 ×).

Table 3 Effect of *Morus rubra* aqueous leaf extract (400 mg/kg) on serum lipid profile of diabetic rats after 21 days of treatment

Group	Serum lipid profile (mg/dl)				
	TG	TC	HDL	LDL	TG/HDL
Normal control	72.2 ± 2.6	65.1 ± 2.3	35.4 ± 1.0	15.3 ± 1.5	2.05 ± 0.05
Diabetic control	137.4 ± 8.5^{+}	108.0 ± 3.9^{+}	23.2 ± 1.6^{+}	57.3 ± 2.1^{+}	5.98 ± 0.35^{+}
Diabetic + glibenclamide	97.4 ± 4.3**	89.4 ± 2.9*	26.0 ± 1.3	43.9 ± 2.9*	3.77 ± 0.19**
Diabetic + *Morus rubra*	86.8 ± 5.0**	88.6 ± 4.0*	30.4 ± 1.6*	40.8 ± 2.4**	2.87 ± 0.18**

TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are expressed as mean ± SEM of five rats in each group and evaluated by one-way analysis of variance followed by Tukey's test. $^{+}P < 0.001$ compared with normal rats; $^{*}P < 0.01$, $^{**}P < 0.001$ compared with diabetic control rats.

biguanides, thiazolidinediones, α-glucosidase inhibitors and so on. Glibenclamide, a sulphonylurea, was used as a reference antidiabetic drug in this study. It has been proposed that sulphonylureas produce their hypoglycaemic effect through increased release of insulin from pancreatic β-cells and enhancement of insulin's action on target tissues.[42] Thus, any plant secondary metabolite or chemical constituent that is capable of affecting the pancreatic β-cells or insulin's action will be a good mimic of sulphonylureas. Due to the minimal, or lack of, side effects of plant based drugs, these can be better candidates for the treatment of diabetes mellitus.

Streptozotocin, a beta cytotoxin, is a commonly used compound for the induction of experimental diabetes.[43] It acts through rapid depletion of pancreatic β-cells, which leads to reduction in insulin secretion. STZ-induced diabetic rats represent a good experimental diabetic state with

Table 4 Effect of *Morus rubra* aqueous leaf extract (400 mg/kg) on oxidative stress in diabetic rats after 21 days of treatment

Group	Blood				Liver		
	MDA (nmol/ml)	SOD (U/g Hb)	CAT (U/g Hb)	GSH (mg/dl)	MDA (nmol/100 g tissue)	CAT (U/g tissue)	GSH (μmol/g tissue)
Normal control	1.59 ± 0.05	2416 ± 57.6	3.34 ± 0.14	38.3 ± 1.44	0.96 ± 0.08	155 ± 8.7	9.46 ± 0.63
Diabetic control	3.62 ± 0.17^{+}	1645 ± 48.3^{+}	1.73 ± 0.17^{+}	26.9 ± 1.16^{+}	2.08 ± 0.10^{+}	103 ± 2.9^{+}	4.87 ± 0.31^{+}
Diabetic + glibenclamide	2.75 ± 0.11***	1950 ± 48.9**	2.44 ± 0.16*	31.6 ± 1.01	1.65 ± 0.08**	121 ± 7.7	6.69 ± 0.26*
Diabetic + *Morus rubra*	2.04 ± 0.16***	2180 ± 70.9***	2.87 ± 0.19***	34.9 ± 1.67**	1.23 ± 0.06***	134 ± 9.1*	7.15 ± 0.32**

MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione. Data are expressed as mean ± SEM of five rats in each group and evaluated by one-way analysis of variance followed by Tukey's test. $^{+}P < 0.001$ compared with normal rats; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with diabetic control rats.

Table 5 Effect of aqueous extract of *Morus rubra* leaves on liver and kidney function tests during acute oral toxicity study in normal rats

Group	Dose (mg/kg)	Liver function tests		Kidney function tests	
		SGPT (IU/ml)	ALP (IU/ml)	Urea (mg/dl)	Creatinine (mg/dl)
Control	n.a.	56.2 ± 3.0	210.0 ± 10.0	38.2 ± 2.3	1.08 ± 0.04
Treated	2000	55.8 ± 2.3	209.8 ± 12.7	40.8 ± 3.3	0.95 ± 0.03
Treated	4000	50.4 ± 3.2	221.8 ± 13.3	33.0 ± 3.8	0.96 ± 0.04
Treated	6000	51.6 ± 2.7	205.2 ± 11.4	36.4 ± 1.9	1.02 ± 0.05

The doses of 2000, 4000 and 6000 mg/kg were 5, 10 and 15 times the effective dose, respectively. n.a., not applicable; SGPT, serum glutamate phosphotransferase; ALP, alkaline phosphatase. Data are expressed as mean ± SEM of five rats in each group and tested by one-way analysis of variance followed by Tukey's test. No significant difference was found between control and treated groups.

residual or remnant insulin production by the β-cells. The diabetic state in these rats is, therefore, not the same as that obtained by total pancreatectomy, as daily administration of insulin is not required for survival of STZ-induced diabetes. Further, the STZ-induced diabetic rats may exhibit most diabetic complications.[44]

The *M. rubra* aqueous leaf extract produced significant reduction in the blood glucose levels of diabetic rats. The anti-hyperglycaemic activity of *M. rubra* leaf extract could be due to its insulinogenic action, as increased levels of insulin and C-peptide were found in extract-treated diabetic rats. Hence, the extract was able to potentiate the release of insulin from pancreatic islets. Histopathological examination of pancreatic sections revealed an increased number of β-cells following treatment with the extract. This might mean that the extract produces its insulinogenic effect through the regeneration of β-cells. In this context, a number of plant extracts have been reported to have anti-hyperglycaemic activity through a stimulatory effect on insulin secretion.[45,46] Kedar and Chakrabarti[47] reported that treatment of diabetic animals with medicinal plant extracts resulted in activation of β-cells and granulation returned to normal, showing an insulinogenic effect.

During diabetes, the excess glucose present in the circulation reacts with haemoglobin to form HbA1c, a glycosylated haemoglobin. The estimation of HbA1c is a well-accepted parameter used in the prognosis of the diabetic state.[48] In this study, administration of the extract for three weeks significantly decreased the level of HbA1c in diabetic rats. As the amount of increase in HbA1c is found to be directly proportional to the FBG level,[49] this suggests that the fall in FBG following supplementation with the extract might be responsible for low levels of HbA1c in extract-fed diabetic rats. Extract-fed diabetic rats also showed signs of recovery in body weight. This might have been due to the improved glycaemic control produced by the extract.

Since lipid abnormalities and increased oxidative stress accompanied by atherosclerosis are the major causes of cardiovascular disease in diabetic patients, the ideal treatment for diabetes should have a favourable effect on the lipid profile and antioxidant system in addition to glycaemic control. In view of this, we investigated the effect of *M. rubra* leaf extract on lipids and the antioxidant system.

The altered lipid and lipoprotein profile (i.e. increase in TG, TC and LDL with fall in HDL) was significantly reversed after repeated administration of the extract for three weeks to the diabetic rats. Insulin insufficiency is responsible for the derangement of lipid and lipoprotein metabolism.[50] Insulin decreases TG levels and increases HDL through activation of lipoprotein lipase, an enzyme responsible for catabolism of TG-rich lipoproteins, which provide a significant portion of HDL.[51,52] Insulin also increases receptor-mediated removal of LDL-C and hence decreased activity of insulin during diabetes leads to increased levels of serum LDL-C and consequently hypercholesterolaemia.[53] This suggests that the significant control in the level of serum lipids in extract-treated diabetic rats might have been due to the improvement in the insulin level upon administration of the extract. On the other hand, decreased glucose disposal during diabetes leads to increased utilization of fatty acids for

energy production, which consequently results in increased formation of acetyl coenzyme A and thus of lipids. Hence, the increased insulin levels brought about by *M. rubra* extract indicates the possible extract effect on sensitizing tissues like skeletal muscles and adipose tissue for uptake of glucose and thus protecting lipid formation.

The increased TG and TC levels and decreased HDL are known factors associated with coronary heart disease (CHD).[54,55] The atherogenic index, TG/HDL, has been used to predict CHD and is a marker of small, dense LDL, which is an atherogenic lipoprotein.[56,57] As the extract produced a favourable effect on these factors, this suggests that it may help to prevent the progression of cardiovascular diseases. Recent studies suggest that TG itself is independently related to CHD[58,59] and that most of the anti-hypercholesterolaemic drugs do not decrease TG levels, but *M. rubra* leaf extract lowered the TG level to near normal after 21 days of treatment. Its strong effect on diabetic hypertriglyceridaemia could be through its control on hyperglycaemia. This is in agreement with the following facts: (1) the level of glycaemic control is the major determinant of TG and VLDL concentrations[60] and (2) improved glycaemic control following sulphonylurea therapy decreases levels of serum TG and VLDL.[61,62]

During diabetes, imbalance between enzymatic and non-enzymatic antioxidant defence and generation of free radicals leads to enhanced oxidative stress. This study also demonstrated increased oxidative stress, as demonstrated by a fall in GSH content and activity of antioxidant enzymes, along with a concomitant increase in lipid peroxidation. GSH, a natural antioxidant, is a potent scavenger of reactive oxygen species (ROS) and helps to maintain the structural and functional integrity of erythrocytes. Treatment with the extract increased the GSH level in both erythrocytes and liver of diabetic rats. SOD and CAT are two major antioxidant enzymes and are involved in the direct elimination of ROS.[63] Reduced activity of these free-radical scavenging enzymes may result in a number of deleterious effects due to accumulation of superoxide radicals and H_2O_2.[64] However, the activity of erythrocytic SOD and CAT and hepatic CAT was found to be partially restored following supplementation with the extract. Elevation in the GSH level and in the activity of SOD and CAT supports the notion that the extract may act as a free radical scavenging agent.

Furthermore, the protective effect of *M. rubra* extract on lipid peroxidation was demonstrated by the significant reduction in the levels of serum and hepatic MDA in extract-treated rats. Reduction of MDA, a by-product of lipid peroxidation, reflected the suppression in lipid peroxidation. Attenuation of lipid peroxidation may be due to blunting of free-radical-mediated lipid peroxidation of the membrane lipids, which in turn is supposed to be due to reduction in ROS formation or increased scavenging of them. This hypothesis can be supported by increased GSH level and upregulation of activity of antioxidant enzymes in diabetic rats following treatment with the extract.

The restoration of altered lipid peroxidation and anti-oxidant defence system following treatment with *M. rubra* extract could be primarily due to the subsequent lowering of blood glucose levels. It has been postulated that free circulating glucose is the proximal source of increased oxidative stress in the hyperglycaemic condition.[65] Glucose has been demonstrated to undergo autoxidation, generating oxygen free radicals. Autoxidation of glucose is directly linked to protein glycation, which is another source of free radicals.[66] Thus, the lowering of blood glucose levels would prevent the formation of ROS and oxidative stress. In addition, it might be due to direct impact of the extract on the antioxidant defence system and pathways leading to free radical generation. In this context, Kaviarasan *et al.*[67] reported that a polyphenol-rich extract from seeds of fenugreek protects diabetic human erythrocytes against hydrogen peroxide-induced oxidative stress.

The anti-hyperglycaemic effect of *M. rubra* aqueous leaf extract was comparable with that of glibenclamide. However, the hypolipidaemic and antioxidant effects of the extract were found to be more potent than those of glibenclamide. Therefore, it can be suggested that the extract produces its lipid-lowering and antioxidant effect not only through glycaemic control but also imparts some direct or indirect effect in controlling lipids and improving antioxidant defence systems.

The rats given a single dose of up to 6 g/kg of the extract survived and appeared active and healthy. No signs of abnormal behaviour, adverse biochemical toxicological modification in liver and kidney or mortality were observed during a 14-day post-treatment period. Therefore, the extract can be considered relatively safe up to the dose of 6 g/kg.

Conclusions

In conclusion, the aqueous extract of *M. rubra* leaves exhibits a potent anti-hyperglycaemic effect in addition to anti-hyperlipidaemic and antioxidant activity. This has clinical implications, in that the relatively non-toxic *M. rubra* extract, if used as an anti-hyperglycaemic agent, may also reverse dyslipidaemia as well as associated complications of diabetes through its antioxidant action. The study also provides support for its ethnomedical use and its relation with the ethnopharmacology of the species.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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